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INDOLE-3-ACETIC ACID (IAA) AND KINETIN (KN) MEDIATED REGULATION OF AsA-GSH CYCLE: A PROTECTIVE STRATEGY OF RICE SEEDLINGS TO COUNTER ARSENIC (As) TOXICITY

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ABSTRACT

This comprehensive study investigated the impact of arsenic (As) exposure on hydroponically grown *Oryza sativa* L. seedlings, with a focus on the mitigating effects of indole-3-acetic acid (IAA) and kinetin (KN) phytohormones. The results unequivocally demonstrated that As exposure at both 50 and 100 mM doses profoundly impaired shoot and root growth, as well as their ratio, underscoring the severity of As-induced stress. Moreover, As exposure had a deleterious impact on photosynthetic activity and protein content, while respiratory O_2 uptake exhibited a contrasting response. Furthermore, As exposure triggered a significant increase in superoxide radicals (SOR) and hydrogen peroxide (H_2O_2) levels, leading to lipid peroxidation and electrolyte leakage. In stark contrast, supplementation with IAA and KN remarkably alleviated As toxicity in rice seedlings, as evidenced by improved growth parameters, enhanced photosynthetic activity, and reduced oxidative stress markers. Both phytohormones augmented the activity of AsA-GSH cycle enzymes, including ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR), as well as their associated metabolites, ascorbate (AsA) and glutathione (GSH), thereby minimizing oxidative stress caused by As toxicity. Notably, a comparative analysis of the responses to both hormone treatments revealed that KN was more efficacious than IAA in counteracting the detrimental effects of As and promoting the growth of rice seedlings under stress conditions. These findings have significant implications for the development of novel strategies to mitigate the adverse effects of As on crop productivity, suggesting that KN may be a more potent phytoprotectant against As toxicity in rice.

***Keywords*:** Arsenic, AsA-GSH cycle, indole-3-acetic acid, Kinetin, *Oryza sativa* L.

Introduction

Arsenic (As) is ubiquitous in the environment and highly toxic to all forms of the life. As mainly introduced in the environment via naturally through dissolution of As compounds adsorbed onto pyrite ores into the water by geochemical factors as well as by anthropogenic activity via use of insecticides, herbicides and phosphate fertilizers, semi-conductor industries, mining and smelting etc. As has fascinated plant biologists since As-contaminated natural water is now a worldwide problem (Argos *et al*., 2010). As loaded groundwater used for agricultural purpose is considered the major source of As penetration into the food chain. Arsenic-induced toxicity has been reported in many plants (Talukdar 2013; Singh *et al*., 2020). Arsenic severely intoxicates plants by reducing their biomass and plant height i.e. root and shoot length. Wilting and necrosis on leaves decrease leaf area and photosynthesis culminating into decrease in plant productivity and total death of the plant may occur (Mishra *et al*., 2016; Ahmad *et al*., 2020). A

burgeoning body of evidence suggests that higher concentrations of As interact with sulphhydryl groups of enzymes and proteins, thereby compromising the structural integrity and functionality of the photosynthetic activity (Ahmad and Gupta, 2013; Pandey and Gupta, 2015).

There is significant experimental evidence that the exposure of plants to As does result in the over-production of active oxygen species (like $^1O_2$; $O_2^{\bullet-}$; and H_2O_2) which is concerned with the valance change from As^V to As^{III} (Talukdar, 2013; Mishra *et al*., 2022). Higher concentration of ROS than the certain threshold level breaks photosynthetic pigments, organic components of membrane system and nucleic acids. Therefore, usual cellular metabolism is disturbed (Talukdar, 2013; Mishra *et al*., 2016; 2022). The equilibrium between the rate of ROS formation and their quenching decides the successful survival of life thus quenching of ROS is performed by a pervasive antioxidant system and in this concern enzymes involved in AsA-GSH cycle and their associated metabolites play crucial role. This intricate cycle involves a quartet of enzymes, namely ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR) (Noctor and Foyer, 1998). Notably, APX assumes a vital role in scavenging hydrogen peroxide (H_2O_2), relying on reduced ascorbate (AsA) as an electron donor, which is meticulously regenerated through the ascorbate-glutathione cycle (Noctor and Foyer, 1998). The enzymes DHAR and GR facilitate the regeneration of reduced ascorbate and glutathione from their oxidized counterparts, while MDHAR catalyzes the reduction of monodehydroascorbate to reduced ascorbate, utilizing NADPH as an electron donor, thus ensuring the seamless operation of the cycle.

Cereals play a crucial role in meeting the nutritional demands of the expanding global population. Among numerous cereals, *Oryza sativa* L. (rice) is of great concern. Despite being a semi-aquatic annual grass plant, rice serves as the staple food for nearly two-thirds of the worldwide population. Rice is revered as the "grain of life" in Asian cultures, where it is deeply intertwined with tradition, identity, and sustenance. In India, many rice-producing regions fall under severely As-polluted areas. Therefore, it must become necessary to develop some tactics to reduce As toxicity in rice plants. In this regard, scientists have been evolved and introduced new methods where the application of phytohormones successfully strengthen the plant growth and productivity against a number of abiotic stresses (Singh and Prasad, 2015; 2016; Singh *et al*., 2018; Khan and Qadir, 2021). IAA and kinetin enhance the capacity of the cycle to handle oxidative stress by promoting the regeneration of reduced forms of ascorbate and glutathione (Tiwari *et al*., 2022). IAA interacts with other signaling pathways that modulate redox homeostasis, leading to the activation of the antioxidant system. This includes enhancing the activity of the AsA-GSH cycle enzymes to maintain a balanced redox state. Reports showed that phytohormone supplementation significantly increased the activity of the enzymes of AsA-GSH cycle in *Haematococcus pluvialis* and *Chorella vulgaris* (Raman and Ravi, 2011; Piotrowska-Niczyporuk *et al*., 2015). Despite the wealth of research highlighting the potential of phytohormones to mitigate the adverse effect of heavy metals on plants, the specific impact of IAA and KN on rice plants grown in As contaminated conditions remain unexplored. This study aims to fill this knowledge gap by evaluating the potential of IAA and KN to alleviate As-induced toxicity in rice plants, specifically by examining the AsA-GSH cycle's performance. By doing so, it aims to shed light on the potential benefits of phytohormone-based approaches for enhancing crop resilience to heavy metal stress.

Material and Methods

Plant materials and culture conditions

Among different cultivars of *Oryza sativa* L. available and grown at the nearby places of Ayodhya (India), Bheem cultivar is known to be the best in terms of hydroponic environment. Certified seeds of Bheem cultivar were purchased from authentic supplier of Ayodhya, India. and were surface sanitized in 0.1% $HgCl_2$ solution, followed by washing them with distilled water and soaked in water for 24 h. The seeds were then transferred on a blotting sheet kept in a plastic tray (30 × 14 cm^2) and then placed in dark at 25 ± 2°C for germination. After the germination of seeds it was shifted in a controlled environment of the growth chamber with a 16:8 h light:dark photoperiod (350 µmol photons m^{-2} s^{-1} of photosynthetic active radiation) and at temperatures of 25 ± 2°C with 60% relative humidity. At 10^{th} day of growth 5 plants per pot were selected for the hydroponic system and transferred to PVC cups (4 × 5 cm^2) containing 1/3 strength Hewitt nutrient medium having 50 and 100 mM of As, and 10 µM of IAA and KN. The PVC cups

were then organized in a simple randomized design and placed in natural environmental conditions, as outlined earlier, to simulate real-world growth conditions. Seedlings growing under only Hewitt nutrient medium regarded as control. At 7 days post-treatment, the seedlings were harvested, and growth, photosynthetic activity, and the performance of AsA-GSH cycle enzymes were evaluated to define treatment effects.

Determination of growth

The seedlings of each set were excised and the length of root and shoot and their ratios was recorded instantly with the help of meter scale. Whereas, the protein content of treated and untreated seedlings was quantified as per the technique outlined by Bradford (1976).

Quantification of photosynthesis and respiratory rate

Photosynthetic oxygen yield and respiratory oxygen uptake were determined in leaf discs obtained from treated and untreated rice seedlings, employing the protocol outlined by Kurra-Hotta *et al*. (1987).

Quantification of oxidative stress markers and indices of damage

Superoxide radical ($O_2^{\bullet-}$) content was computed by monitoring the oxidation of hydroxylamine to nitrite, catalyzed by $O_2^{\bullet-}$, according to the protocol outlined by Elstner and Heupel (1976) whereas the hydrogen peroxide (H_2O_2) content in tested samples was determined using the protocol outlined by Velikova *et al*. (2000). As a result of over produced oxidative stress markers the damage conferred to lipid and membrane of test seedlings was evaluated by assessing the amount of malondialdehyde (MDA) equivalents as described by Heath and Packer (1968).

Estimation of AsA-GSH cycle enzymes activity

The activity of ascorbate peroxidase (APX) was determined in each test sample according to the protocol outlined by Nakano and Asada (1981). APX activity was calculated using the extinction coefficient (ε = 2.8 mM^{-1} cm^{-1}), and one unit of APX activity was defined as the amount of enzyme that oxidizes 1 nmol of ascorbate per minute. The activity of glutathione reductase (GR) was determined in rice seedlings according to the protocol outlined by Schaedle and Bassham (1977). GR activity was calculated using the extinction coefficient (ε=6.2 mM^{-1} cm^{-1}), and one unit of GR activity was defined as the amount of enzyme that oxidizes 1 nmol of NADPH per minute. The monodehydroascorbate reductase (MDHAR) activity in test seedlings was assayed spectrophotometrically as per the method outlined by Hossain *et al*. (1984). The enzyme activity was calculated using an extinction coefficient of ε=6.2 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as nmol NADH oxidized min^{-1}. Dehydroascorbate reductase (DHAR) activity in each test samples was monitored according to the protocol outlined by Nakano and Asada (1981). The activity was quantified using the extinction coefficient (ε=7.0 mM^{-1} cm^{-1}). One unit of DHAR activity was defined as the amount of enzyme that reduces 1 nmol of DHA per minute.

Quantification of total ascorbate (AsA+DHA), reduced ascorbate (AsA) and dehydroascorbate (DHA) contents

The contents of total ascorbate (AsA+DHA), reduced ascorbate (AsA), and dehydroascorbate (DHA) were determined in test seedlings using the spectrophotometric method of Gossett *et al*. (1994). This method exploits the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by ascorbic acid, which subsequently forms a red chelate with 2,2'-bipyridyl, allowing for the quantification of ascorbate contents.

Quantification of total glutathione (GSH+GGSG), reduced glutathione (GSH) and oxidized glutathione (GSSG) contents

Total glutathione (GSH+GSSG), reduced glutathione (GSH), and oxidized glutathione (GSSG) contents were estimated using the enzyme-recycling method described by Brehe and Burch (1976), with minor modifications. This assay relies on the successive oxidation of GSH by 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and the reduction of GSSG in the presence of NADPH and glutathione reductase.

Statistical analysis

The data expressed in this study are of means ± standard error of three independent experiments, each with two replicates, to confirm the data reproducibility. Statistical analysis was performed using one-way ANOVA, followed by Duncan's Multiple Range Test (DMRT) to determine significant differences between control and treatment means at a significance level of $p < 0.05$.

Results and Discussion

Growth

This research conducts an in-depth investigation into the potential benefits of applying exogenous phytohormones, specifically indole-3-acetic acid (IAA), a naturally occurring auxin, and kinetin (KN), a synthetic cytokinin, to counteract the negative impacts of arsenic (As) toxicity on the growth and developmental processes of rice (*Oryza sativa* L.) seedlings. The exposure to As at both 50 and 100 mM doses profoundly impeded the growth of test seedlings, resulting in a marked reduction in root and shoot length, as well as their ratios, and a notable decrease in protein content (Fig. 1). The As-mediated decline in growth parameters could be attributed to a multifaceted interplay of factors, including the higher accumulation of As in tissues, diminished levels of photosynthetic pigments (data published elsewhere) and compromised photosynthetic activity, elevated levels of respiratory O_2 uptake (Fig. 2), increased production of reactive oxygen species (ROS), and subsequent indices of membrane damage (Fig. 3). Our results are in accordance with the earlier study performed by Piacentini *et al*. (2020) where Cd and As mediated disruption in root system, mean root number and mean embryonic adventitious roots were reported in *Oryza sativa* L. seedlings owing to the massive membrane electrolyte leakage of root cells. Similarly, Pandey and Gupta (2015) also reported the decreased shoot length, root length and fresh weight of rice seedlings when exposed to As stress. Further, the supplementation of IAA and KN significantly counteracted As-induced toxicity in test seedlings, likely due to the decreased As accumulation in tissues and oxidative stress (Fig. 3), which were achieved through the augmented activities of the enzymes associated with AsA-GSH cycle and their metabolites (Figs. 4 and Table 1 and 2). Hormones mediated recovery in growth of rice plants could also be clarified on the basis of augmented root activity and plant metabolism, relax the cell wall of root system, vascular tissue formation and facilitate the plant nutrient absorption (Zhu *et al*., 2013; Ronzan *et al*., 2018). Likewise our study, Gangwar *et al*. (2010) elucidated that the synergistic application of 10 µM IAA and Mn effectively mitigated Mn toxicity symptoms and bolstered growth in pea seedlings, surpassing the growth observed in seedlings treated with Mn alone. Similarly, Singh and Prasad (2014) also reported that the exogenous application of KN effectively mitigate the detrimental effects of Cd toxicity on the growth and development of *Solanum melanogena* seedlings. After comparing the responses of both hormones' treatment, it was found that KN always dominant over the IAA, as it much neutralizes the deteriorating impact of As and supports the growth of rice seedlings under stress conditions too.

Photosynthesis and respiratory activity

To gain insight into the mechanisms underlying growth impairment in rice seedlings, this study examined the effects of As alone and in conjunction with KN and IAA on photosynthetic oxygen evolution and respiration. As shown in Fig. 2, the data on photosynthetic activity and respiration reveal that As exposure significantly impaired photosynthetic function in test seedlings at both doses. Nevertheless, the supplementation of IAA and KN, individually or in combination with As, effectively counteracted the adverse effects of As on photosynthesis, thereby minimizing the damage. Opposing, the respiration shows exactly reverse trend. As mediated loss in photosynthetic activity could be correlated with study of Durand *et al*. (2010) where they explained the presumptive factor contributing to the decrease in photosynthesis by interrupting the electron flow at the oxidation side, damaging PS II reaction centre and affecting the performance of Rubisco and other enzymes contributed in Calvin cycle. In contrast to this, when As treated seedling simultaneously supplemented IAA and KN it shows ameliorative behavior (Fig. 2). IAA and KN considerably neutralized the As induced decline in photosynthetic oxygen yield by strengthening photosynthetic apparatus as evidenced by considerable improvement in photosynthetic pigment contents (Data published elsewhere). The improvement in photosynthetic activity mediated by KN is thought to rely largely on the preservation of photosynthetic light reactions, which are functionally coordinated with the enzymes of PSII and PSI. Additionally, the operation of these photosystems is closely tied to the establishment of a trans-thylakoid proton gradient, generating a pH gradient that modulates electron transfer to PSI. This process is reportedly regulated by the PGR5 protein, which controls the proton gradient, as demonstrated by Tikkanen *et al*. (2015). The increased PSII activity in KN-supplemented seedlings may have prevented the generation of singlet oxygen, thereby protecting the chloroplast structure from oxidative stress and enhancing photosynthetic efficiency. Additionally, although As exposure led to a significant increase in

dark respiratory oxygen uptake in test seedlings, the concurrent application of IAA and KN counteracted this negative impact on respiratory processes (Fig. 2). In fact, respiration is generally the first aspect of plant metabolism to be affected by oxygen shortage. The elevated respiratory rate observed in response to As stress is likely a consequence of the excessive production of ROS that possibly consumes more O_2 (actively accepts electron at an intermediate stage of respiratory and photosynthetic ETC) (Singh *et al*., 2018); therefore, an obvious demand for O_2 was observed. Furthermore, rise in respiratory O_2 uptake under As stress was diminished substantially, indicating the appreciable decrease in As mediated fall in growth of rice seedlings under the supplementation of IAA and KN. The observations made in this study are in agreement with the results of Tiwari *et al*. (2018) where KN treatment significantly decreased the oxygen uptake in *Nostoc muscorum* under Cr^{VI} stress. The observed decline in respiration rate may be explained by the decreased production of ROS ($O_2^{\bullet-}$ and H_2O_2) as O_2 acts as a source for ROS during stress.

Oxidative stress and AsA-GSH cycles enzymes

Arsenic is capable to induce oxidative stress by the excessive generation of ROS i.e. superoxide radicals ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet-}$), and hydrogen peroxide (H_2O_2), which can interact with biomolecules and modify their structure and function (Mishra *et al*., 2016; 2022). In case of As reactive oxygen species are also produced through the conversion of As^V to As^{III} in plants (Mascher *et al*., 2002). In the present study too, the rice seedlings exposed to As showed a severe oxidative stress as evident by a sharp increase in the level of $O_2^{\bullet-}$ and H_2O_2 and thereby MDA equivalents contents, which might have occurred as a result of electron leakage from overloaded ETC of chloroplasts and mitochondria, stimulated by As (Singh *et al*., 2020). Our results corroborate the findings of preceding investigations (Talukdar, 2013; Mishra *et al*., 2022), where ROS generation and stress markers were highlighted against the As stress. Conversely, the supplementation with IAA and KN, either alone or in combination, markedly check ROS levels, with a more pronounced effect observed in seedlings treated with KN. The present study revealed a substantial increase in APX activity in seedlings treated with toxic concentrations of As (Fig. 4), aligning with the observations of Singh *et al*. (2016), who reported similar enhancements in APX activity under As stress. Moreover, the supplementation of IAA and KN led to a pronounced increase in APX activity facilitating effective H_2O_2 scavenging and creating an optimal environment for detoxification. This is evident from the decreased levels of MDA equivalents content (Fig. 3). In this investigation, an increment in DHAR activity was observed under 50 mM of As; whereas the activity of the same was found to less under 100 mM of As (Fig. 4). Upon 100 mM of As treatment the fall in the activity of DHAR enzyme implies the inadequate regeneration of AsA from DHA. Our findings are in support of Chao *et al*. (2010) where they explained the reason for decrease in AsA content might be the greater expenditure of AsA in comparison to their synthesis. The observed enhancement in DHAR activity aligns with the results reported by Bashri and Prasad (2016) where Cd stressed *Trigonella foenumgraecum* L. seedlings showed tremendous response under the supplementation of plant growth regulators. Consequently, the transient increase in DHAR activity observed under 100 mM As treatment may be insufficient to enhance the AsA-GSH cycle, ultimately disrupting the cellular redox balance. It is apparent from the sharp fall in AsA content hence, showing decreased AsA/DHA ratios (Table 1). Contrastingly, under the supplementation of IAA and KN improved DHAR activity in As treated rice seedlings by keeping elevated AsA content and thereby AsA/DHA ratio too (Fig. 4; Table 1). These results were supported by Ye *et al*. (2025) who confirmed that reduced ascorbate scavenges ROS directly or indirectly by means of APX. The results indicate that IAA and KN, applied singly or in combination with As, exert a profound influence on AsA recycling and the turnover rate of AsA-GSH cycle enzymes in rice seedlings (Fig. 4). Enhanced AsA recycling, in turn, enables more effective H_2O_2 scavenging. The increase in AsA/DHA and GSH/GSSG (have been discussed later) ratios suggesting an IAA and KN mediates the gradual shift from oxidized cellular redox status towards the homeostatic regulation of cellular redox reactions. Our data corroborate the observations made by Singh *et al*. (2018) where they argued that upon foliar application of KN in Cd treated tomato seedlings the activities of APX, GR, DHAR and MDHAR increased significantly and alleviated the toxic effects of Cd on performance of chloroplast as supported by substantial improvement in photosynthetic oxygen yield along with active reaction centers. Similarly, Ahammed *et al*. (2013) reported enhanced activities of APX and GR in

Solanum lycopersicum seedlings treated with Cd, following exogenous application of 24-epibrassinolide. Notably, the AsA-GSH cycle plays a dual role, not only in detoxifying H_2O_2 but also in redox sensing and signaling, as highlighted by Pastori and Foyer (2002).

The present study revealed a notable decline in reduced glutathione (GSH) content, accompanied by a corresponding increase in oxidized glutathione (GSSG) content, following exposure to both tested doses of As (Table 2). Consequently, the GSH/GSSG ratio declined sharply, indicating an imbalance in the cellular redox state. The possible explanation for this decrease in GSH content can be correlated with its contribution in the synthesis of PCs, a typical metal chelator proteins specialized for the chelation of As like metals (Yadav, 2010; Ruíz Torres *et al*., 2017). Moreover, GSH itself known as a key source of non protein reduced S. The conjugation of GSH to xenobiotics also catalyzed by GST enzyme that enables the efficient removal of harmful substances, highlighting the crucial role of GSH in cellular detoxification processes. Similarly, the elevated GSSG levels under As stress (Table 2) may be due to: (i) decreased GSH recycling efficiency, and (ii) increased GSH catabolism during stress, further compounded by the heightened requirement for GSH in the AsA regeneration pathway, as noted by Foyer and Noctor (2005). Further the condition got reversed under the supplementation of IAA and KN and enhanced the level of reduced GSH in test seedlings. Our data corroborate the observations made by Koprivova *et al*. (2010); Bashri and Prasad (2016) and Singh *et al*. (2018).

In current investigation, the GR enzyme activity was increased significantly under 50 mM of As treatment whereas per cent accumulation under 100 mM of As it was less recorded (Fig. 4). The increased GR enzyme activity advocates that the metalloid induced GR activity was inappropriate to deal with massive GSH consuming effects of the metalloid, such as direct metalloid GSH binding, GSH oxidation, GST synthesis and PCs synthesis (As-PCs complex) (Jozefczak *et al*., 2012). Thus, the value of GSH by GSSG was also decreased. Notably, the exogenous application of IAA and KN, either individually or in tandem, elicited a pronounced increase in GR activity, GSH content, and the GSH/GSSG ratio in rice seedlings subjected to As stress. This synergistic effect suggests that IAA and KN supplementation can effectively bolster the GSH pool in rice seedlings, thereby enhancing their resilience to As-induced oxidative damage. Our findings are in accordance with the Cd-treated *Trigonella foenum-graecum* L. seedlings (Bashri and Prasad, 2016), where foliar application of 10 µM of IAA considerably increased the GSH pool. Consistent with our findings, Singh *et al*. (2018) observed that KN application significantly enhanced GSH content in Cd-stressed tomato seedlings. The underlying mechanism for this increase in GSH content may be attributed to its crucial role in protecting cells against As toxicity. Specifically, GSH can directly conjugate with As via its sulfhydryl (-SH) group, thereby neutralizing its harmful effects. Additionally, GSH serves as a substrate for the synthesis of phytochelatins (PCs), which are essential for As detoxification (Niczyporuk *et al*., 2020).

Conclusion

This investigation unequivocally demonstrates the deteriorating impact of As exposure on rice seedlings, with both 50 and 100 mM doses profoundly impairing growth and development. Specifically, As treatment triggered a precipitous decline in photosynthetic activity, while concomitantly stimulating a surge in ROS production, thereby inducing oxidative stress and compromising the seedlings' overall health. Notably, despite the AsA-GSH cycle's compensatory mechanisms, including enhanced enzyme performance and metabolite accumulation, As exposure still inflicted considerable damage on the rice seedlings, underscoring the severity and complexity of As-induced stress in rice. In stark contrast, supplementation with IAA and KN remarkably alleviated As-induced toxicity in rice seedlings, highlighting the potential of phytohormone-mediated stress mitigation strategies. This mitigation was manifested through improved photosynthetic activity, enhanced enzyme activities of the AsA-GSH cycle, and increased levels of their metabolites, which collectively limited the damage caused by ROS and restored cellular homeostasis. The overall findings of this study suggest that supplementation with both IAA and KN may enhance the yield and productivity of rice seedlings by modulating stress responses and promoting tolerance to As-induced toxicity. Moreover, the comparative analysis reveals that KN supplementation may be more effective in As-contaminated sites, providing a potential strategy for mitigating the adverse effects of As on rice crops and ensuring food security in regions affected by As contamination.

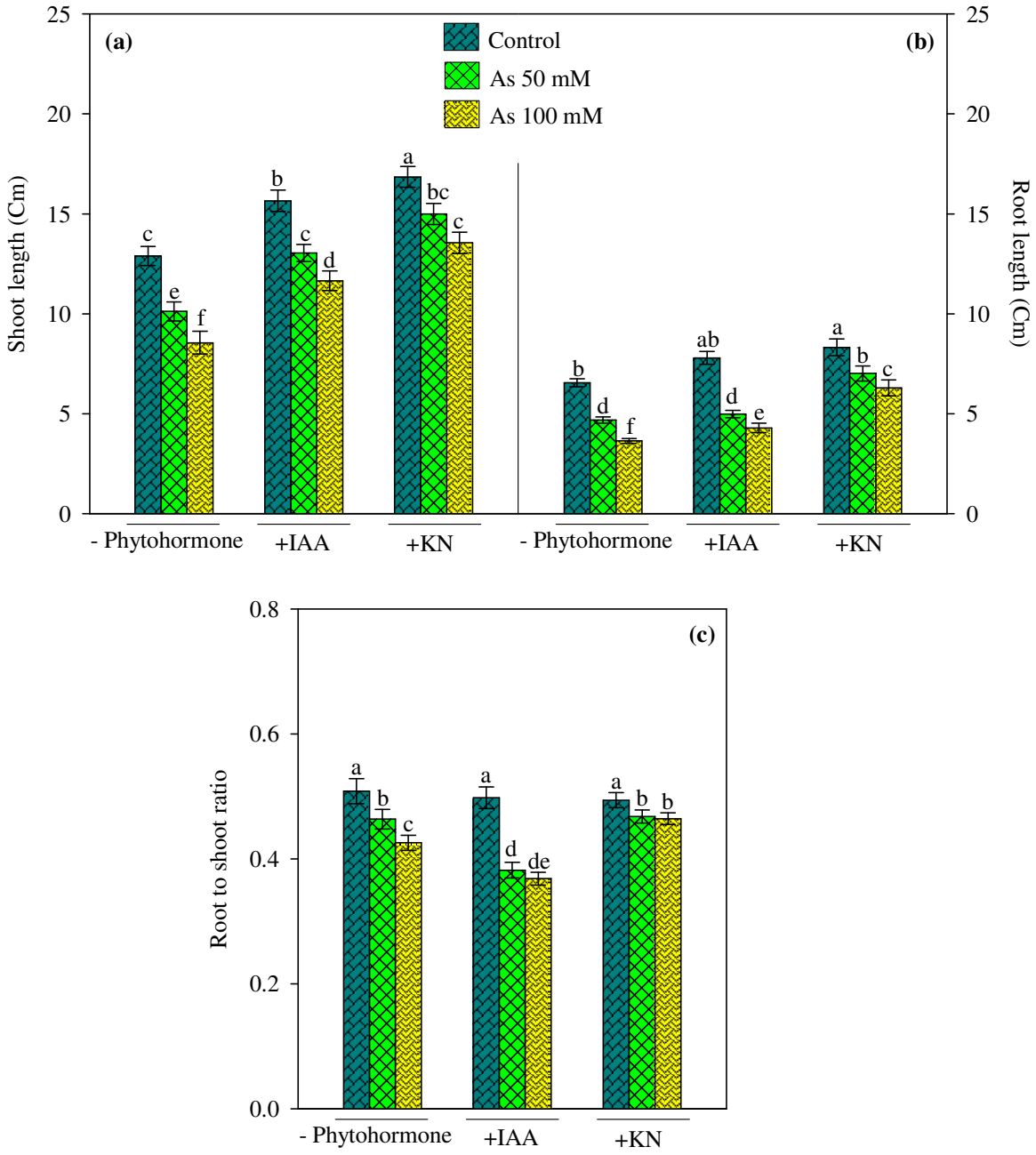

Fig. 1: Impact of IAA and KN on shoot (a) and root (b) length and root to shoot ratio (c) of rice seedlings under As toxicity. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at P<0.05 between treatments according to Duncan's multiple range test.

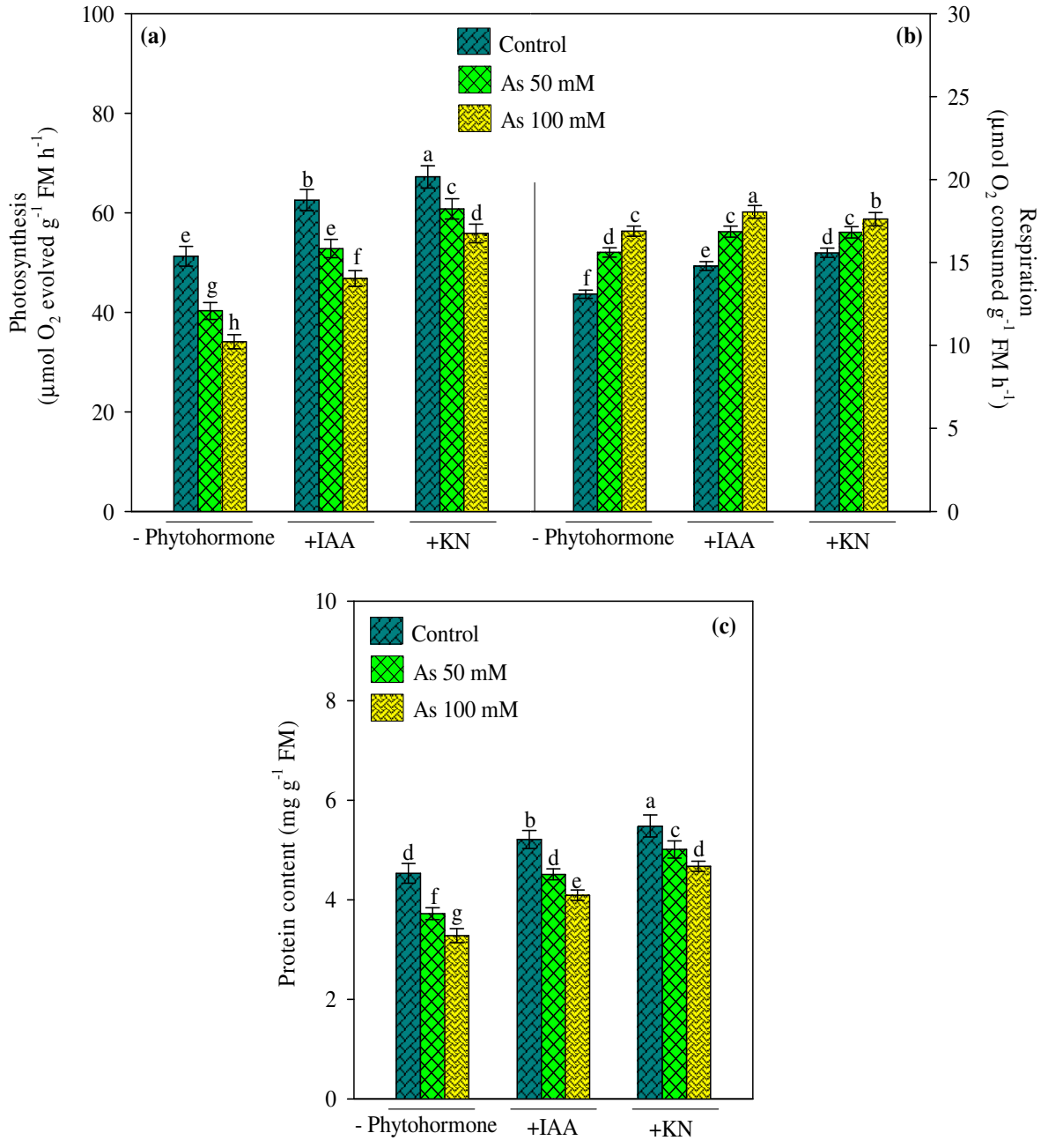

Fig. 2: Impact of IAA and KN on photosynthesis (a), respiration (b) and protein content (c) of rice seedlings under As toxicity. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at P<0.05 between treatments according to Duncan's multiple range test.

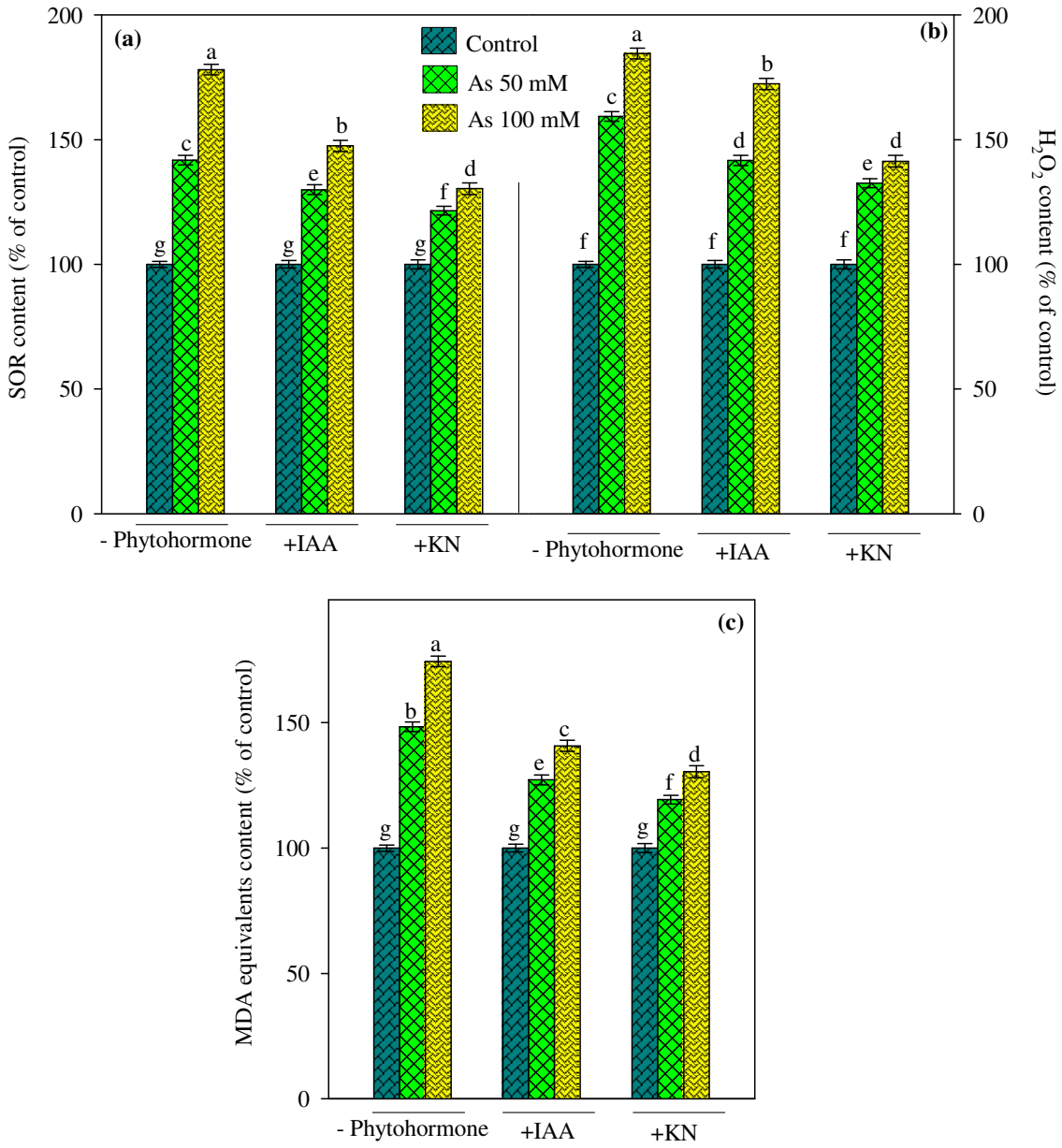

Fig. 3: Impact of IAA and KN on SOR (a), H_2O_2 (b) and MDA equivalents content (c) of rice seedlings under As toxicity. The 100 per cent value corresponds to control for SOR were 107.8 ± 3.87, 622.75 ± 24.31 for H_2O_2 and 49.27 ± 1.24 nmol g^{-1} FM. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at $P<0.05$ between treatments according to Duncan's multiple range test.

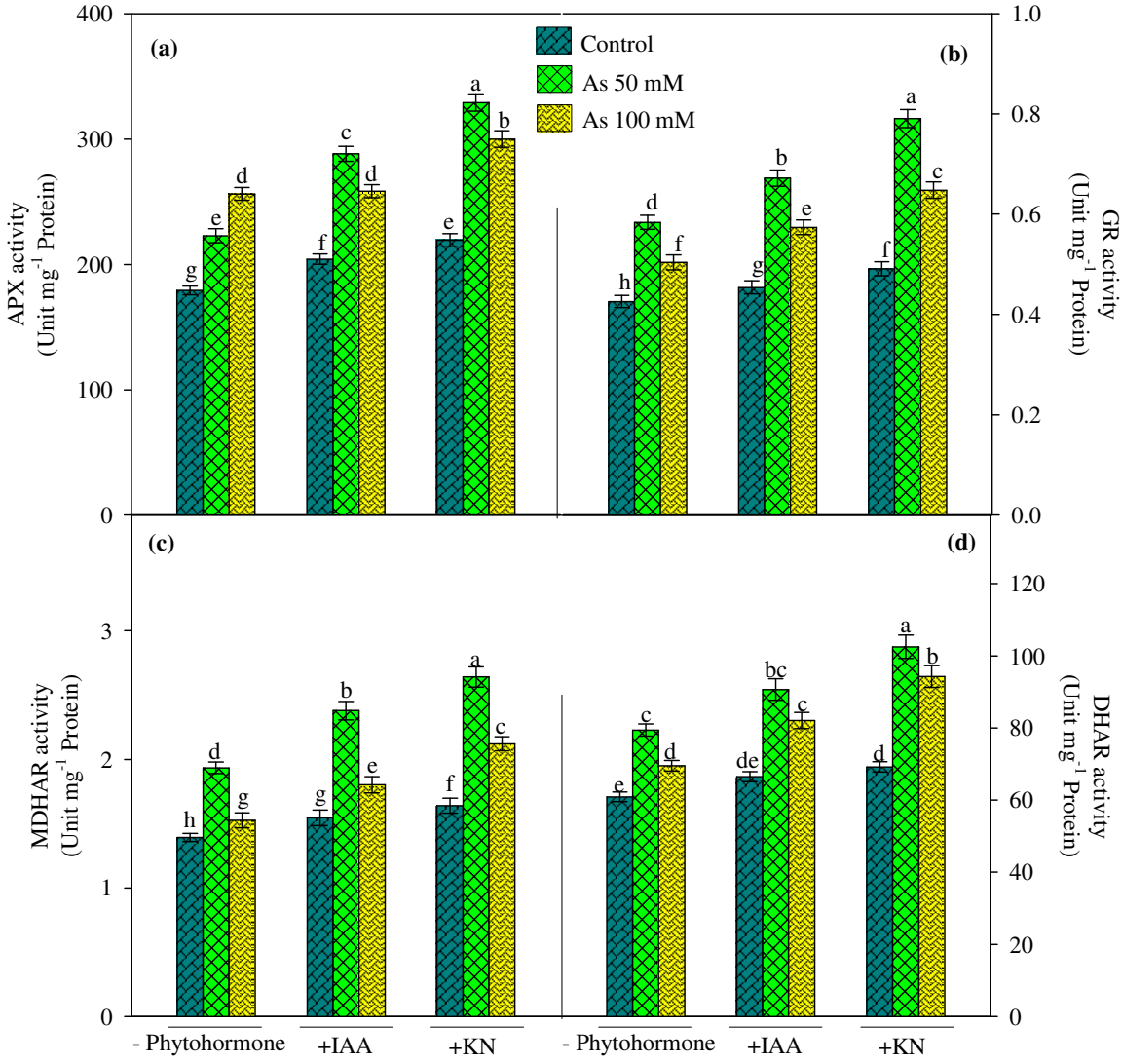

Fig. 4: Impact of IAA and KN on ascorabate glutathione cycle enzymes APX (a), GR (b), MDHAR (c) and DHAR (d) activities of rice seedlings under As toxicity. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at P<0.05 between treatments according to Duncan's multiple range test.

Table 1: Impact of IAA and KN on metabolites associated with AsA-GSH cycle i.e. reduced ascorabte (AsA), dehydroascorbate (DHA), total ascorbate (AsA+DHA) and their ratio (AsA/DHA) of rice seedlings under As toxicity.

Treatments	Content (µmol g^{-1} FM)			Ratio of AsA to DHA
	AsA	DHA	AsA+DHA	AsA/DHA
Control	8.56 ± 0.284^{d}	2.87 ± 0.087^{f}	11.43 ± 0.351^{e}	2.98 ± 0.089^{a}
As 50 mM	6.39 ± 0.198^{g}	3.79 ± 0.116^{de}	10.18 ± 0.317^{g}	1.69 ± 0.068^{e}
As 100 mM	5.08 ± 0.176^{h}	4.37 ± 0.144^{a}	9.45 ± 0.269^{h}	1.16 ± 0.041^{g}
+ IAA 10 µM	9.58 ± 0.314^{b}	3.11 ± 0.097^{e}	12.69 ± 0.398^{c}	3.08 ± 0.091^{a}
As 50 mM + IAA 10 µM	7.89 ± 0.257^{e}	3.82 ± 0.126^{d}	11.71 ± 0.364^{e}	2.07 ± 0.070^{c}
As 100 mM + IAA 10 µM	6.98 ± 0.182^{f}	4.35 ± 0.141^{a}	11.33 ± 0.347^{f}	1.60 ± 0.074^{f}
+ KN 10 µM	10.19 ± 0.327^{a}	3.39 ± 0.117^{a}	13.58 ± 0.521^{a}	3.01 ± 0.089^{a}
As 50 mM + KN 10 µM	8.98 ± 0.292^{c}	3.91 ± 0.139^{c}	12.89 ± 0.414^{b}	2.30 ± 0.080^{b}
As 100 mM + KN 10 µM	8.19 ±0.271^{d}	4.21 ± 0.124^{b}	12.40 ± 0.387^{d}	1.95 ± 0.082^{d}

Data present here are mean ± standard error of three independent experiments. Values within same column followed by different letters show difference at P<0.05 level between treatments according to Duncan's multiple range test.

Table 2: Impact of IAA and KN on metabolites associated with AsA-GSH cycle i.e. reduced glutathione (GSH), oxidized glutathione (GSSG), total glutathione (GSH+GSSG) and their ratio (GSH/GSSG) of rice seedlings under As toxicity.

Treatments	Content (µmol g^{-1} FM)			Ratio of AsA to DHA
	GSH	GSSG	GSH+GSSG	GSH/GSSG
Control	6.03 ± 0.114^{d}	2.26 ± 0.052^{g}	8.29 ± 0.157^{f}	2.67 ± 0.079^{a}
As 50 mM	5.05 ± 0.068^{g}	3.08 ± 0.069^{d}	8.13 ± 0.151^{g}	1.64 ± 0.038^{e}
As 100 mM	3.97 ± 0.047^{h}	3.78 ± 0.094^{a}	7.75 ± 0.121^{h}	1.05 ± 0.019^{g}
+ IAA 10 µM	6.82 ± 0.122^{b}	2.54 ± 0.071^{f}	9.36 ± 0.188^{c}	2.69 ± 0.088^{a}
As 50 mM + IAA 10 µM	5.94 ± 0.107^{e}	3.22 ± 0.098^{c}	9.16 ± 0.176^{d}	1.84 ± 0.044^{d}
As 100 mM + IAA 10 µM	5.29 ± 0.092^{f}	3.65 ± 0.101^{b}	8.94 ± 0.167^{e}	1.45 ± 0.027^{f}
+ KN 10 µM	7.01 ± 0.139^{a}	2.77 ± 0.088^{e}	9.78 ± 0.204^{a}	2.53 ± 0.068^{b}
As 50 mM + KN 10 µM	6.54 ± 0.127^{c}	3.11 ± 0.099^{d}	9.65 ± 0.191^{ab}	2.10 ± 0.051^{c}
As 100 mM + KN 10 µM	5.97 ± 0.111^{e}	3.59 ± 0.104^{b}	9.56 ± 0.189^{b}	1.66 ± 0.039^{e}

Data present here are mean ± standard error of three independent experiments. Values within same column followed by different letters show difference at P<0.05 level between treatments according to Duncan's multiple range test.

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