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ORIGINAL PAPER

Arsenic-induced oxidative stress in *Brassica oleracea*: Multivariate and literature data analyses of physiological parameters, applied levels and plant organ type

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Abstract Plant redox homeostasis governs the uptake, toxicity and tolerance mechanism of toxic trace elements and thereby elucidates the remediation potential of a plant. Moreover, plant toxicity/tolerance mechanisms control the trace element compartmentation in edible and non-edible plant organs as well as the associated health hazards. Therefore, it is imperative to unravel the cellular mechanism involved in trace element toxicity and tolerance. The present study investigated the toxicity and tolerance/detoxification mechanisms of four levels of arsenic (As(III): 0, 5, 25 and 125 μM) in *Brassica oleracea* under hydroponic cultivation. Increasing As levels significantly decreased the pigment contents (up to 68%) of *B. oleracea*. Plants under As stress showed an increase in H_2O_2 contents (up to 32%) in roots while a decrease (up to 72%) in leaves because As is mostly retained in plant roots, while less is translocated toward the shoot, as evident from the literature. Arsenic treatments caused lipid peroxidation both in the root and leaf cells. Against As-induced oxidative stress, *B. oleracea* plants mediated an increase in the activities of peroxidase and catalase. Contradictory, the ascorbate

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peroxidase and superoxide dismutase activities slightly decreased in the As-stressed plants. In conclusion and as evident from the literature data analysis, As exposure (especially high level, 125 µM) caused pigment toxicity and oxidative burst in *B. oleracea*. The ability of *B. oleracea* to tolerate As-induced toxicity greatly varied with applied treatment levels (As-125 being more toxic than lower levels), plant organ type (more toxicity in leaves than roots) and physiological response parameter (pigment contents more sensitive than other response variables). Moreover, the multivariate statistical analysis appeared to be a useful method to estimate plant response under stress and trace significant trends in the data set.

Keywords Arsenic · *Brassica oleracea* · Oxidative damage · Redox homeostasis · Antioxidant enzymes · Phytoremediation

Introduction

Arsenic is a toxic metalloid of global concerns (Ahmed et al., 2020; Wu et al., 2020). Environmental As contamination is a worldwide health and agricultural problem because of its persistent and hazardous nature (Shabbir et al., 2020b; Shahid et al., 2018). Besides its natural occurrence in different environmental components, As contamination of the environment is increasing owing to its high use in different industries, e.g., chemical industry, infrared detectors, light-emitting diodes, and microelectronic/semiconductors, paints, dyes, veterinary drugs and soaps manufacturing. Furthermore, the use of sewage sludge in agricultural land could also provoke soil contamination. The natural/geogenic sources due to the dissolution of As-minerals also contribute to its environmental contamination (Bech, 2020; Hussain et al., 2020; Natasha et al., 2020b; Natasha et al., 2021)

Arsenic is well-known to disrupt the metabolic functioning of the plants by enhancing the level of reactive oxygen species (ROS) (Rafiq et al., 2018; Shahid, 2020). These As-induced ROS are very unstable and reactive molecules (Ghassemi-Golezani et al., 2020). Arsenic-induced higher ROS production in plants may result in stunted growth, reduction in pigment content, mutagenicity and oxidation of lipid macromolecules (Yu et al., 2020). The increased ROS production is very toxic for several biochemical, physiological and metabolic processes occurring in plants (Shahid et al., 2014). Moreover, As stress conditions may activate the plant protective system by increasing the accumulation of compatible solutes, thereby causing an inhibition or activation of antioxidative enzymes (Rafiq et al., 2018; Yu et al., 2020). However, the induction and/or inhibition of antioxidative enzymes under As stress may vary with its applied form, dose, the duration of exposure and the type of plant species/organ (Table S1, S2). Nevertheless, these aspects are not fully specified for different trace elements and plant species. Phytohormones including auxin, abscisic acid, ethylene, gibberellins, cytokinin, salicylic acid, strigolactones, brassinosteroids and nitrous oxide are upregulated during stress and affect plant functioning. The hormones help to tolerate various environmental cues as most of them are involved in the phytochelatin biosynthesis (Bücker-Neto et al., 2017). Plant hormones can also interact with redox signaling to control responses to abiotic stress (He et al., 2021).

One of the prerequisites of phytoremediation (more precisely phytoextraction) is the production of high plant biomass by tolerating trace element stress. Moreover, the trace element toxicity and tolerance mechanisms also govern the compartmentation of trace elements in edible/non-edible plant organs and associated health hazards (Paithankar et al., 2020). The recent development in plant physiology at cellular levels has revealed that the variations in the biophysiochemical attributes (ROS, antioxidative enzymes, pigment contents, lipid peroxidation, genotoxicity) of plant species determine the plant potential to tolerate trace element stress (Natasha et al., 2020a). Hence, the extent of trace element-induced variations in biophysiochemical attributes determines the capability of a plant species to tolerate trace element stress. Despite considerable progress in phytoextraction potential of various plant species, there is still limited data about the role of various biophysiochemical attributes in phytoremediation, compartmentation of trace elements in edible/non-edible tissues and allied health hazards. The compartmentation of trace elements in plant tissues is equipotent for phytoremediation as the risk assessment. The cultivation of edible leafy plants and accumulation of trace elements in root tissues is of

dual importance for phytoremediation and food production (Natasha et al., 2020c).

Some plant species, mostly termed as hyperaccumulators, are capable of tolerating high levels of trace elements by maintaining their biophysiochemical attributes via activation of specific detoxification mechanisms (Souri et al., 2020; Zhang et al., 2020). Contrarily, other plant species, characterized as sensitive, do not have well-developed tolerance mechanisms and undergo severe trace element toxicity due to high variations in biophysiochemical attributes consequently affecting food chain (Rafiq et al., 2018). Therefore, understanding the mechanism of redox homeostasis in plants under trace element stress is important with respect to its tolerance, remediation and compartmentation in different plant organs. This research aimed to demonstrate the variations in biophysiochemical attributes of *B. oleracea* against various As(III) concentrations by discussing the underlying mechanism at the cellular level. In this study, As(III) was used to evaluate As toxicity in plants as As(III) is 60 times more toxic than As(V). The study especially compares the As-induced toxicity and tolerance for different physiological parameters and applied levels of As and plant organ type (leaf vs roots).

Materials and methods

Brassica oleracea growth and arsenic treatments

Dry and certified seeds of *B. oleracea*, purchased from Ayub Agriculture Research Institute Faisalabad, Pakistan, were grown for one week in sand culture. The germinated plants were transferred to a nutrient solution (NS). The NS was prepared as previously stated (Natasha et al., 2020c). All the pots were properly aerated during the experiment. The volume of the NS in the pots was properly maintained throughout the experiment. The solution was changed twice during the entire experiment (18 days).

Two weeks after vegetative growth in hydroponics, *B. oleracea* plants were treated for four days with four levels of As(III): Control treatment containing NS only with no As, As-5 treatment containing NS + 5 µM As(III), As-25 treatment containing NS + 25 µM As(III) and As-125 treatment containing NS + 125 µM As(III). Arsenite treatments were prepared using sodium arsenite salt ($NaAsO_2$) (sigma). After treatment exposure, the physiological status of plant samples was conserved by immediately freezing the samples in liquid nitrogen. Six replications were used for each treatment.

Physiological analysis of *Brassica oleracea*

Leaves and roots (~ 1 g) of As(III) treated and untreated *B. oleracea* plants were ground under liquid nitrogen, and the extract was prepared using 1:4 hydro-acetone buffer (v/v). The sample centrifugation was performed at 3000 g, for 10 min (Anwar et al., 2021). The supernatant was stored for further analysis.

Reactive oxygen species (H_2O_2)

For the quantification of ROS, the absorbance mixture was prepared using *B. oleracea* extract (1 mL), 10 mM potassium phosphate buffer (1 mL) and 1 M potassium iodide (2 mL) (Islam et al., 2008). The absorbance of the assay mixture was recorded on a spectrophotometer (AA, Solar -Series) at 390 nm.

Lipid peroxidation

Leaf and root extract of the *B. oleracea* plants was incubated at 95 °C in the water bath with butyl hydroxytoluene (0.01%) and trichloroacetic acid (20%). The incubation was carried out in the absence and presence of thiobarbituric acid (0.65%) (Hodges et al., 1999). The mixture was then centrifuged at 3000 g for 10 min, and the absorbance of the mixture was recorded at 440, 532 and 600 nm using spectrophotometer.

Analysis of chlorophyll contents

The chlorophyll contents of *B. oleracea* leaves were determined by recording the absorbance of the mixture at 663.2 and 646.8 nm against a buffer control (1:4 hydro-acetone buffer (v/v)(Lichtenthaler, 1987)).

Analysis of Antioxidant enzymes

About 100 mg of leaf samples of *B. oleracea* plants was ground under liquid nitrogen in 0.1 M phosphate buffer of pH-7.0. The extract was centrifuged twice for

30 min at 3000 g, and the supernatant was stored for enzymic determinations.

The activity of superoxide dismutase (SOD) was calculated using 0.25 mL of *B. oleracea* extract, 0.1 mM EDTA, 50 mM phosphate buffer (pH-7.8), 13 mM methionine, 60 µM riboflavin and 75 µM NBT. The mixture was kept below a light source (30 W fluorescent lamps) for 10 min to start the reaction. The assay mixture was run on the spectrophotometer to record absorbance at 560 nm.

For determining catalase (CAT) activity, 0.25 mL of *B. oleracea* extract was mixed with 15 mM H_2O_2 and 50 mM phosphate buffer (pH-7.0). The assay mixture was run on the spectrophotometer to record absorbance at 240 nm for 45 s. The degradation of H_2O_2 µM min^{-1} mg^{-1} protein represents CAT activity.

For determining guaiacol peroxidase (POD) activity, 0.25 mL of *B. oleracea* extract was mixed with 15 mM H_2O_2, 12 mM guaiacol and 50 mM phosphate buffer (pH-6). The assay mixture was run on the spectrophotometer to record absorbance at 470 nm for 90 s. The oxidation of guaiacol µM min^{-1} mg^{-1} protein represents POD activity.

Ascorbate peroxidase (APX) was determined using 0.25 mL *B. oleracea* leaf extract, 0.25 mM H_2O_2 and 0.25 mM ascorbic acid in 50 mM phosphate buffer (pH 7.0). The absorbance of the mixture was recorded at 290 nm. The H_2O_2 degradation per min^{-1} mg^{-1} protein represents APX activity.

Statistical analysis

The experiment was completely randomized with four treatments and six replications. XLSTAT (ver. 19.4) was run to perform analysis of variance following Duncan's multiple range test (DMRT) at 5% level of significance, Pearson correlation and principal component analysis (PCA). All the graphs were plotted using Microsoft Excel 2019.

Results

Arsenic-induced production of ROS in *Brassica oleracea*

Exposure of *B. oleracea* to different As(III) treatments showed a significant decrease in H_2O_2 contents over the control in the leaves of *B. oleracea* (Fig. 1). This decrease in H_2O_2 contents was 49%, 72% and 15%, respectively, for As-5, As-25 and As-125 over the control (Table 1). However, the roots of *B. oleracea* showed an increase in H_2O_2 content except for As-25. The variation in H_2O_2 content over the control in roots was 23%, −6% and 32%, respectively, for As-5, As-25 and As-125. This showed that As toxicity differed in different plant organs (leaves vs roots) of *B. oleracea*. Among the three As treatments, the highest H_2O_2 contents were observed in the As-125 treated plants. However, the H_2O_2 contents remained higher in the control group compared to As treated plants probably due to the activation of the detoxification mechanism of As-treated plants.

Arsenic-induced lipid peroxidation

High As dose (As-125) caused lipid peroxidation (TBARS) both in leaves and roots of *B. oleracea* (Fig. 2). While the lower applied levels of As (5 and 25 µM) either decreased or did not significantly affect TBARS contents in leaves. In roots, the As treatments significantly affect the TBARS contents, and the significantly high TBARS was observed in As-125 treated plants. The variation in TBARS contents was -19%, −46% and 88% for leaves and −9%, 18% and 124% for roots, respectively, for As-5, As-25 and As-125 over the control (Table 1). This shows that different applied levels of As have significantly different effects on TBARS contents of *B. oleracea*.

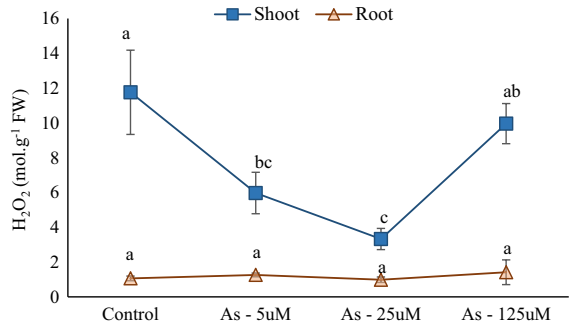

Fig. 1 Arsenic-induced H_2O_2 (nmol g^{-1} FW) productions in root and leaves of *B. oleracea*. Values delineate an average of six replications. Different lettering specifies the significant difference

Table 1 Percent variation (decrease or increase) response variables of *B. oleracea* under As stress

Arsenic levels	Chl-a	Chl-b	Chl-a + b	Shoot H_2O_2	Root H_2O_2	Shoot TBARS	Root TBARS	T. Protein	POD	CAT	SOD	APX
Control	–	–	–	–	–	–	–	–	–	–	–	–
As-5 uM	28	55	39	−49	23	−19	−9	1	175	100	−5	101
As-25 uM	53	69	58	−72	−6	-46	18	8	125	0	−27	−10
As-125 uM	68	78	72	−15	32	88	124	23	575	100	−29	−23
Overall	−149	−203	−169	−135	49	23	132	32	875	200	−61	68

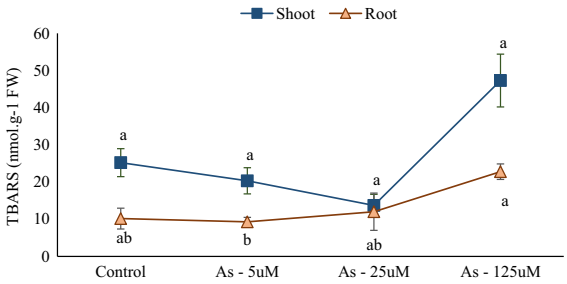

Fig. 2 Arsenic-induced TBARS (nmol g^{-1} FW) productions in root and leaves of *B. oleracea*. Values delineate an average of six replications. Different lettering specifies the significant difference

Arsenic-induced toxicity to chlorophyll contents of *Brassica oleracea*

Exposure to As treatments significantly decreased the chlorophyll contents compared to control (Fig. 3). A linear decrease in pigment contents was observed in As-treated plants over the control, and As-125 treatment caused a maximum reduction in pigment contents. The decrease in Chl-a contents was 28%, 53% and 68%, respectively, for As-5, As-25 and As-125 than the control (Table 1). The trend was same for Chl-b and Chl-a + b. This shows that As exposure can induce significant variations in pigment contents of *B. oleracea*.

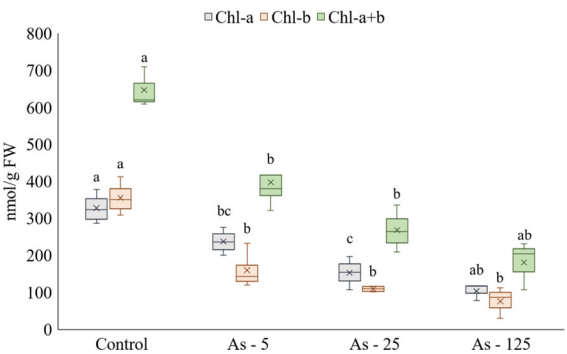

Fig. 3 Arsenic-induced toxicity to pigment content (μg g^{-1} FW) in *B. oleracea* leaves

Arsenic-induced changes in antioxidant enzyme activities

Exposure of *B. oleracea* to different As treatments differently affected the activities of antioxidative enzymes, and the effect varied with the type of antioxidative enzyme (Table 2). Overall, As treatments significantly increased the activities of CAT and POD enzymes, while lessened the activities of SOD and APX enzymes. The total protein contents also increased significantly in a dose-dependent manner under As treatments. Moreover, the decrease/increase in the activities of antioxidative enzymes was not dose-dependent with respect to the applied levels of As. This showed that As-induced activation/suppression of antioxidant enzymes varied with its applied levels and the type of antioxidative enzyme.

Arsenic-induced activation of CAT was 100%, 0.1% and 100%, while that of POD was 175%, 125% and 575%, respectively, for As-5, As-25 and As-125 over the control (Table 1). Significant high levels of SOD and POD were found in As-125-treated plant leaves. The significant decrease in SOD activity was 5%, 27% and 29% for increasing levels of As. In case of APX, As-5 increased its activity by 101%, while As-25 and As-125 decreased its activity, respectively, by 10% and 23%. It revealed that As-applied levels contrarily affect the activation of different antioxidative enzymes. Furthermore, the intensity of activation or suppression also varied with the type of these antioxidative enzymes.

Table 2 Arsenic-mediated modification in antioxidants in the leaves of *B. oleracea*. Values delineate an average of six replications ± SD. Different lettering specifies the significant difference

Treatments	T. Protein	SOD	CAT	POD	APX
Control	9.36 ± 0.3 b	49.2 ± 1.6 a	0.01 ± 0.002 b	0.04 ± 0.01 b	13.18 ± 10.1 a
As-5 uM	9.5 ± 0.8 b	46.6 ± 3.8 a	0.02 ± 0.003 b	0.11 ± 0.02 b	26.5 ± 3.4 a
As-25 uM	10.1 ± 0.4 ab	35.8 ± 4.9 b	0.01 ± 0.001 b	0.09 ± 0.01 b	11.9 ± 2.3 a
As-125 uM	11.5 ± 0.8 a	35.0 ± 3.9 b	0.02 ± 0.004 a	0.27 ± 0.05 a	10.1 ± 5.3 a

Multivariate analysis

A multivariate analysis was performed to develop a set of observations of possibly correlated variables and to establish a correlation between the biophysiochemical responses of *B. oleracea* under different levels of As(III) (Figs. 4, 5, 6, Figure S1, S2, Table S5, S6). The PCA of data separated all the attributes into eleven component factors (F1 to F11) (Table S5). The contributions of F1, F2, F3, F4 and F5 were 31%, 25%, 14%, 8% and 4%, respectively, in total variance. For F1, Chl-a, Chl-b, Chl-a + b and ROS-Root were the main parameters. The POD and CAT were the key contributors to F2. Similarly, F3 had a main contribution of LPO-Shoot, SOD and ROS-Root contents. However, APX and LPO-Root were the major contributors to F4 and F7, respectively (Table S5).

The PCA graph showed three major groups (Fig. 4). The PCA grouped APX and SOD together, which was due to a decrease in their activities against As stress. Similarly, POD and CAT were grouped together along with the stress biomarkers (ROS-R, LPO-R and T. protein) showing their similar variation trend (increase) under As stress (Fig. 4).

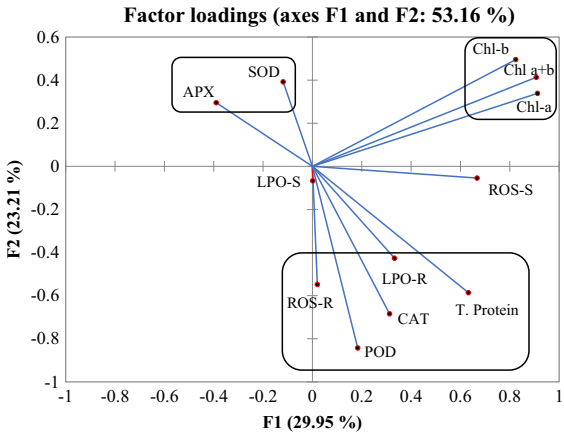

Fig. 4 PCA graph for different response parameters of *B. oleracea*

The Pearson correlation is indicated by developing a hot–cold heat map (red-blue) (Fig. 6). Different colors exhibited the intensity of the correlating variables. Most of the biophysiochemical variables of the *B. oleracea* showed a moderate-strong correlation (Fig. 6, Table S6, and Figure S2). This was probably due to the varied effects of different applied levels of As to different plant attributes.

The different treatments of As were also compared using PCA. The PCA graph showed generally a separate grouping of control and three applied levels of As(III) (Fig. 5). It means the overall effects of these treatments on various plant biophysiochemical responses were different from each other. Largely, the four treatments were grouped in an anti-clockwise manner starting from control to As-5 to As-25 and As-125. However, some replications did not exactly follow this trend.

Discussion

Arsenic-induced oxidative stress and tolerance mechanisms

The As-induced imbalance between ROS production and scavenging in plants and the ultimate happening of oxidative stress is well-known (Abbas et al., 2018; Irem et al., 2019; Rodríguez-Ruiz et al., 2019). Similarly, ROS-induced toxic effects on different plant biomolecules and biochemical processes (pigments and photosynthesis) have been extensively demonstrated in the literature (Abbas et al., 2018; Rafiq et al., 2018). Moreover, the activation of the plant defense system (upregulation of antioxidant enzymes) has also been reported in the literature (Yu

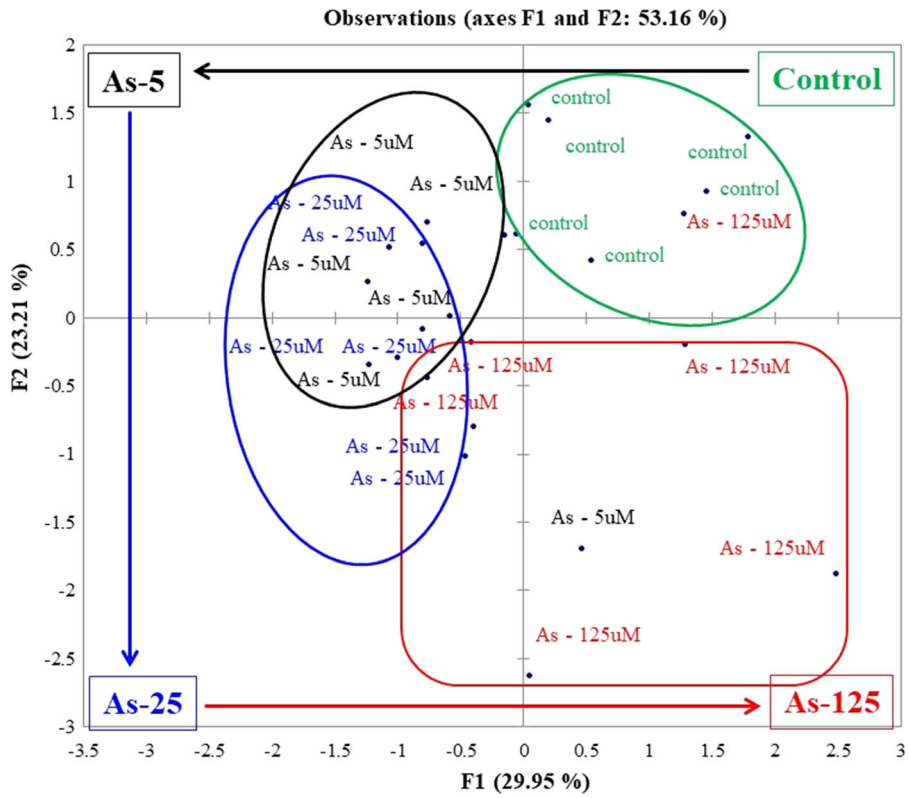

Fig. 5 PCA graph comparing different treatments of As. An anti-clockwise trend was observed starting from control, As-5, As-25 and As-125

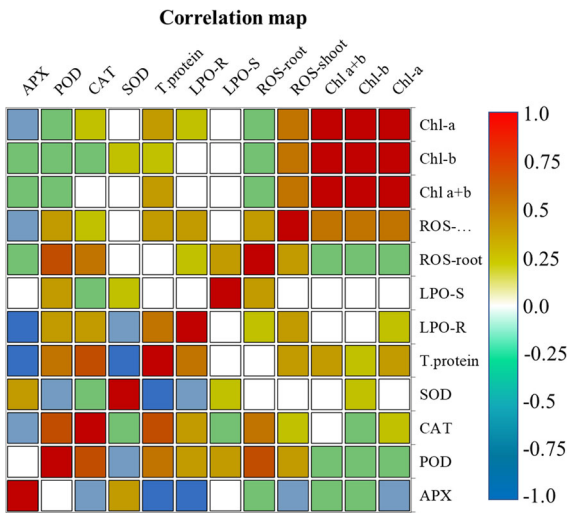

Fig. 6 Red-blue (hot–cold) scale correlation map among various biophysiochemical variables of *B. oleracea*

et al., 2020). The current study also confirmed the As-induced enhanced production of ROS, lipid peroxidation, toxicity to pigments and activation of antioxidants in *B. oleracea*.

However, this study revealed that As-induced oxidative stress and tolerance mechanisms greatly varied as follows:

- For different As-applied levels (As-125 vs As-5 and As-25)
- Induction or suppression of antioxidant enzymes
- For different plant organs: root vs leaves

Low vs high applied doses of As

In this study, the higher and lower applied levels of As mediated opposite trends of H_2O_2 generation. The As-125 treated plant leaves showed enhanced H_2O_2 generation, while As-5 and As-25 treatments showed decreased H_2O_2 generation compared to control. Similarly, Coelho et al. (2020) reported that increasing the levels of As(III) in growth medium (2–7 mg L^{-1}) decreased the production of H_2O_2 in plants, while the

highest level of As(III) (8 mg L^{-1}) increased H_2O_2 content in *Lemna valdiviana* plants. They also showed a linear reduction in $O_2^{\cdot-}$ content when the As concentration in the growth medium was increased up to 8 mg L^{-1}. Likewise, Rodríguez-Ruiz et al. (2019) reported a slight reduction in ROS production at 50 µM As in *Pisum sativum* as compared to 0 µM As.

The possible reason for the decreased ROS production under low As levels and enhanced ROS production under high As level could be due to the hormetic effect. Generally, the hormetic interactions demonstrate an increase in positive effects at lower doses, while a decrease in positive effects at higher doses of toxic substances (Shahid et al., 2020). The tendency reverts for toxic effects; increase in negative effects at lower doses, while a decrease in negative effects at higher doses.

In fact, recently, it has been revealed that low-applied doses of toxic substances (such as trace elements) may spur an increase in beneficial effects or a decrease in toxic effects. For example, Shahid et al. (2020) reviewed and revealed the trace element-induced phytohormesis for various plant responses of different plant species. It was anticipated that low levels of a toxic substance can promote the activation of defense system and also increase the levels of plant hormones (e.g., auxin, cytokinin, brassinosteroids, salicylic acid, gibberellic acid, abscisic acid, ethylene and jasmonic acid) (Shabbir et al., 2020a; Shahid et al., 2020). Together these phytohormones and tolerance mechanisms either enhances beneficial responses or alleviate the toxic effects.

Hence, in this study, it is quite possible that low levels of As (As-5 and As-25) may had improved the plant tolerance mechanism which scavenged enhanced ROS and alleviated possible oxidative stress (lipid peroxidation). While higher As level may have more seriously affected the tolerance mechanism, and thereby enhanced ROS generation and induced lipid peroxidation. Indeed, plant defense/detoxification mechanism can tolerate trace element stresses up to a certain threshold level. However, this threshold level of stress may vary with plant type, plant tissues (root/leaves) and the applied dose of trace element (Table S1).

Induction or suppression of antioxidant enzymes

The current study delineated an increase in the activities of POD and CAT, while a decline in the activities of SOD and APX. This revealed that different tolerance mechanisms/variables may respond differently under stress conditions. This can be due to their varied roles under stress conditions. Overall, the stimulation of POD and CAT confirmed the possible tolerance response of *B. oleracea* to As toxicity. The H_2O_2 is a very strong oxidizing agent, which can provoke numerous toxic effects such as lipid peroxidation in plants. Similar to the current findings, Saha et al. (2017) reported the induction in CAT and GPX activity while a linear suppression in APX enzyme in rice seedlings with increasing soil As concentration from 25 to 100 µM. Generally, CAT, POD and APX are considered as strong scavengers of H_2O_2 in the plants (Fig. 7) (Shahid, 2020). Elevated

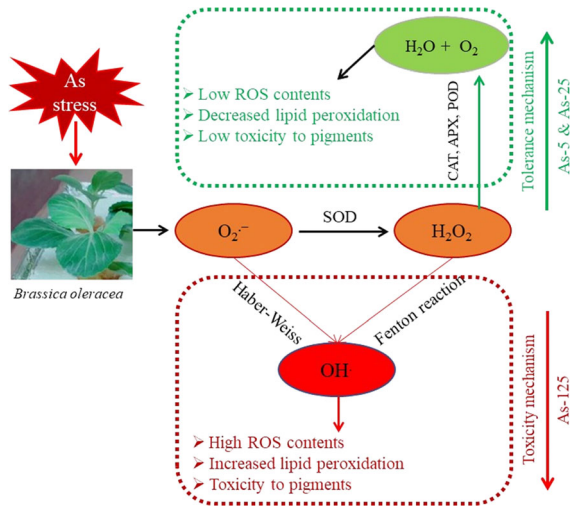

Fig. 7 Comparison of the possible mechanism of As toxicity or tolerance in sensitive and tolerant/hyperaccumulator plants. Generally, As can provoke $O_2^{\cdot-}$ production which is converted to H_2O_2 by SOD enzyme. This H_2O_2 can be scavenged by various antioxidant enzymes (CAT, APX and POD) to produce water and oxygen. This mechanism, highlighted green in the figure, is most common in tolerant/hyperaccumulator plant species. Alternatively, $O_2^{\cdot-}$ and H_2O_2 can be transformed to $OH^{\cdot}$, which is the most toxic form of ROS. This $OH^{\cdot}$ can induce oxidative stress resulting in lipid peroxidation, activation/inactivation of enzymes and toxicity to pigments. This mechanism, highlighted red in the figure, is most common in sensitive plant species. Overall, these mechanisms define the potential of a plant species to tolerate metal stress and the ability to remediate a metal-contaminated site

POD activities in this study can be for scavenging As-induced overproduction of H_2O_2.

In the case of APX, activity increased at low As level (As-5) but slightly reduced at higher As-applied levels. It is possible that APX activation in *B. oleracea* may occur only at lower applied levels of As or at low cellular level of H_2O_2. On the other hands, SOD is efficient to detoxify $O_2^{\cdot -}$ (Fig. 7). Therefore, it is possible that $O_2^{\cdot -}$ may have already been converted to H_2O_2 in this study; therefore, the activity of SOD did not show any upregulation.

This reduction in the activities of antioxidant enzymes can also be due to trace element toxicity to the precursors of enzymes (Paithankar et al., 2020; Peralta et al., 2020). Moreover, several antioxidant enzymes act simultaneously to scavenge ROS and convert them to other forms of ROS or into a neutral product such as oxygen and water (Shabbir et al., 2020a). Under such simultaneous phenomenon of ROS scavenging, certain enzymes may get more activated than others, as evidenced in this study. This variation in the activation of different antioxidant enzymes can be due to the reason that these antioxidant enzymes scavenge different types of ROS (Fig. 7). Therefore, during interconversion of ROS from one form to another form, the activities of respective scavenging antioxidant enzymes may also get affected.

Several previous studies also reported the alteration (up- and down-regulation) in the activities of antioxidant enzymes under As stress (Table S2). Hence, the difference among enzyme activation could be due to the changes in ROS production patterns, different species of ROS and plants species (Shahid et al., 2014). However, genetic level studies representing the expression of associated genes can better verify the associated mechanisms.

Roots vs leaves

In contrast to shoot, the roots of all the As-treated plants showed low variations in stress parameters (ROS and lipid peroxidation). This showed that the intensity of metal(loid)-induced toxicity may differ greatly in different plant organs. Despite, high As accumulation in roots, Singh et al. (2019) reported high membrane degradation in leaves compared to roots. However, in contrast, high ROS production and induction of lipid peroxidation were reported in roots compared to leaves of *Vigna radiata* in response to increasing As levels (1–2 µM) (Shabnam et al., 2019). This can be due to the varied activation of tolerance mechanisms in different plant organs.

Overall, plant leaves are the site greatly equipped with physiological mechanisms/apparatuses such as photosynthesis/chloroplast (El Yamani et al., 2020). These mechanisms/apparatuses are highly sensitive to stress conditions. Moreover, ROS are mainly generated in the electron transport chain of light reactions taking place in chloroplast/leaves (Rafiq et al., 2018). These variations may cause enhanced production of ROS and causing lipid peroxidation in leaves than roots. Many previous studies also reported variations in physiological attributes between roots and leaves (Table S1, S2).

Meta-analysis of recent data (2017–2020)

In order to compare the results of current study with the literature data, we performed out a meta-analysis of recently published data (2017–2020) about As-induced overproduction of ROS, lipid peroxidation and activation of antioxidant enzymes (Table S1-S2). The observations of studies were grouped into (i) root vs shoot and (ii) low vs high As-applied levels. The data analysis was carried to trace percent variation in ROS, lipid peroxidation and antioxidant enzyme activities under As stress compared to control. Moreover, the number of positive and negative observations was also calculated to show increase or decrease in ROS, lipid peroxidation and antioxidant enzyme activities.

Low vs high applied levels

In case of different applied levels of As, meta-data were divided into four categories as follows: < 10 µM, 10–50 µM, 50–100 µM and > 100 µM. Generally, the As-induced % change in ROS production and lipid peroxidation increase linearly in a dose-dependent manner. The % increase in ROS contents was 67%, 58%, 84% and 134%, respectively, for < 10 µM, 10–50 µM, 50–100 µM and > 100 µM As levels (Table 3). The corresponding % increase for TBARS contents was 27%, 88%, 125% and 123% (Table 3). In case of antioxidants, the trend in % increase was not linear for these applied levels of As. However, the % increase in the activities of all the

Table 3 Data analysis of arsenic-mediated percent variations of ROS contents and lipid peroxidation (LPO) in plant leaves and roots. Full data available in Table S1

Parameter	Leaves ROS	Roots LPO	Roots + Leaves ROS	LPO	ROS	LPO
Mean	97	109	70	48	86	87
Minimum	4	2	−1	−93	−1	−93
Maximum	825	600	238	225	825	600
Total # of observations	38	29	25	17	63	46
Negative observations	0	0	1	2	1	2
% negative observations	0	0	4	12	2	4
Positive observations	38	29	23	15	61	44
% positive observations	100	100	92	88	97	96
Effect < 10%	1	4	3	5	4	9
Effect < 25%	7	7	7	5	14	12
Effect < 50%	15	13	12	10	27	23
No effect	0	0	1	0	1	0

antioxidant enzymes was higher for > 100 μM As level compared to < 10 μM As level (Table S4 a-d).

Leaves vs roots

Data analysis revealed that the mean values of percent changes in ROS contents under As stress were higher in leaves (97%) than roots (70%) (Table 4). The trend was same for TBARS contents (109% in leaves vs 48% in roots) (Table 4). The overall (root + leaves) increase in ROS and TBARS content was 86 and 87%, respectively. Similarly, As-induced % change in the activities of SOD, CAT, POD, GPX, APX and GR was higher in leaves than roots (Table 5). This variation was 74% and 32% for SOD, 66% and 65% for CAT, 84% and 25% for POD, 29% and 27% for GPX, 91% and 25% for APX and 58% and 36% for GR, respectively, for leaves and roots (Table 5). These observations confirmed the results of current study where As mediated higher toxicity was observed in leaves than roots. The overall (root + leaves) increase in the activities of antioxidant enzymes in plants under different As levels was 65% for SOD, 66% for CAT,

Table 4 Data analysis of arsenic-mediated percent variations of ROS contents and lipid peroxidation (LPO) in plant under different applied levels. Full data available in Table S1

Parameter	< 10 μM ROS	10–50 μM LPO	50–100 μM ROS	> 100 μM LPO	ROS	LPO	ROS	LPO
Mean	67	27	58	88	84	125	134	123
Minimum	4	−93	0	8	−1	4	17	15
Maximum	155	158	288	414	238	450	825	600
Total # of observations	15	15	14	7	19	11	15	13
Negative observations	0	2	0	0	1	0	0	0
% negative observations	0	13	0	0	5	0	0	0
Positive observations	15	13	13	7	18	11	15	13
% positive observations	100	87	93	100	95	100	100	100
Effect < 10%	1	7	2	1	1	1	0	0
Effect < 25%	4	8	4	2	5	1	1	1
Effect < 50%	7	10	9	5	6	3	5	5
No effect	0	0	1	0	0	0	0	0

Table 5 Data analysis of arsenic-mediated percent variations in the activities of antioxidant enzymes in plant leaves (5a) and roots (5b). Full data available in Table S2

Parameter	SOD	CAT	POD	GPX	APX	GR
Mean	74	66	84	29	91	58
Minimum	−4	−36	−36	−45	−55	−16
Maximum	438	400	400	150	775	263
Total # of observations	23	33	15	16	24	16
Negative observations	1	5	2	5	8	1
% negative observations	4	15	13	31	33	6
Positive observations	22	28	13	11	16	15
% positive observations	96	85	87	69	67	94
Effect < 10%	3	9	2	8	8	1
Effect < 25%	9	14	3	8	9	6
Effect < 50%	13	18	6	11	16	9
No effect	0	0	0	0	0	0
b						
Parameter	SOD	CAT	POD	GPX	APX	GR
Mean	32	65	25	27	25	36
Minimum	9	−5	−58	−65	−18	−58
Maximum	67	270	153	194	144	162
Total # of observations	7	19	7	6	11	15
Negative observations	0	2	3	3	6	3
% negative observations	0	11	43	50	55	20
Positive observations	7	17	4	3	5	12
% positive observations	100	89	57	50	45	80
Effect < 10%	1	2	3	3	6	4
Effect < 25%	3	2	3	3	7	7
Effect < 50%	6	10	5	4	8	10
No effect	0	0	0	0	0	0

66% for POD, 28% for GPX, 70% for APX and 46% for GR (Table S3).

Induction vs suppression of antioxidant enzymes

Meta-analysis also showed both increase and decrease in the activities of antioxidant enzymes under As stress. Overall, 97%, 87%, 77%, 65%, 61% and 88% observations showed an increase in the activities of SOD, CAT, POD, GPX, APX and GR, respectively (Table S3). This shows that enzymes may get activated or suppressed under As stress.

Multivariate analyses

A number of recent studies used the multivariate analysis technique to find out general trends in a data set based on overall correlation and covariance among different treatments and variables. In the current study, PCA graph showed that different plant variables and As treatments were grouped in different sections (Figs. 4 and 5). This confirmed that different As treatments (control, As-5, As-25 and As-125) caused overall different effects, i.e., toxicity and detoxification. Similarly, biophysiochemical responses (POD and CAT vs SOD and APX) also varied in terms of their activation or suppression against various As treatments and were therefore grouped separately in PCA graph. The heat map indicated Pearson correlation among response variable of *B. oleracea*. The biophysiochemical variables of the *B. oleracea* showed a moderate-strong correlation (Fig. 6, Table S6, and Figure S2) probably due to the varied effects of different applied levels of As. The

multivariate analysis is highly useful in distinguishing plant's biophysiochemical attributes with respect to their importance toward the phytoremediation process (Anwar et al., 2020).

Conclusions

This study indicated that overall, more toxicity in terms of TBARS and ROS contents was observed in plant leaves than roots. Similarly, higher As-applied level (As-125) was more toxic compared to lower levels (As-5 and As-25). Likewise, pigment contents were more sensitive to As-induced oxidative stress than other plant responses. It is concluded that As-induced toxicity and tolerance in terms of variation in biophysiochemical attributes of *B. oleracea* greatly varied with As-applied levels, plant physiological response and plant organ type (leaf/root). The data analysis of the literature also revealed similar trends with low toxicity and high tolerance at lower As levels and vice versa. Therefore, the generalization of results must be made keeping in view the applied levels of a trace element, the type of studied organ (leaf or roots) and the response variables. Moreover, the plant species (serving root/shoot as edible part) should be well identified in terms of trace element accumulation and partitioning in the tissues that can be used for the remediation of contaminated sites.

Perspectives

- More studies are required at the cellular and genetic level to elucidate the underlying mechanisms of antioxidant activation or suppression under specific stress conditions.
- The physiological plant response to stress conditions may greatly vary with applied stress level, plant type, plant parameter type, plant organ type, etc. These aspects warrant further investigations.
- The role of plant tolerance mechanisms toward trace element compartmentation inside plants and associated health hazards is not still fully developed.

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Declarations

Conflict of interest Authors declare no conflict of interest.

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