Journal of Plant Process and Function, Vol. 7, No. 25, Year 2018

Interactive of arsenate and phosphate on arsenic-induced oxidative stress in root of *Isatis cappadocica* Desv.

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(Received: 22/09/2017-Accepted: 21/08/2018)

Abstract

In present study, growth, arsenic accumulation, and antioxidant responses in root of *Isatis cappadocica* were investigated in response to application of arsenate and phosphate. Reduction in root dry weights was significant with 1200 µM arsenate and 5 µM phosphate treatments. Phosphate had a steady effect on root dry weight improvement, especially in high arsenate treatments ≥ 200 µM. As concentrations in roots increased with increasing arsenate supply levels, growth decreased markedly with increasing phosphate in each arsenate treatment. Applied arsenate induced oxidative stress, which caused increasing the concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). The activities of antioxidant enzyme such as catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) in *Isatis cappadocica* roots increased significantly from 200-800 µM arsenate and 5 µM phosphate treatments while their activities were decreased at 1200 µM arsenate and 5 µM phosphate, compared with the control plants. The phosphate mediated decline of CAT, POD and APX activities, which could be an important factor in regulating As-induced oxidative stress in roots. Our results concluded that, *I. cappadocica* has a great capability to tolerance and accumulation As. Also, application of phosphate has a clear vital role in oxidative stress reduction in roots of exposed *I. cappadocica* to arsenate.

Key words: Antioxidant enzymes, Arsenate, *Isatis cappadocica*, Oxidative stress, Phosphate

Introduction

In past decades, the widespread accumulation of heavy metals and metalloids in the environment is increasingly becoming a problem for animals and plants (Munzuroglu and Geckil, 2002; Tripathi *et al.,* 2007). Arsenic (As) is widely distributed into the nature in the form of either metalloids or chemical compounds (Karimi *et al*., 2010; Sayantan and Shardendu, 2016), which is listed as the most hazardous substance by Agency for Toxic Substances and Disease Registry (ATSDR 2012). This metalloid is a potent toxin and highly carcinogenic, the exceptionally high concentrations in the environment have drawn much public attention (Matschullat, 2000). Consequently, remediation of these contaminated sites through a concerted effort by researchers has become a top priority (Bondada *et al.,* 2004). Recently, use of phytoremediation technique to clean up As polluted sites is gaining attention due to the environmental concerns and remediation costs associated with conventional remediation processes (Singh and Ma, 2006: Karimi *et al.,* 2009; Souri *et al.,* 2017a, b).

Hyperaccumulator plants can grow on metalliferous soils to accumulate extraordinarily high amounts of heavy metals in the aerial organs, far in excess of the levels found in the majority of species, without suffering phytotoxic effects (Karimi *et al.,* 2009; Rascio and Navari-Izzo, 2011). The discovery of the As-hyperaccumulator capability not only opened the possibility for the phytoremediation, but also offered a unique model to study all aspects of plant–As interactions (Huang *et al.,* 2011). *Isatis cappadocica,* the identified As- hyperaccumulator (Karimi *et al.,* 2010; Karimi and Souri, 2013, 2015), belongs to the family of brassicaceae, has accumulated As in the shoots to concentrations as high as 700.93 mg/kg when grown in hydroponic medium at 1200 µM arsenate treatment (Karimi and Souri, 2015). Studies dealing with the uptake of heavy metals by hyperaccumulators have examined heavy metals and metalloids uptake only via the root system. This is because most of the heavy metals and metalloids linger in the soil system, and after plant uptake, they are often confined in the roots (Bondada *et al.,* 2004; Singh and Ma, 2006).

Arsenic and phosphorus belong to the VA family in the periodic table of chemical elements. The main route of arsenate uptake in plants is through the phosphate transporters as a phosphate analogue, whereas arsenite (III) and undissociated methylated arsenic species enters through nodulin 26–like intrinsic (NIP) aqaporin channels (Zhao *et al.,* 2010; Souri *et al.,* 2017a). Based on previous studies, translocation of As from roots to shoots in hyperaccumulators are highly efficient, and efflux levels are insignificant (Chen *et al.,* 2016). The extremely efficient As translocation in As hyperaccumulators is probably because of the effective

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reduction of AsV to AsIII in roots; to the high AsIII efflux from cortical cells to the xylem; limited thiol compound complexation of AsIII and sequestration in root vacuoles; in addition to minimal AsIII efflux from roots to the external medium (Karimi *et al.,* 2009; Zhao *et al.,* 2009; Indriolo *et al.,* 2010; Souri *et al.,* 2017). Also, one of the important As detoxification strategy in hyper-accumulating plants is the synthesis of glutathione (GSH) and phytochelatines (PCs) which produces complexe compounds with As that facilitate its transport into the vacuoles in shoots (Karimi *et al.,* 2009; Zhao *et al.,* 2009; Souri *et al.,* 2017).

An increase in phosphate level leads to reduced arsenate uptake in plants and vice versa (Meharg and Macnair, 1992). The competition of uptake and translocation between phosphate and arsenate have been shown in lots of plants (Chen *et al.,* 2004; Liu *et al.,* 2004; Rosa *et al.,* 2006; Lihong and Guilan, 2009). In previous studies, we have reported the competitive effect between arsenate and phosphate on As accumulation and growth parameters in *I. cappadcica* (Karimi and Souri 2015). Furthermore, maintaining a greater ratio of P/As in the roots may also be one of the reasons for the high As tolerance of the hyperaccumulators, *Pteris vittata* and *I. cappadocica* (Singh and Ma, 2006; Karimi and Souri, 2015). Therefore, the interaction between phosphate and arsenate affect arsenate resistance and it might be an important factor to affect As-induced oxidative stress in plants.

Accumulation of As interferes with various metabolic processes and thereby adversely affects the plant metabolism and leads to reduced plant growth (Sharma, 2012).When, plant is subjected to As stress, some reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), is produced. Also, arsenic damages the chloroplast membrane and disorganizes the membrane structure (Miteva and Merakchiyska, 2002) and inhibits the growth and fresh and dry biomass accumulation (Stoeva *et al.,* 2003/4). To counteract and neutralize this toxicity, cells are equipped with enzymatic and non-enzymatic mechanisms to eliminate or reduce their damaging effects. The importance of antioxidant enzymes is their ability to scavenge ROS and thereby prevent oxidative damage (Krüpper *et al.,* 2007; Stancheva *et al.,* 2010; Sayantan and Shardendu, 2016).

Antioxidant enzymes, including catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7) and ascorbate peroxidase (APX; EC 1.11. 1.11) provide the first line of defense against ROS (Mallik *et al.,* 2011; Sayantan and Shardendu, 2016). CAT, POD and APX constitute a main H_2O_2 scavenging system in cells, and the changes of these enzyme activities can regulate intracellular H_2O_2 levels (Mittler, 2002). The stimulation of enzymatic activities, e.g. CAT and APX has been considered as basic defense mechanisms against As-induced oxidative stress in some higher plants (Sharma *et al.,* 2007). Thus, to mitigate the harmful effects of free radicals, plant cells have developed an antioxidant defense mechanism such as enzymatic antioxidants, which act as the scavengers of free radicals (Michalak, 2006; Sharma *et al.,* 2012).

Most of the previous studies on interaction of arsenate and phosphate were mainly carried out in As accumulation and transport. However, limited reports have shown the effects of phosphate on As induced oxidative stress on root of the plants so far. The phosphate may partially protect the membrane from As induced oxidative stress in chickpea (Geng *et al.,* 2006). Wang *et al* (2007) have shown that, phosphate starvation causes decline in antioxidant enzyme activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in wheat plants exposed to As. However, it has to be noted that most of the studies have been carried out on the soil, where As toxicity is influenced by environmental factors such as soil type, availability of phosphate and arsenate, nutrient supply and soil pH (Tu and Ma, 2003). So far, less is known about As toxic mechanisms in relation to interactions with P in the roots of As hyperaccumulator plants. The present study was designed to investigate the effects of arsenate and phosphate on the growth, As accumulation, oxidative stress marker (H_2O_2), and the activities of enzymatic antioxidants (CAT, POD and APX) in roots of *I. cappadocica* for the first time, . The results would be meaningful to elucidate the As detoxification mechanisms of these As hyper accumulating plants.

Materials and methods

Plant material, growth conditions and treatments: The seeds of *I. cappadocica* collected from a population growing at the gold-As Zarshuran deposit mine (36°43′04″N 47°08′02″E, 40 km north of the town of Takab in the West Azarbaijan province, northwest of Iran). The total soil As concentration in this deposit area ranged from 145 to 6,525 mg kg^{-1} (Karimi *et al.,* 2010). Seeds of *I. cappadocica* were surface sterilized in 50% (v/v) commercial bleach (LODA; 4% NaOCl) for 2 mins., followed by rinsing three times for 5 mins. in sterilized distilled water. Seeds were germinated on distilled water moistened filter paper in Petri dishes for 4 d at room temperature, <25°C. After germination, seedlings were transferred to 1 liter polyethylene pots (four seedlings per pot) which filled with a perlite and sand and added nutrient solution composed of 0.5 mM KNO_3, 0.75 mM Ca $(NO_3)_2$, 0.2 mM $MgSO_4$, 15 µM H_3BO_3, 2 µM $MnCl_2$, 1 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM Na_2MoO_4. $2H_2O$ and 50 µM Fe-EDTA (pH 6.0) (Karimi et al. 2009) every 10 days. Afterwards, plants were amended for 10 days, the solutions with different Na2HAsO4 Na_2HAsO_4 (0, 50, 200, 800 & 1200 µM) and KH_2PO_4 (5, 50, 200, 800 & 1600 µM) concentrations added for another 20 days. During this period, the nutrient medium and these treatments was renewed twice per a week. Plants were kept in a growth chamber (Conviron model CG72, Canada) with 14/10 h light/dark cycles; temperature was kept at 26 °C during the day and 20 °C during the night. Light intensity was

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around 280 µmol m^{-2} s^{-1}. Each treatment was replicated three times and each time, the pots were randomly arranged during the growth period.

Dry weight and arsenic accumulation: The harvested plant roots were washed with tap water followed by rinsing in ice-cold phosphate buffer containing 1 mM Na_2HPO_4, 10 mM MES, and 0.5 mM $Ca(NO_3)_2$ to ensure desorption of As from material surface and the root free space. Thereafter, the plants were rinsed in tap water followed by deionized water. The root biomass $plant^{-1}$ was measured after oven drying at 65 °C for 3 days. For determination of total As concentration, dried plant sample were ground of which 0.2 g was mixed with 2 ml of HNO_3 (67% suprapur) and 2 ml of H_2O_2 (30% by volume), and then microwave-digested at 95 °C. The digest was diluted with a solution containing 10% HCl, 5% ascorbic acid and 10% KI, and then analyzed using hydride generation–atomic absorption spectrometry with a flow injection hydride generator interfaced with a Shimadzu AA-6200 atomic absorption spectrometer (HG-AAS, Japan). Reference standard for calibration of the AAS was made using 1000 mg l^{-1} (Beech leaves material FD8, Commission of the European Communities, Joint Research Centre ISPRA).

Bioaccumulation factor (BF) and tolerance index (TI): The bioaccumulation factor (BF) and tolerance index (TI) were calculated to determine the heavy metals phytoextraction efficiency (Zayed *et al.,* 1998, Mattina *et al.,* 2003). The BF expresses the ability of a plant to accumulate As from medium and tolerance index (TI) based on the dry weight (dry weight of the plants grown in arsenate solution/dry weight of the plants grown in control solution) was chosen as indicator of the toxic effects of As on plants under different dose of arsenate and phosphate treatments. In the current study, the TF and TI of root values for As are given by:

BF_{root} = As concentration in harvested plant material (mg kg^{-1})/As concentration in the medium (mg kg^{-1})

TI_{root} = dry weight of the plants grown in arsenate solution/ dry weight of the plants grown in control solution

Hydrogen peroxide (H_2O_2) content: Hydrogen peroxide content was determined as method described by Velikova *et al* (2000). Fresh roots (0.5 g) were homogenised in ice bath with 5 mL 0.1% (w/v) Trichloroacetic acid (TCA). The homogenate were centrifuged at 12,000 × g for 15 min then 0.5 mL of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI. H_2O_2 concentration was estimated based on the absorbance of the supernatant at 390 nm.

Enzyme extractions and assays: Enzyme extractions were carried out at 4°C. Frozen root tissues were ground to a fine powder in liquid nitrogen in a pestle and mortar and extracted at a ratio 1:3 (w/v) fresh weight to extraction buffer (Tris-HCl buffer (pH 7.8) containing 0.1 mM EDTA, 3 mM DTT and 5% (w/v) insoluble PVPP). The homogenate was centrifuged at 14,000 rpm for 20 mins. and the resulting supernatant was used for enzyme assays and protein extraction.

Catalase (CAT, EC 1.11.1.6): Catalase (CAT) activity was estimated by the method of Aebi (1974). For the measurement of the CAT, 2 mL reaction mixture comprised of 50 mM sodium phosphate buffer (pH7.0), 20 mM H_2O_2 and a suitable aliquot of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm and calculated on the basis of extinction coefficient 39.4 mM^{-1} cm^{-1}.

Peroxidase (POD, EC 1.11.1.7): Peroxidase (POD) activity was measured according to Chance and Maehly (1995). The reaction mixture (3.0 ml final volume) consisted of 50 µL of 10 mM guaiacol, 2.9 mL of 50 mM K-phosphate buffer, pH 7.0, 10 µL of 40 mM H_2O_2. A 40 µl aliquot of the crude enzyme extract was then added to start the reaction. The activity of the mixture was determined spectrophotometerically at 470 nm after 1 mins. at 20◦C. Enzyme activity was calculated using the extinction coefficient of (26.6 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX, EC 1.11.1.11): Ascorbate peroxidase (APX) activity was assayed in presence of ascorbate by following the decline in absorbance of the oxidized ascorbate at 290 nm, according to Chen and Asada (1989). Enzyme activity was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbate.

Total protein: Protein concentration was determined as described by Bradford (1976). The extracts of CAT, POD and APX (100 µL) were mixed with 5 ml of protein reagent containing 0.01% (w/v) Coomassie Brilliant Blue G-250 (as dye), 4.7% (w/v) ethanol and 8.5% (w/v) phosphoric acid. After mixed thoroughly for 2 mins., the absorbance was measured at 595 nm. The Bovine V albumin was used as standard protein.

Statistical analysis: Each experiment was repeated at least thrice independently. To confirm the variability of data and validity of the results, all the data were subjected to an analysis of variance (ANOVA). To determine the significant difference between treatments, the Duncan's multiple range test (DMRT) was applied to see the significant level wherever required. Difference from the control was considered significant as $P < 0.05$ or very significant as $P \leq 0.001$. All the values presented in this paper were expressed as the means of three replicates ± standard error (S.E).

Results and discussions

Plant growth and arsenic accumulation: Plant root biomass was measured after 1 month as dry weight (DW). There was a net decrease in root biomass being indicative of as toxicity in plants (Figure. 1). The decline in biomass production could explain, in part, due to inhibition of both cell elongation and division by heavy metal (Arduini *et al.,* 1994). Also, Ouzounidou *et*

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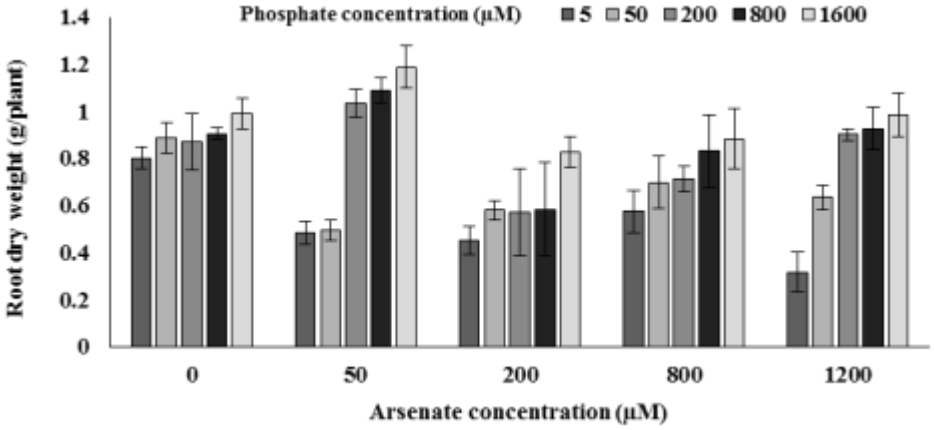

Figure 1. Root dry weight (g plant^{-1}) in *I. cappadocica* grown for 4 weeks in arsenate and phosphate amended medium. The data are means ± S.D for 3 individual measurements in each group.

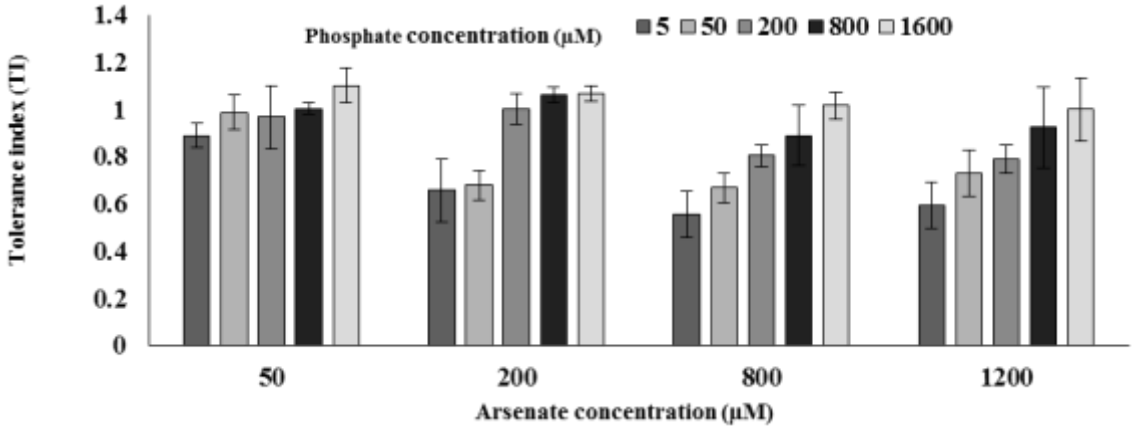

Figure 2. Tolerance index of roots in *I. cappadocica* grown for 4 weeks in arsenate and phosphate amended medium. The data are means ± S.D for 3 individual measurements in each group.

al (1992) reported that, heavy metals or metalloids affect the ultra-structure of meristematic cells, altering the ribosomal RNA precursor biosynthesis, thus affecting the plant growth. There was a severe decrease in root biomass at 1200 µM as.

Increasing phosphate supply, increased biomass markedly in all of the studied arsenate levels (50 -1200 µM), which approved the interaction effects (As × P) on growth of *I. cappadocica* (Figure. 2). Also, the application of phosphate showed a relatively increase in TI value. Maximum TI was found in the 1600 µM phosphate- treated that showed a significant difference at 5% level. The values of TI=1, TI>1 and TI<1 mean no influence, positive and negative impacts on growth by treatments, respectively (Zaier *et al.,* 2010). In hydroponic systems with arsenic hyperaccumulator plants, phosphate has long been reported to suppress plant arsenic uptake (Tu and Ma, 2003). Our results suggest that, an increased supply of phosphate provided protection to the plant system and that effect appears in the form of a reduced amount of growth inhibition and increase of tolerance during As toxicity.

The bioaccumulation factor (BF) is defined as the ratio of metal concentration in the plant to metal concentration in the medium. As BFs for *I. cappadocica* showed highly efficient accumulation of As from the medium. BF was increased by as treatment but, the two highest levels of P supply (800 and 1600 µM) gave lower BF than the two lowest rates of P supply (Figure. 3). With the 800 µM As treatment the 1600 µM P treatment gave lower BF than the lowest rates of P supply.

To evaluate the extent of As-induced oxidative stress and the associated As detoxification systems in *I. cappadocica*, it is important to know the plant As concentration. Among As species, arsenate acts as a phosphate analog and is transported across the plasma membrane via phosphate co-transport systems (Tripathi *et al.,* 2007). The root uptake of As in *I. cappadocica* was increased with the augment of its concentration in the medium (Figure. 4). When *Isatis* was exposed to 1200 µM of arsenate, the concentration of accumulated As reached to the maximum (464 mg kg^{-1}) in roots. These results implied that, *I. cappadocica* had a high ability to absorb arsenate by roots. In each arsenate treatment, low phosphate levels (up to 50 µM) led to highest As accumulation which, could be related to their interaction. However, the increasing phosphate supply decreased As uptake markedly, at high phosphate levels (800 and 1600 µM) (Figure. 1). These data is in good agreement with the finding of Meharg and Macnair

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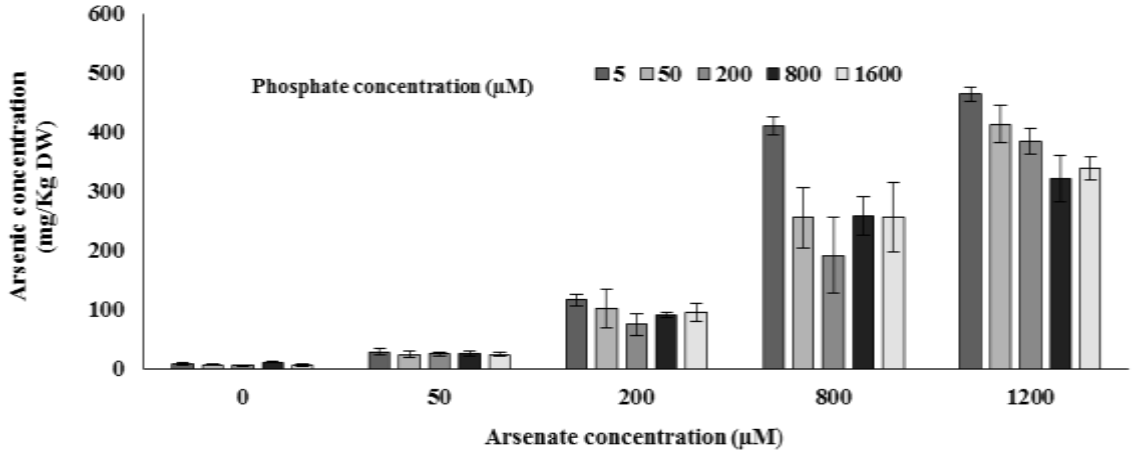

Figure 3. Bioaccumulation factor of roots in *I. cappadocica* grown for 4 weeks in arsenate and phosphate amended medium. The data are means ± S.D for 3 individual measurements in each group.

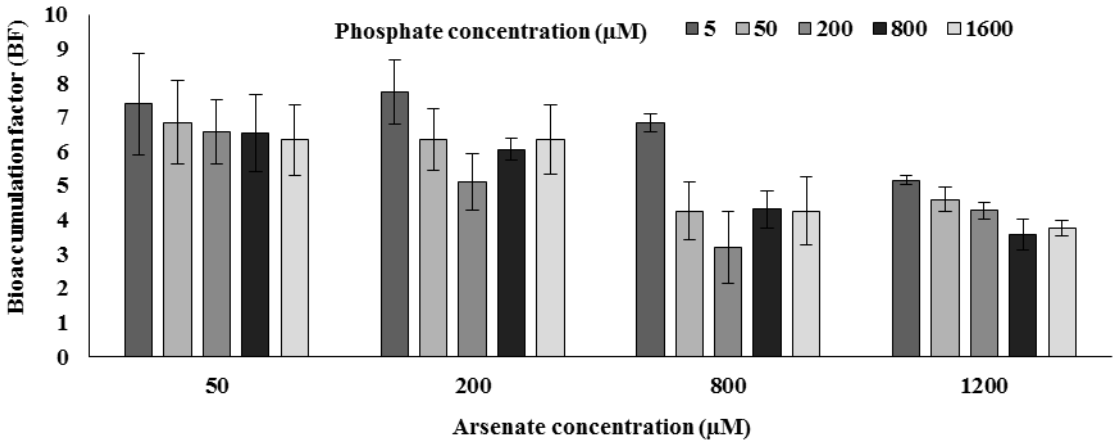

Figure 4. Total arsenic concentrations (mg kg^{-1}, DW) in *I. cappadocica* grown for 4 weeks in arsenate and phosphate amended medium. The data are means ± S.D for 3 individual measurements in each group.

(1994) that, as phosphate and arsenate compete for the high-affinity phosphate transporter, and phosphate binds to it more efficiently than arsenate, high phosphate concentration in the medium favors uptake of phosphate rather than arsenate.

The ROS is normal products of plant cellular metabolism. Various environmental stresses lead to the overproduction of ROS in plants, which are highly reactive, toxic and cause progressive oxidative damage. The amount of H_2O_2 (a product of the superoxide dismutase reaction) showed a significant increase in the roots of *I. cappadocica* treated with As particularly at higher concentrations, compared to untreated plant (Figure. 5). However, H_2O_2 content was reduced following the increase in phosphate which, led to decrease in As stress.

The formation of ROS most probably occurs through the conversion of As (V) to As (III) then ROS formation process leads to the activation of antioxidant enzymes (Srivastava, 2014). ROS being a strong oxidizing agent induces oxidative stress to the biomolecules and finally brings about cell death (Srivastava, 2014; Sayantan and Shardendu, 2016). Rapid increase in H_2O_2 content during the high levels of As (800-1200 µM), was related with As accumulation in the roots, suggesting a severe oxidative stress (Figure. 4 and Figure. 5). Therefore, high as treated plants showed severe H_2O_2 production, which plays roles as signals in the induction and regulation of antioxidant enzymes such as POD, APX and CAT (Sharma, 2012).

Activities of antioxidant enzymes (CAT, POD and APX): Plants are normally protected against ROS by the operation of intricate antioxidant systems, comprising enzymatic systems such as CAT, POD and APX. In fact, plants with enhanced activities of antioxidant enzymes have been shown to be tolerant to oxidative stress (Mittler, 2002). In the current study, potential involvement of some antioxidant enzymes (CAT, POD and APX) activities in the tolerance of *I. cappadocica* roots were investigated in response to application of arsenate and phosphate. The antioxidant enzyme activities increased gradually in response to the increasing levels of arsenate, and were maximal at 800 µM As and 5 µM phosphate treatment (Figure. 6). These enzymatic activities nearly followed similar patterns to the plant biomass, increasing with medium arsenate concentrations and then reduction at medium arsenate >800 µM (Figure. 6). A remarkable decline in

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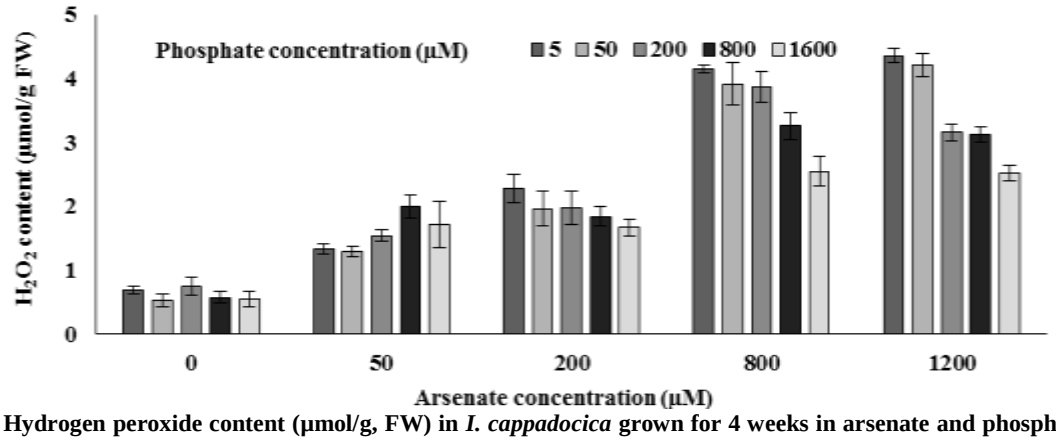

Figure 5. Hydrogen peroxide content (µmol/g, FW) in *I. cappadocica* grown for 4 weeks in arsenate and phosphate amended medium. The data are means ± S.D for 3 individual measurements in each group.

antioxidant enzyme activities was observed in *I. cappadocica* which exposed to high phosphate concentrations (800 and 1600 µM phosphate) in each arsenate treatment (from 50-800 µM).

Regarding to the interaction of arsenate with phosphate, it has to be noted that phosphate (from 200 to 800 µM) can practically protect the plant against as toxicity. This finding is in accordance with previous studies in other plants, and also, supporting that increased phosphate supply leads to, alteration of antioxidant enzyme activities in wheat, chickpea and pearl millet (Wang *et al.,* 2007; Gunes *et al.,* 2009; Sharma and Travlos, 2012; Sayantan and Shardendu, 2016).

CAT activity of the *I. cappadocica* roots were negligible by the As levels below 800 µM (Figure. 6a). The CAT activity in roots was remained almost unchanged up to 200 µM As, but increased significantly at 800 µM As treatment which, reach 3 fold of its respective control (Figure. 6a). Increase of the POD activity in roots was notable in plants treated with 200 and 800 µM As (Figure. 6b). The APX activity did not vary significantly in roots when, treated with 50-200 µM As but, its activity was further increased markedly in 800 µM As treatment (Figure. 6c).

CAT can directly catalyze the reaction of H_2O_2 to H_2O without any electron donor, and POD needs the help of certain phenolic substrate to complete the process, while APX can convert H_2O_2 to H_2O mainly through the ascorbate/glutathione cycle (Qiu *et al.*, 2008). Comparison of the three enzyme activities showed that, the lower CAT, APX and POD activities in 50 -200 µM As implies that in this concentration range of As exposure, H_2O_2 accumulation was reduced by As and consequently the oxidative stress was alleviated. It was not necessary for these three enzymes consume excessive energy to maintain high activity in roots. Furthermore, more induction of CAT activity in high As treatments, quenches H_2O_2, thus, enhancing the continuous accumulation of As. Therefore, the high CAT activity might play a key role in tolerance of the roots of *I. cappadocica* against As stress. The activities of these three antioxidative enzymes were either decreased or leveled off upon high levels of As exposure (1200 µM As). So, it is possible that these antioxidative enzymes failed to capture H_2O_2 at high As exposure. Similar induction of CAT, APX and POD activities with exposure to As has previously been reported in As hyperaccumulator and sensitive fern species, (Cao *et al.*, 2004 Souri *et al.*, 2017b) and *Pteris* spp. (Srivastava *et al.*, 2005; Singh *et al.*, 2006).

The ability of higher plants to neutralize the toxic effects of reactive oxygen species seems to be very important determinant of their tolerance to heavy metal and metalloid stresses (Namdjoyan *et al.,* 2011; Sayantan and Shardendu, 2016; Souri *et al.,* 2017b). When studying the behavior of CAT, and the principal H_2O_2-scavenging enzyme, we observed an increase of its activity in roots. It should be noted that, POD participates in lignin biosynthesis, which in turn could build up a physiological barrier against toxic heavy metals (Hegedus *et al.,* 2001). In plants, the detoxification of H_2O_2 has been known to be an important function of the peroxidases, which uses ascorbate as the hydrogen donor (Hegedus *et al.*, 2001). The APX activity was found to increase in *I. cappadocica* with increasing concentrations of externally supplied As.

As the application of P increased, the activity of CAT, APX and POD decreased in plants supplied As. Furthermore, H_2O_2 had a low tendency for As-supplied plants grown in the high P levels. On the other hand, the *I. cappadocica* roots supplied with 800 and 1600 µM P had lower MDA values at 1200 µM supply of As componed to the plants grown in 50 and 200 µM P despite the higher internal As concentrations. Our previous study showed that, plants supplied with high P accumulated more phosphate in the roots than shoots (Karimi and Souri 2015), which might have had a protective effect against oxidative damage. However, these strategies were not sufficient to prevent lowered shoot growth in plants supplied with 1200 µM As. Possibly the accumulated phosphate influenced cellular pH, and limited the conversion of arsenate to arsenite, and lowered the formation of ROS. Also, an increased supply of P decreased activities of CAT and APX in response to arsenic toxicity in chickpea and *Pteris*

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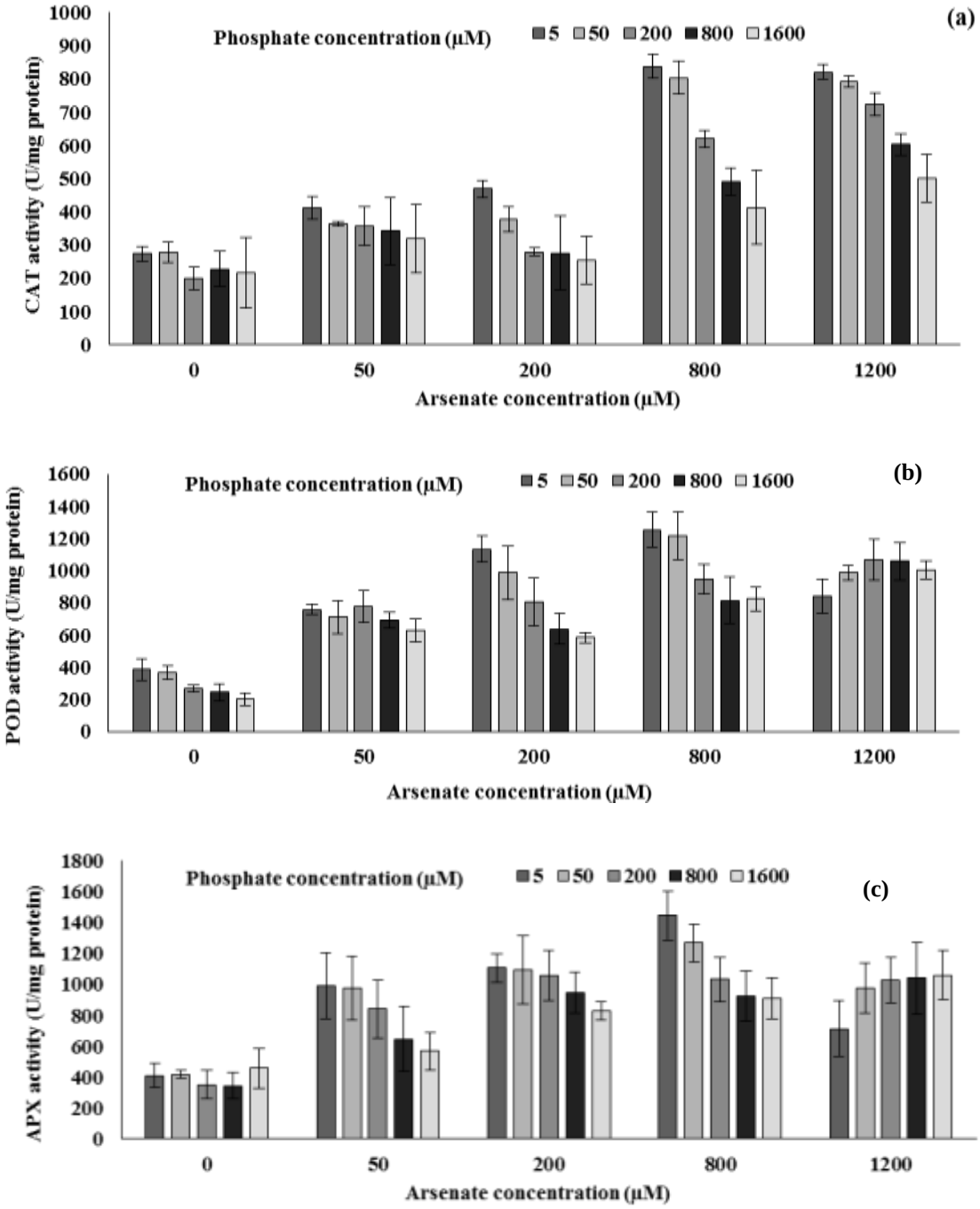

Figure 6. Catalase (a), peroxidase (b) and ascorbate peroxidase (c) activity (U mg protein^{-1}) in *I. cappadocica* grown for 4 weeks in arsenate and phosphate amended medium. The data are means ± S.D for 3 individual measurements in each group.

vittata (Srivastava *et al.*, 2005; Gunes *et al.*, 2009).

Conclusions

According to the results, it can be concluded that toxic concentrations of As cause oxidative damage as evidenced by increased H_2O_2 content and decreased root biomass. The data revealed that, As accumulation in the roots of *I. cappadocica* increased all the studied antioxidant enzyme activities from 50 to 800 µM As treatments. However, at high levels of As (1200 µM As) these activities were decreased which indicate severe oxidative stress. Also, these results suggest that, this antioxidant enzyme defense mechanism may contribute to extraordinary remediation of As and explain, why *I. cappadocica* is tolerant to high As concentrations and is capable of hyperaccumulating As. Moreover, phosphate mediation significantly decreased As concentrations in *I. cappadocica* roots, and it had some effects on decreasing oxidative damages in As stressed plants despite giving lower activity of some antioxidant enzymes specifies that status of phosphate in the medium plays an important role in the mitigation of As-induced oxidative stress.

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