



Influence of Phosphate on Arsenic Uptake and Activities of Different Phosphatase Enzymes in Growing Rice (*Oryza sativa* L.) Seedlings



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Abstract: The effect of arsenate on the levels of phosphate contents and activities of different phosphorolytic enzymes were studied in ten days of rice seedlings var. MTU-1010. Total arsenic contents were increased both in root and shoot of rice seedlings treated with various concentrations of arsenate and increment was linear with increasing concentrations of arsenate. The effect of arsenate was manifested via a decline in phosphate contents and inhibition in the activities of phosphatase enzymes in the rice seedlings. The activities of both acid and alkaline phosphatases were inhibited with increasing concentrations of arsenate. Similarly, inorganic pyrophosphatase and ATPase activities also declined, along with an increasing concentration of arsenate. The results suggest that exposure of rice seedlings to arsenate leads to lowering of the phosphate pool and alteration in the activities of major phosphohydrolytic enzymes, which contribute to metabolic disturbance and a decrease in the growth of rice seedlings. During combined application of arsenate with phosphate exhibited better growth of the seedlings and significant alteration of different phosphatase enzymes activities. Whereas the combined application of arsenate and phosphate altered the level of arsenic accumulation in the test seedlings, which was very little in the root but high in the shoot with respect to arsenate treatment alone thus phosphate inhibits transport of external arsenate within seedlings. However, when arsenate was applied in conjunction with phosphate, the seedlings exhibited improved growth and significant changes in the activities of different phosphatase enzymes. Notably, this combined application altered arsenic accumulation levels, resulting in lower arsenic concentrations in the roots but higher levels in the shoots compared to treatments with arsenate alone. This suggests that phosphate may inhibit the transport of external arsenate within the seedlings, thereby mitigating some of the negative impacts associated with arsenate exposure. Overall, the study highlights the complex interplay between phosphate availability and arsenate toxicity in rice cultivation, emphasizing the potential benefits of managing nutrient levels in arsenic-affected soils.

Introduction

Phosphorus is an essential nutrient for plants and an important component in cell metabolism. It has a vital functional role in energy transfer and acts as modulator of enzyme activity and gene transcription; hence, its assimilation, storage and metabolism are of major importance to plant growth and development (Khan et al., 2023). Hydrolytic breakdown of phosphate esters is brought about by phosphatases, which is a critical process

in energy metabolism, metabolic regulation and a wide range of signal transduction pathways in plants (Hassan et al., 2017 ; Khan et al., 2023). Phosphatases catalyze reactions that result in inorganic phosphorus (Pi) liberation from various substrates in a thermodynamically favourable process, which occurs in both acidic and alkaline medium (Khan et al., 2023). Inorganic phosphorus is a nutrient that often limits plant growth in a natural environment. Acid phosphatases (EC 3.1.3.2,



orthophosphoric-monoester phosphohydrolases) having broad and overlapping substrate specificities are ubiquitous and abundant enzymes in plants and catalyze non-specific hydrolysis of Pi from phosphate monoesters in pH ranges from 4 to 6 and play a major role in the supply and metabolism of phosphate in plants (Khan et al., 2023; Tabaldi et al., 2007). Similarly alkaline phosphatases (EC 3.1.3.1) have a potential role in utilization of phosphomonoesters as the source of Pi required for maintenance of cellular metabolism (Lv et al., 2020). Inorganic pyrophosphatase (EC 3.6.1.1) catalyzes pyrophosphate hydrolysis and synthesis and enriches the phosphate pool in plants by hydrolyzing inorganic pyrophosphate to two molecules of Pi (Grzechowiak et al., 2019; Srivastava et al., 2021). Inorganic pyrophosphate is a by-product of several biosynthetic reactions and is essential for regulating many biochemical reactions in plant cells (Srivastava et al., 2021). Adenosine triphosphatases (ATPases) have wide occurrence in plant tissues and participate in active transport of molecules and ions across membranes and in cell biosynthetic processes (Luo et al., 2022). Stressful conditions of the environment adversely affect P nutrition and its metabolism in plants. Activities of the phosphorolytic enzymes acid phosphatase, alkaline phosphatase, and ATPase show significant alteration in plants exposed to abiotic stressful conditions of the environment such as soil salinity (Guiza et al., 2022), osmotic stress (Guo et al., 2018), excess of heavy metals (Chen et al., 2022), etc. Elevated levels of metals in the soil environment cause toxicity in growing plants and reduce crop productivity. Arsenic is a highly toxic metal to all forms of life. Southeast Asia's vast areas are threatened by arsenic-contaminated groundwater (Jayasumana et al., 2015; Biswas and Saha, 2021). Irrigation with arsenic-laden water gradually adds this metal to the soil surface layers (Siddiqui et al., 2024). Deposition of arsenic in the soil environment leads to toxicity in growing plants. Background levels of arsenic in contaminated soils generally reach 4 to 8 mg As kg⁻¹ but may reach as high as 83 mg As kg⁻¹ (Siddiqui et al., 2024), whereas ground water used for irrigation may contain up to 80-180 µg As L⁻¹ (Alam et al., 2024). In recent years, arsenic has emerged as a potent metal poison and an alarming increase in its content in agricultural soils and different parts of crop plants has set a challenge to crop production in many parts of the world (Siddiqui et al., 2024; Geng et al., 2024; Pal et al., 2024; Kashyap et al., 2018). Rice is a staple food crop for a majority of the world population and is cultivated in many regions of the world where ground water is

severely contaminated with arsenic (Bera and Choudhury, 2023; Siddiqui et al., 2024; Geng et al., 2024; Touhami et al., 2020). The redox-active forms of arsenic that cause intoxication in plants are arsenite (As III) and arsenate (As V). Both these soluble forms can be found in soil and water, but arsenite predominates under growth conditions of paddy fields due to the partial anaerobic conditions. Consequently, the +3 oxidation state is regarded as responsible for arsenic toxicity in this environment (Jayasumana et al., 2015). Although it has been shown that excess arsenic in the soil affects a number of physiological and biochemical reactions in plants (Geng et al., 2024; Mishra et al., 2020), the precise mechanisms underlying arsenic phytotoxicity are poorly understood (Zhou et al., 2023). Earlier studies conducted in our laboratory using two rice cultivars indicated that, after the uptake, arsenite is translocated to different parts of the plant, although more was found in roots than in shoots (Geng et al., 2024). In order to identify arsenic phytotoxicity targets related to P metabolism in rice plants, the present study was undertaken to examine the effects of increasing levels of As₂O₃ in the growth medium on the size of the phosphate pool and the activities of the phosphohydrolase enzymes, namely acid phosphatase, alkaline phosphatase, inorganic pyrophosphatase and chloroplastic as well as mitochondrial isoforms of adenosine triphosphatases, in growing rice seedlings.

Material and Methods

Plant Material and Treatment Conditions

Rice (*Oryza sativa* L.) seeds cv. MTU 1010 obtained from the State Rice Research Station, Chinsura, Hooghly, West Bengal were surface sterilized with HgCl₂ (0.1%, w/v). About 50 seeds for each batch were spread over in petridishes (ϕ 10cm) lined with filter papers containing various concentrations of sodium arsenate (Na₂HAsO₄·7H₂O) solution. The seeds were kept in dark for 48 hours in distilled water at 30°C for germination. Different batches of germinating seeds were exposed to test solutions with 16 hours photoperiod (260 µmol m⁻² s⁻¹ PFD). The possible reversal of arsenate toxicity was determined by treating the rice seeds with suitable phosphate concentrations (potassium dihydrogen orthophosphate; KH₂PO₄) and sodium arsenate solutions. After ten days, the seedlings were harvested and following experiments were performed to characterize the toxic effects of arsenate on the growth and metabolism of rice seedlings and its possible reversal by phosphate. Standard methodologies were used to carry out all the experiments.

Extraction and Estimation of Arsenic Content

Total arsenic contents were measured from arsenate-treated roots and shoots of rice seedlings after ten days of growth by acid digestion of about 500 mg oven-dried (70°C for 3 d and then 100°C for 2 d) samples. The dried samples were digested over a hot plate using 7 ml of HNO₃ (65%) and 2 ml of H₂O₂. Arsenic concentrations of the samples were determined by atomic absorption spectrophotometer with flow injection hydride generation system (Perkin Elmer, Analyst 700), using standard arsenic solution to prepare standard curve.

Extraction and Estimation of Phosphate

Total phosphate was extracted from roots and shoots of rice seedlings after ten days of growth by acid digestion of about 500 mg oven-dried (70°C for 3 d and then 100°C for 2 d) samples. The final volume was made up of 10 ml with deionized water. Phosphate was estimated spectrophotometrically (Fiske et al., 1925) and absorbance was measured at 660 nm in an Hitachi-2000 spectrophotometer, using KH₂PO₄ as a phosphate standard.

Extraction and Assay of Phosphatase Enzymes Activities:

About 500 mg root and shoot samples from freshly harvested rice seedlings were used for enzyme extractions and assays. All three phosphatase enzymes' activities were assayed (Sharma et al., 2023).

Acid phosphatase activity

For acid phosphatase assay, samples were extracted in 5 ml of 100 mM sodium acetate buffer (pH 4.5) using a chilled mortar and pestle. Homogenates were centrifuged at 22,000 x *g* at 4°C for 10 min. and the supernatants were placed in cellophane membrane tubing and dialyzed against extraction buffer in the cold for 8 h with 3-4 changes of buffer. The assay mixture contained 5 mM disodium *p*-nitrophenyl phosphate as substrate, 50 mM acetate buffer (pH 4.5) and 0.2 ml dialyzed enzyme extract in a total volume of 1 ml. After incubation for 30 min at 30°C, the reaction was terminated by the addition of 4.0 ml of 100 mM NaOH. The amount of *p*-nitrophenol liberated was measured by recording the absorbance at 400 nm. One nkat of enzyme activity is defined as one nmol *p*-nitrophenol liberated s⁻¹ and specific activity as nkat mg⁻¹ protein.

Alkaline phosphatase activity

The procedure employed for the assay of alkaline phosphatase was similar to acid phosphatase except that for enzyme extraction and incubation 100 mM sodium bicarbonate buffer (pH 10.0) was used.

Inorganic pyrophosphatase activity

For the assay of inorganic pyrophosphatase, roots and shoots were homogenized in 5 ml of 0.1 M glycine-NaOH buffer (pH 8.8). After centrifugation, the supernatant was dialyzed. The assay mixture contained 4 μmol Na₄P₂O₇, 100 μmol glycine-NaOH buffer (pH 8.8), 10 μmol MgCl₂ and enzyme in a total volume of 2 ml. After incubation for 20 min at 30°C the reaction was stopped by adding 0.5 ml of 30% trichloroacetic acid. Any precipitate formed was removed by centrifugation, and the supernatant was used to determine Pi according to Fiske and Subbarow's method (1925). Enzyme specific activity was expressed as nk at mg⁻¹ protein.

Assay of ATPase activity

The activity of ATPase was assayed (Shoji et al., 2018) in enzyme preparations from fresh roots and shoots. The assay mixture contained 80 μmol Tris-HCl buffer (pH 7.5), 10 μmol Na₂ATP, 20 μmol MgCl₂, 1mM EDTA and 0.2 ml enzyme in a total volume of 2 ml. After incubation for 20 min at 30°C, the reaction was terminated by the addition of 0.5 ml ice-cold 30% TCA. After centrifugation Pi was estimated in the supernatant by the method of Fiske and Subbarow (1925). Specific activity of the enzyme was expressed as nk at mg⁻¹ protein.

Statistical analysis

Supplementary material for statistical analysis is accessible online. The experiments followed a completely randomized design (CRD) with five replicates. Each replicate consisted of a single Petri dish containing approximately 50 seeds. Descriptive statistics, including standard error (± SE), were used to assess differences among mean values. All data were analyzed using Student's *t*-test, with a significance threshold set at *P* < 0.05 for all statistical tests.

Results and Discussion

Arsenic and Phosphate Content of Rice Seedlings

There was an increase in the levels of total arsenic contents both in root and shoot of rice seedlings treated with various concentrations of arsenate and the increment of arsenic contents was linear with increasing concentrations of arsenate (Figure 1a). The phosphate contents were decreased in the root as well as in the shoot of arsenate-treated seedlings. Joint application of arsenate and phosphate in rice seedlings induced less arsenate absorption in the root but more in shoot (Figure 1b). The phosphate accumulation was increased both in shoot and root when test seedlings were treated with phosphate and arsenate jointly (Table 1).



Figure 1a. Effect of different concentrations of arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) on the growth of ten days old rice (cv. MTU 1010) seedlings.



Figure 1b. Effect of arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and phosphate (KH_2PO_4) applied either singly or in combination on the growth of ten days old rice (cv. MTU 1010) seedlings.

Table 1. Total arsenic and phosphate contents in root and shoot of ten days old rice seedlings (cv. MTU-1010) treated with arsenate and phosphate applied singly or combined.

Treatments	Arsenic Content ($\text{mg kg}^{-1} \text{ f w}$)		Phosphate Content ($\text{g kg}^{-1} \text{ f w}$)	
	Root	Shoot	Root	Shoot
Control	BDL	BDL	5.31 ± 0.07	6.05 ± 0.08
Arsenate (μM)				
20.0	44.0 ± 0.054	12.4 ± 0.014	4.85 ± 0.05	5.45 ± 0.06
100.0	76.0 ± 0.029	14.0 ± 0.020	3.77 ± 0.07	4.08 ± 0.07
Phosphate (2mM) + Arsenate (μM)				
20.0	BDL	BDL	6.17 ± 0.07	6.74 ± 0.09
100.0	16.0 ± 0.017	1.5 ± 0.011	7.53 ± 0.05	6.81 ± 0.05
100.0	62.0 ± 0.024	12.6 ± 0.013	9.08 ± 0.04	7.47 ± 0.06

The values are means of 5 replicates \pm SE.
BDL = Below Detection Level i.e. $> 0.01 \text{ mg kg}^{-1}$

Phosphate metabolism in growing rice seedlings was impaired by increasing arsenate treatment. The present study indicates that arsenate toxicity leads to alteration of total phosphate level and also inhibits the activities of different phosphorolytic enzymes viz., acid phosphatase, alkaline phosphatase, inorganic pyrophosphatase and ATPase. Acid and alkaline phosphatase are the key phosphorolytic enzymes which regulate inorganic phosphorus levels in growing plants (Imani et al., 2023; Ciereszko et al., 2011).

Phosphate plays a critical role in cellular metabolism and bioenergetics. It is one of the most important mineral nutrients for plant growth. Anionic phosphorus (PO_4^{2-}) form is usually the assimilatory form for plant uptake (Imani et al., 2023). In the present experiment, the total phosphate concentration in the root and shoot of growing rice seedlings was significantly increased by higher arsenate levels applied singly or in combination with phosphate. According to (Jayasumana et al., 2023), these results could explain in such a way that plant roots accumulate Pi and arsenate via the same uptake system and that the Pi-arsenate uptake system is much more efficient in accumulating Pi compared with arsenate. Arsenic is analogous to phosphate; therefore, arsenic can substitute for phosphorus in plants but is unable to carry

out energy transfers like phosphate. Thus, the plant may react by increasing phosphate uptake as plant arsenic increases (Zulfiqar et al., 2023).

There was an increase in the levels of total arsenic contents both in the root and shoot of rice seedlings treated with various concentrations of arsenate, and the increment of arsenic content was linear with increasing concentrations of arsenate. The phosphate contents were decreased in root as well as in shoot arsenate-treated seedlings. The joint application of arsenate and phosphate in rice seedlings induced less arsenate absorption in the root but more in the shoot. The phosphate accumulation was increased both in the shoot and root when test seedlings were treated with phosphate and arsenate jointly.

Studies on Arsenate toxicity have shown that plant species not resistant to As suffer considerable stress upon exposure, with symptoms ranging from inhibition of root growth through to death (Pallavi et al., 2024; Meadows 2014). Arsenate acts as a phosphate analogue and is transported across the plasma membrane via a phosphate co-transport system (Azeem et al., 2017). Once inside the cytoplasm, arsenate competes with phosphate, such as replacing phosphate in ATP to form unstable ADP-As, disrupting cell energy flows (Xu et al., 2022). Non-resistant plants can be more resistant to arsenate by raising their phosphorus status, as the phosphate is taken more effectively than arsenate (Monroy-Licht et al., 2022; Pan et al., 2020). Also, in arsenate-resistant plants with high phosphate status, a reduced arsenate sensitivity has been observed, which is not due to a difference in arsenate influx but is presumably a result of higher cytoplasmic phosphate status, decreasing arsenate toxicity within the cell (Xu et al., 2022). The effects of phosphate nutrition on arsenate metabolism could be - high plant phosphate status leads to down regulation of the arsenate/phosphate plasma-lemma transporters, and high cellular phosphate levels will result in greater competition with arsenate for biochemical processes where arsenate substitutes for phosphate (Zhang et al., 2024).

Effect of Arsenate on Phosphate Metabolizing Enzymes

Acid phosphatase

The toxic effect of arsenate on ten-day-old rice seedlings showed decreased activity of acid phosphatase in both root and shoot. The activity was little decreased by 3% and 9% in roots at 20 μ M and 100 μ M arsenate treatments respectively. In contrast, in shoots, about 15% and 24% decrease in enzyme activity were recorded under the same concentrations of arsenate (Figure 2).

When the seedlings were treated with 2 mM phosphate along with the same doses of arsenate, the acid phosphatase activity was found to be reduced in roots by about 26% and 22%. In contrast, in shoots, the enzyme activity was decreased by about 9% and 2% in 20 μ M and 100 μ M arsenate treatments along with phosphate, respectively (Figure 2).

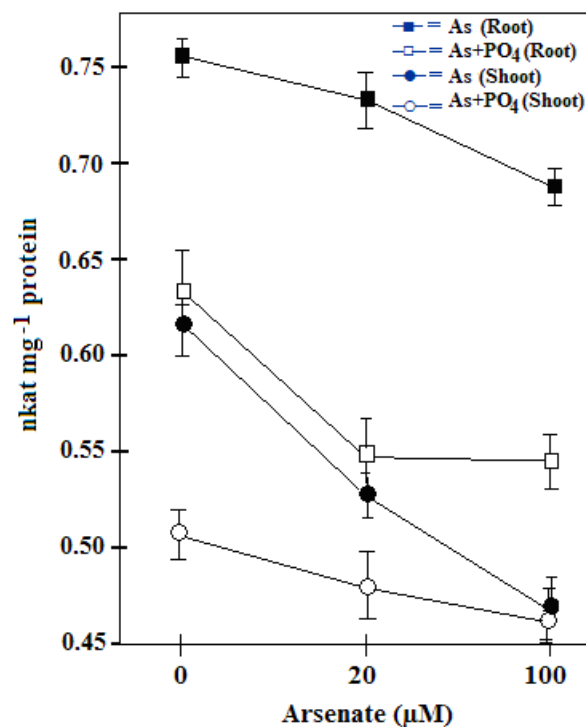


Figure 2. Effect of different concentrations of arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and phosphate (KH_2PO_4) applied either singly or in combination on the activity of acid phosphatase enzyme of ten days old rice (cv. MTU 1010) seedlings. Each data point is expressed as mean value \pm SE (n=5).

Acid phosphatase (ortho-phosphoric monoester phosphohydrolases, EC.3.1.3.2) and alkaline phosphatase (EC.3.1.3.11) are a group of enzymes that are widely distributed in plants (Khan et al., 2023), which non-specifically catalyses the hydrolysis of a variety of phosphate ester in an acidic and alkaline environment (Sharma et al., 2023) which enable the plant to maintain an adequate phosphorus level (Imani et al., 2023; Hassan et al., 2017). Due to various metal toxicities acid phosphatase activity in plants has been altered (Chen et al., 2022; Gupta et al., 2024). Zn and Mg ions are activators, whereas Cd, Cu, and Hg ions act as inhibitors of acid phosphatase *in vitro* reported by (Bossa et al., 2024). The requirement and toxicity of metallic ions for acid phosphatase activity were higher in the shoot than in the root. In spite of that, under *in vitro* phosphate application, acid phosphatase activity was much reduced than non-phosphate treatment. According to (Girault et al., 2021), low Pi status induced acid phosphatase activity. Earlier studies reveal that arsenate with other

phosphate analogous like molybdate, ascorbate, tartrate and vanadate function as a potent competitive inhibitors for plant acid phosphatase (Sharma et al., 2023; Azeem et al., 2017).

Alkaline phosphatase

In this rice cultivar, alkaline phosphatase activity was increased in response to 20 μ M and 100 μ M arsenate in roots, which were 97% and 169%, respectively, over water control. But alkaline phosphatase activity decreased in a shoots by about 4% and 19% at 20 μ M and 100 μ M arsenate treatments, respectively (Figure 3). By phosphate application along with 20 μ M and 100 μ M arsenate treatments, the amount of promotion caused by arsenate alone was slightly induced in roots whereas, in shoots there was 20% increment in enzyme activity when 100 μ M arsenate was combined with phosphate (Figure 3).

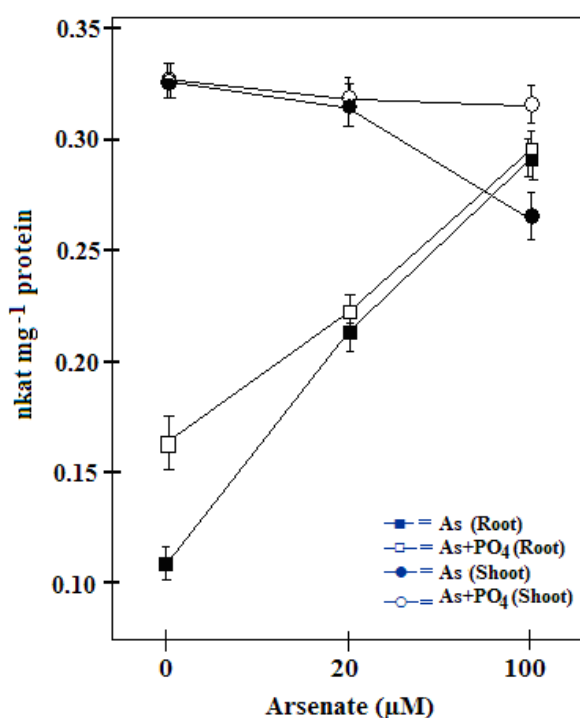


Figure 3. Effect of different concentrations of arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and phosphate (KH_2PO_4) applied either singly or in combination on the activity of alkaline phosphatase enzyme of ten days old rice (cv. MTU 1010) seedlings. Each data point is expressed as mean value \pm SE (n=5).

In the case of alkaline phosphatase, a variable activity behaviour has been noticed in growing rice seedlings with increasing arsenate treatment. An increased activity of alkaline phosphatase in the root was observed, and a declining activity was observed in the present study in shoot. At higher concentrations of the metals, alkaline phosphatase activity was inhibited by competitive inhibition, resulting in changes in enzyme conformation (Lane et al., 2000). Alkaline phosphatase is an inducible enzyme which synthesized at low external Pi

concentration (Girault et al., 2021). For maintaining an adequate supply of Pi in the plant cells alkaline phosphatase hydrolyse suitable organic phosphate ester to release inorganic phosphate. There is no specific reason for this altered behaviour of alkaline phosphates activity but sequestration of potentially toxic compounds possibly may lead to altered activity of these enzymes when subjected to environmental constrains (Mondal et al., 2022).

Inorganic pyrophosphatase

The activity of inorganic pyrophosphatase was found to decrease in roots, but in shoots, activity was almost unchanged on arsenate exposure in ten days old rice seedlings. In roots, about 33% and 53% decrease in enzyme activity were recorded at 20 μ M and 100 μ M arsenate treatments, respectively, from water control at the same levels of arsenate treatments (Figure 4). On the contrary, the phosphate application, along with 20 μ M and 100 μ M arsenate treatments, increased enzyme activity to about 47% and 117%, respectively, in roots. In shoots, the inorganic pyrophosphatase activity was found to be increased by about 183% and 268% when 20 μ M and 100 μ M arsenate were combined with phosphate in respect to arsenate treatment alone (Figure 4).

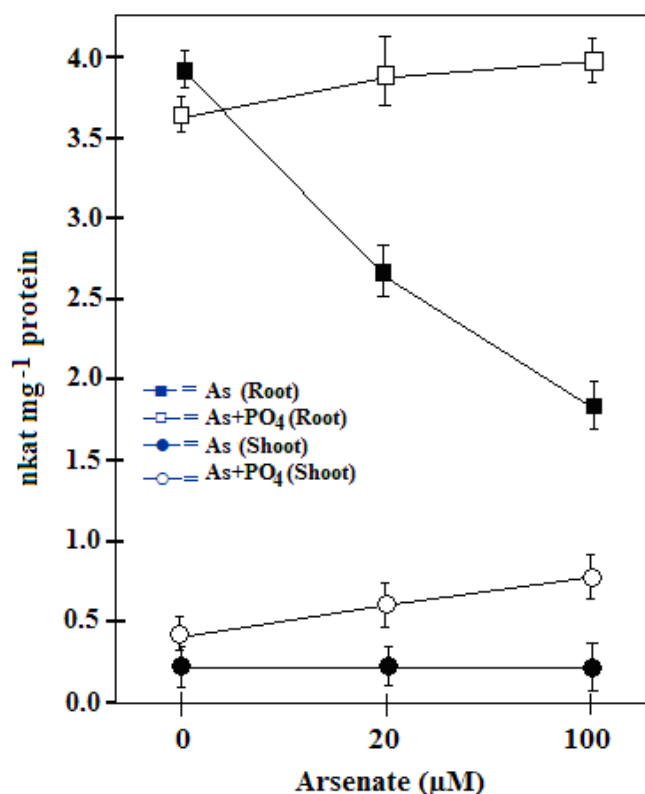


Figure 4. Effect of different concentrations of arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and phosphate (KH_2PO_4) applied either singly or in combination on the activity of inorganic pyrophosphatase enzyme of ten days old rice (cv. MTU 1010) seedlings. Each data point is expressed as mean value \pm SE (n=5).

Inorganic pyrophosphatase (EC.3.6.1.1) catalyses pyrophosphate hydrolysis and synthesis, which is a simple compound with an energy-rich phosphoanhydride bond. It is essential for the regulation of many biochemical reactions in plant cells. In present studies, the inhibition of inorganic pyrophosphatase activity in both the shoot and root of arsenate-stressed rice seedlings was observed. In earlier studies, the inhibition of some metals like Cd^{2+} , Co^{2+} , and Cu^{2+} has been shown in pyrophosphatase activity (Shoji et al., 2018). According to (Saeed et al., 2021), the increased pyrophosphatase activity leads to increased biosynthetic reactions in growing plant tissues.

ATPase

Rice seedlings showed decreased activity of ATPase in both roots and shoots due to arsenate toxicity after ten days. In roots, about 24% and 37% decrease in enzyme activity were observed in 20 μM and 100 μM treatments, respectively, while in shoots, about 17% and 49% decrease in enzyme activity was found (Figure 5). When the seedlings were treated with 2 mM phosphate along with the same concentrations of arsenate, an increased enzyme activity was found. In roots, phosphate with 20 μM arsenate treatment exhibits 43% increased enzyme activity, whereas the activity was increased up to 21% with 100 μM arsenate. In shoots, phosphate application combined with arsenate caused increased enzyme activity by up to 38% on average (Figure 5).

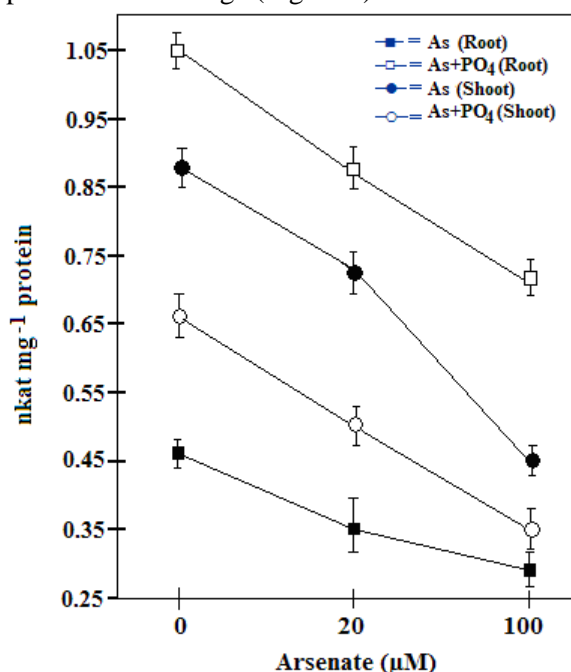


Figure 5. Effect of different concentrations of arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and phosphate (KH_2PO_4) applied either singly or in combination on the activity of ATPase enzyme of ten days old rice (cv. MTU 1010) seedlings. Each data point is expressed as mean value \pm SE (n=5).

Adenosine triphosphatase (ATPase) is widely distributed in plant tissues and participates in the active transport of ions and molecules across the plasma membranes and in cell biosynthetic processes (Luo et al., 2022). The activities of ATPase exhibit marked alteration in plant tissues when exposed to abiotic stress full conditions of the environment such as soil salinity (Guiza et al., 2022), osmotic stress (Guo et al., 2018), excess heavy metals (Chen et al., 2022). In present experiments, a decline in ATPase activity in shoot and contrary in root on enhanced ATPase activity was noticed under arsenate stressed condition of rice seedlings. In the presence of phosphate and arsenate, ATPase activity was enhanced both in root and shoot due to Cd toxicity in oat roots and decreased ATPase activity (Morales-Cedillo et al., 2015).

Conclusion and Future Scope

This study offers important insights into how nutrient availability influences heavy metals' absorption and the mechanisms plants use to tolerate them. The findings suggest that the presence of phosphate can reduce arsenic uptake, thereby diminishing its harmful effects on rice seedlings. Additionally, the research indicates that different levels of phosphate significantly influence the activities of various phosphatase enzymes, which are essential for phosphorus metabolism and the mobilization of inorganic phosphates during nutrient stress. Since phosphate ions compete with arsenate for uptake in the root system, increasing soil phosphate levels may effectively lower arsenic toxicity in rice, especially in regions where arsenic is a concern. The study also highlights that different phosphatase enzymes react uniquely to varying concentrations of phosphate, suggesting potential targets for breeding programs aimed at enhancing phosphorus efficiency and arsenic tolerance. These results emphasize the importance of customized fertilization strategies that account for soil nutrient profiles to maximize rice growth and reduce arsenic absorption, thereby enhancing food safety.

The conclusions had drawn from this research open several avenues for future exploration. Further studies into the genetic mechanisms behind the interactions of phosphate and arsenic could lead to the development of rice varieties that are more tolerant to arsenic and better at absorbing phosphorus. Additionally, long-term field trials assessing the impact of phosphate fertilization on arsenic uptake across different soil types would yield valuable insights into sustainable agricultural practices.

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Conflict of interest

There is no conflict of interest.

References

- Alam, S. M., Bhattacharya, P., & Panaullah, G. (2023). Arsenic contamination in groundwater and its implications for rice production in Bangladesh. *Environmental Science and Pollution Research*, 30(1), 2567-2580. <https://doi.org/10.1007/s11356-022-23357-x>.
- Azeem, W., Ashraf, M., & Shahzad, S.M. (2017). Phosphate-arsenate relations to affect arsenic concentration in plant tissues, growth, and antioxidant efficiency of sunflower (*Helianthus annuus* L.) under arsenic stress. *Environmental Science and Pollution Research*, 24, 24376–24386. <https://doi.org/10.1007/s11356-017-9977-2>.
- Bera, A., & Choudhury, B. (2023). Arsenic Uptake, Transport, Accumulation in Rice and Prospective Abatement Strategies – A Review. *Int. J. Exp. Res. Rev.*, 30, 388-401. <https://doi.org/10.52756/ijerr.2023.v30.036>
- Biswas, S., & Saha, S. (2021). A report groundwater arsenic contamination assay in the delta area of West Bengal. *Int. J. Exp. Res. Rev.*, 25, 84-88. <https://doi.org/10.52756/ijerr.2021.v25.008>
- Bossa, R., Di Colandrea, M., Salbitani, G., & Carfagna, S. (2024). Phosphorous Utilization in Microalgae: Physiological Aspects and Applied Implications. *Plants*, 13, 2127. <https://doi.org/10.3390/plants13152127>.
- Chen, Q., Hu, Y., Yang, L., Zhu, B., & Luo, F. (2022). Phosphorus Regulates the Level of Signaling Molecules in Rice to Reduce Cadmium Toxicity. *Current Issues in Molecular Biology*, 44, 4070-4086. <https://doi.org/10.3390/cimb44090279>.
- Ciereszko, I., Żebrowska, E., & Ruminowicz, M. (2011). Acid phosphatases and growth of barley (*Hordeum vulgare* L.) cultivars under diverse phosphorus nutrition. *Acta Physiologiae Plantarum*, 33, 2355–2368. <https://doi.org/10.1007/s11738-011-0776-y>.
- Fiske, C.H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus, *J. Biol Chem.*, 66, 375. [https://doi.org/10.1016/S0021-9258\(18\)84756-1](https://doi.org/10.1016/S0021-9258(18)84756-1)
- Geng, A., Lian, W., Wang, Y., Liu, M., Zhang, Y., Wang, X., & Chen, G. (2024). The Molecular Mechanism of the Response of Rice to Arsenic Stress and Effective Strategies to Reduce the Accumulation of Arsenic in Grain. *International Journal of Molecular Sciences*, 25(5), 2861. <https://doi.org/10.3390/ijms25052861>.
- Girault, M., Siano, R., Labry, C., Latimier, M., Jauzein, C., Beneyton, T., Buisson, L., Del Amo, Y., & Baret, J.C. (2021). Variable inter and intraspecies alkaline phosphatase activity within single cells of revived dinoflagellates. *Multidisciplinary Journal of Microbial Ecology*, 15(7), 2057-2069. <https://doi.org/10.1038/s41396-021-00904-2>.
- Grzechowiak, M., Ruszkowski, M., Sliwiak, J., Szpotkowski, K., Sikorski, M., & Jaskolski, M. (2019). Crystal structures of plant inorganic pyrophosphatase, an enzyme with a moonlighting autoprolytic activity. *Biochemical Journal*, 476, BCI20190427. <https://doi.org/10.1042/BCI20190427>.
- Guiza, M., Benabdelrahim, M.A., & Brini, F. (2022). Assessment of Alfalfa (*Medicago sativa* L.) Cultivars for Salt Tolerance Based on Yield, Growth, Physiological, and Biochemical Traits. *Journal of Plant Growth Regulation*, 41, 3117–3126. <https://doi.org/10.1007/s00344-021-10499-9>.
- Guo, R., Shi, L., Jiao, Y., Li, M., Zhong, X., Gu, F., Liu, Q., Xia, X., & Li, H. (2018). Metabolic responses to drought stress in the tissues of drought-tolerant and drought-sensitive wheat genotype seedlings. *Annals of Botany*, 10(2), 016. <https://doi.org/10.1093/aobpla/ply016>.
- Gupta, M., Dwivedi, V., Kumar, S., Patel, A., Niazi, P., & Yadav, V.K. (2024). Lead toxicity in plants: mechanistic insights into toxicity, physiological responses of plants and mitigation strategies. *Plant Signaling & Behavior*, 19(1), 2365576. <https://doi.org/10.1080/15592324.2024.2365576>.
- Hassan, H.A., Rani, S., Fatima, T., Kiani, F.A., & Fischer, S. (2017). Effect of protonation on the mechanism of phosphate monoester hydrolysis and comparison with the hydrolysis of nucleoside triphosphate in biomolecular motors. *Biophysical Chemistry*, 230, 27-35. <https://doi.org/10.1016/j.bpc.2017.08.003>.

- Imani, M., Lydia, G., Jasmine, P., Flavio, G., Lisa V. B., Jacob, L J., & Rosangela, S., (2023) Phosphate starvation: response mechanisms and solutions, *Journal of Experimental Botany*, 74(21), 6417–6430. <https://doi.org/10.1093/jxb/erad326>.
- Jayasumana, C., Paranagama, P., & Fonseka, S. (2015). Presence of arsenic in Sri Lankan rice. *Food Contamination*, 2, 1. <https://doi.org/10.1186/s40550-015-0007-1>.
- Kashyap, L., & Garg, N. (2018). Arsenic Toxicity in Crop Plants: Responses and Remediation Strategies. In: Hasanuzzaman, M., Nahar, K., Fujita, M. (eds) *Mechanisms of Arsenic Toxicity and Tolerance in Plants*. Springer, Singapore. https://doi.org/10.1007/978-981-13-1292-2_6.
- Khan, A., Kanwal, F., Shahzad, M., Naz, S., Jalil, S., & Zhang, G. (2024). Interactions of arsenic and phosphorus in their uptake and transportation in plants: Advances and prospective research on the mechanisms and approaches for alleviating arsenic stress. *Journal of Integrative Agriculture*. <https://doi.org/10.1016/j.jia.2024.07.022>.
- Khan, F., Siddique, A.B., Shabala, S., Zhou, M., & Zhao, C. (2023). Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. *Plants (Basel)*.12(15), 2861. <https://doi.org/10.3390/plants12152861>.
- Lane, T.W., & Morel, F.M. (2000). A biological function for cadmium in marine diatoms. *Proc Natl Acad Sci.*, 97(9), 4627-31. <https://doi.org/10.1073/pnas.090091397>.
- Luo, Y., Ma, L., Du, W., Yan, S., Wang, Z., & Pang, Y. (2022). Identification and Characterization of Salt and Drought - Responsive AQP Family Genes in *Medicago sativa* L. *International Journal of Molecular Sciences*, 23, 3342. <https://doi.org/10.3390/ijms23063342>.
- Lv, Y., Zhang, Y., Song, J., & Xu, G. (2020). Regulation of the activity and kinetic properties of alkaline phosphatase in eutrophic water bodies by phosphorus and heavy metals. *Desalination and Water Treatment*, 177, 131-138. <https://doi.org/10.5004/dwt.2020.24924>.
- Meadows, R. (2014). How Plants Control Arsenic Accumulation. *PLOS Biology*, 12(12), e-1002008. <https://doi.org/10.1371/journal.pbio.1002008>.
- Mishra, S., & Dubey, R. S. (2020). Molecular Mechanism of Arsenic Toxicity in Plants and Its Mitigation Strategies. *Plant Physiology and Biochemistry*, 156, 178-186. <https://doi.org/10.1016/j.plaphy.2020.08.002>.
- Mondal, S., Pramanik, K., & Ghosh, S.K. (2022). Molecular insight into arsenic uptake, transport, phytotoxicity, and defense responses in plants: a critical review. *Planta*, 255, 87. <https://doi.org/10.1007/s00425-022-03869-4>.
- Monroy-Licht, A. (2023). Effect of phosphate on arsenic species uptake in plants under hydroponic conditions. *Journal of Plant Research*. 136, 729–742. <https://doi.org/10.1007/s10265-022-01381-0>
- Morales-Cedillo, F., González-Solís, A., & Gutiérrez-Angoa, L. (2015). Plant lipid environment and membrane enzymes: the case of the plasma membrane H⁺-ATPase. *Plant Cell Report*, 34, 617–629. <https://doi.org/10.1007/s00299-014-1735-z>
- Pal, S., Shankar, T., Majumder, S., Adhikary, R., & Pal, S. (2024). Impact of Agronomic Zinc Biofortification on Yield Attributes, Yield and Micronutrient Uptake of Rice (*Oryza sativa* L.) in Southern Odisha. *International Journal of Experimental Research and Review*, 40(Spl Volume), 90-103. <https://doi.org/10.52756/ijerr.2024.v40spl.007>
- Pallavi, S., Vandana, A., Jasvinder, K., Sonal, S., Satish, K., Verma, A. N., Pankaj, K. S., & Suchi, S. (2024). Mitigation of arsenic toxicity in wheat by the inoculation of methyltransferase containing *Pseudomonas oleovorans* NBRI-B4.10. *International Biodeterioration & Biodegradation*, 193, 105851.
- Pan, D., Yi, J., & Li, F. (2020). Dynamics of gene expression associated with arsenic uptake and transport in rice during the whole growth period. *BMC Plant Biology*, 20, 133. <https://doi.org/10.1186/s12870-020-02343-1>
- Saeed, Q., Xiukang, W., Haider, F.U., Kučerik, J., Mumtaz, M.Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M., & Mustafa, A. (2021). Rhizosphere Bacteria in Plant Growth Promotion, Biocontrol, and Bioremediation of Contaminated Sites: A Comprehensive Review of Effects and Mechanisms. *International Journal of Molecular Sciences*, 22(19), 10529. <https://doi.org/10.3390/ijms221910529>.
- Siddiqui, K., Babar, M., & Jamail, I. (2024). Silicon-Mediated Arsenic Tolerance: Restriction of Arsenic Uptake and Modulation of Antioxidant Defense System in Rice Seedlings. *Journal of Plant Growth Regulation*. <https://doi.org/10.1007/s00344-024-11472-y>
- Sharma, L., Kahandal, A., Kanagare, A., Kulkarni, A., & Tagad, C.K. (2023). The multifaceted nature of

- plant acid phosphatases: purification, biochemical features, and applications. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 38(1), 2282379. <https://doi.org/10.1080/14756366>.
- Shoji, S., Mariko, A., Satoru, K., Mayu, F., Yoichi, N., & Masayoshi, M. (2018). Biochemical, Structural and Physiological Characteristics of Vacuolar H⁺-Pyrophosphatase, *Plant and Cell Physiology*, 59(7), 1300–1308. <https://doi.org/10.1093/pcp/pcy054>
- Srivastava, R., Basu, S., & Kumar, R. (2021). Phosphorus starvation response dynamics and management in plants for sustainable agriculture. *Journal of Plant Biochemistry and Biotechnology*, 30, 829–847. <https://doi.org/10.1007/s13562-021-00715-8>.
- Tabaldi, L.A., Ruppenthal, R., Cargnelutti, D., Morsch, V.M., Pereira, L.B. and Schetinger, M.R.C. (2007). Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings, *Environmental and Experimental Botany*, 59, 43-48. <https://doi.org/10.1016/j.envexpbot.2005.10.009>
- Touhami, D., McDowell, R.W., & Condon, L.M. (2020). Role of Organic Anions and Phosphatase Enzymes in Phosphorus Acquisition in the Rhizospheres of Legumes and Grasses Grown in a Low Phosphorus Pasture Soil. *Plants* (Basel), 9(9), 1185. <https://doi.org/10.3390/plants9091185>.
- World Bank, Water and Sanitation Programme (WSP) (2005). Arsenic contamination of ground water in South and East Asian countries: towards a more effective operational response, Washington D.C., USA, World Bank, 2, Report No. 31303
- Zhang, L., Liu, Z., Song, Y., Sui, J., & Hua, X. (2024). Advances in the Involvement of Metals and Metalloids in Plant Defense Response to External Stress. *Plants*, 13, 313. <https://doi.org/10.3390/plants13020313>.
- Zhou, H.Y., Nian, F.Z., Chen, B.D., Zhu, Y.G., Yue, X.R., Zhang, N.M., & Xia, Y.S. (2023). Synergistic Reduction of Arsenic Uptake and Alleviation of Leaf Arsenic Toxicity in Maize (*Zea mays* L.) by Arbuscular Mycorrhizal Fungi (AMF) and Exogenous Iron through Antioxidant Activity. *Journal of Fungi*, 9, 677. <https://doi.org/10.3390/jof9060677>.
- Zulfiqar, U., Jiang, W., Xiukang, W., Hussain, S., Ahmad, M., Maqsood, M.F., Ali, N., Ishfaq, M., Kaleem, M., Haider, F.U., Farooq, N., Naveed, M., Kucerik, J., Brtnicky, M., & Mustafa, A. (2022). Cadmium Phytotoxicity, Tolerance, and Advanced Remediation Approaches in Agricultural Soils; A Comprehensive Review. *Frontier in Plant Science*, 13, 773815. <https://doi.org/10.3389/fpls.2022.773815>.
- Xu, H., Jia, Y., Sun, Z., Su, J., Liu, Q.S., Zhou, Q., Jiang, G. (2022). Environmental pollution, a hidden culprit for health issues. *Eco-Environment & Health*. 1(1), 31-45. <https://doi.org/10.1016/j.eehl.2022.04.003>.

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