



Selenium Seed Priming Adjusts Photosynthesis, Metabolic Constituents and Gene Expression Profiling in *Vicia faba* L to Outstand Lead Stress

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Abstract

Selenium (Se) is necessary for plants in trace amounts, whereas lead (Pb) is a hazardous that hinders their growth. To fill a gap in the existing literature, we intentionally subjected plants to 50 mM Pb concentration to investigate their immediate molecular, physiological, and biochemical responses, as well as the potential of Se seed priming to mitigate the anticipated adverse response. *Vicia faba* seeds were primed by soaking in a 100 μ M Se solution for 12 h or in water for control. The two-week-old plants experienced a transient stress for three successive days by Pb foliar spray (50 mM). The molecular, physiological, and biochemical responses of the four-weeks-old plants were examined. The short-term exposure to 50 mM Pb *via* foliar spray resulted in minimal visible phytotoxic effects; however, decreased growth parameters were observed, indicating that while acute toxicity is not apparent, physiological stress exists. This was evidenced by a marked reduction in photosynthetic pigment content and capacity, alongside an increase in stress markers: malondialdehyde (48.56%), H₂O₂ (68.11%), electrolyte leakage (22.36%), and lipoxigenase (35.43%). Se priming significantly reduced all these stress markers. Under Pb stress, Se priming resulted in significant increases in shoot length (20.5%), fresh weight (31.3%), and leaf area (27.6%), along with enhancements in gas exchange parameters, including photosynthetic rate (68.2%) and chlorophyll a and b contents (17.5%). Furthermore, it significantly enhanced the levels of antioxidant compounds by increasing total phenolics (16.6%), proline (28.3%), glycine betaine (10.9%), total thiol (13.6%), and total antioxidant capacity (33.3%) compared to Pb stress alone. Significant upregulation of stress-signaling genes was observed in Se-primed plants exposed to Pb stress, specifically in the ribulose-bisphosphate carboxylase oxygenase, P-type plasma membrane H⁺-ATPase, and calcium-dependent protein kinase. Additionally, Se seed priming reduced Pb translocation by lowering the translocation factor to 0.57 from 0.7 in non-Se-primed Pb-stressed plants. This experimental approach illuminates the rapid physiological defense mechanisms, transcriptional response of certain stress and signaling genes, and resilience to Pb stress *via* Se seed priming to support plant development in Pb-contaminated environments.

Key Message

Se priming increased defensive compounds, antioxidant synthesis, and stress signaling genes such *rbcl*, PM H⁺-ATPase, and CDPK to reduce Pb stress and boosts plant growth and photosynthetic capacity.

Keywords Calcium-dependent protein kinase · P-type plasma membrane H⁺-ATPase · Pb toxicity · Ribulose-bisphosphate carboxylase oxygenase gene · Selenium · Stability index · Stomatal conductance

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Abbreviations

APX	ascorbate peroxidase
CDPK	Calcium-dependent protein kinase
CAT	catalase
EC	electrical conductivity
EL	electrolyte leakage
A/gs	intrinsic water-use efficiency

LOX	lipoxygenase
MDA	malondialdehyde
MSI	membrane stability index
A	net photosynthetic rate
PM H ⁺ -ATPase	P-type plasma membrane H ⁺ -ATPase
POX	peroxidase
PPO	polyphenol oxidase
ROS	reactive oxygen species
rbcl	Ribulose-bisphosphate carboxylase oxygenase large subunit
gs	stomatal conductance
SRI	stress resistance index
TAC	total antioxidant capacity
ci/ca	the ratio of internal to atmospheric CO ₂ concentration
E	transpiration rate

1 Introduction

Throughout the globe, legume crops are cultivated as a sustainable source of high-protein food. Faba bean (*Vicia faba* L.) is the third most significant fodder grain legume cultivated on a global scale after soybean (*Glycine max*) and pea (*Pisum sativum*) (FAOSTAT 2024; Mínguez and Rubiales 2021). It is an exceptional source of minerals, carbohydrates, cellulose, vitamin C, and protein, with approximately 25% of its content in desiccated seeds (Dhull et al. 2022). Despite its nutritional and agricultural importance, faba bean production is confronted with a variety of environmental challenges, such as heavy metal stress that resulted from the drastic increase in industrialization and urbanization led to substantial growth in pollutants globally. These pollutants can have an adversely substantial impact on crop yield and quality. Consequently, it is imperative to investigate and comprehend the effects of these pollutants on this significant legume crop. A robust correlation was observed between the specific metabolic reactions observed and the type of metal, as well as its concentration (Sumalan et al. 2023). Lead (Pb) is one of the most prevalent and highly hazardous heavy metals due to its persistence in soil and lack of natural remediators (Collin et al. 2022). The accumulation of Pb in plant tissues results in a variety of physiological processes, such as the inhibition of electron transport during photosynthesis and respiration, alterations in cell membrane permeability, water stress and disruptions in cell division (Collin et al. 2022; Gupta et al. 2024). One of the visible symptoms of Pb toxicity in plants is growth inhibition and leaf chlorosis (Abdelhameed et al. 2024a). Research indicated that high concentrations of Pb contamination in soil (800 mg Kg⁻¹) adversely affected maize growth (Wyszkowska et al. 2024), while a concentration of 300 mg Kg⁻¹ diminished

the growth of *Aegilops tauschii* (Wang et al. 2024a, b). The Pb toxicity in the soil significantly alters the diversity and metabolic functions of plants, resulting in the production of reactive oxygen species (ROS) and stress markers such as H₂O₂, [•]OH, and O₂^{•-}, malondialdehyde (MDA), and electrolyte leakage. In an effort to adjust to Pb stress, plants can produce antioxidants that are both enzymatic (e.g., catalases and peroxidases) and non-enzymatic (e.g., glutathione, phenolics, and carotenoids) (Gupta et al. 2024).

The primary mechanisms for the management of heavy metals in plants are purification, transportation, and sequestration. Recent research has revealed that plants respond to heavy metal stress by undergoing heritable modifications in gene expression, which lead to gene-specific stress responses (Cong et al. 2019). The stress response of a plant is fundamentally influenced by these rapid and efficient changes in gene expression, which are mediated by complex intracellular signaling networks that convert extracellular stimuli into cellular responses. These signaling cascades enhance the resilience of plants to applied stressors by modulating downstream targets. Metallic transporters, calcium signaling pathways, mitogen-activated protein kinase (MAPK) cascades, and hormone signaling are the most important signaling components in this process (Jalmi et al. 2018). Heavy metals are responsible for encouraging the production of ROS as well as the accumulation of calcium, which subsequently activates calcium-dependent protein kinases (CDPKs) and MAPKs (Jalmi et al. 2018). The adaptation of morphological and biochemical characteristics is necessary for plants to ensure their survival and reproductive success in the presence of stress. Molecular techniques can be employed to identify and quantify these adaptations. Although the molecular changes (Cao et al. 2018) and biochemical responses (Mroczek-Zdyrska et al. 2017; Layachi et al. 2018) of plants to Pb and Se have been extensively studied, the precise effects of Se priming on the expression of signaling coded genes under Pb stress remain to be elucidated. Practically, the detrimental effects of Pb toxicity on plants can be mitigated through eco-friendly technological approach, including chelation and biological strategies (Gupta et al. 2024).

Selenium (Se) is a potential antagonist of a variety of heavy metal toxicities, including Hg, Pb, Cd, Zn, Cu, and Ag (Ul Hassan et al. 2019; Abdelsalam et al. 2025). Se is not a critical element in the growth of plants; however, it is absorbed and metabolized by plants in their tissues, resulting in improved growth and resistance to environmental stimuli. Multiple studies have indicated that Se has a beneficial impact on antioxidant properties at low concentrations. This effect is achieved by enhancing the assimilation pigment, maintaining the integrity of cell membranes, and rebuilding chloroplasts, thereby promoting plant growth

and development and alleviating oxidative stress (Abdel-salam et al. 2025). Se's impact on plants is contingent upon its concentration in the nutrient solution and its chemical form. Ulhassan et al. (2018) demonstrated that selenite exhibited antioxidant properties at low concentrations (25 μM) in *Brassica napus*, but it exhibited prooxidant properties at higher concentrations (100 μM). By limiting the absorption and translocation of heavy metal within plant tissue, Se can alleviate this stress, thereby influencing the total heavy metal content. The biosynthesis of phytochelatins is facilitated by Se fertilization. Due to their abundance of thiol groups, these phytochelatins exhibit a high affinity for heavy metals and chelate with them, thereby decreasing their transfer coefficient in plants (Jia et al. 2025). Layachi et al. (2018) demonstrated that 50 mM Pb decreased chlorophyll content and increased lipid peroxidation synthesis in *V. faba* that were cultivated on the Hoagland medium for two weeks. The negative effects of Pb were reversed by the addition of Se in the form of selenate at 1.5 and 6.0 M. This was accomplished by significantly increasing the antioxidant enzyme activities in roots and shoots, reducing the accumulation of lipid peroxidation, and increasing the pigment content to mitigate Pb stress.

Although the effects of persistent soil Pb contamination have been extensively studied, there is still a lack of comprehension regarding the physiological consequences of transient, high-concentration foliar Pb exposure and the potential protective mechanisms that may be induced by Se seed priming in plants. As a result, the purpose of this study is to address this critical knowledge gap by examining the response of *V. faba* to an acute, high-concentration Pb challenge (50 mM) following Se seed priming (100 μM). The morphological, physiological, and biochemical responses of the plants were investigated. This research also concentrates on the possibility of molecular changes that underlie the resilience to Pb stress induced by Se-seed priming. This is achieved by analyzing the changes in gene expression of specific stress-signaling-related genes, such as Ribulose-bisphosphate carboxylase oxygenase (rbcL), P-type plasma membrane (PM) H^+ -ATPase (PM H^+ -ATPase), and Calcium-dependent protein kinase (CDPK5). The results of this study will provide valuable insights into the potential strategies for enhancing the resilience of plants to heavy metal stress, specifically Pb.

2 Materials and Methods

2.1 Plant Material and Experimental Layout

Seeds of the *V. faba* L. cultivar “Giza 716” were acquired from the Crop Institute, Agricultural Research Center, Giza,

Egypt, and subsequently planted following the acquisition of authorization. The seeds were divided into two groups, with each group consisting of six separate pot replicas. Group 1 seeds were immersed in distilled water for 12 h, whereas group 2 seeds were submerged in a solution of 100 μM sodium selenite (Na_2SeO_3). Each pot containing five soaked seeds was sown in clay soil (pH: 7.5) gathered from the garden at Helwan University. The containers were irrigated biweekly with tap water in equal quantities for two weeks. Half of the pots in group 1 (3 replicas) were foliar sprayed with 50 mM lead acetate on three consecutive days and labeled as “Pb”. While the other half was sprayed with distilled H_2O and labelled “Control”. Similarly, the plants of group 2 were treated, in which the 3-replicas sprayed with Pb was labelled “Se+Pb”, the other 3 replicas sprayed with distilled H_2O were labelled “Se”. After treatment, the plants were watered as described above till the plant aged 4 weeks, then imaged, and samples were collected for further analysis. Figure 1 shows a diagrammatic representation of the experiment timeline (Fig. 1a) and different treatment groups (Fig. 1b).

2.2 Data Recording and Related Procedures

2.2.1 Agronomic Traits

The collected plants of four weeks old were separated into shoots and roots that were measured with a graduated ruler to determine their lengths. Then, shoots and roots were weighed to record their fresh weights. The collected plant parts were oven-dried at 65 $^{\circ}\text{C}$ for two days until their weights were constant, after which their dry weights were determined. The leaf area of the 3rd leaf from the shoot apex was imaged, and their area was calculated using ImageJ software (Abramoff et al. 2004). Moreover, stress resistance indexes (SRI), of each growth parameter were calculated.

$$SRI = \frac{\text{value of stressed plants}}{\text{value of control plants}} \times 100$$

2.2.2 Quantitative gene expression analysis

2.2.2.1 RNA Extraction, DNase Treatment and cDNA Preparation Leaf tissues were collected from each group and finely ground in liquid nitrogen then the total RNA was extracted using Zymo Direct-Zol RNA Miniprep kit (Zymo Research, R2050) according to manufacturer's instruction. The isolated RNA was DNase treated to remove any DNA contaminant using DNase I (ThermoScientific cat # EN0521). Successful DNase treatment was confirmed by the absence of any PCR product after 39 cycles using rRNA gene primers and DNase-treated RNA as a template. 2 μg of

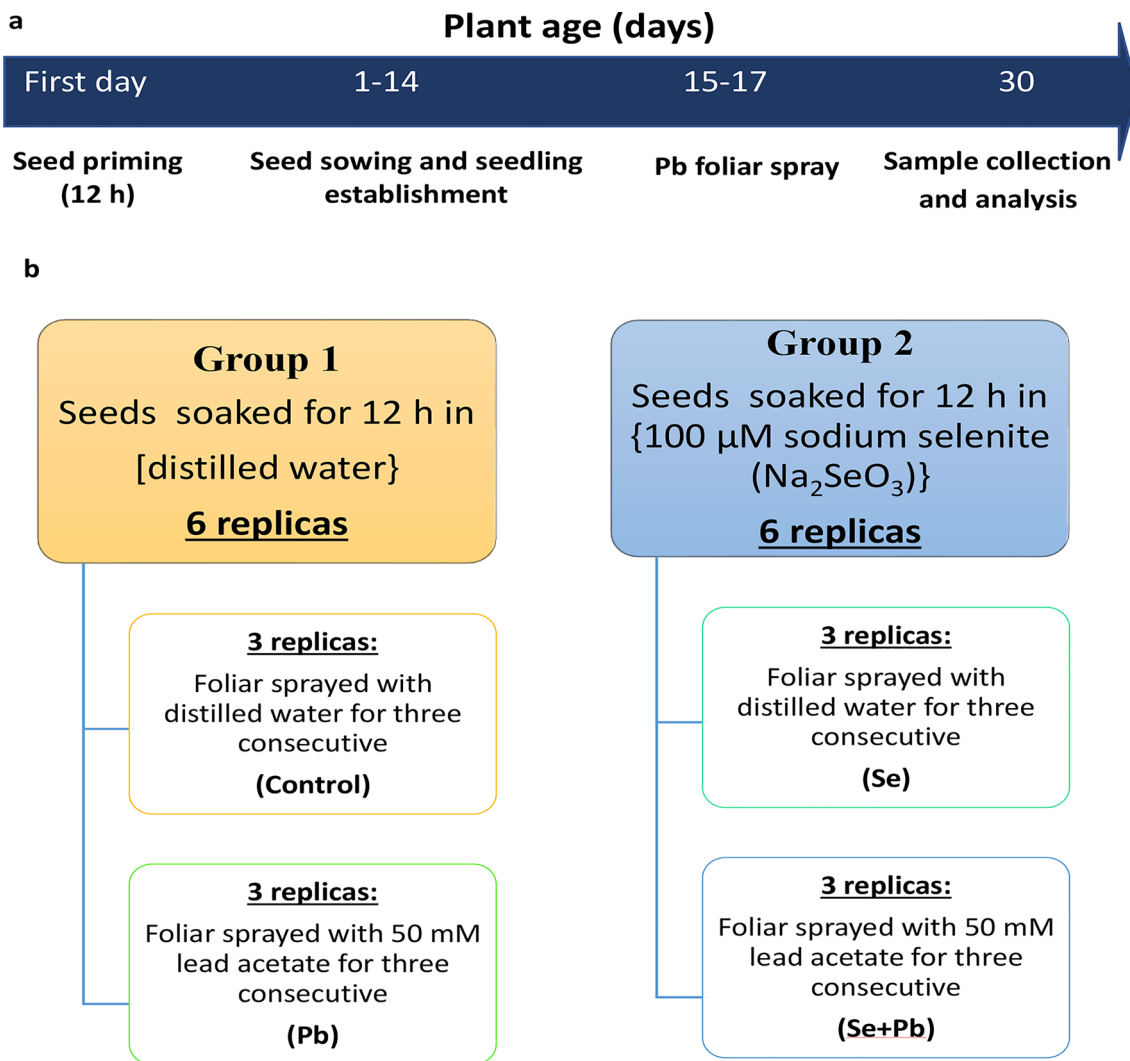


Fig. 1 Diagrammatic representation of the experimental layout. **(a)** Timeline outline of the experiment duration. The experiment passes through 4 stages: stage 1 for 12 h of seed priming with 100 μ M Se, stage 2; seed sowing and seedlings establishment for 14 days, stage 3; stress application by 50 mM Pb foliar spray for 3 consecutive days, stage 4; continuous regular irrigation with tap water till the plant aged 30 days, then the experiment terminated and samples collected for analysis. **(b)** The experimental groups and treatments. The experiment comprises 4 different groups; each has three replicas: control (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plants)

DNase-treated RNA was intended for cDNA synthesis with SuperScript™ II reverse transcriptase and oligo (dt)₁₈ primers (cat.# 18064-014, Life technologies-Invitrogen), following the manufacturer's instructions.

2.2.2.2 Quantitative Real-Time PCR (qPCR) HERA PLUS SYBR green qPCR master mix (cat# 600882, Agilent Technologies-Stratagene) and PCR was achieved using a QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA) with cycling parameters: 95 °C (2 min) then 39 cycles of 95 °C (15 s), and 60 °C (20s). Each reaction was performed in duplicate using rRNA gene as an internal control. The gene sequences (Ammar et al. 2017) and their IDs

that were used are scheduled in Table 1. The expression differences were assessed by the comparative Ct method using $\Delta\Delta C_t$ according to (Livak and Schmittgen 2001).

2.3 Physiological Traits

2.3.1 Leaf Chlorophyll and Carotenoid Determination

As demonstrated by Metzner et al. (1965), faba bean leaf tissues were crushed in cooled acetone (85%) before being tested for carotenoids, chlorophyll a, and chlorophyll b, which were reported as mg g⁻¹ fresh weight.

Table 1 Primers' list used for quantitative gene expression analysis in *V. faba* plants using real-time PCR

Description	F-primer Sequence (5'→3')	R-primer Sequence (5'→3')
Ribulose-bisphosphate carboxylase oxygenase large subunit (rbcL) gene	ATTCGCAAATCT TCCAGACG	GAGCCTGTTC CTGGAGAAGA
P-type plasma membrane (PM) H ⁺ -ATPase	ACTGGCCATAG CAAAGGAGA	GAAATACCCC AGCAAATCCA
Calcium-dependent protein kinase sk5-like	CTCTGCACACA CAACCCAAC	GTCCCATGGA TCCTGACAAC
rRNA encoding gene	CTTGCAGTCAA GCTCCCTTC	CCTTGTCCTCA AGACAGACCA

2.3.2 Quantification of Photosynthetic Rate and Gas Exchange Characteristics

Gas exchange parameters and photosynthetic rate of the plants were performed in vivo using an LC pro-SD infrared gas analyzer at the Ecology laboratory, Faculty of Science, Helwan University, Egypt. The measured parameters include leaf net photosynthetic rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intrinsic water use efficiency (WUE_i) (A/g_s , $(\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O})$), and the ratio of internal to atmospheric CO_2 concentration (ci/ca).

2.3.3 Index of Membrane Stability (MSI)

Following Sairam et al. (2005) protocol, four leaves were collected from each treatment, sliced, and placed into two sets of tubes, each having 30 mL of double distilled water. After 4 h of shaking at 25°C, the electrical conductivity (EC1) of the first set of samples was measured with an Oakton PC2700 conductivity meter. After autoclaving the second set for 20 min at 121°C to extract all the ions from the tissues, it was cooled to 25°C, and the conductivity (EC2) was assessed. The MSI were given using the formula:

$$\text{MSI} = [1 - (\text{EC1}/\text{EC2})] \times 100$$

2.4 Metabolic Traits

2.4.1 Soluble Protein

A spectrophotometer was used to measure soluble protein using bovine serum albumin as standard at 750 nm, following the procedure outlined by Lowry et al. (1951), then the protein content was given as mg g^{-1} fresh weight.

2.4.2 Total Free Amino Acids

Using the ninhydrin procedure (Lee and Takanashi 1966), fresh leaves of the bean plant were extracted in ethanol (80%) to determine the free amino acids. To calculate free amino acids (mg g^{-1} fresh weight), a glycine standard curve was developed.

2.4.3 Stress Markers Determination

The total amount of inorganic ions that seeped out of the leaves (electrolyte leakage, or EL) was calculated using the technique developed by Sullivan and Ross (1979). To record ECa, ECb, and ECc, EL was measured in a solution of 20 leaf discs (0.5 cm) treated to ambient temperature, 45°C–55°C for 30 min, and 100°C for 10 min respectively.

$$\text{EL (\%)} = [(\text{ECb} - \text{ECa}) / \text{ECc}] \times 100$$

Using the Hodges et al. (1999) approach, the content of MDA was used to determine the amount of lipid peroxidation. Using the extinction coefficient of $155 \text{ mmol}^{-1} \text{ cm}^{-1}$, the MDA concentration was computed and reported as nmol g^{-1} fresh weight.

The H_2O_2 content was measured using Velikova et al. (2000) methodology. Each sample's H_2O_2 content was calculated as mg g^{-1} fresh weight by comparing its absorbance at 390 nm to a standard calibration curve.

According to Boyes et al. (1992), lipoxygenase (LOX) activity was determined by observing the shift in absorbance at 234 nm after linoleic acid was oxidized.

2.4.4 Enzymatic and Non-Enzymatic Antioxidants

2.4.4.1 Free Proline and Glycine Betaine Content Free proline was measured using the procedure stated by Bates et al. (1973). The absorbance was conducted at 520 nm, and by using a standard curve for L-proline, the proline was stated as $\mu\text{mol g}^{-1}$ fresh weight.

Based on the protocol of Senthilkumar et al. (2021), glycine betaine was extracted in 2 N H_2SO_4 then the extract was heated in a water bath at 60 °C for 10 min to extract the quaternary ammonium compounds. Using cold KI-I_2 , crystals of glycine betaine were formed attached to the wall and bottom of the tube, which were dissolved by 1, 2-dichloroethane, and the absorbance was computed at 365 nm, and the concentration was given as $\mu\text{g g}^{-1}$ fresh weight.

2.4.4.2 Total Phenolics The Folin-Ciocalteu reagent was utilized in the colorimetric approach (Jindal and Singh 1975) to determine the total phenolic component content of

fresh leaves. After measuring the absorbance at 650 nm, the data were reported as ($\mu\text{g g}^{-1}$ fresh weight), using the gallic acid curve as a reference.

2.4.4.3 Determination of the Total SH Content Using Ellman's reagent, the total content of SH groups was ascertained, following the methodology outlined by Sedlak and Lindsay (1968). The total SH groups were identified using an extinction coefficient of 13,600 and reported as mg g^{-1} fresh weight.

2.4.4.4 Enzymatic Antioxidant Determinations In order to extract antioxidant enzymes, leaf samples (1000 mg fresh weight) were crushed in 10 mL of 50 mM potassium phosphate buffer (pH 7.8). The homogenate was centrifuged for 15 min at 4 °C at 8000 rpm. Supernatant was gathered for catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and polyphenol oxidase (PPO) tests. CAT activity was carried out in fresh supernatant (0.5 mL) in accordance with Aebi's (1984) instructions. The procedure proposed by Maehly and Chance (1954) was used to measure POX activity. At 290 nm, the activity of APX was measured using the Nakano and Asada (1981) technique. The purpurogallin synthesis was tracked, and the absorbance was recorded at 495 nm using the Lavid et al. (2001) approach to determine the activity of the enzyme PPO.

2.4.4.5 Total Antioxidant Capacity The total antioxidant capacity (TAC) of methanolic extracts of fresh leaves under all treatments was determined using the phosphomolybdenum procedure (Prieto et al. 1999).

2.4.5 Activity of Phenylalanine Ammonia Lyase (PAL)

Phenylalanine was used as a substrate to assess the PAL activity of leaf extract, according to McCallum and Walker (1990).

2.5 Quantification of Pb Concentration in Plant Tissue

The plant tissue was prepared to quantify Pb concentration using the recommended protocol of Ukpebor et al. (2010). Half a gram of four-weeks-old dry plant tissue was ashed at 550°C in a muffle furnace for 3 h and then digested overnight with 10 mL nitric acid (2.8%). Utilizing ultra-pure distilled water, the sample volume was increased to 50 mL. The Agilent 4210 MP-AES (Microwave Plasma-Atomic

Emission Spectrometer, Agilent Inc.) was used at the Ecology Laboratory, Faculty of Science, Helwan University, Egypt, to measure the content of Pb in the plant tissue.

2.6 Statistical Data Analysis

The data gathered for all attributes were analyzed statistically by one-way analysis of variance (one-way ANOVA) followed by Duncan test to reveal the effects of Se and Pb on *V. faba* plants using SPSS program. The Pearson correlation among various agronomic, metabolic, and molecular traits was investigated using multivariate analysis. Hierarchical cluster analysis between the four different treatments (control, Se, Pb and Se+Pb) was designed using Past program.

3 Results and Discussion

3.1 Se Seed Priming Enhances Agronomic Traits and Membrane Stability Under Pb Stress

As shown in Fig. 2; Table 2, the application of a foliar spray containing 50 mM of Pb to bean plants led to a considerable reduction in all agronomic characteristics, including the lengths of the shoots and roots (Fig. 2a and b), the fresh and dry weights, and the leaf area. When compared to

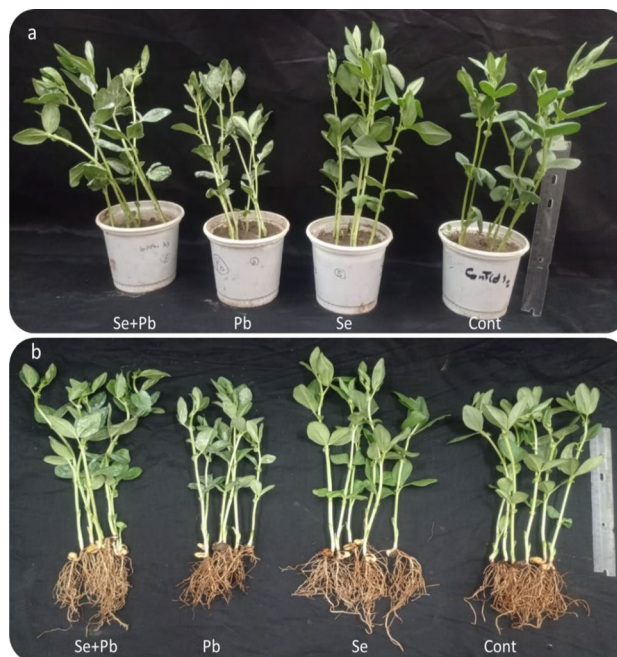


Fig. 2 Effect of Se seed priming and/or Pb stress on the morphology of *V. faba* plants. **(a)** Showing the effect on shoot length **(b)** Showing the effect on both shoots and roots. The experiment comprises 4 different groups; each has three replicas: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plants)

Table 2 Changes in growth traits of *V. faba* plants as a result of Se priming under Pb stress. The experiment comprises 4 different groups; each has three replicates: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plant). Data are the mean $\pm$ standard error of 3 replicates. Within each column, different small letters indicate significant differences among treatments using a one-way ANOVA followed by the Duncan's test ($p < 0.05$)

	Shoot length (cm)	Root length (cm)	Shoot FW (g)	Root FW (g)	Shoot DW (g)	Root DW (g)	Leaf area (mm)	MSI (%)
Control	25.2 $\pm$ 0.666ab	12.8 $\pm$ 0.338b	3.738 $\pm$ 0.098a	1.512 $\pm$ 0.04b	0.3956 $\pm$ 0.01a	0.139 $\pm$ 0.0037a	1547.84 $\pm$ 40.95b	33.33 $\pm$ 0.88b
Se	26.8 $\pm$ 0.709a	14 $\pm$ 0.37a	3.764 $\pm$ 0.099a	1.75 $\pm$ 0.046a	0.4168 $\pm$ 0.011a	0.15 $\pm$ 0.0039a	1741.22 $\pm$ 46.06a	50.00 $\pm$ 1.032a
Pb	20 $\pm$ 0.529d	8.6 $\pm$ 0.227d	2.604 $\pm$ 0.069c	0.9136 $\pm$ 0.024d	0.2481 $\pm$ 0.006c	0.102 $\pm$ 0.0027c	1004.53 $\pm$ 26.57d	17.07 $\pm$ 0.45d
Se+Pb	24.1 $\pm$ 0.637c	11.6 $\pm$ 0.307c	3.414 $\pm$ 0.091b	1.187 $\pm$ 0.031c	0.3238 $\pm$ 0.008b	0.116 $\pm$ 0.0028b	1282.84 $\pm$ 33.94c	27.03 $\pm$ 0.71c

plants that were not subjected to Pb stress, the shoot height and root length were both reduced by 20.6% and 32.8% on average, respectively. However, Se-primed plants grew better than control plants. Most interestingly that under Pb stress, the shoot height of plants that were primed with Se increased by 20.5%, while fresh weights increased by 31.1% and dried weights increased by 30.5%. In addition, the leaf area increased by 27.7%. While root length, fresh and dry weights increased by 34.8, 29.9, and 13.5%, respectively, compared to Pb stressed plants only. Remarkably, Se priming lessened Pb harm, as seen by upraising in SRI (stress resistance index values) of shoot, root, and leaf growth parameters (Fig. 3). The MSI of bean leaves significantly declined ($17.07 \pm 0.45d$) under Pb stress compared to the control ($33.33 \pm 0.88b$) (Table 2). This effect was alleviated in the Se primed bean plants, which had a high MSI value ($50.00 \pm 1.032a$) that is comparable with the control. Nevertheless, in the Pb-stressed plants, Se allowed the maintenance of a higher MSI ($27.03 \pm 0.71c$). Several studies (Saleem et al. 2018; Abdelhameed et al. 2024a) found reduced fresh and dried weights for the roots and

shoots of *Helianthus annuus* and *V. faba* plants exposed to Pb. This could be attributed to a deficiency of macro-elements (K, P, Ca, and Mg), as Pb exposure inhibited their assimilation, altered the permeability of the plasma membrane, and disrupted the ultrastructure of the chloroplast (Rahman et al. 2024). According to Jia et al. (2025), Se can modify the absorption and accumulation of other elements that are essential for plant metabolism, thereby improving the quality of nutrition. Through the enhancement of plant nutrition and antioxidant activity, Hussain et al. (2023) demonstrated that Se seed stimulation promoted the growth of salt-stressed *Brassica rapa* L. Hussain et al. (2020) demonstrated that the foliar application of Se mitigates the toxicity of both Cd and Pb in rice. As per a study conducted by Azizi et al. (2020), the development of *Satureja hortensis* plants grown under Cd stress was induced by trace amounts of Se up to 40 μ M. This was achieved by enhancing the proline content, stimulating CAT and POX enzymes, limiting cell membrane leakage, and improving chlorophyll content. Layachi et al. (2018) found that the production of lipid peroxidation increased and chlorophyll was diminished in *V. faba* that was grown on the Hoagland medium for two weeks after exposure to 50 mM Pb. At 1.5 and 6.0 M, selenate supplementation reversed the detrimental effects of Pb, where the pigment content was increased, lipid peroxidation was reduced, and the activity of root and shoot antioxidant enzymes was enhanced. Se seed priming alters gene expression levels to withstand Pb stress.

3.2 Se Seed Priming Alters Gene Expression Levels to Withstand Pb Stress

The *rbcL* gene encodes the major subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme, an essential enzyme that is necessary for the process of carbon fixation during photosynthesis. In contrast to the control group, the relative gene expression levels of *rbcL* decreased by 0.67-fold in Se-primed plants and 0.72-fold in Pb-treated plants, despite the absence of a statistically significant difference. This decline was accompanied by another substantial decrease in both chlorophyll content and net CO₂ assimilation rates in Pb-stressed plants. In contrast,

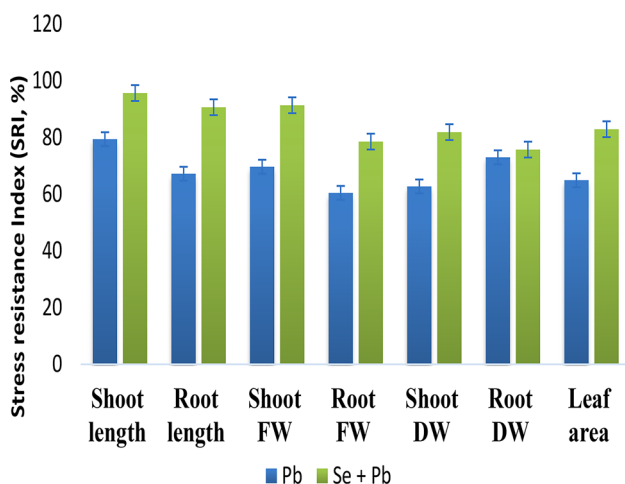


Fig. 3 Stress resistance index (SRI) of different growth parameters of *V. faba* plants under different treatments. (SRI), of each growth parameter were calculated as follow= {(Value of stressed plants)/(Value of control plants) $\times$ 100}. The experiment comprises 4 different groups; each has three replicates: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plant)

the relative expression of Pb-treated plants that were initially primed with Se increased substantially by 1.85-fold relative to the group that served as the control (Fig. 4a). This increase occurred along with an increase in chlorophyll content and net CO₂ assimilation rates of the plants. The results of *rbcL* gene expression suggested that Se priming may induce the upregulation of the RuBisCO gene in response to Pb stress. This gene is responsible for fixing an increased amount of CO₂, the purpose of which is to mitigate the detrimental effects of lead stress on chlorophyll concentration and the rate of photosynthesis. Ohmiya et al. (2014) and Martins et al. (2023) have previously validated the robust correlation between *rbcL* gene expression and chlorophyll content. The *rbcL* gene expression was employed as a reliable heavy metal contamination monitor in soil for Zn, Pb, and Cd. The response was specific to the applied concentration and plant species, as *rbcL* relative expression was downregulated in response to all heavy metal stresses (Jaskulak et al. 2018). The RuBisCO gene was downregulated in *Brassica chinensis* seedlings in response to Cd stress, as evidenced by Shaari et al. (2023). However, the expression of the gene was maintained at nearly the same level as the control when the plants were treated with a mixture of 100 ml/L *Sargassum polycystum* and *S. platensis* extracts. The expression of the *rbcL* gene in *Chlorella vulgaris* was observed to be concentration-dependent in response to Se. When compared to the control group, the gene's expression is upregulated by 1.76 and 0.86-fold at moderate and high concentrations of 10 and 100 µM, respectively, and downregulated by 0.36-fold at a concentration of 1 µM. Additionally, an increase in chlorophyll content and antioxidant stress indicators, such

as MDA, was observed in conjunction with gene expression. In the presence of 10 µM Se, glutathione levels increased, while a substantial decrease was observed in the presence of 100 µM Se (Ozakman et al. 2018). It is presumed that the increased expression of *rbcL* in Se-primed plants treated with Pb is a result of selenium's ability to enhance the antioxidant system of the plant and reduce the oxidative stress initiated by Pb stress. Excessive oxidative stress can compromise chloroplast integrity and reduce photosynthesis (Jaskulak et al. 2018).

Plasma membrane H⁺-ATPases (PM H⁺-ATPases) are membrane proteins that function as proton pumps, transporting protons across cell membranes through the process of ATP hydrolysis. They assist in the regulation of cytosolic pH, membrane potential, and secondary transport processes. In plants, PM H⁺-ATPases are essential for mineral nutrition, stomatal opening, cell elongation, and adaptation to environmental stresses (Zhang et al. 2017; Ding et al. 2022; Michalak et al. 2022). The present data indicate that P-type H⁺-ATPase was non significantly downregulated in response to Se and Pb independently. Conversely, plants that were primed with Se and stressed with Pb experienced a substantial increase (Fig. 4b). The downregulation of PM H⁺-ATPase activity is anticipated to disrupt physiological processes that rely on proton gradients. It is possible to effectively boost Al-resistance in faba bean (Chen et al. 2015) and soybean (Wang et al. 2016) by activating the PM H⁺-ATPase by its phosphorylation (Haruta et al. 2015), applying magnesium, IAA, or employing transgenic techniques. The amount of ATPase activity that is present in the cell membranes of cucumber and rice is reduced by

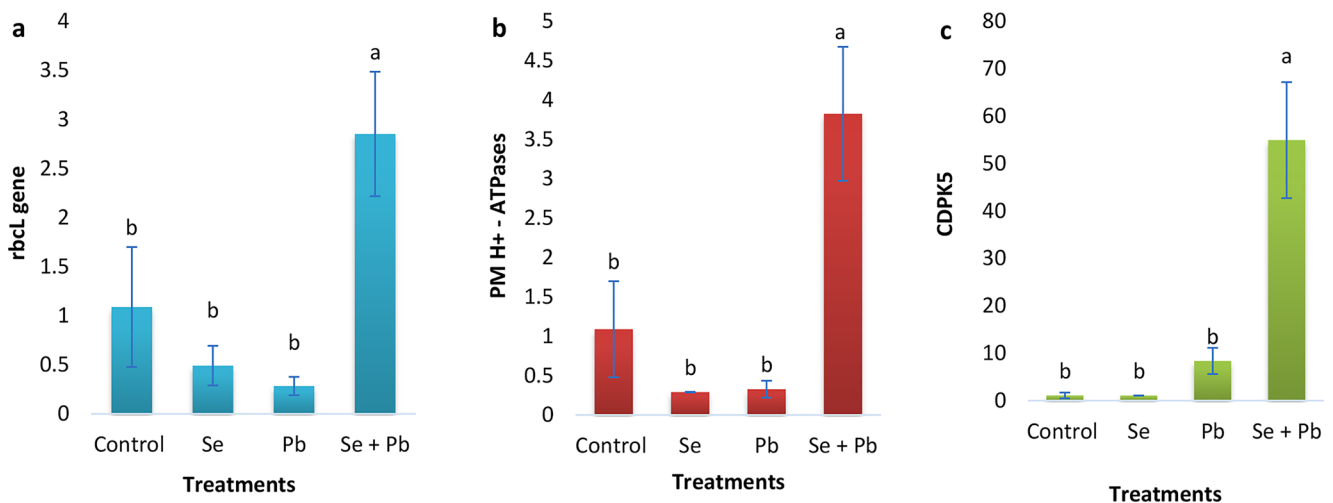


Fig. 4 Fold change in relative gene expression analysis of (a) large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme (*rbcL*), (b) P-type plasma membrane H⁺-ATPase gene (PM H⁺-ATPases) and (c) calcium-dependent protein kinase 5 (CDPK5). Data are the mean ± standard error of 3 replicas. The same letter above each column indicates no significant difference between the treatments as analyzed by a one-way ANOVA followed by Duncan's test ($p < 0.05$). The experiment comprises 4 different groups; each has three replicas: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plant)

Cd toxicity in plants, as demonstrated by Janicka-Russak et al. (2012) and Ding et al. (2022). The expression of the PM H⁺-ATPase gene in *Aeluropus littoralis* was reduced by 100 μ M Pb, Ag, and Hg treatments for varying durations (6, 48, and 72 h). Jam et al. (2014) proposed that the *A. littoralis* provides control over the H⁺-ATPase gene to manage its capacity for the removal of metals, specifically Pb and Hg. One of the heavy metal P-type ATPases, OsHMA4, is responsible for the prevention of copper accumulation in rice grains. According to Huang et al. (2016), this is accomplished by depositing copper in root vacuoles, which subsequently restricts the quantity of copper that accumulates within the grain. In a recent study, the activity of PM H⁺-ATPase in *V. faba* did not substantially change in response to 50 mg Kg⁻¹ Cr stress, but it was significantly upregulated in response to 10 mg Kg⁻¹, similar to the current finding. This suggests that the concentration adjustments are dose-dependent (Soliman et al. 2025). Se priming in Pb-treated plants reduced its uptake and accumulation in faba bean plants, as confirmed by the current data. This resulted in an upregulation of the PM H⁺-ATPase gene, which increased proton pumping activity and supported plant adaptation to Pb stress.

The CDPK5 gene expression was upregulated in response to Se priming and Pb treatment independently. Furthermore, plants that were simultaneously primed with Se along with the stress of lead exhibited a significant increase (Fig. 4c), indicating a potential synergistic effect of these treatments on the gene's regulation. These findings are consistent with prior research that has examined the involvement of protein kinases in response to the stress caused by heavy metals. For instance, the expression of genes that encode protein kinases, including DUF26, RLCK, and LRK10L-2, is induced by a variety of heavy metals, including Cr(VI), Cu, and Cd, as demonstrated by microarray data analysis and qPCR validation. Intriguingly, the function of CDPKs in the response to different heavy metal stress is consistent across a diverse array of plant taxa. For instance, chromium, lead, and arsenic have been reported to induce CDPK gene expression in rice seedlings (Huang et al. 2014; Trinh et al. 2014), in *Cucurbita pepo* leaves under nickel stress (Valivand et al. 2019), in the seedlings of *Setaria italica* and

V. faba that were exposed to chromium (Fang et al. 2014; Soliman et al. 2025).

The upregulation of CDPK5 may be essential for the activation of downstream targets through phosphorylation, including mitogen-activated protein kinases (MAPKs) like AtMPK3 and AtMPK6 (Kobayashi et al. 2007; Galletti et al. 2011). This activation has the potential to initiate a series of defense mechanisms, such as the biosynthesis of cell walls for the immobilization of Pb. The ability of the cell wall to bind heavy metal ions at locations that are negatively charged, including (COOH, OH, and SH), leads to modifications in its composition, which may contribute to metal tolerance (Lin et al. 2024). Additionally, seed priming with Ca²⁺ and NaHS has been demonstrated to improve physiological parameters and enhance CDPK upregulation in plants under Ni stress (Valivand et al. 2019). The observed upregulation of CDPK5 in our study may be a component of a more extensive signaling network that involves the influx of calcium and the subsequent activation of a variety of downstream targets. This network is likely to encompass various transcription factors, membrane channels, and NADPH oxidase; that is, all of it is crucial to the plant's adaptive response to the stress caused by heavy metal (Kobayashi et al. 2007; Jalmi et al. 2018).

3.3 Se Seed Priming Modifies the Photosynthetic Components to Withstand Pb Stress

Leaf chlorophyll contents of faba bean plants were greatly impacted by the application of Se and Pb, as displayed in Table 3. As shown in this table, the exposure of faba bean plants to Pb stress decreased all the measured plant pigments. Pb's detrimental effects on photosynthetic pigments are the primary cause of its detrimental effects on faba bean vegetative growth (Abdelhameed et al. 2024a). Noticeably, by using exogenous Se, this effect could be reduced. Exogenous Se application demonstrated notable rises in the chlorophyll a, b and carotenoids of 10.6, 21.7 and 7.4%, respectively, when compared with the control. Besides, Pb stress showed lessening effects for the evaluated photosynthetic parameters (Table 4), where the net photosynthetic rate (A), transpiration rate (E), water-use efficiency (WUE, A/gs) and stomatal conductance (gs) levels were reduced by

Table 3 Changes in chlorophyll (Chl) and carotenoid (mg g⁻¹ fwt) in *V. faba* plants as a result of Se priming under Pb stress. The experiment comprises 4 different groups; each has three replicas: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plant). Data are the mean $\pm$ standard error of 3 replicas. Within each column, different small letters indicate significant differences among treatments using a one-way ANOVA followed by the Duncan's test ($p < 0.05$)

	Chl a	Chl b	Total Chl	Carotenoids	Total pigments
Control	2.107 $\pm$ 0.0557b	0.840 $\pm$ 0.0222b	2.947 $\pm$ 0.0779b	1.489 $\pm$ 0.039a	4.437 $\pm$ 0.117b
Se	2.330 $\pm$ 0.0616a	1.022 $\pm$ 0.027a	3.352 $\pm$ 0.088a	1.599 $\pm$ 0.042a	4.951 $\pm$ 0.131a
Pb	1.718 $\pm$ 0.0454c	0.580 $\pm$ 0.015c	2.297 $\pm$ 0.061d	1.182 $\pm$ 0.031c	3.479 $\pm$ 0.092d
Se+Pb	1.874 $\pm$ 0.0496c	0.825 $\pm$ 0.0218b	2.699 $\pm$ 0.071c	1.352 $\pm$ 0.036b	4.051 $\pm$ 0.107c

Table 4 Changes in gas exchange parameters of *V. faba* plants as a result of Se priming under Pb stress. The experiment comprises 4 different groups; each has three replicas: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se + Pb (Se seed primed and Pb sprayed plant). Data are the mean $\pm$ standard error of 3 replicas. Within each column, different small letters indicate significant differences among treatments using a one-way ANOVA followed by the Duncan's test ($p < 0.05$)

	Stomatal conductance (gs, mmol H ₂ O m ⁻² s ⁻¹)	Photosynthetic rate (A, μmol CO ₂ m ⁻² s ⁻¹)	Water-use efficiency (WUE) (A/gs, μmol CO ₂ mmol ⁻¹ H ₂ O)	The ratio of internal to atmospheric CO ₂ concen- tration (ci/ca)	Transpiration rate (E, mmol H ₂ O m ⁻² s ⁻¹)
Control	7.630 $\pm$ 0.202b	1.818 $\pm$ 0.048b	0.239 $\pm$ 0.0063b	0.310 $\pm$ 0.0082c	0.7592 $\pm$ 0.02a
Se	8.771 $\pm$ 0.23a	2.282 $\pm$ 0.06a	0.260 $\pm$ 0.0068a	0.262 $\pm$ 0.0069d	0.7471 $\pm$ 0.0197a
Pb	6.913 $\pm$ 0.18c	0.784 $\pm$ 0.021d	0.113 $\pm$ 0.0029d	0.724 $\pm$ 0.019a	0.3412 $\pm$ 0.009c
Se + Pb	7.559 $\pm$ 0.21bc	1.319 $\pm$ 0.035c	0.175 $\pm$ 0.0046c	0.583 $\pm$ 0.015b	0.5708 $\pm$ 0.015b

56.8, 55.1, 52.7 and 9.4% relative to the control. However, the use of Se seed primed bean plants raised the level of photosynthetic pigments and gas exchange parameters more than the control plants where the percent increase for gs was 14.9%, 25.5% for A, and 8.8% for A/gs (Table 4). Under Pb stress conditions, Se seed priming increased A, E, and gs levels by 68.2, 67.3, and 9.3%, respectively. As a result, Pb stress dramatically reduced the level of photosynthetic traits in bean leaves compared to non-stressed plants, but Se application significantly mitigated this deleterious impact. Metal toxicity had several detrimental consequences on a plant's physiological characteristics, including the activity of photosynthetic enzymes, gs, A, and E, and chlorophyll functioning. In *B. oleracea*, gs and E were found to be reduced under Cd stress (Shah et al. 2020). By inhibiting RuBisCO's activity or by interacting with its thiol group, toxic metals decrease CO₂ fixation. The increased synthesis of tiny starch grains and bigger osmiophilic globules in Cd-treated plants causes damage to the chloroplast structure, resulting in the condensation of grana and lamella (Guo et al. 2016).

In line with this study, low Se raised the chlorophyll content of lettuce and sweet potato seedlings (Xue et al. 2001; Chen et al. 2022). The addition of Se effectively scavenges ROS, resulting in a rise in chlorophyll concentration. By safeguarding chloroplast enzymes and promoting the formation of photosynthetic pigments, low Se concentration can improve photosynthetic pigment biosynthesis (Hasanuzzaman et al. 2020; Jia et al. 2025). The physiology of photosynthetic plants is extremely vulnerable to environmental stresses, and Se can control environmental stresses in plants. Zhang et al. (2014) demonstrated that the Se application improved rice transpiration efficiency, intercellular CO₂ concentration, and photosynthetic rate. Huang et al. (2018) found that applying Se solutions to strawberry seedlings reduced the amount of chlorophyll degradation and the levels of MDA and H₂O₂ accumulation. This helped to preserve the stability of the photosynthetic apparatus and membrane system in plants, improve gas exchange parameters, and increase the seedlings' resistance to chilling. Se may take part in the synthesis of Se-amino acids, which

are precursors of chlorophyll (Abdelsalam et al. 2025). Se increased the production of carotenoids in faba bean plants under Pb stress in our experiment, a result that was also reported by Mroczek-Zdyrska et al. (2017) in the literature. Carotenoids are non-enzymatic antioxidant compounds that support plant resistance to environmental stimuli by smothering triplet chlorophyll, limiting peroxidation, and preventing the destruction of the chloroplast membrane (Wang et al. 2024a, b).

3.4 Se Seed Priming Affects Stress Markers of Pb Stressed *V. faba* Plants

The cell membrane, as a selective semipermeable membrane, serves as a crucial barrier to reduce or stop the entry of metals into cells. An overabundance of metals can disrupt cell homeostasis and affect the integrity of the plasma membrane (Rahman et al. 2024). As a result, damage to the plasma membrane identified by MDA creation can be used to identify metal toxicity and lipid peroxidation, and it is typically associated with a rise in ROS generation (Rahman et al. 2024). In this study, the stress markers were assessed in order to confirm the theory that Se treatments could reduce Pb-induced oxidative stress (Fig. 5). Our results showed that the electrolyte leakage (EL) increased under Pb stress (46.34 $\pm$ 1.226a) while in Se seed applied bean plant, EL decreased significantly (30.00 $\pm$ 0.793c) (Fig. 5a). Besides, The MDA (nmol/g fwt) and H₂O₂ (mg/g fwt) content decreased significantly in Se-applied plants (11.456 $\pm$ 0.29c and 4.946 $\pm$ 0.131d) compared to the control (12.48 $\pm$ 0.311c and 6.248 $\pm$ 0.165c) (Fig. 5b and c). Moreover, significant changes in the LOX activity were observed (Fig. 5d). Numerous stressors result in an excessive build-up of H₂O₂ and the generation of more ROS, which disrupt membranes and trigger lipid peroxidation (Abdelhameed et al. 2024b, 2025; Abdelhameed and Metwally 2024). Similar observation of increasing lipid peroxidation and H₂O₂ in the shoots of *V. faba* under Pb treatment was considered by Mroczek-Zdyrska et al. (2017); Abdelhameed et al. (2024a).

According to the study of Huang et al. (2018), the MDA and H₂O₂ contents were greatly reduced by Se treatment,

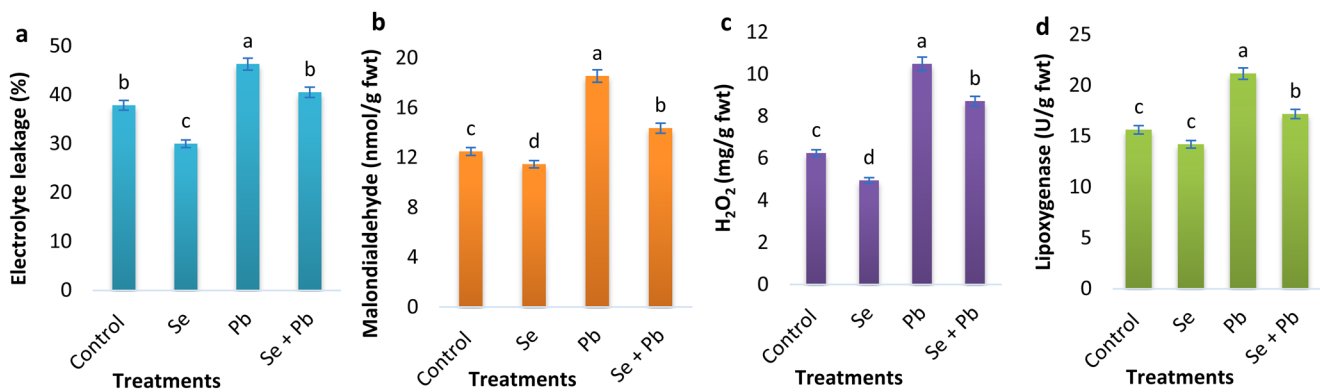


Fig. 5 Changes in stress markers (**a**: EL: electrolyte leakage, **b**: MDA: malondialdehyde, **c**: H₂O₂: hydrogen peroxide, and **d**: LOX: lipoxigenase) in *V. faba* plants as a result of Se priming under Pb stress. Data are the mean $\pm$ standard error of 3 replicates. The same letter above each column indicates no significant difference between the treatments as analyzed by a one-way ANOVA followed by Duncan's test ($p < 0.05$). The experiment comprises 4 different groups; each has three replicates: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plant)

which also shielded the plant membrane from stress. The concentration of H₂O₂ determines its binary (or dual) effects, as it is a crucial regulator for numerous processes related to growth, development, and stress protection. It organizes programmed cell death and causes oxidative stress at high concentrations, while at mild concentrations, it functions as a signaling molecule required for the tolerance mechanisms to biotic and abiotic stimuli. Following Se priming, a decrease in MDA, H₂O₂, and membrane damage was found, which is consistent with earlier findings of Djanaguiraman et al. (2010) and Habibi (2017). Another finding by Hasanuzzaman et al. (2020) revealed that greater chlorophyll and lower MDA concentration were caused by the modest dosage of Se employed in foliar spraying, which also reduced the H₂O₂ level. Most recently, Ahmad et al. (2025) stated that Se application helped chili plants to cope with higher levels of Cr stress by limiting lipid peroxidation and membrane damage. This protective action of Se could be explained by its ability to scavenge free radicals, improve physiological potential, and preserve proteins. These outcomes are explained by Se's antioxidative qualities, particularly at lower concentrations (Ul Hassan et al. 2018), where its supplementation is suggested to reduce excessive ROS production by strengthening the antioxidant defense system in stressed plants (Hasanuzzaman et al. 2020; Ahmad et al. 2025).

3.5 Protein, Glycine Betaine and Total Free Amino Acids as Affected by Se and Pb Application

Protein content in *V. faba* plants was much lower under Pb stress than they were in control plants (Fig. 6a). Conversely, total free amino acids revealed a distinct pattern

as Pb increased their contents in *V. faba* plants (Fig. 6b). Comparable findings were made by Sidhu et al. (2017), who found that *Coronopus didymus* L's protein content slightly decreased following exposure to Pb. An associated investigation by Bharwana et al. (2014) showed that the soluble protein content in the roots and leaves of the cotton plants was considerably decreased upon the addition of Pb at 50 and 100 μ M to the nutrition solution. This discovery may be clarified by a decrease in protein synthesis during Pb stress, Pb-induced protein degradation, or the breakdown of proteins by protease activity, which increases the degree of protein denaturation. Furthermore, it has been demonstrated that Pb stress in plants results in ROS generation, which directly modifies and alters proteins by oxidizing amino acid side chains (Mishra et al. 2006), perhaps causing protein breakage.

Clearly, under Pb stress as well as control conditions, Se efficiently improved the protein and glycine betaine contents. Under Pb stress conditions (Fig. 6c), Se priming showed a 27.6 and 12.2% of increase in protein and glycine betaine compared with the non-treated one. Our findings support the idea that the increased protein and glycine accumulation is a general adaptive mechanism that plants have developed to combat stressors and protect their structural integrity. Specifically, these mechanisms are thought to improve cell membrane integrity by lowering ROS and improving tissue water content through osmotic adjustment. In addition to maintaining membrane stability and cellular osmotic adjustment, glycine-betaine functions as an appropriate osmolyte, boosts antioxidant activity, and protects the function of the enzymes RuBisCO and photosystem II during photosynthesis (Masood et al. 2016).

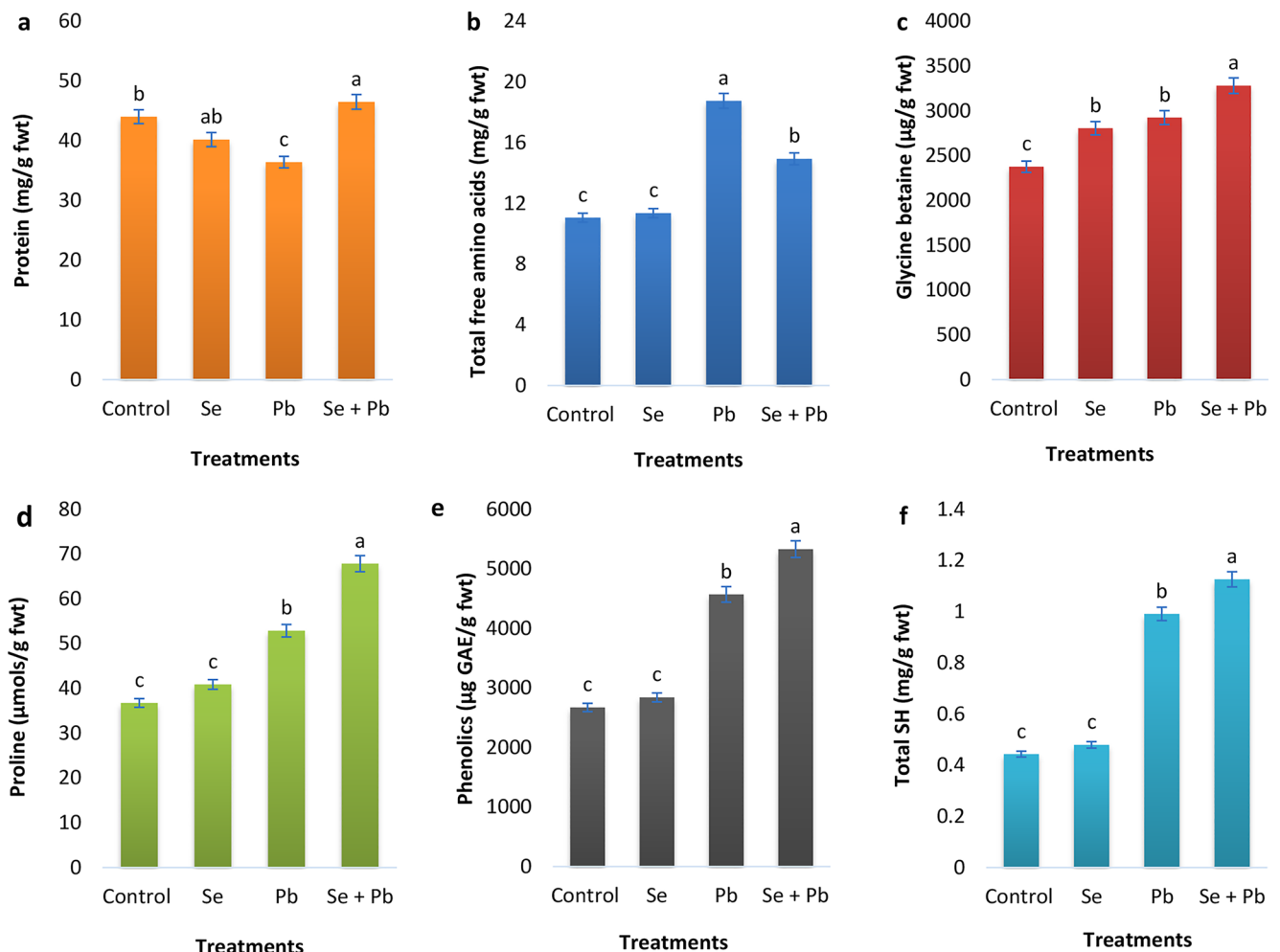


Fig. 6 Changes in biochemical traits in *V. faba* plants as a result of Se priming under Pb stress. (a) protein, (b) total free amino acids, (c) Glycine betaine contents, (d) proline, (e) phenolics and (f) total amount of sulfhydryl groups (Total SH). Data are the mean $\pm$ standard error of 3 replicates. The same letter above each column indicates no significant difference between the treatments as analyzed by a one-way ANOVA followed by the Duncan's test ($p < 0.05$). The experiment comprises 4 different groups; each has three replicates: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plant)

3.6 Non-Enzymatic Antioxidants as Affected by Se and Pb Application

Data in Fig. (6d and e) exhibited that the quantity of non-enzymatic antioxidants (total phenolics and free proline) was greatly raised in bean plants stressed by Pb where an upsurge of 71% for total phenolics and 43.9% for free proline was observed under Pb stress relative to control. Remarkably, Se seed priming caused further enhancement in total phenolics (99.5%) and free proline (84.6%) under Pb stress comparable to non-stressed plants. This improvement might be viewed as a way to shield plants from Pb since proline and phenolics act as non-enzymatic antioxidants. According to Khan et al. (2015), changes in proline and glutathione production resulted in a decrease in Cd-induced oxidative stress in wheat, because proline functions as an osmotic

regulator, a nitrogen storage material, a ROS detoxifier that maintains the stability of membranes, antioxidant enzyme activities, and protein structure. Elevated proline content after osmotic adaptability and intercellular macromolecule protection results from Se application (Ikram et al. 2024). Proline oxidation may have decreased, and its production increased, leading to this elevation (Hayat et al. 2012). Phenolics shield plant cells because they can function as water-soluble, non-enzymatic antioxidants. According to Ashraf et al. (2010), this feature is attained *via* quenching ROS and free radicals. Phenolics decrease oxidative stress and lessen its negative effects by preventing ROS formation and buildup. According to Raza et al. (2024), Se considerably reduced the negative effects of drought and enhanced the accumulation of phenolics in quinoa leaves.

3.7 Total SH, TAC, PAL, and Enzymatic Antioxidants as Affected by Se and Pb Application

As previously mentioned in the present study, Pb causes oxidative stress and the formation of ROS. Plants can effectively scavenge oxygen species to counteract the effects of Pb. This mechanism involves both non-enzymatic antioxidants and enzymatic antioxidants, such as peroxidases and catalases (Gupta et al. 2024). Faba bean plants have many enzymatic antioxidant mechanisms to safeguard them from the harm brought on by oxidative stress, which were analyzed, and the data were presented in Fig. (7). The findings in Figs. (6 and 7) demonstrated that total SH (Fig. 6f), TAC (Fig. 7a), and the appraised enzymes were elevated in faba bean under Pb stress. Relative to control, Pb stress affectedly boosted total SH, TAC, CAT (Fig. 7b), PPO (Fig. 7c), POX (Fig. 7d) and APX (Fig. 7e) activities by 54.1, 20.1, 32.6, 36.5, 35.8 and 41.7%, respectively. In divergence, priming

of *V. faba* plants with Se under Pb stress increased the total SH, TAC, and PPO activities by 13.6, 33.3, and 22.1% compared with those under Pb stress only. These results show that Se priming has a beneficial effect on enhancing Pb tolerance in *V. faba* plants *via* altering the antioxidant activities and detoxifying ROS. Since Se is a necessary component of antioxidant enzymes like glutathione peroxidase and thioredoxin reductase, its roles are mostly related to its antioxidant qualities. They play crucial roles in controlling the quantity of free radicals created during metabolic reactions, including reactive oxygen and nitrogen species, and scavenging them to prevent damage and oxidative stress to cells (Sentkowska and Pyrzynska 2019).

One typical plant response to Pb stress is an increase in the activity of antioxidant enzymes (Gupta et al. 2024). Additionally, applying foliar or soil-applied Se (Silva et al. 2018) enhanced the production of chlorophyll and photosynthesis, as well as the antioxidant capacity and yield of

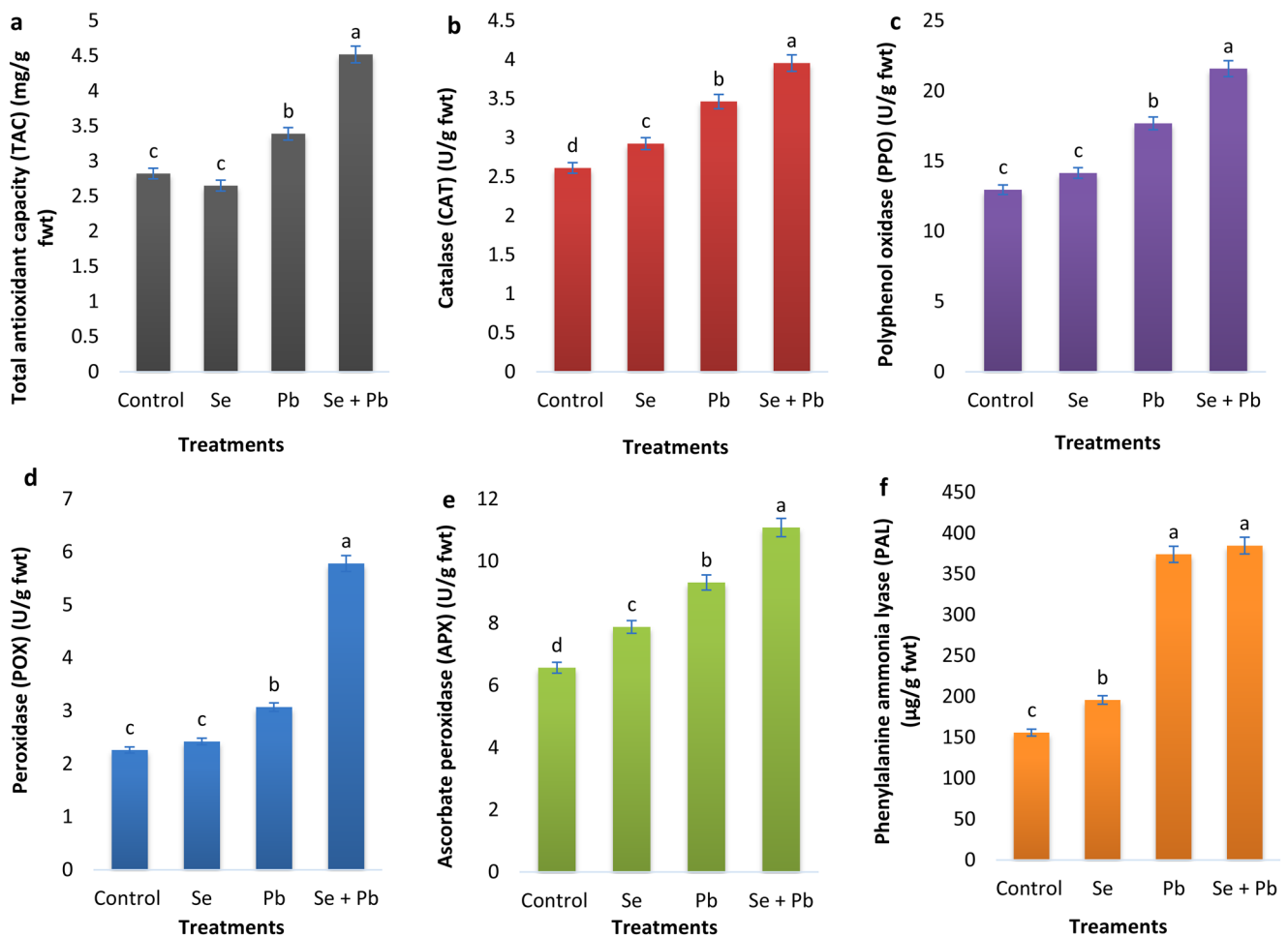


Fig. 7 Changes in (a) total antioxidant capacity (TAC), (b) catalase (CAT), (c) polyphenol oxidase (PPO), (d) peroxidase (POX), (e) ascorbate peroxidase (APX), and (f) phenylalanine ammonia-lyase (PAL) in *V. faba* plants as a result of Se priming under Pb stress. Data are the mean $\pm$ standard error of 3 replicates. The same letter above each column indicates no significant difference between the treatments as analyzed by a one-way ANOVA followed by Duncan's test ($p < 0.05$). The experiment comprises 4 different groups; each has three replicates: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se + Pb (Se seed primed and Pb sprayed plant)

cowpea plants. In a different study, quinoa treated with Se under normal circumstances showed increased growth, photosynthesis, proline, antioxidants, and a decrease in ROS generation (Khalofah et al. 2021). Thus, after receiving Se treatment, there may be a notable increase in antioxidant enzymatic activities, which could counteract the detrimental effects of Pb and show that Se has a greater effect on faba bean plants.

Most clearly, in the present study, to combat these adverse effects of Pb, faba bean plants increased the activity of PAL. Also, Se augmented PAL activity under Pb conditions (Fig. 7f). This result was consistent with observations made by Astaneh et al. (2018) that Se administration strongly increased the particular activity of PAL. PAL is involved in secondary metabolic biosynthesis and an important phenolic biosynthetic enzyme (Cui et al. 2014), which could explain the upregulation in the total phenolics detected (see Fig. 6e). According to Huang et al. (2010), phenylpropanoid compounds are precursors of a broad variety of phenolic compounds that have a variety of roles in plants; these substances have significant roles in a number of distinct processes, including signal transduction, protection from abiotic stressors, plant defense against pathogens, and regulation. Therefore, the phenylpropanoid pathway's evolutionary appearance in plants is a crucial adaptation that permits plant defense against both biotic and abiotic stressors.

3.8 Pb Contents in *V. faba* Plants

Pb is one of the most prevalent and hazardous poisonous metal in the environment. It may bind tightly to protein sulfhydryl groups, causing structural proteins and enzymes to change and plants to become phytotoxic (Abdelhameed et al. 2024a). Pb content was measured in both shoots and roots, and its translocation factor was calculated (Table 5). It was obvious that Pb content was greater in the root (558.2 mg Kg^{-1}) than the shoot (340.4 mg Kg^{-1}) in bean plants exposed to Pb. Interestingly, under Pb, the application

of Se by priming reduced the uptake and translocation of Pb, recording $394.95 \text{ mg Kg}^{-1}$ in roots and 195.3 mg Kg^{-1} in shoots. Regarding the translocation factor, Se reduced it significantly from 0.708 (without Se application) to 0.574 (with Se application). According to Gao et al. (2018), Se shields plants from harmful metals by decreasing their absorption and movement from the roots to the top sections of the plant, such as the shoots and leaves, which is one of the vital stress tolerance mechanisms. According to Fargašová et al. (2006), there have been reports in the literature on different degrees of reduction in heavy metal (Pb, Cd, Cr, Co, Ni, Zn) uptake and accumulation in the roots and shoots of plants when there is Se present in the growing medium. The plant's age, species, portion (shoots or leaves), and supplementation method could all be contributing factors to this variation. The most recent study by Yu et al. (2025) stated that application of Se reduced the Cd content in the above-ground parts of *Artemisia argyi* seedlings which could represent a promising strategy to contribute to the development and sustainability of crop production on soils contaminated with Cd. According to some theories, Se can shield plants from harmful metals by directly blocking their adsorption and translocation, changing the location of metal ions, creating less bioavailable metal complexes, reducing oxidative stress, and controlling the activity of antioxidant enzymes (Jia et al. 2025). Furthermore, the way that Se affects the build-up of heavy metals in plants is very dependent on the ratio of the elements (Fargašová et al. 2006).

Collectively, data inter-relationship analysis (Fig. 8) revealed a correlation between different physiological, biochemical, and molecular traits (Pearson correlation; Fig. 8a). Figure 8b displayed the clustering between control and Se treatments, which was in a close distance with Se+Pb treatment, while the Pb treatment was the farther. This data inter-relationship analysis confirmed the role of Se in alleviating the Pb stress.

4 Conclusion

It can be concluded the efficacy of selenium (Se) as an effective priming agent that alleviates stress in faba bean plants and improves the performance of faba bean plants under conditions of lead (Pb) stress. Se improved plant growth compared with the control treatment by increasing a variety of indices about agronomic, physiological, and biochemical attributes, emphasizing the possibility of a synergistic action of Se. The current gene expression analysis data has confirmed that Se seed priming can establish a stress memory for subsequent stresses, thereby enhancing resilience. This can be accomplished by modulating the extensive signaling network regulated

Table 5 Changes in Pb content in shoots and roots of *V. faba* plants and its translocation factor (Tf) as a result of Se priming grown under Pb stress. The experiment comprises 4 different groups; each has three replicates: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se+Pb (Se seed primed and Pb sprayed plant). Data are the mean $\pm$ standard error of 3 replicates. Within each column, different small letters indicate significant differences among treatments using a one-way ANOVA followed by the Duncan's test ($p < 0.05$)

	Pb root (mg Kg^{-1})	Pb shoot (mg Kg^{-1})	Tf
Control	0c	0c	-
Se	0c	0c	-
Pb	558.2a	394.95a	0.708
Se+Pb	340.4b	195.3b	0.574

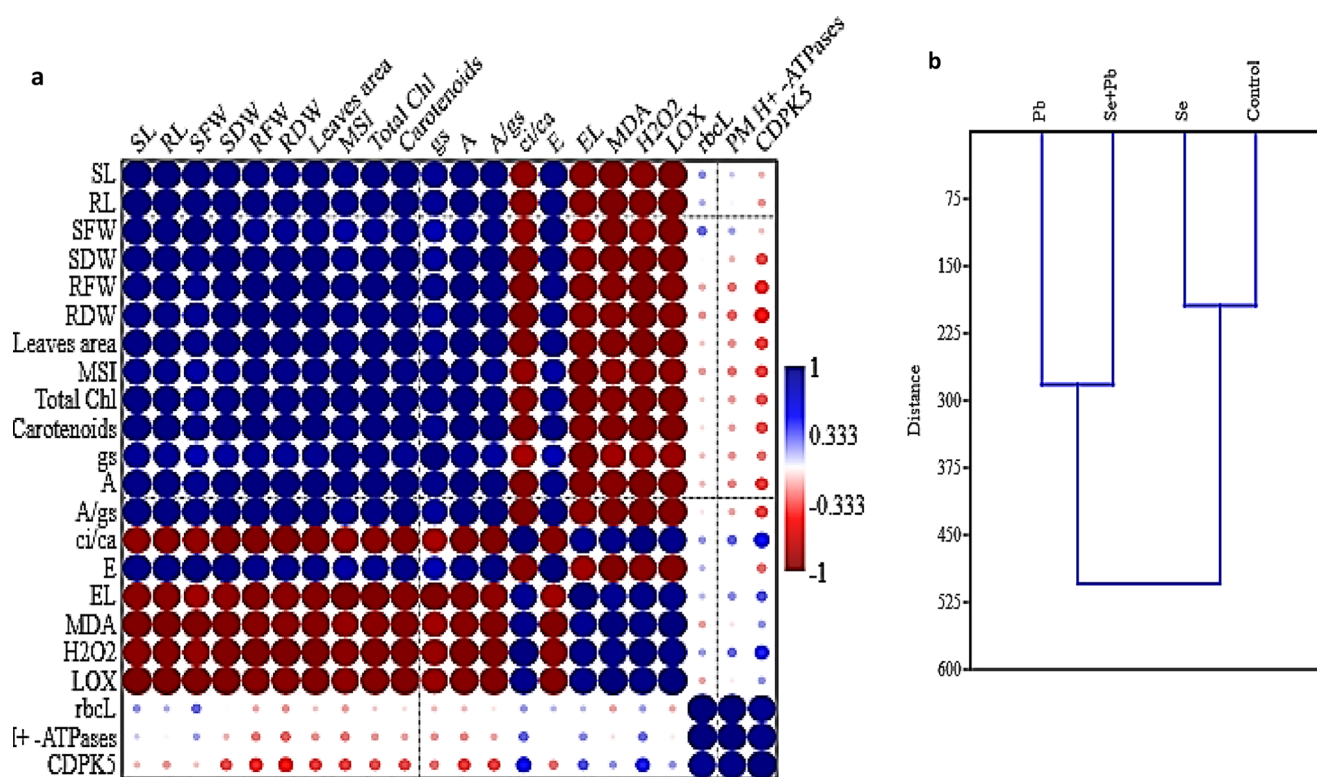


Fig. 8 (a) Pearson correlation between different parameters and (b) Hierarchical cluster analysis between different treatments. The experiment comprises 4 different groups; each has three replicates: cont. (no treatment), Se (selenium seed primed), Pb (lead foliar sprayed plants), and Se + Pb (Se seed primed and Pb sprayed plant). SL: shoot length, RL: root length, SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight, RDW: root dry weight, MSI: membrane stability index, Total chl: total chlorophyll, gs: stomatal conductance, A: net photosynthetic rate, A/gs: intrinsic water-use efficiency, ci/ca: the ratio of internal to atmospheric CO₂ concentration, E: transpiration rate, EL: electrolyte leakage, MDA: malondialdehyde, H₂O₂: hydrogen peroxide, LOX: lipoxygenase, rbcL: Ribulose-bisphosphate carboxylase oxygenase large subunit, PM H⁺-ATPases: P-type plasma membrane H⁺-ATPase, CDPK5: Calcium-dependent protein kinase

by calcium-dependent protein kinase (CDPK5) and the P-type plasma membrane (PM) H⁺-ATPase gene. Furthermore, it undermines the reduction in photosynthesis that is provoked by Pb stress by elevating the level of activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme (rbcL). It is clear from the findings of this investigation that Se seed priming possesses the capacity to serve as a stress memory inducer that can enhance performance after heavy metal exposure, particularly Pb.

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Data Availability The relevant datasets supporting the results of this article are included within the article.

Declarations

Ethics Approval and Consent to Participate plant materials were attained after permission from the Department of Agricultural Research Center, Giza, Egypt. This article does not contain any studies with human participants or animals performed by any of the authors.”

Consent for Publication Not applicable.

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