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RESEARCH ARTICLES

Arsenic induced chromosomal aberrations, biochemical and morphological changes in *Vigna radiata* L. (Mung bean) seedlings and its amelioration by Curcumin

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Abstract
The responses to arsenic (As) and curcumin (Cur) were examined in *Vigna radiata* L. (Mung bean) seedlings in order to evaluate the role of Cur in reducing the effects of As stress. To aim this, As, Cur and their interactions were investigated on growth, biochemical, cytological and histochemical traits in the plant seedlings. The findings demonstrated that As stress causes chromosomal abnormalities such as C-mitosis, Laggard chromosome, clumped metaphase, and Bridge in Anaphase. Additionally, some cells' nucleoli did not vanish during metaphase. The morphological traits like germination percentage and seedling growth were altered with a simultaneous aggravation in amylase and antioxidant activity under As stress. The application of Cur significantly improved morphological traits, and antioxidant activity. Chromosomal aberrations, mitotic index, oxidative stress marker, and histochemical parameters were also altered in the interaction study of As and Cur. The investigation revealed that the consequences of As-induced phytotoxicity and chromosomal aberrations in *Vigna radiata* seedlings were ameliorated by Cur by perking up the antioxidative enzymes and augmenting amylase activity in a dose-dependent manner.

Keywords *Vigna radiata* L. · Arsenic stress · Chromosomal aberration · Antioxidant · Curcumin · Amelioration

Introduction

Arsenic (As) is one of the fastest dissipating toxic contaminants in earth's crust (Cooper et al. 2020). Arsenic occurs in the environment as an organic, inorganic, and gaseous forms and its contamination seriously menaces the quality and safety of agricultural products and its sustainable development (Zeeshan et al. 2021). Consumption or exposure to more than safe levels of As contaminated food for humans causes bronchiectasis, cardiac coronary diseases, damaged intellectual function, diabetes, gangrene, skin, and lung cancers, etc. (Singh et al. 2015). The growth and productivity of crops are sternly affected by As stress through alteration of physiological processes and disruption of osmotic potential. The excessive ROS provoked under As stress causes membrane lipid injury, altered carbohydrate metabolism, damaged proteins and nucleic acids, reduced water, and nutrient uptake that result phytotoxic effects like cell dysfunction and death in plants (Alvarenga et al. 2020). The genotoxic effects of this chemical mutagen are induced by DNA damages or chromosomal aberrations that lead to reduced mitotic index in plants (Hani et al. 2020). Gupta et al. (2020) reported on the phytotoxic and genotoxic effects of As in *Vicia faba* and it was evident through significant increases in hydrogen peroxide (H_2O_2), malondialdehyde (MDA) levels and carbonyl groups in root and shoot of *V. faba*. The cyto-genotoxic effects of As stress were seen through decreased mitotic index (MI), relative abnormality rate (RAR) as well as other chromosomal abnormalities along with micronuclei in root meristematic cells of *V. faba*. Picchi et al. (2021) demonstrated the phytotoxic effects of As contaminated soils in *Cannabis sativa* L. and *Brassica juncea* L and the effects were seen in antioxidant enzymatic activities and photosynthetic performance that lead to a reduction in biomass by 50 and 25% in *C. sativa* and *B. juncea*. González-Moscoso et al. (2022) also reported the

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phytotoxic effects of As on tomato plants as evidenced by reduced growth, reduced number of leaves, decreased photosynthetic pigments, yield, enzymatic and non-enzymatic antioxidant contents of tomato plants. To counteract the toxicological effects of As stress, plants are furnished with a complex network of defense systems including antioxidant defense systems that protect their cellular system from harmful effects. Hasanuzzaman et al. (2020) reported that plants activate their antioxidant defense system to alleviate the adverse effects of oxidative stress or ROS generated under metal stress, and it varies among plant species, concentration and duration of stress. Supplementations of various compounds are reported to boost the antioxidant defense system of plants to cope up with As stress. The study of Asgher et al. (2021) described that the application of an adequate amount of H_2O_2 modulates the expression and activity of antioxidant enzymes and protects the photosynthetic activity from As stress. They reported that the activities of antioxidant enzymes like ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathione reductase (GR) were elevated by the application of H_2O_2 on As stressed plants compared to the control plants.

Curcumin or diferuloylmethane is a polyphenol extracted from the rhizome of the Turmeric (*Curcuma longa*) plant, known for its high antioxidative property (Tang et al. 2021). Several pharmacological and clinical studies have been conducted on curcumin over time because it decreases oxidative stress by inhibiting peroxidation of lipids and neutralizing hydrolyzed and superoxidized radicals. *Vigna radiata* or Mung bean is one of the major legumes of Asia that is well known for its rich constituents of carbohydrate, proteins, dietary fiber, minerals, and vitamins (Mekkara et al. 2021). This legume has antioxidative, anticancerous, anti-inflammatory, hypolipidemic, nutraceutical and prebiotic properties and can be used as a meat alternative for vegetarians as it is a very rich source of protein. *V. radiata* has the potential for nitrogen fixation capability, fast growth, soil reinforcement, and prevention of soil erosion, etc. In India, mung bean is consumed almost in every house mainly as Prasad or as a potent source of protein (Katiyar et al. 2020). The purpose of this study was to evaluate the phytotoxic and genotoxic effects of As on growth, biochemical and cytological traits of *V. radiata* (Mung) seedlings and its amelioration by the antioxidative property of Cur. Our work is the first to report As induced chromosomal alleviating property of Cur in *V. radiata* seedlings.

Material and methods

From a local, certified shop, Mung bean seeds were purchased. Surface-sterilization was done with 0.1% (w/v) $HgCl_2$ solution and then rinsed thrice with distilled water. Sodium arsenate heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$) solution was used as the source of As. The test solution consists of two concentrations of As (25, 50 µM), two concentrations of Cur (10 and 25 µM), and an interaction of As and Cur i.e. 50 µM As + 10 µM Cur and 50 µM As + 25 µM Cur. The sterilized seeds were kept in test solutions for one hour in the shaker. After one hour, the seeds were washed with distilled water and set for germination on filter paper in Petri dishes containing the test solution for 48 h. The filter paper was previously soaked in water and each petri dish contained 20 seeds sprayed with 3 mL of test solutions. Otherwise in control, 3 ml of distilled water was used. Based on the literature study on seed priming and optimization of our work, we have used 3 ml of test solutions in treated seeds and distilled water was used in control. The plates were kept at 28 ± 1 °C in the incubator. After 48 h, sampling was done to determine growth, cytological, and biochemical parameters.

Ten randomly selected seedlings were taken from each petri dish to measure plumule, radical length, and fresh mass. The length of plumule and radical was measured using thread and scale. Radicle and plumule elongation percentage was calculated by dividing the value of desired concentration from the compared concentration, after that subtracting that value with the compared concentration and finally multiplying it with 100. For dry mass estimation, plumule and radical were wrapped separately with aluminum foil and were dried in the oven at 80° C for 48 h. Histochemical parameters were analyzed to detect membrane lipid injury and can be analyzed by using the Schiff reagent. For cytological parameters, Acetocarmine squash preparation was made by following the method of Sharma and Sharma (1980). The alpha-amylase activity and lipid peroxidation was determined by following the method of Swain and Dekker (1966) and Heath and Packer (1968). The superoxide dismutase (SOD) activity was assayed using the Giannopolitis and Reis (1977) method. Catalase (CAT) and GPX activity was measured according to the method of Chance and Maehly (1955). In addition to this, the method of Griffith (1980) was followed to determine Glutathione reductase (GR) activity.

Statistical analysis

The data obtained were analyzed by using a one-way analysis of variance (ANOVA) ($P \leq 0.01$). Fisher's least significant difference (LSD) method at $P \leq 0.05$ was used to compare the difference between the means of every assay. Mean ± standard error of three experiments are represented by each bar.

Results

The result of our present study revealed varied results of germination percentage under 25 µM As, 50 µM As, 10 µM Cur, 25 µM Cur, and in their interactions [50 µM As + (10 + 25 µM Cur)], which are represented in Table 1. Germination percentage was lowest in 50 µM As treatment (84%) relative to the control (98%). Moreover, 50 µM As treatment reduced plumule and radicle length of seedlings by 59.67% and 82.40% respectively compared to the control. The plumule and radicle of 50 µM As + 10 µM cur interactions elongated by 40.32% and 105.26% concerning 50 µM As. The fresh and dry mass of the plumule and radicle followed the same hierarchy with the length being lowest at 50 µM As and highest at 25 µM Cur (Table 1 and Fig. 1).

The effect of different concentrations of As, Cur and their interactions on the mitotic index (MI) in *V. radiata* has represented in Table 2. With respect to 50 µM As treatment, the mitotic index of 50 µM As + 10 µM Cur interactions escalated by 30.87%. Our results showed that the application of Cur improved the MI. The genotoxic effect of As cause chromosomal abnormalities in *V. radiata* seedlings. In our present study, chromosomal aberrations were progressively elevated with the increase of As treatments by 7% and 11.33% in 25 µM and 50 µM As treatments. No chromosomal aberrations and nuclear abnormalities were observed at the control and Cur treatments. As treatment-induced different types of chromosomal aberrations like clumped mitosis, c-mitosis, chromosomal bridges, laggard chromosomes, binucleated cells, etc.in different frequencies and are given in Table 2 and Fig. 2. In addition to this, our results showed that in some cells under 50 µM As treatment, nucleolus did not disappear during metaphase cell division. The structures of normal chromosomes are given in Fig. 3. Lipid peroxidation in plant roots can be visualized with Schiff's reagent by the appearance of pink coloration that indicated the accumulation of lipid peroxidation products (malondialdehyde). The results related to the histochemical analysis of *V. radiata* roots under As stress is depicted in Fig. 4. Oxidation of membrane lipids or pink coloration occurred in every As treatment and was especially pronounced in 50 µM As treated plant root tips (Fig. 4Dd, Ee). Moreover, the As treated roots appeared hard, thick and shorter. Accumulation of malondialdehyde (MDA) was least significant in control and Cur treated plants (Fig. 4Aa, Bb. However, in

Table 1 Affect of arsenic (25 and 50 µM As), curcumin (10 and 25 µM Cur) and their interaction [50 µM As + Cur (10 and 25 µM)] on *Vigna radiata* L. during germination. Data presented are mean ± SE (n = 3) respectively

Treatment	Germination %	Moisture content %	Plumule length (cm)	Radicle length (cm)	Fresh mass of plumule (mg)	Fresh mass of radicle (mg)	Dry mass of plumule (mg)	Dry mass of radicle (mg)
0	98 ± 0.01	93.99 ± 0.005	2.48 ± 0.08	2.16 ± 0.04	78.5 ± 3.07	9.58 ± 0.186	1.03 ± 0.129	0.6 ± 0.091
25 µM As	87 ± 1.86	75.48 ± 0.005	1.24* ± 0.04	0.64* ± 0.07	59.0* ± 0.85	4.63* ± 0.159	0.34* ± 0.028	0.00* ± 0.00
50 µM As	84 ± 2.32	74.39 ± 0.005	1* ± 0.02	0.38* ± 0.03	46.7* ± 0.649	3.52* ± 0.398	0.23* ± 0.078	0.00* ± 0.00
10 µM Cur	98 ± 0.01	82.44 ± 0.002	2.50* ± 0.05	2.17* ± 0.04	79 ± 0.468	8. 9 ± 0.322	1.06 ± 0.033	0.59 ± 0.005
25 µM Cur	100 ± 0.00	83.74 ± 0.002	2.89 ± 0.04	2.72 ± 0.05	81.7 ± 2.22	9 ± 0.174	1.1 ± 0.051	0.62 ± 0.035
50 µM As + 10 µM Cur	92 ± 1.95	79.96 ± 0.002	1.48* ± 0.02	0.78* ± 0.05	63. 2* ± 2.29	5.56* ± 0.517	0.56* ± 0.0575	0.15* ± 0.034
50 µM As + 25 µM Cur	89 ± 1.29	76.61 ± 0.002	1.37* ± 0.02	0.65* ± 0.03	60.8* ± 3.64	4.9* ± 0.044	0.44* ± 0.034	0.22* ± 0.029

An asterisk (*) represents significant difference from the control ($p < 0.05$)

Fig. 1 Changes in germinating mung seeds (*Vigna radiata*) subjected to Arsenic (As) stress, curcumin (CUR) and their interaction. **A** 0 µM (Controlled), **B** 25 µM As, **C** 50 µM As, **D** 10 µM Cur, **E** 25 µM Cur, **F** 50 µM As + 10 µM Cur, **G** 50 µM As + 25 µM Cur. A bar with an asterisk (*) represents significant difference from the control ($p < 0.05$)

Table 2 Chromosomal aberrations and Mitotic index percentage in root tip cells of mung bean (*Vigna radiata* L.) under different concentrations of Arsenic (25 and 50 µM As), curcumin (10 and 25 µM Cur) and their interactions [50 µM As + Cur (10 and 25 µM)]. Data presented are mean ± SE (n = 3) respectively

Treatment	Total cells	Dividing cells	Aberrant cell %	Mitotic index %	Aberrations					
					Clumped mitosis	C-mitosis	Laggard chromosome	Bridge	Binucleated cell	Stickiness
0	474 ± 12.99	371 ± 12.20	0.00 ± 0.00	77.90 ± 4.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25 µM As	481 ± 12.11	220 ± 5.17	7.00* ± 1.03	45.89* ± 2.10	2.66 ± 0.78	1.00 ± 0.51	2.00* ± 0.51	2.00 ± 0.51	1.00 ± 0.51	2.00* ± 0.51
50 µM As	488 ± 6.49	177 ± 5.66	11.33* ± 2.08	36.37* ± 0.91	14* ± 2.06	4.66* ± 1.07	5.33* ± 0.78	5.33* ± 0.78	3.00* ± 1.03	5.00* ± 1.03
10 µM Cur	454 ± 24.88	360 ± 12.03	0.00 ± 0.00	79.29 ± 7.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25 µM Cur	451 ± 19.10	370 ± 3.62	0.00 ± 0.00	82.03 ± 3.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50 µM As + 10 µM cur	492 ± 5.68	234 ± 12.79	3.00 ± 0.51	47.60* ± 2.33	3.00* ± 0.51	1.00 ± 0.51	1.66* ± 0.78	2.00 ± 1.03	1.66* ± 0.29	1.66 ± 0.78
50 µM As + 25 µM cur	490 ± 5.19	225 ± 8.62	4.33* ± 0.59	46.11* ± 2.23	3.66* ± 0.51	1.00 ± 0.51	1.33 ± 0.29	2.33 ± 0.59	2.00* ± 0.51	3.33* ± 0.29

An asterisk (*) represents significant difference from the control ($p < 0.05$)

the roots exposed to the interaction of As and Cur, staining was comparatively weaker than the 50 µM As treatment revealing the ability of Cur to downregulate membrane damage under As stress conditions (Fig. 4Ff, Gg.

An elevated level of alpha-amylase activity was observed in *V. radiata* seeds with an increasing value of Cur by 2.08% at 10 µM and 21.73% at 25 µM relative to the control as shown in Fig. 5. It signifies the positive role of Cur in endospermic starch digestion and better seed germination of *V. radiata* seeds. The alpha-amylase activity was lowest at 50 µM As (39.13%) treatment in comparison with control. It increased by 19.42% in 50 µM As + 25 µM Cur interactions in comparison with 50 µM As treatment. The lipid peroxidation product or MDA has escalated progressively with the increase of As indicating the severe damaging effect of As on membrane lipids (Fig. 6). In our present study, almost all the antioxidant enzymes are slightly turned on under moderate As concentration (25 µM As) but gradually decreased at 50 µM As concentration. In our experiment, the SOD activity of plumule declines under As stress (25, 50 µM As) over control, 10 µM cur, 25 µM cur and in their interactions. But the radicle of 25 µM As showed the same SOD activity as the control whereas it significantly declines at 50 µM As treatments. The remarkable increase in SOD activity was observed both in plumule, and radicle in the interaction study of 50 µM As + 25 µM Cur in comparison with 50 µM (Fig. 7). Catalase (CAT) can abolish H_2O_2 by degenerating it into water H_2O and O_2. The CAT activity has increased at a low concentration of As (25 µM) by 22.30% in plumule relative to the control, signifying the As-induced increase in CAT activity and aforesaid total soluble protein content as a defense mechanism under moderate As stress. However, at 50 µM As treatment, the CAT activity reduced, and that could be due to the inactivation of the enzyme by the higher accumulation of H_2O_2 resulting from quenching of O^{2-} by SOD (Fig. 7). The GPX activity of different concentrations were found in the series of 25 µM cur > 10 µM cur > control > 50 µM As + 10 µM Cur > 50 µM As + 25 µM Cur > 25 µM As > 50 µM As and are represented in Fig. 8. Glutathione reductase (GR) activity of both plumule and radicle slightly escalated at 25 µM As stress compared to control which may be due to augmentation of GR activity under As-inflicted oxidative stress. But under 50 µM As, GR activity of both plumule and radicle declined compared to the control that might be due to the generation of excessive ROS (Fig. 8). Application of Cur boosts GR activity which is crucial for detoxification of ROS, and improved defense against oxidative stress under As stress.

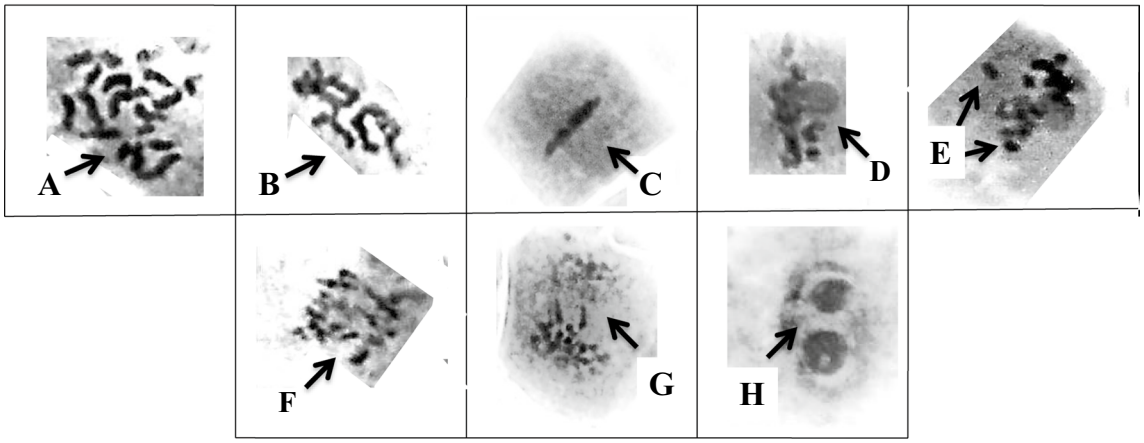

Fig. 2 Chromosomal aberrations were found at different stages of the cell cycle on root tip cells of mung (*Vigna radiata*) under As stress. **A** Cmitosis, **B** laggard chromosome, **C** clumped metaphase, **D** nucleolus did not disappear in metaphase, **E** unequal division of chromosome at anaphase, **F** multiple bridges in anaphase, **G** bridge in anaphase, **H** binucleated cell

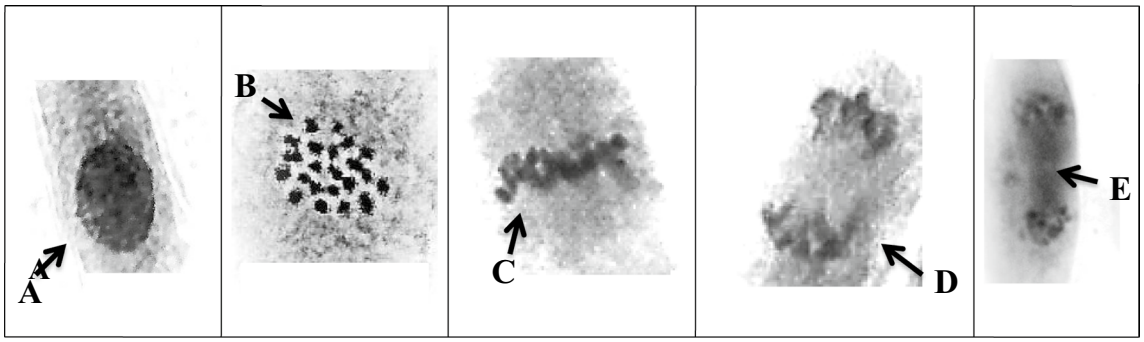

Fig. 3 Normal mitosis stages of cell cycle in mung (*Vigna radiata*) root tips. **A** Prophase, **B** early metaphase, **C** metaphase, **D** anaphase, **E** telophase

Discussions

Arsenic pollution is one of the major threats to all living organisms that may interfere with the growth and development of plants by altering the structure of chromosomes (aberration) resulting in changes in biochemical and morphological parameters. In this study, we have provided insight into how Cur regulates As stress to furnish protection to *V. radiata* seedlings. Higher concentration of As stress resulted in a decrease in the overall growth of plants, feasibly by inducing chromosomal aberrations, disturbing the antioxidative defense system and increasing H_2O_2 and MDA contents. However, Cur exposure provides protection and helps in the growth and development of *V. radiata* seedlings against As toxicity. It is reported that As exerts negative effects on plants by interacting with their chromosomal structures, disrupting their signalling mechanism and cell division that leads to changes in morphological and biochemical traits. It generates chromosomal aberrations by hampering the normal cell cycle (prophase, metaphase, anaphase, and telophase) of plants by damaging nucleic acids, and inhibiting DNA repair systems by binding to the thiol group. Sharma (2012) reported that metalloids decreased root turgor pressure, reduced cyclin and Cdk production which are crucial for regulating the cell cycle and lead to inhibition of cell enlargement and generate abnormalities in cell cycles. The degree of As stress was seen with the induced levels of chromosomal aberrations. The interactions of 50 µM As + 10 µM Cur showed fewer chromosomal aberrations than other respective concentrations of As, Cur and their interactions. Arsenic-treated chromosomal aberrations also had been reported in *Allium cepa* and *V. faba* roots (Gupta et al. 2018, 2020). But Cur-dependent mitigation of As toxicity by analyzing cytological parameters in *V. radiata* seedlings has not been reported yet. Our result is as per the study on animal model (mice) by Liju et al. (2021) in which Cur mitigated chromosomal aberrations in mice.

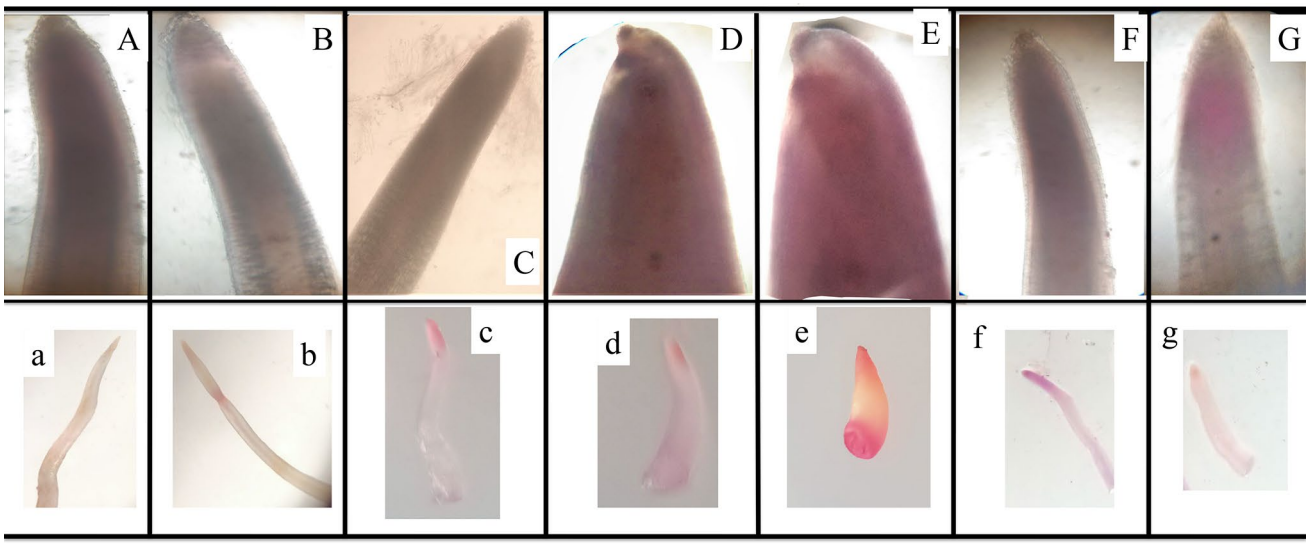

Fig. 4 Histochemical studies of roots of germinating mung seeds (*Vigna radiata*) subjected to As stress, Cur, and their interactions were observed under the simple microscope (a–g) and compound microscope (**A–G**). **A** a—Control, **B** b—10 µM Cur, **C** c—25 µM Cur, **D** d—25 µM As, **E** e—50 µM As, **F** f—50 µM As + 10 µM Cur, **G** g—50 µM As + 25 µM Cur

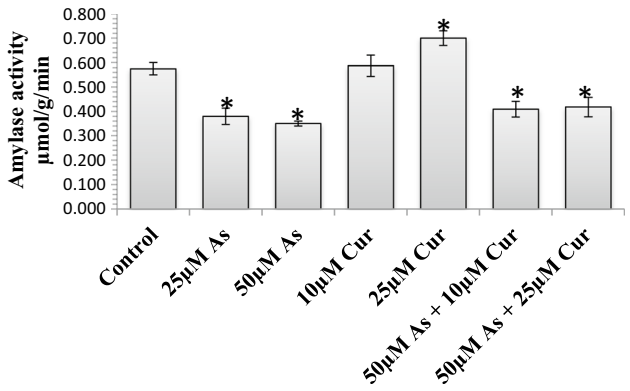

Fig. 5 Changes in alpha-amylase activity of germinating mung seeds (*Vigna radiata*) subjected to As stress, Cur, and their interactions. Data presented are mean ± SE (n = 3). A bar with an asterisk (*) represents significant difference from the control ($p < 0.05$)

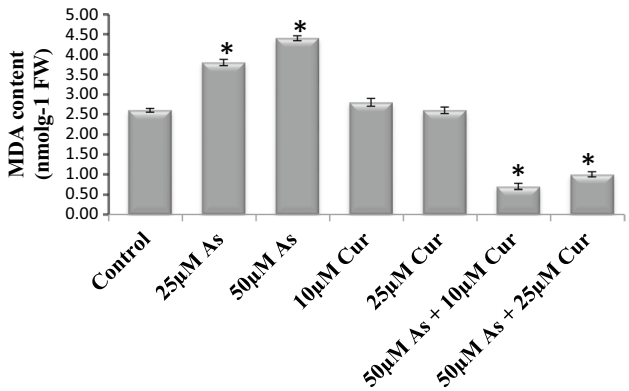

Fig. 6 Changes in MDA content of germinating mung seeds (*Vigna radiata*) subjected to As stress, Cur, and their interactions. Data presented are mean ± SE (n = 3). A bar with an asterisk (*) represents significant difference from the control ($p < 0.05$)

The progressive reduction of mitotic index (MI) with an increase in As concentration might be due to the reduction of cell division, and decreased extensions of the cell cycle under As stress. Our result is following the study on *Allium sativum* by Chatterjee and Chatterjee (2021) under As stress. Several studies demonstrated that chromosomal aberrations have been related to MI, and seedling length. Inhibition of mitosis due to the generation of chromosomal aberrations might be the reason for suppressing the elongation of cells by As that lead to the reduction of plant growth (Aidid and Okamoto 1992). The result is per the study by Tiwari and Rao, (2010) that reported the As-induced genotoxicity ameliorating property of Cur.

The first physiological process affected by As stress is observed in seed germination due to improper uptake of water and nutrients (Chandrakar et al. 2017; Yadu et al. 2019). The results of our present study showed gradual inhibition of germination rate with an increase in As concentration relative to the control. Similar outcomes were reported in *Oryza sativa* by Mridha et al. (2021) and in *Lepidium sativum* by Nouri and Haddioui (2021) under As stress. The decrease in moisture content of the seed might have inhibited the germination rate under As stress. Azad et al. (2021) demonstrated that seed germination is influenced by the reduction in the water potential gradient between seeds and their surrounding media. The application of Cur at

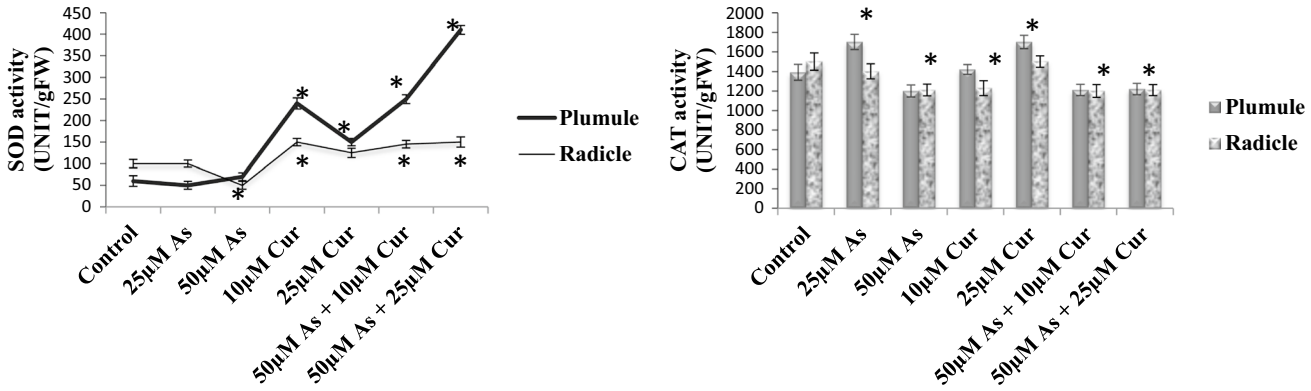

Fig. 7 Changes in SOD and CAT activity of plumule and radicle of germinating mung seeds (*Vigna radiata*) subjected to As stress, Cur and their interaction. Data presented are mean ± SE (n = 3). A bar with an asterisk (*) represents significant difference from the control ($p < 0.05$)

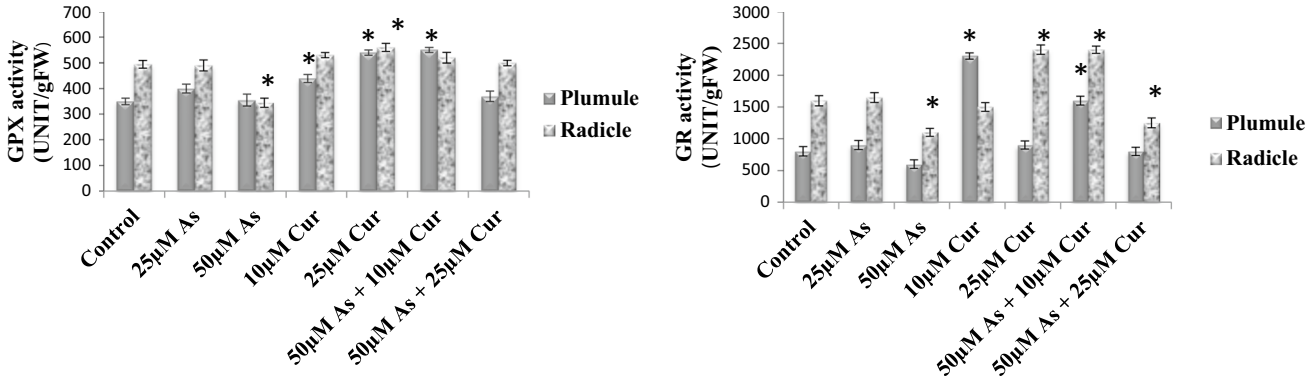

Fig. 8 Changes in GR and GPX activity of plumule and radicle of germinating mung seeds (*Vigna radiata*) subjected to As stress, Cur, and their interaction. Data presented are mean ± SE (n = 3). A bar with an asterisk (*) represents significant difference from the control ($p < 0.05$)

lower concentrations alleviated the reduction of germination rate under As stress. These results are per the study of Upadhyaya et al. (2014). In the current study, the increase of germination percentage under As stress by Cur treatment suggests enhanced moisture content and proper hydration of the seeds that facilitated germination. The fundamental consequences of metalloid toxicity include inhibition of germination rate, decreased growth, root elongation, and reduced biomass accumulation as reported by Chandrakar et al. (2016). It may be due to the maximum use of accessible energy for the production of stress-linked crucial compounds (e.g. antioxidants, phytochelatins, etc.), enhanced leakage of cellular constituents, water loss, and protein degradation (Chandrakar et al. 2018; Agnihotri and Seth 2016). The concentration-dependent declines of plumule and radicle length suggested the correlation between seedling lengths with As concentration. The pronounced toxicity of As stress on radicle over plumule may be attributed to less translocation of As to plumule and more accumulation of As on radicle and it indicates the enhanced lipid peroxidation resulted from high oxidative stress under As stress (Singh et al. 2007). Wang et al. (2004) also reported exclusion mechanism adoption in which the metals are accumulated in roots (radicle) inhibiting their transport to the shoots (plumule). Our result following the study on wheat by Kumar et al. (2021) under As stress. Nevertheless, Cur treatment especially at 10 µM, improved all measured growth attributes under As stress because Cur is a potent antioxidant that prevents protein and membrane lipid degradation due to oxidative damage caused by excessive ROS (Mekkara et al. 2021).

Histochemical analysis with Schiff's reagent represents a useful tool for the indirect detection of membrane lipid injury. Gilbert and Martin (2015) described that Schiff's reagent produces a magenta or purple-colored Schiff base or imine by reacting with aldehydes originating from lipid peroxides downstream of reactive oxygen species. Arsenic treatment increased the lipid peroxidation of *V. radiata* seedling roots, with the strongest effects induced by 50 µM As treatment. In this treatment, strong and widespread histochemical staining was observed signifying the profound damage of the cell membrane lipids under As stress conditions in a dose-dependent manner. This demonstrated

the increased production of free radicles under As stress in *V. radiata* roots. However, Cur-treated root tips weakly showed histochemical staining in comparison with the control. Interestingly, the interactions of As and Cur showed significantly faint staining compared to the 50 µM As treatment. Moreover, in the interaction study, the roots appeared thinner and longer than both of the As treatments. The result of our present study showed concentration-dependent declination of alpha-amylase activity with As concentration that may be contributed by the reduction in soluble sugars, and proteins (raw materials of structural components) under As stress that might diminish the growth of developing axis in germinating seed. The reduction of alpha-amylase activity under As stress is in agreement with the study on *Oryza sativa* (Choudhury et al. 2010), and mung (Ismail 2012). Our result is also in accordance with the study on medicinal pumpkins under salinity stress by Farsaraei et al. (2021). The application of Cur enhanced alpha-amylase activity at 25 µM concentration and also the interactions of 50 µM As + 25 µM Cur show a slight increase of alpha-amylase activity over As stress (25 and 50 µM), and interactions of 50 µM As + 10 µM Cur. The feasible mechanism for increasing alpha-amylase activity by Cur under As stress can be explained that Cur augments soluble sugars and proteins contents and thereby, increased the alpha-amylase activity under As stress.

Lipid peroxidation is considered a biomarker for the evaluation of oxidative damage to the membrane by ROS. In the current study, the degree of As stress can be seen with the increased levels of lipid peroxidation product or malondialdehyde (MDA) suggesting cell membrane damage due to exaggerated oxidative stress. The result of our study is following the study on *Camellia sinensis* by Li et al. (2021), and the study on barley and maize by AbdElgawad et al. (2021). The application of Cur (especially 25 µM) significantly reduced lipid peroxidation levels but the interactions of 50 µM As + 10 µM Cur showed better results in downregulating lipid peroxidation level over 50 µM As + 25 µM Cur interactions. Further, decreased MDA content under Cur supplementation might be closely associated with cellular ROS level under As stress. Our result is in agreement with the study on [*Vigna radiata* (L) Wilczek] by Upadhyaya et al. (2014) and on an animal model (rats) by Ahmadabady et al. (2021). Following the substantial increase in MDA under As stress, the result of our study demonstrated that increased levels of MDA were correlated with As-mediated oxidative burst in *V. radiata* seedlings, which indicated severe membrane damage by As stress.

Upon As stress, plants stimulate an efficient antioxidative defense system to cope with ROS and evade oxidative damages (Blokhina and Fagerstedt 2010). But depletion in antioxidant enzyme activities indicated oxidative injury and their insufficiency to counteract the As-induced oxidative stress (Yadu et al. 2018; Chandrakar et al. 2017; Hasanuzzaman and Fujita 2013). The first line of defense against reactive oxygen species (ROS) is established by the enzymatic antioxidant, super oxide dismutase (SOD) which scavenges the radical superoxide (O^{2-}) to hydrogen peroxide (H_2O_2) and oxygen (O_2) (Thounaojam et al. 2012). The decrease in SOD activity under 50 µM As may be due to the reduction in essential micronutrient constituents of SOD-like manganese (Mn), copper (Cu), and iron (Fe) under As stress as reported by Farnese et al. (2014). A similar result of reduced SOD activity under As stress was shown by the experiment of Katiyar et al. (2020). However, Cur's treatment increased in SOD activity, which implied that Cur provided a safeguard against ROS under As stress. In addition to this, moderate stress (25 µM As) condition elevated CAT activity but at 50 µM As stress, its activity was downregulated which may be due to the induction of excessive oxidative stress. The result of our study following the study on wheat (*Triticum sativum*) under As stress by Sil and Biswas (2020). Reduction of GPX activity was more prominent in radicle particularly in 50 µM As stress. A decrease in GPX activity implies a higher accumulation of H_2O_2 and indicates abortive detoxification that leads to increased ROS-induced damages (Sil and Biswas 2020). Moreover, As induced growth retardation was reported to be more in roots than shoots (Ahmad et al. 2020). Similar results were shown in the study on Tomato plants by González-Moscoso et al. (2022). Some studies suggested that elevated activities of GPX conferred tolerance to heavy metal stress. The result of our present study revealed that the enhanced activities of GPX due to Cur treatment might be due to GSH-dependent peroxide scavenging that led to reduce oxidative damage. Glutathione reductase (GR) is a flavoprotein which involves in the detoxification of ROS, contributes to the pertinent functioning of the antioxidant systems, maintenance of a higher GSH to GSSG ratio, and phytochelatin synthesis in plants (Thounaojam et al. 2012). GR activity was enhanced under moderate As stress (25 µM) but its activity decreased at 50 µM As stress revealing the exaggerated generation of ROS under high As stress. Arsenic stress-induced reduction of GR activity was also reported in *Brassica juncea* by Ahmad et al. (2021) and in *V. radiata* by Sadeghipour and Monem (2021).

Abatement in plant growth under stress conditions has been regarded as an effect of decreased photosynthetic content and altered biochemical parameters in plants. The result of our study revealed the reduced activity of SOD, CAT, GPX, and GR of both plumule and radicle (especially radicle) under excessive (50 µM) As stress might be due to destruction of antioxidative defense mechanism under high As stress (Yan et al. 2021), Which thereby hindered the ROS detoxification mechanism and subsequently increased the oxidative stress. Sharma (2012) mentioned that As binds to thiol groups of antioxidant enzymes and directly affects

biochemical reactions and reduced the overall growth of the plant. These findings suggested that Cur sturdily provides tolerance towards As stress in *V. radiata* seedlings. Moreover, the present study revealed that the elevation in growth occurred due to enhanced stimulation in antioxidative activity and reduced chromosomal aberrations and lipid peroxidation by Cur, which ultimately resulted in improved growth, biochemical, and histochemical traits in *V. radiata* seedlings.

Conclusion

In *Vigna radiata* L. (Mung), As stress significantly reduced germination, length of plumule, radicle, biomass, mitotic index, and amylase activity. Arsenic toxicity hampered water uptake or moisture content of seeds, increased lipid peroxidation and induced different types of chromosomal aberrations that may lead to the reduction of germination percentage, shortening of plumule, and radical length. The application of Cur alleviated As stress in the plant, which was revealed by the cytological, physiochemical, and antioxidative responses of the seedlings. The beneficial property of Cur may overcome the phytotoxic and genotoxic effects of As stress and help in the sustainable development of agriculture in a dose-dependent manner.

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Data availability statement The data that support this study are available in the article.

Declarations

Conflict of interest The authors declare no conflict of interest.

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