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Sananda Mondal

sananda.mondal@visva-bharati.ac.in

Visva-Bharati University

Smrutirekha Dash

Visva-Bharati University

Rabindranath Acharya

Visva-Bharati University

Debasish Panda

Visva-Bharati University

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Magnesium sulfate seed priming mitigates lead acetate induced stress in mungbean during vegetative growth

[*1]Sananda Mondal, [1]Smrutirekha Dash, [1]Rabindranath Acharya and [1]Debasish Panda

[1]Department of Crop Physiology, Institute of Agriculture, Visva-Bharati, Sriniketan-731236, WB, India [[*]Correspondence: sananda.mondal@visva-bharati.ac.in]

Abstract

Lead (Pb) contamination as heavy metal in soil is a limiting factor for mungbean (*Vigna radiata* L.) by reducing seed germination, seedling establishment, and photosynthetic efficiency through oxidative damage. A two-phase study was conducted to evaluate the potentiality of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) as seed priming agent to ameliorate the Pb acetate-induced stress in mungbean var. Samrat. Seeds primed with 5 mM and 10 mM $MgSO_4$ were compared with non-primed controls under varying Pb concentrations (0–2000 mg L^{-1}). Lead stress significantly reduced germination percentage, plumule and radicle length, root number, biomass accumulation, chlorophyll content, and plant height, while enhancing proline and sugar content. $MgSO_4$ priming alleviated these adverse effects, with 5 mM $MgSO_4$ consistently outperforming 10 mM in maintaining higher germination (68–71% under severe stress), seedling vigor, leaf area, root volume, and chlorophyll content. Enhanced osmolyte accumulation and improved antioxidant responses were observed in primed seedlings, indicating better osmotic adjustment and stress tolerance. Statistical analysis confirmed highly significant improvements across morpho-physiological and biochemical traits. The findings suggest that $MgSO_4$ seed priming, particularly at 5 mM, is a cost-effective strategy to enhance mungbean resilience against Pb-induced heavy metal stress, thereby supporting sustainable crop production in contaminated soils; whereas, among the variable concentration range of lead acetate stress up to 1000mg L^{-1} is the tolerance limit for better growth and development of variety Samrat.

Keywords Mungbean, Seed Priming, Magnesium Sulfate, Lead Acetate, Osmolytes

Highlights

- Lead acetate stress severely inhibited mungbean germination, seedling vigor, plant height, leaf area, and chlorophyll content in a dose-dependent manner.
- Magnesium sulfate seed priming (5 mM) consistently alleviated Pb-induced damage, outperforming 10 mM across morpho-physiological and biochemical traits.
- Germination percentage under severe Pb stress (2000 mg L^{-1}) improved from ~54–56% in non-primed seeds to 68–71% in 5 mM primed seeds.
- Primed seedlings showed enhanced osmolyte accumulation (proline and sugars) and improved antioxidant responses, supporting osmotic adjustment and stress tolerance.
- Variety Samrat demonstrated a tolerance threshold up to 1000 mg L^{-1} Pb acetate, beyond which growth and development were severely impaired.

1 Introduction

Mungbean (*Vigna radiata* L.*),* is widely grown in India and in all the seasons like Zaid, kharif, and rabi [1]. It is rich source of protein, vitamins, minerals and dietary fiber and contains significant amounts of bioactive compounds. Vigna crop is also grown under crop rotation regimes for its capacity to enhance soil fertility [2,3]. Currently, it is estimated that approximately 7.3 to 8 million

hectares (Mha) of agricultural land worldwide are under mungbean cultivation with nearly 90% of this production are found in Asia. India and Myanmar are the major producers of mung bean, with each country accounting for about 30% of global output. The reduction in productivity is attributed to various biotic stresses such as insect, bacterial and viral infections and abiotic stresses like salinity, drought, high heat, heavy metals and waterlogging [4,5].

Rapid increase in trends of urbanization and industrialization has led to more heavy metals in the environment during the past decades [6]. The origins of these heavy metals are natural or anthropogenic activities [7]. General increases in the level of heavy metals pose a pervasive threat to the natural ecosystem. The presence of heavy metals in the environment is a serious ecological problem, because these elements can enter food chains and the biological cycle. The plants under stress conditions are most likely to be adversely affected by high concentration of heavy metals. The possible adverse effects of heavy metal pollution and their phytotoxic effects have been reported in *Shorea robusta, Capsicum annum, Albizia procera, Sinapis arvensis, Triticum aestivum* [8]. The term "heavy metal" refers to the high density (>5gm/cm^3) of any metallic elements [9]; common ones are zinc (Zn), lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni), present in the soil in a trace amount as a result of natural weathering [10], but its excessive accumulation ensues through the discharge from the industries, mine tailings, fertilizers, pesticides, coal combustion residues, and atmospheric deposition [11].

Among the metals, lead particularly has become a cosmopolitan environmental pollutant. Lead is a toxic environmental contaminant that induces many biochemical and structural changes in biological systems. The process of photosynthesis is adversely affected by Pb toxicity. One of the phytotoxic effects of Pb appears to be induction of oxidative stress in growing plant parts due to enhanced production of reactive oxygen species (ROS) resulting in an unbalanced cellular redox status. A number of different ROS, including the superoxide anion (O_2^-), singlet oxygen (O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) are produced during normal oxidative metabolism in aerobic organisms, but these ROS can pose a severe threat when produced in larger amounts. Although the ROS generating processes are slow under normal conditions, Pb accelerates them [12]. In plants, Pb toxicity disrupts many metabolic activities like organelles stability, electron transport chain, membrane integrity, photosystems complex, mineral metabolism, enzymatic activity and oxygen-evolving complex [13]. Exogenous Pb causes increases leaf H_2O_2, lipid peroxidation, growth reduction, pigments reduction and photosynthesis decline due to oxidative stress [14].

Magnesium (Mg) as an essential macronutrient plays multifarious roles in plants and is a forgotten element in crop production [15]. Most specifically, Mg is the central part of chlorophyll molecule with functions in plant photosynthetic processes. As a cofactor for several enzymes, Mg regulates the functions of these enzymes [16], involved in the stabilization of structure of nucleic acid and protein synthesis [17]. Mg is also involved in the partitioning of carbohydrates in plants and seed reserves mostly starch from the source to sink organs by regulating the activity of enzyme complexes such as H^+-ATPase with role in the phloem loading and the distribution of sugars from leaves to stem and roots [18]. Magnesium (Mg^{2+}) has been shown to reduce the physiological and biochemical stress in plants caused by heavy metals. To date our understanding of how Mg^{2+} ameliorates the adverse effects of heavy metals in plants is scarce. The potential effect of Mg^{2+} on lead (Pb^{2+}) toxicity in plants has not yet been studied. Furthermore, Mg^{2+} addition increased root growth and oxidative activity, and protected the root ultrastructure [19].

Sulfur (S) is an important element that plays a crucial role to mitigate abiotic stresses in crop plants. Metabolism of S results in the formation of several S-containing defense compounds such as methionine, cysteine, glutathione, and phytochelatins that are involved in plant survival during various abiotic stresses including drought [20]. The interplay of S with phytohormones helps to

regulate crucial metabolic processes in plants [21]. Deficiency of S may result in impaired growth, loss in plant production, and susceptibility to environmental stresses due to reduced protein synthesis, chlorophyll degradation and decreased photosynthetic rate [22].

According to Granata et al., [23], seed priming is a simple, environment friendly and effective technique to enhance seed germination, seedling establishment, and overall plant performance in the field. Seed priming is a value-added technique applied to a specific seed lot and to promote faster germination and uniform emergence. Seed priming (SP) technology can be a feasible, low-cost, easily deployable option to boost plant's tolerance against HMs toxicity and to also obtain good growth and yield [24,25]. According to Paul et al. [26], seed priming offers several advantages, such as accelerated and synchronized germination, enhanced nutrient uptake, alleviation of phytochrome-induced photo- and thermos-dormancy, a wider range of germination temperatures, enhanced water use efficiency, and synchronous crop maturity. Priming allows seeds to complete all metabolic processes in the early phases of germination, even under extreme soil and environmental conditions [27].

Seed priming with Magnesium sulphate ($MgSO_4$) is an effective, low-cost strategy to mitigate the detrimental effects of heavy metal stress, such as lead (Pb) acetate, in mungbean (*Vigna radiata*). Priming helps to overcome Pb-induced inhibition of germination and seedling growth by enhancing metabolic activity and strengthening antioxidant defences. Seed priming with $MgSO_4$ involves controlled soaking and re-drying, which initiates germination-related metabolic activities without allowing radicle protrusion. It prepares the seed to handle stress upon sowing by activating defense genes. The pre-loading of Mg^{2+} ions helps the developing radicle maintain a proper ionic balance immediately upon germination in Pb contaminated soil. Mg^{2+} ions compete with Pb ions for binding sites on the root surface and within transporter proteins, reducing the amount of Pb that enters the root [28].

Realizing the importance of the problem, an investigation was undertaken to study the germination physiology, morpho-physiological as well as biochemical traits of Mungbean (*Vigna radiata* L.) var Samrat during vegetative growth phase under lead acetate trihydrate induced heavy metal stress ameliorated by magnesium sulfate heptahydrate primed and non-primed seeds.

2 Materials and methods

2.1 Geographical situation and location

The experiment was conducted in two phases; firstly, a laboratory experiment which was carried out in the Crop Physiology Laboratory, and secondly, a polyhouse experiment was conducted in the Department of Crop Physiology of the Institute of Agriculture, Palli Siksha Bhavana, Visva-Bharati, Sriniketan – 731236, WB, during kharif season (rainy season) in the year 2025.

2.2 Experimental details

The design of experiment was Completely Randomized Design with 3 replications, where the crop was mungbean variety Samrat; the mungbean seeds were collected from the Department of Genetics and Plant Breeding of the Institute of Agriculture, Visva-Bharati. The priming agent used for experiment was magnesium sulphate heptahydrate (commonly known as Epsom salt) and the concentrations used was 0, 5 and 10mM; whereas, the lead acetate trihydrate was used as heavy metal stress inducing agents in various concentrations of 0, 250, 500, 1000, 1500 and 2000 mg L^{-1}.

The non-primed control and primed seeds were used for germination study as well as related initial vegetative growth parameters. Petri-dishes of 3.0-inch diameter were used to conduct germination studies. The control and air-dried primed seeds were placed equidistantly (25 seeds in each Petri-dish) and 5 mL of distilled water was poured in control one as well as 5mL of Lead acetate Trihydrate solution of different concentrations was poured in rest of the petri-dishes followed by

the design of experiment; and whenever required the double distilled water was added in the petri dishes during the study period of germination physiology (up to 8 DAS).

2.3 Sterilization and seed priming

Before sowing the seeds in the petridishes/pots, seeds need to be disinfected/sterilized. To disinfect the seeds, seeds were washed in 70% ethanol for 1 min and then rinse the seeds with distilled water. After that the seeds were sterilized in 0.01mM mercuric chloride solution for 1 min and rinse the seeds upto 4-5 times by using distilled water. The sterilized seeds were soaked for 4 hours in 100 ml solutions of 5mM and 10mM magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) having molecular weight of 246.47g. After 4 hours, seeds were taken out from the beaker and rinsed once in distilled water; then dried in bloating paper under the fan, until the original weight was received. After drying, the seeds were sown in both petridishes and pots.

2.4 Germination and growth attributes

The parameters considered for germination physiology were germination percentage, plumule and radicle lengths, root number, fresh and dry weights of seedlings, total sugar and proline content, recorded at 4^{th} and 8^{th} days after sowing (DAS); whereas, total sugar and proline content was also noted at 15 and 30DAS in pot culture. Germination was observed daily up to 3 days with a 24 hrs of interval (i.e., 24, 48 and 72h).

Germination percent (%) = (Number of seeds germinated / Number of seeds present in the petri dish) × 100

Fresh weight of seedlings: The fresh weights of 3 seedlings were considered for each treatment at 4 and 8 DAS for 3 replications; by using the electronic balance (ER-200A).

Dry weight of seedlings: To take the dry weight of seedlings (3 seedlings were taken into consideration for each treatment per replication at both 4 and 8DAS), samples were kept for an hour in an oven pre-set at 100 - 110ºC. Thereafter it was placed in oven, which was set at 60±2ºC till to get constant weight.

Plumule / shoot length: The length of the plumule/ shoot was taken by using centimeter scale by placing it on the junctions of plumule and radicle to the tip of the plumule in case of Petri plates grown seedlings at 4 and 8DAS (3 seedlings were taken into consideration for each treatment per replication).

Radicle / root Length: The centimeter scale was placed at the base of radicle to the tip of the radicle to measure the radicle length at 4 and 8DAS (3 seedlings were taken into consideration for each treatment per replication).

Root Number: Root Number was recorded for each treatment per replication (for 3 seedlings) at 4 and 8DAS as an indicator of rooting efficiency and plant establishment.

2.5 Pot Culture Experiment

In the second phase of experiment, total 54 pots were taken into consideration (where, 3 level of magnesium sulphate primed concentrations, 6 level of lead acetate trihydrate heavy metals concentrations and 3 replications were considered respectively). Green gram var. Samrat were sown

in pots inside Polyhouse on 15th July, 2025 followed by the Completely randomized design and statistical significance was calculated at CD value of 1% ($p < 0.01$) and 5% ($p < 0.05$) level of significance.

2.5.1 Preparation of soil and sowing of seeds

To conduct the present heavy metal stress induced pot culture experiment, soil was analyzed and only cow-dung manure was applied as a source of nutrients. The ratio of soil: cow-dung was 3:1; and no chemical fertilizer as well as herbicide, pesticide, insecticide was applied. The pot was filled with soil mixed with cow-dung and kept in the polyhouse. Before sowing, the soil was prepared by putting required amount of lead acetate solution on the basis of design of experiments and kept it for 15 days so that the lead acetate gets mixed with the soil properly. After 15 days the soil was loosened up with khurpi and 5 seeds per pot was sown and applied water. The observation of the essential parameters was observed at 15, 30, 45 DAS. The induced heavy metal stress i.e., lead acetate trihydrate solution was applied 3 times at 10, 25 and 40 DAS respectively. Watering was done every 2 days interval. Morpho-physiological parameters [i.e. plant height (at 15, 30 & 45DAS); leaf area (Leaf Area Meter 211), leaf fresh & dry weight (at 15 and 30 DAS); root length, root volume, shoot and root dry weight (at 45 DAS)] and biochemical parameters (i.e. chlorophyll content, proline content, and total sugar content at 15 and 30DAS) were recorded as DAS mentioned.

2.5.2 Root volume: To get the volume of roots at 45 days, the seedlings were first watered vigorously and then uprooted very gently to avoid any type of injury to root system of the seedlings. These were kept in a beaker of 1 L capacity filled with distilled water. The soil present on root surface was washed thoroughly for its proper cleaning. The volume of roots of plants was also calculated by placing the root part on a 100 ml measuring cylinder. The changes in volume in the measuring cylinder were recorded as root volume.

2.5.3 Biochemical Parameters

Total Chlorophyll Content

Total chlorophyll content of green gram plants in the field condition at 15 and 30 DAS was measured by Chlorophyll meter [Soil and plant analysis development (SPAD) meter, MC 100]. The reading was recorded from top most 3rd and 4th leaves of five randomly selected plants and mean values were reported. The value measured by the chlorophyll meter (MC-100 chlorophyll meter) related to the amount of chlorophyll present in the leaf of plant. The light, which passes through the sample leaf, strikes the receptor which convert the transmitted light to analog electrical signals, these signals are boosted by the amplifier and are converted into digital signals by the A/D converter. These digital signals are then used by the micro process to calculate the SPAD value, which is shown on the display and stored in the memory.

Proline Content

Proline content was determined by the method described by Bates et al. [29]. Dry weight of seedlings at 4 and 8DAS/ 3rd and 4th top most leaf tissues collected from 15 and 30 DAS (50 mg) were extracted in 10.0 mL sulphosalicyclic acid (SSA 3% aqueous) using mortar-pestle at room temperature. The homogenate was centrifuged for 10 minutes at 5000 rpm by using model R-8C of REMI. 2.0 mL of supernatant was taken in separate test tube for each treatment, in addition with that 2 mL of glacial acetic acid was mixed, followed by adding 2.0 mL acid ninhydrin reagent and mixed well. The tubes containing assay mixtures were incubated in a boiling water bath (100ºC) for 30 minutes till the reddish pink colour developed and then cooled in room temperature. After cooling the reaction mixture, 4 mL of toluene solvent was added to each tube. Toluene fractions were collected and intensity of pink colour read at 520 nm on a spectrophotometer (LMSP UV-1200) against toluene as blank. A standard curve was prepared using L-Proline (0.1 mg mL^{-1}) and contents in sample aliquots determined. Total proline was expressed as mg g^{-1} dry weight of seedlings/ leaf sample.

Total Sugar Content

Total sugar content was determined from 50mg fresh samples of 4 and 8DAS seedlings as well as 500mg fresh samples of top most 3rd and 4th leaf of plant collected at 15 and 30DAS; followed by the method proposed by Morris [30]. Sample was homogenized with 5 mL ethanol (80 percent). The extract of the sample was centrifuged at 4,000 rpm for 15 minutes by using model R-8C of REMI. The supernatant was separated and remaining residue was extracted twice with 5mL of 80 percent ethanol. Supernatants were pooled and total volume was maintained to 50 mL with 80 per cent ethanol. Residue was saved and used for the extraction and estimation of starch. 0.5 mL of the extract was taken in a test tube, dried in oven and the volume was made to 1.0 mL with distilled water. 5 mL anthrone reagent was added to it. The tubes were placed in a boiling water bath for 10 minutes, after which they were allowed to cool immediately in running water. A blank was prepared in the similar way, but in lieu of 0.5 mL substrate (extracted sugar), 0.5 mL of distilled water was added. The absorbance was measured at 620 nm by using a spectrophotometer (LMSP UV-1200). The amount of the sugar present in the mungbean plant samples was calculated by standard curve. Total sugar was expressed as mg g^{-1} fresh weight of seedling/ leaf samples.

2.5.3 ROS Detection: For detection of ROS, freshly collected 3rd and 4th fully expanded leaf samples at 45 DAS were immersed in 0.2% NBT solution and left for incubation for 8hr to detect deep brown spots indicative of H_2O_2 and dark blue spots representing O_2^-, respectively. Later, photographs of the stained leaves were captured using a camera.

3. Results

Lead acetate stress significantly reduced germination, seedling growth, plant growth, and biochemical performance of mungbean in a dose-dependent manner.

3.1 Germination Percentage

The germination percentage (Table 1) of mungbean seeds was significantly influenced by induced lead acetate stress and magnesium sulfate seed priming. The non-primed control i.e., P_1T_1, the germination percentage remained highest under the control condition, recording 98% at 24, 48 and 72 h. However, germination declined progressively with increasing lead acetate concentration, reaching the lowest value of 54–56% under the highest stress level (P_1T_6, 2000 mg L^{-1}). In contrast, magnesium sulfate primed seeds showed improved germination under stress conditions. Among the priming treatments, 5 mM $MgSO_4$ (P_2) maintained the highest germination under severe stress, recording 68–71% at T_5 and 59–61% at T_6, whereas 10 mM $MgSO_4$ (P_3) also improved germination compared with the non-primed treatment at higher stress levels. Under control conditions, germination in primed seeds ranged from 89–93% in P_2 and 88–91% in P_3. The corresponding CD (1%) values of 3.73, 3.90 and 3.73 confirmed that the differences among treatments were statistically significant.

3.2 Seedling Growth at 4 DAS

Lead acetate stress significantly affected the early growth of mungbean seedlings by reducing plumule length, radicle length, root number, fresh weight and dry weight in a concentration-dependent manner presented in Table 2. In the non-primed treatment (P_1), the highest values were recorded under the control (T_1), with plumule length (4.26 cm), radicle length (4.27 cm), root number (2.78), fresh weight (0.156 g) and dry weight (0.0222 g). Progressive increase in lead acetate concentration markedly suppressed seedling growth, and the lowest values were observed in the highest stress treatment (P_1T_6). Magnesium sulfate seed priming alleviated these adverse effects by maintaining higher seedling growth under stress. Among the priming treatments, 5 mM $MgSO_4$ (P_2) exhibited the best performance, producing the highest plumule length (4.59 cm), radicle length (4.54 cm), fresh weight (0.193 g) and dry weight (0.0231 g) under control conditions, while sustaining greater growth than the non-primed seedlings across increasing stress levels. Although 10 mM $MgSO_4$ (P_3) also improved seedling growth compared with the control, its performance was generally inferior to P_2 under severe stress. Statistical analysis revealed that all growth parameters differed significantly among treatments. The CD (1%) values were 0.284, 0.208, 0.327, 0.010 and 0.0015 for plumule and radicle lengths, root number, fresh and dry weights respectively, confirming that the observed improvements due to magnesium sulfate priming were highly significant.

3.3 Seedling Growth at 8 DAS

Seedling growth at 8 DAS was markedly influenced by lead acetate stress, with increasing concentrations causing progressive reductions in plumule length, radicle length, root number and biomass accumulation (Table 3). Seed priming with magnesium sulfate substantially improved seedling growth under stress. Among the treatments, 10 mM $MgSO_4$ priming (P_3) recorded the highest plumule length (25.92 cm), radicle length (7.65 cm), root number (7.33), fresh weight (0.253 g) and dry weight (0.023 g) in without stress condition, while 5 mM $MgSO_4$ (P_2) consistently maintained superior growth under moderate and severe induced lead acetate stress compared with the non-primed seedlings. The lowest growth performance was consistently observed in P_1T_6, indicating severe inhibition caused by lead toxicity. The CD (1%) values were 1.32, 0.45, 0.97, 0.024 and 0.004 for plumule and radicle lengths, root number, fresh and dry weights respectively.

3.4 Total sugar and proline content at 4 and 8 DAS

Lead acetate stress induced a significant accumulation of total sugars and proline in mungbean seedlings, and the magnitude of accumulation increased with increasing stress intensity presented in Table 4. Magnesium sulfate priming further promoted osmolyte accumulation. Among the treatments, 10 mM $MgSO_4$ (P_3) recorded the highest sugar content, reaching 16.30 mg g^{-1} FW at 4 DAS and 40.61 mg g^{-1} FW at 8 DAS, whereas the highest proline content at 4 DAS (1.55 mg g^{-1} DW) was also observed in P_3. At 8 DAS, however, proline estimation could not be performed under the highest stress levels (P_2T_6 and P_3T_5–T_6) because insufficient seedling biomass was available for analysis. The lowest sugar and proline contents were recorded in the non-primed control treatment (P_1T_1). Statistical analysis confirmed highly significant treatment effects with CD (1%) values of 0.698 and 1.59 (4 and 8 DAS sugar), 0.070 and 0.192 (4 and 8 DAS proline).

3.5 Plant Height

Plant height was significantly influenced by induced lead acetate stress throughout the experimental period, with increasing lead acetate concentrations causing a progressive reduction in plant growth at 15, 30 and 45 DAS (Table 5). In the non-primed treatment (P_1), plant height decreased from 28.42, 40.18 and 45.83 cm under the control (T_1) to 18.32, 27.48 and 29.53 cm, respectively, under the highest lead concentration (T_6). Magnesium sulfate seed priming effectively alleviated the inhibitory effects of lead toxicity on plant growth. Among the priming treatments, 5 mM $MgSO_4$ (P_2) consistently produced the tallest plants, recording 29.75, 42.50 and 47.43 cm at 15, 30 and 45 DAS, respectively, under the control treatment (without stress), and maintained significantly greater plant height than the non-primed treatment under all stress levels. Although 10 mM $MgSO_4$ (P_3) also improved plant height compared with the non-primed treatment, its performance was generally slightly lower than P_2 under severe stress conditions. The lowest plant height was consistently observed in P_1T_6. The CD (1%) values were 3.04, 3.22 and 2.36 at 15, 30 and 45 DAS, respectively, confirming that magnesium sulfate priming significantly improved plant growth under induced lead acetate heavy metal stress.

3.6 Leaf Growth Parameters

Lead acetate stress significantly reduced leaf area, fresh weight and dry weight of mungbean plants at both 15 and 30 DAS (Table 6). Under non-primed conditions, leaf area declined progressively from 95.60 cm² at 15 DAS and 118.43 cm² at 30 DAS under the control treatment to 55.80 and 90.30 cm², respectively, at the highest lead concentration. Similar reductions were observed in fresh and dry weights, indicating substantial impairment of leaf development and biomass accumulation under lead toxicity. Magnesium sulfate seed priming markedly improved leaf area. The 5 mM $MgSO_4$ treatment (P_2) maintained the largest leaf area under heavy metal stress and recorded the highest value (123.37 cm²) at 30 DAS, whereas 10 mM $MgSO_4$ (P_3) produced the highest fresh and dry weights under the control treatment. Nevertheless, under increasing lead stress, P_2 consistently maintained greater leaf area and biomass than both the non-primed and 10 mM-primed treatments. The lowest values for all leaf growth parameters were recorded in P_1T_6, reflecting severe inhibition of leaf expansion under maximum lead stress. The differences among treatments were highly significant, with CD (1%) values of 5.32 and 5.52 (leaf area at 15 and 30 DAS), 0.467 and 0.572 (fresh weight at 15 and 30 DAS), 0.086 and 0.111 (dry weight at 15 and 30 DAS) respectively.

3.7 Biochemical Parameters at 15 and 30 DAS

The biochemical characteristics of mungbean plants were significantly affected by lead acetate stress and magnesium sulfate seed priming (Table 7). Chlorophyll content decreased progressively with increasing lead concentration, indicating impairment of the photosynthetic apparatus under heavy metal stress. In contrast, total sugar and proline contents increased with increasing stress intensity, suggesting enhanced osmotic adjustment. Among the priming treatments, 5 mM $MgSO_4$ (P_2) maintained the highest chlorophyll content, recording 24.45 and 28.03 CCI at 15 and 30 DAS under the control treatment, while also sustaining higher chlorophyll levels than the non-primed treatment under stress. The highest total sugar content (56.40 mg g^{-1} FW) was recorded in P_2 under moderate lead stress, whereas 10 mM $MgSO_4$ (P_3) recorded the highest proline accumulation (1.93 mg g^{-1} DW), indicating enhanced osmoprotective responses. Conversely, the lowest chlorophyll content occurred in P_1T_6, while the lowest sugar and proline contents were observed in the non-stressed control treatment, where osmotic stress was minimal. The treatment effects were highly significant, with CD (1%) values of 6.82 and 1.13 (chlorophyll at 15 and 30 DAS), 0.392 and 1.027 (sugar at 15 and 30 DAS), 0.040 and 0.069 (proline at 15 and 30 DAS).

3.8 Root and Shoot Traits at 45 DAS

Lead acetate stress significantly inhibited root growth and biomass accumulation at harvest. In the non-primed treatment, root length, root volume, shoot dry weight and root dry weight progressively declined with increasing lead concentration, reaching their lowest values in P_1T_6 (Table 8). Magnesium sulfate seed priming substantially improved root architecture and biomass production

under stress conditions. The 5 mM $MgSO_4$ treatment (P_2) recorded the highest root volume (14.00 ml), shoot dry weight (3.265 g) and root dry weight (0.276 g), while 10 mM $MgSO_4$ (P_3) produced the maximum root length (31.67 cm) under the control treatment. Both priming treatments maintained significantly greater root growth and biomass than the non-primed plants across all lead concentrations, with 5 mM $MgSO_4$ showing the greatest overall improvement under stress. The corresponding CD (1%) values were 3.88 cm, 1.63 ml, 0.649 g, 0.037 g for root length, root volume, shoot dry weight and root dry weight respectively, statistically significant. These findings indicate that magnesium sulfate seed priming effectively alleviated the detrimental effects of lead toxicity on root development and dry matter accumulation, thereby contributing to improved overall plant growth and stress tolerance.

4 Discussion

Heavy metals (HMs) and metalloids contamination represent a serious hazard to both ecosystems and human health, as these elements are non-biodegradable and readily accumulate within food chains [31]. Crops grown in heavy metal (HM) polluted sites have been reported to display altered metabolism, biochemical and physiological processes leading to growth reduction, lower biomass production and HM accumulation [32]. In this context, Pb is not necessary for plant growth; a higher concentration of Pb causes a magnitude of negative effects causing inhibition of growth and germination, suppressing photosynthesis, altering membrane structure and permeability [33]. Pb toxicity destroys the chloroplast substructure and mitochondria, reduction in ascorbic acid and chlorophyll contents, production of reactive oxygen species (ROS) and decline in the activity of antioxidative enzymes causing an overall reduction in plant growth and development [34]. The present piece of work deals with the lead acetate as heavy metal stress inducing agent and magnesium sulfate priming as stress ameliorating agents. Moreover, mung bean is an important pulse crop across South and Southeast Asia, valued for its short growth cycle, high protein content, and ability to fix atmospheric nitrogen. It plays a key role in crop diversification and sustainable agriculture, fitting well into multiple cropping systems, improving soil fertility, and providing an affordable protein source [35]. Furthermore, Mg^{2+} is key in fortifying plants against environmental stressors and diseases by reinforcing cell walls and spurring the synthesis of defence substances [36]. Magnesium is essential for chelating nucleotidyl phosphate forms and supporting the function of many enzymes and accumulated sugars in leaves under heavy metal stress [37]. Sulphur is the fourth major essential nutrient and vital for regulating growth and development of crops at multiple levels. It also participates in defence mechanisms to abiotic stresses, particularly for HMs. To decrease the toxic effects by environmental stressors, plants activate different ROS-scavenging mechanisms, including enzymatic antioxidative enzymes and many more [38]. Together magnesium sulphate very commonly known as Epsom salt, used as fertilizer/ foliar spray for ornamental plants or indoor plants. In this current study, magnesium sulphate as seed priming agent

was considered to ameliorate the toxic effects of lead acetate used in different concentration range (0-2000 mg L^{-1})

4.1 Lead acetate (heavy metal) induced germination physiology of green gram ameliorated by magnesium sulfate seed priming

Seed priming offers a controlled process of seed germination by placing the seeds either in pure water or in various types of solutions, having low water potential, for a restricted but safe period of time to avoid the radicle protrusion before sowing in the field [39, 40]. During germination, the storage reserves of seeds are converted into its monomeric forms which nourishes the embryo. With these changes, primed seeds can starts germinating by radicle protrusion, which happens when environmental conditions are favourable [41]. The reduction in seed germination as observed in the present study under heavy-metal stress may be due to the disturbance in the activities of hydrolysing enzymes, like amylases and proteases, which hydrolyse starch into sugars and proteins into amino acids, essential to supply the required metabolites/ energy; similar kind of observation also reported by Shafiq et al. [42] and Ashraf et al. [43]. In this context, Ashraf et al. [44] depicted that heavy-metal stress is believed to accelerate protein and starch degradation which increases the levels of total free amino acids and soluble sugars. The ability to maintain high sugar levels in plants growing under heavy-metal stress was reported to be due to the activation of amylases [45], but contrasting reports are also available in the literature indicating the reduction in the activity of hydrolytic enzymes in plants grown under heavy-metal stress [43,46]. Furthermore, lead uptake significantly reduced total root length, surface area and volume compared with the control plants. However, the application of Mg^{2+} increased the indices of root morphological traits of *Vigna radiata* seedlings under lead toxicity. Root oxidative activity implies the degree of root development and metabolic status [47]. In the present experiment, under induced lead acetate stress (0-2000 mg L^{-1}) germination percentage, plumule and radicle length, root number, fresh and dry weight at 4^{th} and 8^{th} days after sowing were observed where the reduced trend for all the growth attributes was noted with increasing concentration of lead acetate as compared to control one. Moreover, while studying the total sugar content and proline content under same situation in petridish, an increasing trend with the increment of lead acetate concentration was noted. However, 5 and 10mM of magnesium sulphate primed seedlings recorded more sugar and proline content in comparison to the control one; showed similar responses with the previous work. In the higher concentrations of lead acetate i.e., 1500 and 2000mg L^{-1} mostly seeds failed to germinate in treatment P_2T_5, P_2T_6 and P_3T_5, P_3T_6; and the germination percentage range was 50-70% as well as the performance in respect of growth were poor enough that could not meet up the demand required to measure the total sugar and proline content. In this study, within the petridish the higher range of lead acetate concentrations hamper the germination physiology of green gram even in primed seeds.

4.2 Heavy metals uptake and transport in plants regulates the vegetative growth phase of green gram

The solubility and bioavailability of HMs in the soil are controlled by the adsorption/desorption, precipitation/dissolution, and are also associated with root respiration and root activity, mycorrhizal interaction, rhizoids secretions, ion transporters, etc. [48,49]. In growth and agronomical characteristics, the result concluded that the magnesium sulphate priming of green gram has positive effects on most of the characteristics it includes plant height at 15, 30 and 45 DAS, leaf area, fresh and dry weights of 3rd and 4th top most leaves at 15 and 30DAS, root length, root volume, shoot and root dry weight at 45DAS. Among the primed and non-primed plants responses P_2 i.e., 5mM magnesium sulphate priming treatment showed better responses for all the growth parameters in comparison to control (P_1) and 10mM magnesium sulphate primed (P_3) plants in effect of induced lead acetate stress (range from 0-2000 mg L^{-1}). Present study depicts that the tolerance level of induced lead acetate toxicity for mungbean survivability was 1000mg L^{-1}, more than this concentration the growth and development was hampered, which leads to decreased production and productivity of mung bean. For instance, Cai et al. [50] demonstrated that effect of cadmium on tomato growth including effects on plant height, root length, early flowering more pollen viability the effect was found simultaneously in all tomato plants. Nouariri et al. [51] demonstrated heavy metal accumulation and effects on growth, biomass and physiological processes in mustard which led heavy metal toxicity induced changes on growth and antioxidative enzymes level in water which is result of negative effect of heavy metal on plant growth and development. Furthermore, Sandalio et al. [52] analysed the effect of Cadmium (Cd) on some agronomical characteristics of the pea plant and suggested that heavy metal stress also effected all the agronomical characters of the plant. These studies are well in lineage with our current findings and strengthen our study.

4.3 Lead acetate (heavy metal) induced ROS generation, sugar and proline accumulation as well as degradation of chlorophyll content

In continuation with that, ROS generation, an expected consequence of abiotic stress, causes several metabolic disorders, including membrane damage, ion toxicity, oxidative stress, protein denaturation, and, finally, cell death [53,54]. Moreover, Alharbi et al. [55] align with previous research conducted on pea plants, Khan et al. [56] on tomato, and Islam et al. [54,57] on Indian mustard. Since Pb is the least active heavy element in the soil and accumulates in the surface layer of the soil, so it is easily absorbed through the roots and enters the aerial parts of plants as revealed by Baldi et al. [58]. However, in the present piece of work, the intensity of oxidative stress was measured in green gram by using NBT solution and dipping the 45 DAS old 3rd or 4th top most leaf from each and every treatment and wait for 8 hours to develop the dark blue and brown colour spots, indicates the location specific intensity of $O_2^{-\cdot}$ and H_2O_2 generation based on the stressor

level; with this histochemical analysis helps to identify the intensity of the ROS generation and location of ROS which depicts stress level (Fig 1(Plate1, 2, 3)). Osmo-protectants, also known as compatible solutes, generally accumulate in plants during stressful conditions and serve an important role in improving plant stress tolerance level by maintaining osmotic adjustment [53]. This present study showed an increased accumulation of sugar and proline content to the rise of lead acetate concentration. This increased accumulation of osmo-protectants is possibly attributed to osmotic adjustment initiated by heavy metals, as is evident in primed green gram plants also; where with increasing concentrations of magnesium sulphate the osmolytes were also increased which depicts the resistance capability of the primed plants to cope up with the lead acetate stress in comparison to control one. Increased concentration of sugar and proline protects the primed green gram plants from stress induced cellular osmotic pressure by maintaining the water uptake balance from the rhizospheric region of the plants. Similar kind of results was also found where Pb and Cd stress caused the production and accumulation of proline in the aerial parts of mung bean. The production of proline is a common response among plants as a result of heavy metals-induced stresses [59]. Proline is a cell membrane protector, which reduces the adverse effects of abiotic stresses on plants. It increases the plant tolerance to stress through mechanisms such as adjusting the osmotic pressure and protecting enzymes against denaturation of protein synthesis [60]. The most important destructive effect of heavy metals in plants is the induction of oxidative stress, which produces reactive oxygen radicals. Plants use proline as a non-enzymatic antioxidant to fight these free radicals. Proline reduces the toxicity of heavy metals in plants by forming a heavy metal-proline complex [61]. However, increasing soluble sugars under heavy metal stress is a plant adaptation method to regulate the cell water potential in the cytosolic fraction to counteract the high concentration of metal ions accumulated in the vacuole [62]. Interestingly, the presence of Pb in the root environment reduces photosynthesis in plants. So, the growth parameters decrease through reduced water uptake by the roots. However, in the present experiment with induced lead acetate stress was agree with the concept depicted by Mahapatra et al. [63], where the chlorophyll content was increased to some extent but decreased in presence of higher concentrations of heavy metals in case of primed and non-primed seedlings of 15 and 30DAS. In this respect plants obtained from primed seeds having more chlorophyll content as the priming agent was magnesium sulphate in absence of stress. Literature suggested that magnesium is the central atom in every chlorophyll molecule. It ensures plants maintain lush, green foliage and effectively convert sunlight into energy; however, without any stress highest chlorophyll content was recorded in primed plants. Whereas, non-primed plants (P_1T_1 to P_1T_6) were the poor performer, having less chlorophyll content in comparison to primed plants in heavy metal stress induced condition in both 15 and 30DAS. In continuation with that Yizong et al. [64] worked on phytotoxicity attenuation in rice under heavy metal stress, where plant growth retardation, reduction of chlorophyll content, formation of necrotic lesions are the main symptoms were recorded due to heavy metal stress.

5 Conclusion

In a nutshell the present investigation, lead acetate stress was found to exert profound negative impacts on the germination physiology, vegetative growth, and biochemical attributes of green gram (*Vigna radiata* L.), manifesting as reduced germination percentage, shorter plumule and radicle length, diminished biomass, and impaired chlorophyll content. These effects were accompanied by enhanced ROS generation and altered osmolyte accumulation, indicating severe oxidative and osmotic stress under heavy metal exposure. However, magnesium sulfate seed priming, particularly at 5 mM (P_2) concentration followed by 10 mM (P_3), significantly ameliorated these deleterious effects by improving germination traits, root morphology, chlorophyll retention, and osmolyte accumulation, thereby enhancing the plant's tolerance to Pb toxicity. The primed seedlings demonstrated better physiological resilience, with increased sugar and proline levels contributing to osmotic adjustment and antioxidative defense, ultimately supporting improved growth and development under stress conditions. The dual role of magnesium in chlorophyll synthesis and Rubisco activation, along with sulfur's contribution to defense regulation and ROS scavenging, has not been comprehensively explored in this crop under heavy metal stress. This novel contribution not only fills a critical research gap but also provides a foundation for future investigations into the broader applicability of $MgSO_4$ priming in enhancing stress tolerance across pulse crops and other food systems.

Author contributions

SRD conducted field experiments, collected morphophysiological data, and performed preliminary statistical analyses. SM conceptualized and designed the experiment, supervised the research work, and critically reviewed the manuscript. RA and DP contributed to experimental planning, data interpretation, advanced statistical analysis, and manuscript editing. SM and RA assisted in laboratory analysis, interpretation of physiological parameters, and literature compilation. All authors read, revised, and approved the final version of the manuscript and agreed to its submission.

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Data availability Statement

All data generated or analysed during this study are included in this published article

Declarations

Ethics approval consent to participate

Plant Guideline

The crop used for this experiment was mungbean variety 'Samrat' which is a cultivated species and procured from the Department of Genetics and Plant Breeding, Institute of Agriculture, Visva-Bharati. Yes, we have followed 'The Biological Diversity Act, 2002', comes under the National Guidelines and Law; where it was depicted that permission is not required while using cultivated species for research purpose.

Consent for publication

All authors have reviewed and approved the manuscript for publication in the Journal of Discover Plants.

Competing interests

The authors declare no competing interests.

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Table 1: Effect of induced lead acetate stress on germination percentage (%) in magnesium sulfate primed and non-primed seeds of mungbean (*Vigna radiata* L.) var. Samrat

Treatments		Germination Percentage (%)		
*Seed Priming (mM)	**Heavy Metal Stress- Lead Acetate (mg L^{-1})	24h	48h	72h
		Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	98±2	98±2	98±2
	T_2	94±2	94±2	94±2
	T_3	86±2	86±2	87±1
	T_4	79±1	79±1	79±1
	T_5	62±0	65±1	68±2
	T_6	54±0	56±2	56±2
P_2	T_1	89±1	93±1	93±1
	T_2	83±3	83±3	83±3
	T_3	76±4	76±4	76±4
	T_4	73±1	73±1	73±1
	T_5	68±2	70±4	71±3
	T_6	59±3	61±1	61±1
P_3	T_1	88±2	89±1	91±1
	T_2	84±0	86±0	87±1
	T_3	77±1	77±1	77±1
	T_4	76±0	76±0	76±0
	T_5	61±1	61±1	61±1
	T_6	56±2	56±2	56±2
SEm (±)		1.53	1.60	1.53
CD at 5%		2.58	2.70	2.58
CD at 1%		3.73	3.90	3.73

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor; 25 seeds were considered for seed germination

Table 2: Effect of induced lead acetate stress on plumule and radicle length (cm), root number, fresh and dry weight (g) in magnesium sulfate primed and non-primed seedlings of mungbean (*Vigna radiata* L.) var. Samrat at 4DAS

Treatments		Growth Parameters at 4 DAS				
*Seed Priming (mM)	**Heavy Metal Stress-Lead Acetate (mg L^{-1})	Plumule Length	Radicle Length	Root Number	Fresh Weight	Dry Weight
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	4.26±0.125	4.27±0.055	2.78±0.050	0.156±0.005	0.0222±0.0014
	T_2	3.67±0.083	2.74±0.090	2.80±0.400	0.122±0.008	0.0184±0.0004
	T_3	3.56±0.092	2.39±0.108	2.23±0.133	0.124±0.011	0.0204±0.0003
	T_4	2.87±0.183	1.35±0.018	2.23±0.067	0.119±0.009	0.0175±0.0014
	T_5	1.58±0.057	0.55±0.000	0.30±0.300	0.094±0.000	0.0106±0.0004
	T_6	1.21±0.108	0.44±0.010	0.00±0.000	0.072±0.001	0.0075±0.0003
P_2	T_1	4.59±0.058	4.54±0.123	4.22±0.117	0.193±0.004	0.0231±0.0009
	T_2	4.37±0.133	3.53±0.080	3.65±0.050	0.184±0.006	0.0220±0.0010
	T_3	4.23±0.83	2.89±0.342	3.33±0.167	0.162±0.000	0.0217±0.0008
	T_4	3.52±0.183	1.78±0.007	2.68±0.117	0.150±0.007	0.0203±0.0012
	T_5	2.18±0.182	1.45±0.025	0.00±0.000	0.099±0.003	0.0175±0.0007
	T_6	1.52±0.093	0.71±0.000	0.00±0.000	0.079±0.005	0.0160±0.0003
P_3	T_1	4.38±0.092	4.57±0.130	4.23±0.133	0.194±0.002	0.0216±0.0007
	T_2	4.52±0.165	3.65±0.097	2.95±0.317	0.190±0.000	0.0215±0.0002
	T_3	3.43±0.350	2.48±0.070	2.47±0.133	0.173±0.002	0.0201±0.0001
	T_4	3.22±0.052	2.70±0.027	2.23±0.133	0.158±0.003	0.0179±0.0006
	T_5	2.33±0.150	1.43±0.050	0.00±0.000	0.107±0.001	0.0160±0.0004
	T_6	1.53±0.017	0.78±0.015	0.00±0.000	0.070±0.000	0.0157±0.0002
SEm (±)		0.117	0.085	0.134	0.004	0.0006
CD at 5%		0.197	0.144	0.227	0.007	0.0010
CD at 1%		0.284	0.208	0.327	0.010	0.0015

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor

Table 3: Effect of induced lead acetate stress on plumule and radicle length (cm), root number, fresh and dry weight (g) in magnesium sulfate primed and non-primed seedlings of mungbean (*Vigna radiata* L.) var. Samrat at 8 DAS

Treatments		Growth Parameters at 8 DAS				
*Seed Priming (mM)	**Heavy Metal Stress-Lead Acetate (mg L^{-1})	Plumule Length	Radicle Length	Root Number	Fresh Weight	Dry Weight
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	23.42±0.42	6.58±0.25	5.83±0.17	0.233±0.007	0.020±0.003
	T_2	20.92±0.75	5.33±0.17	7.00±1.00	0.206±0.017	0.025±0.001
	T_3	17.88±0.55	2.58±0.25	8.60±1.06	0.137±0.033	0.018±0.002
	T_4	12.10±0.23	1.75±0.08	4.50±0.17	0.090±0.013	0.016±0.002
	T_5	7.26±0.43	1.30±0.13	2.17±0.17	0.110±0.007	0.006±0.000
	T_6	5.65±0.49	1.33±0.17	0.00±0.00	0.082±0.005	0.010±0.001
P_2	T_1	25.83±0.83	7.58±0.25	7.00±0.33	0.244±0.002	0.023±0.003
	T_2	24.83±1.67	5.67±0.17	11.33±0.33	0.267±0.003	0.026±0.000
	T_3	21.93±0.93	2.82±0.15	11.33±0.33	0.248±0.022	0.022±0.003
	T_4	16.32±1.02	1.95±0.15	5.25±0.25	0.178±0.002	0.018±0.001
	T_5	7.58±0.08	1.80±0.10	3.50±0.50	0.128±0.007	0.009±0.001
	T_6	6.52±0.28	1.80±0.10	0.00±0.00	0.110±0.002	0.007±0.001
P_3	T_1	25.92±0.42	7.65±0.35	7.33±0.33	0.253±0.013	0.023±0.003
	T_2	23.75±0.08	6.00±0.50	10.83±0.83	0.283±0.010	0.033±0.004
	T_3	23.24±0.40	3.15±0.35	11.25±0.25	0.277±0.003	0.025±0.004
	T_4	16.33±0.67	2.20±0.20	5.50±0.50	0.165±0.005	0.020±0.001
	T_5	8.08±0.33	1.95±0.05	2.50±0.50	0.115±0.003	0.008±0.001
	T_6	6.35±0.05	1.75±0.05	0.00±0.00	0.106±0.001	0.006±0.001
SEm (±)		0.54	0.18	0.40	0.010	0.002
CD at 5%		0.91	0.31	0.67	0.016	0.003
CD at 1%		1.32	0.45	0.97	0.024	0.004

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor

Table 4: Effect of induced lead acetate stress on total sugar content (mg g^{-1} fresh weight) and proline content (mg g^{-1} dry weight) in magnesium sulfate primed and non-primed seedlings of mungbean (*Vigna radiata* L.) var. Samrat at 4 and 8 DAS

Treatments		Total Sugar Content		Total Proline Content	
*Seed Priming (mM)	**Heavy Metal Stress-Lead Acetate (mg L^{-1})	4 DAS	8 DAS	4 DAS	8 DAS
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	2.85±0.25	7.40±0.78	0.36±0.010	1.757±0.042
	T_2	3.51±0.27	9.14±0.48	0.61±0.037	2.277±0.058
	T_3	3.69±0.17	15.85±1.23	1.00±0.020	2.641±0.022
	T_4	4.20±0.12	28.82±1.08	1.33±0.033	2.927±0.104
	T_5	5.59±0.02	38.70±0.5	1.35±0.015	3.973±0.131
	T_6	5.82±0.18	35.38±1.22	1.41±0.004	4.866±0.189
P_2	T_1	5.07±0.69	15.17±0.55	0.67±0.001	2.209±0.023
	T_2	9.62±0.18	21.32±0.18	1.10±0.006	2.773±0.056
	T_3	13.89±0.37	30.79±0.79	1.14±0.022	3.115±0.018
	T_4	14.71±0.11	35.59±1.53	1.36±0.004	3.579±0.027
	T_5	13.87±0.59	39.90±1.22	1.54±0.089	3.970±0.058
	T_6	14.57±0.33	0.00±0.00	1.54±0.065	0.000±0.000
P_3	T_1	7.23±0.19	15.02±0.12	0.87±0.047	1.306±0.296
	T_2	8.21±0.05	16.46±0.28	1.06±0.004	2.849±0.015
	T_3	14.42±0.04	26.67±0.65	1.20±0.031	4.273±0.020
	T_4	15.05±0.91	35.97±0.71	1.43±0.010	4.458±0.026
	T_5	15.44±0.06	40.61±0.61	1.48±0.053	0.000±0.000
	T_6	16.30±0.10	0.00±0.00	1.55±0.022	0.000±0.000
SEm (±)		0.286	0.65	0.029	0.079
CD at 5%		0.483	1.10	0.049	0.133
CD at 1%		0.698	1.59	0.070	0.192

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor [For sugar estimation 50mg fresh seedlings sample was taken; and in proline estimation 50mg dry seedling sample was taken into consideration]

Table 5: Effect of induced lead acetate stress on plant height (cm) in magnesium sulfate primed and non-primed plants of mungbean (*Vigna radiata* L.) var. Samrat at 15, 30 and 45 DAS

Treatments		Plant Height (cm)		
*Seed Priming (mM)	**Heavy Metal Stress- Lead Acetate (mg L^{-1})	15 DAS	30 DAS	45 DAS
		Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	27.50±0.50	42.00±0.33	45.38±1.13
	T_2	29.00±0.33	33.00±0.33	36.42±1.00
	T_3	26.00±0.67	31.27±2.27	34.93±1.39
	T_4	25.75±0.92	29.83±0.83	34.35±0.70
	T_5	24.50±0.17	29.17±0.83	31.37±1.15
	T_6	20.67±0.33	26.67±2.00	29.53±1.11
P_2	T_1	29.75±0.58	42.50±4.17	47.43±1.98
	T_2	29.17±0.83	42.83±0.50	46.37±0.87
	T_3	28.50±0.17	41.67±0.67	44.77±0.46
	T_4	28.92±0.58	38.83±1.83	42.53±1.05
	T_5	28.17±0.83	30.00±1.00	33.91±1.00
	T_6	27.75±0.75	31.00±1.00	33.73±0.57
P_3	T_1	27.17±1.50	45.33±0.33	48.13±0.73
	T_2	27.83±0.17	41.33±0.33	45.26±0.79
	T_3	27.00±2.00	39.17±0.83	42.73±0.75
	T_4	25.08±3.58	37.50±2.50	41.33±1.45
	T_5	24.83±3.50	31.17±1.50	33.99±1.28
	T_6	20.17±2.17	27.17±1.83	30.05±2.27
SEm (±)		1.24	1.32	0.97
CD at 5%		2.10	2.23	1.63
CD at 1%		3.04	3.22	2.36

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor

Table 6: Effect of induced lead acetate stress on leaf area (cm^2), leaf fresh and dry weight (g) in magnesium sulfate primed and non-primed plants of mungbean (*Vigna radiata* L.) var. Samrat at 15, and 30 DAS

Treatments		Growth Parameters					
*Seed Priming (mM)	**Heavy Metal Stress- Lead Acetate (mg L^{-1})	Leaf Area		Leaf Fresh Weight		Leaf Dry Weight	
		15 DAS	30 DAS	15 DAS	30 DAS	15 DAS	30 DAS
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	73.23±1.21	103.40±1.65	1.55±0.17	2.10±0.08	0.320±0.061	0.479±0.067
	T_2	73.47±3.62	111.60±1.11	1.77±0.13	2.39±0.42	0.318±0.031	0.458±0.041
	T_3	74.07±0.93	108.10±3.47	1.87±0.14	2.32±0.51	0.296±0.079	0.422±0.084
	T_4	66.73±3.47	104.80±4.50	1.83±0.12	2.32±0.37	0.240±0.012	0.337±0.037
	T_5	74.60±2.62	103.57±1.24	1.46±0.24	2.25±0.13	0.224±0.060	0.355±0.018
	T_6	55.80±0.72	90.30±5.02	0.72±0.15	1.72±0.21	0.152±0.032	0.267±0.022
P_2	T_1	88.53±1.72	116.87±2.40	2.01±0.26	2.64±0.21	0.354±0.016	0.556±0.031
	T_2	81.57±1.31	123.37±2.10	2.27±0.58	3.06±0.14	0.385±0.055	0.523±0.095
	T_3	72.17±0.81	121.53±3.29	1.86±0.14	2.55±0.12	0.339±0.079	0.463±0.008
	T_4	75.27±2.67	105.00±2.38	1.91±0.18	2.65±0.27	0.275±0.036	0.373±0.099
	T_5	65.03±2.06	107.30±2.87	1.80±0.17	2.26±0.42	0.263±0.029	0.367±0.046
	T_6	65.73±3.18	101.33±3.01	1.15±0.03	1.70±0.10	0.245±0.036	0.326±0.010
P_3	T_1	80.20±3.92	110.13±2.76	2.95±0.45	2.96±0.58	0.402±0.039	0.480±0.074
	T_2	75.97±4.38	105.83±1.45	2.77±0.16	2.94±0.15	0.332±0.004	0.443±0.014
	T_3	74.80±4.16	103.07±0.91	1.98±0.14	2.90±0.06	0.345±0.056	0.390±0.054
	T_4	69.07±0.86	102.23±1.78	2.01±0.18	2.34±0.34	0.241±0.008	0.374±0.014
	T_5	64.93±2.41	95.63±3.76	1.85±0.11	2.76±0.17	0.256±0.022	0.373±0.039
	T_6	63.00±2.86	97.87±1.80	1.47±0.22	2.89±0.02	0.278±0.005	0.399±0.088
SEm (±)		2.18	2.26	0.191	0.234	0.035	0.045
CD at 5%		3.68	3.82	0.323	0.396	0.060	0.077
CD at 1%		5.32	5.52	0.467	0.572	0.086	0.111

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor; Top most 3rd and 4th leaf was taken into consideration for observation.

Table 7: Effect of induced lead acetate stress on chlorophyll content (CCI), sugar content (mg g^{-1} fresh weight) and proline content (mg g^{-1} dry weight) in magnesium sulfate primed and non-primed plants of mungbean (*Vigna radiata* L.) var. Samrat at 15, and 30 DAS

Treatments		Biochemical Parameters					
*Seed Priming (mM)	**Heavy Metal Stress- Lead Acetate (mg L^{-1})	Chlorophyll Content		Sugar Content		Proline Content	
		15 DAS	30 DAS	15 DAS	30 DAS	15 DAS	30 DAS
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	21.35±0.91	25.30±0.83	12.64±0.06	28.34±0.36	0.556±0.022	0.83±0.06
	T_2	20.03±0.38	22.87±0.62	16.39±0.41	32.29±0.21	0.929±0.029	1.11±0.04
	T_3	19.45±0.57	21.60±1.24	17.06±0.08	38.68±0.26	1.094±0.021	1.07±0.04
	T_4	18.12±0.51	21.23±0.61	17.28±0.06	38.73±0.05	1.041±0.039	1.35±0.06
	T_5	18.24±0.45	20.64±0.47	17.04±0.08	38.41±0.03	1.031±0.020	1.66±0.03
	T_6	17.48±0.19	19.88±0.30	17.25±0.15	39.73±0.11	1.208±0.015	1.84±0.02
P_2	T_1	24.45±0.78	28.03±0.56	15.58±0.32	38.29±0.23	0.712±0.004	1.19±0.03
	T_2	24.19±0.43	27.79±0.45	16.53±0.17	50.85±0.19	0.991±0.026	1.37±0.04
	T_3	23.67±0.39	26.86±0.47	17.90±0.16	56.47±0.49	1.170±0.011	1.46±0.06
	T_4	22.06±0.84	26.16±0.16	18.69±0.21	51.14±0.00	1.269±0.010	1.57±0.01
	T_5	20.00±0.20	24.16±0.95	19.39±0.17	56.89±0.25	1.247±0.015	1.65±0.02
	T_6	19.62±0.46	22.80±0.24	19.58±0.13	56.31±0.19	1.487±0.025	1.82±0.03
P_3	T_1	16.57±0.53	27.30±0.20	16.23±0.03	39.61±0.83	0.968±0.010	1.16±0.02
	T_2	23.94±0.25	27.60±0.41	16.58±0.32	50.17±0.35	1.083±0.010	1.82±0.03
	T_3	23.00±0.69	26.20±0.57	19.42±0.08	51.39±0.11	1.175±0.009	1.70±0.01
	T_4	20.35±0.62	24.18±0.31	18.97±0.33	55.48±0.12	1.269±0.010	1.76±0.03
	T_5	19.76±0.65	24.05±0.24	19.36±0.12	56.40±1.80	1.393±0.009	1.93±0.01
	T_6	17.81±0.40	21.51±0.37	19.85±0.09	55.80±0.14	1.646±0.037	1.82±0.02
SEm (±)		2.80	0.46	0.161	0.421	0.017	0.028
CD at 5%		4.73	0.79	0.272	0.711	0.028	0.048
CD at 1%		6.82	1.13	0.392	1.027	0.040	0.069

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor; Top most 3rd and 4th leaf was taken into consideration for estimation. [For sugar estimation 500mg fresh leaf sample (top most 3rd and 4th leaf) was taken; in proline content 50mg dry leaf sample (top most 3rd and 4th leaf) was taken]

Table 8: Effect of induced lead acetate stress on root length (cm), root volume (ml), shoot dry weight (g) and root dry weight (g) in magnesium sulfate primed and non-primed plants of mungbean (*Vigna radiata* L.) var. Samrat at 45 DAS

Treatments		45 DAS			
*Seed Priming (mM)	**Heavy Metal Stress-Lead Acetate (mg L^{-1})	Root Length	Root Volume	Shoot Dry Weight	Root Dry Weight
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
P_1	T_1	16.23±0.50	8.00±1.00	1.979±0.588	0.160±0.009
	T_2	30.00±3.12	8.00±1.00	2.834±0.643	0.192±0.30
	T_3	26.67±0.76	7.33±0.58	3.221±0.322	0.250±0.013
	T_4	19.47±0.90	7.00±0.00	2.443±0.317	0.197±0.016
	T_5	22.37±2.05	5.67±0.58	2.226±0.226	0.156±0.013
	T_6	19.67±3.21	4.33±0.58	2.301±0.222	0.234±0.013
P_2	T_1	24.50±1.50	13.33±1.15	3.265±0.464	0.219±0.023
	T_2	30.23±2.40	14.00±1.00	2.959±0.497	0.266±0.030
	T_3	30.67±1.82	10.00±1.00	2.793±0.332	0.272±0.015
	T_4	28.17±0.76	9.00±1.00	2.672±0.243	0.236±0.028
	T_5	23.17±0.76	8.67±0.58	2.499±0.180	0.227±0.023
	T_6	23.33±1.53	7.33±0.58	2.446±0.097	0.276±0.009
P_3	T_1	20.67±0.58	13.83±1.26	2.960±0.132	0.241±0.008
	T_2	31.67±2.08	11.17±0.76	2.918±0.336	0.247±0.013
	T_3	29.00±1.32	10.33±0.58	2.893±0.078	0.226±0.011
	T_4	26.00±1.50	9.00±1.00	2.619±0.115	0.248±0.018
	T_5	24.60±4.15	7.33±0.58	2.386±0.059	0.236±0.013
	T_6	23.50±1.32	4.50±0.50	2.590±0.131	0.215±0.025
SEm (±)		1.59	0.67	0.266	0.015
CD at 5%		2.69	1.13	0.449	0.026
CD at 1%		3.88	1.63	0.649	0.037

*P_1 = Control, P_2 = 5mM $MgSO_4.7H_2O$, P_3 = 10mM $MgSO_4.7H_2O$ as priming agent; **T_1 – T_6 = 0, 250, 500, 1000, 1500, 2000mg L^{-1} Lead Acetate Trihydrate as stressor.

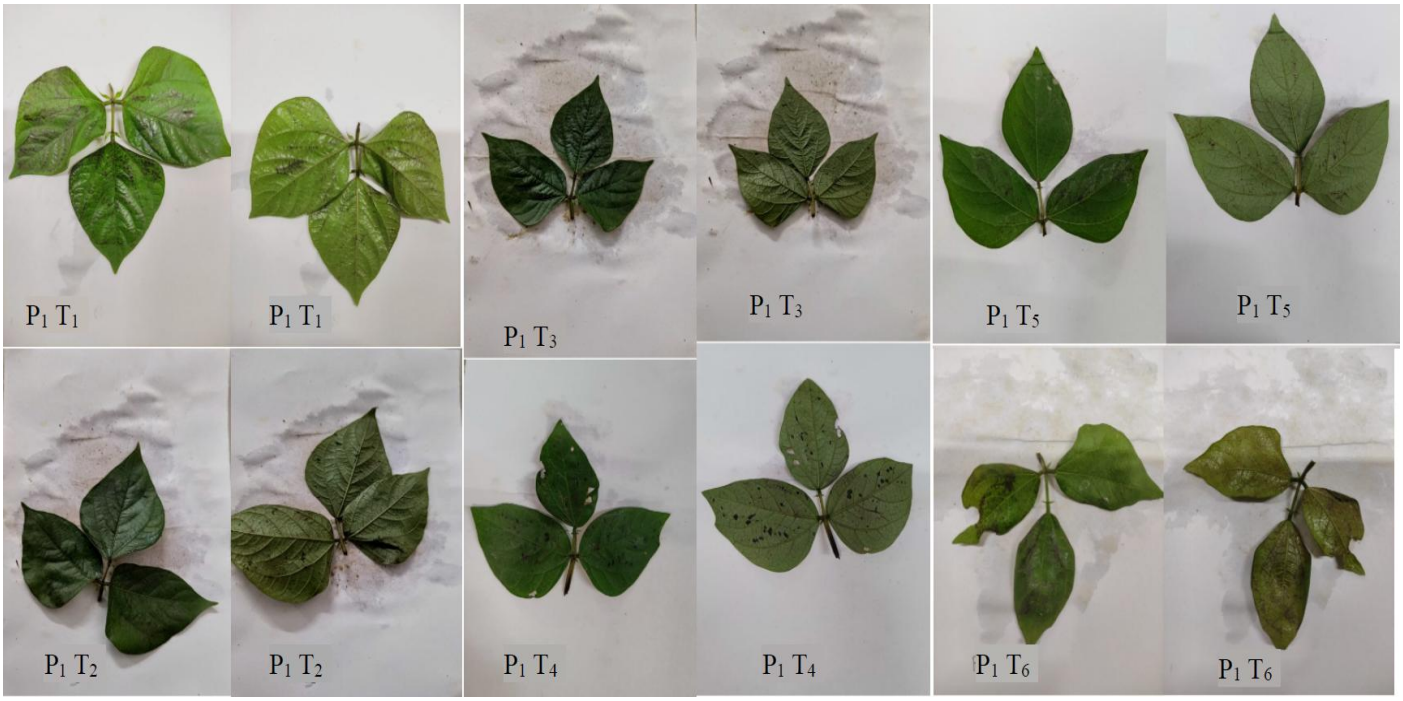


Plate 1



Plate 2



Plate 3

Fig 1: Detection of ROS on the adaxial and abaxial surfaces of mung bean leaves

***P_1= Control (Plate 1), P_2 = 5mM $MgSO_4.7H_2O$ (Plate 2), P_3= 10mM $MgSO_4.7H_2O$ (Plate 3)**

#T_1-T_6 = 0, 250, 500. 1000, 1500, 2000 mg L^{-1} Lead Acetate Trihydrate.