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Arsenic in plants

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Abstract: Currently, the world population is 7 billion plus and the major challenge of the third millennium is global food security, which is continuously being arrested by abiotic stresses arise due to the extreme changes in the climate and rapid increase in the population. On an estimated view, more than 50 % crop yield is being lost by abiotic stresses. Arsenic (As) is playing major roles in looming global food security especially in South—East Asia; where rice is an important staple food. Arsenic tends to affect the growth, pigment and all the important growth-regulating processes. Herein, this review a brief highlight of As and its interaction with plant system has been provided.

Introduction:

Highlights of arsenic challenges worldwide: a burning problem in South—East Asia

Currently, the world population is 7 billion plus and the major challenge of the third millennium is global food security, which is continuously being arrested by abiotic stresses arise due to the extreme changes in the climate and rapid increase in the population [1]. On an estimated view, more than 50 % crop yield is being lost by abiotic stresses [2] every year. Among several heavy metals are the most brutal environmental issues limiting crop productivity. During the last few decades, arsenic (As) is playing major roles in looming global food security especially in South—East Asia; where rice is an important staple food. The 50 % of the worlds' people are facing severe risk of As toxicity due to its high As uptake property [3]. Arsenic contamination in drinking water intimidates more than 150 million

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individuals worldwide; among them about 110 million individuals are living in the countries of South and South—East Asia [4], where more than 3200 μg L^{-1} As have been reported in ground water against the permissible limit *i.e.* 0.10 mg L^{-1} [5]. This As—laden groundwater is mostly being used for the irrigation and drinking purposes, which significantly adds a huge amount of As in agricultural fields that causes severe loss in crop productivity and subsequently through food chain also causes serious health disorders in other life forms [6,7].

Impact of arsenic on growth attributes

Morphological characters are the external appearance of the plants against any environmental stimuli. Almost all the life driven processes *i.e.* seed germination, seedling growth, stomatal conductance, water status, nutrients uptake, chlorophyll synthesis, photosynthetic rate, carbohydrate metabolism, protein and DNA suffer from As toxicity [8]. According to Niazi et al. [9] different As concentrations *i.e.* 25, 50 and 75 mg As kg^{-1} soil inhibited plant height, leaf area, number of leaves and root and shoot dry biomass in *Brassica juncea* and *Brassica napus* seedlings, that declines crop yield and may culminate into plant death. Ruíz—Torres et al. [10] have reported that As^{V} at 200 μM dose majorly decreased the growth of root than shoots in *Allium sativum*. Weight water content (WWC) is another less studied growth parameter that has been reported to be decreased under higher As doses [11]. Anjum et al. [12] have reported that 200 μM As reduced the number of leaves $plant^{-1}$, plant height, stem diameter, leaf area, fresh weight (FW) and DW of the shoot and yield attributes.

In medicinal plant *Artemisia annua*, the most emblematic symptoms were the extreme red coloration of leaflets principally at the apex at 150 μM As exposure [13]. Further, As showed inhibitory effect on number of inflorescences, their branches and plant height. In contrast, the size of capitula, number of florets, ratio of capitula/ inflorescence and number of oil glands were increased under similar conditions [13]. Radicle, root and shoot length (RL and SL) and DW were reduced by As treatment in rice (*Oryza sativa*) and fenugreek (*Trigonella foenum_graecum*) L. seedlings [14,15]. While working on *O. sativa*, Das et al. (2013) observed that increasing doses of As reduces the number of active seedlings pot^{-1}, number of tillers $plant^{-1}$, dry matter yield (g pot^{-1}), number of matured grains $panicle^{-1}$ and grains yield (g pot^{-1}) in a dose—dependent manner.

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Impact of arsenic on mineral nutrients status

Arsenic interferes with the uptake and translocation of essential mineral nutrients [7]. Under As stress, no definite trend in macro (Mg, Ca and K) and micro (Mn, Fe and Cu) elements in roots of *B. juncea* was observed by Pandey et al. [17]. In contrast, Parsons et al. [18] while working on corn seedlings reported that Fe concentration increased by 7 folds under As^{III} treatment, while 4 folds in As^{V} treated growth media as compared to control (500 ppm). Whereas, the total Fe levels in shoots was decreased by 125 ppm *i.e.* same amount for both As treatments than 360 ppm in control. At 150 µM As, a significant decline in Na content was reported by Pandey et al. [17], while under similar conditions no significant changes in P content of roots were observed. Contrastingly, P contents were decreased noticeably in both root and shoots [18] in maize suggesting that As inhibited the phosphate metabolism by substituting iP in plants [19]. Further, S level was improved by 560 ppm and 800 ppm under As^{III} and As^{V} treatments, respectively as compared to control (358 ppm) in the root, while in case of shoots no significant change was found. However, the levels of K were increased in roots and decreased in shoots [20].

Impact of arsenic on photosynthetic pigments

Photosynthetic pigments are the first observable target against any environmental change, earlier to any effect on photosynthetic performance or to oxidative stress; hence, are considered as a sensitive indicator of the metabolic status of the cell. Several studies have witnessed the decrease in chlorophylls (Chls) content under As stress. The 5 and 50 µM doses of As were shown to decline the contents of both Chl *a* and *b* and the reduction in Chl *b* was greater than Chl *a*; therefore, higher Chl *a*/*b* ratio was reported in *Luffa* seedlings [21]. Further, 5 and 50 µM of As treatment also declined the carotenoids (Car) content; however, the decreasing impact was less than that of Chls [21]. Contrastingly, in the leaves of maize cultivars, reduction in Chl *a* and *b* was observed after As treatment where greater reduction of Chl *a* was found over Chl *b* [12]. Singh et al. [22] observed a continuous decreasing trend for the total Chl (Chl *a*+*b*) content under 25 and 50 µM As doses, while Cars content showed a reverse trend. Decline in the contents of Chl *a*, *b*, total Chl and Car, and Chl *a*/*b* ratio has also been reported in other studies [23]. In bean plant, Miteva and Merakchiyska [24] have reported that excess As in the soil, declined Chls content due to alteration in chloroplast

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structure with the appearance of the concave membrane. In one of the studies in *Hydrilla verticillata*, the contents of Chl *a*, *b* and Car were significantly decreased after 24 h of 100 and 500 µM As^V treatments; while Chl *a*/*b* was reduced under 500 µM As^V [25].

Similarly, in the leaves of aquatic macrophyte *Ceratophyllum demersum*, Mishra et al. [26] have reported that at 0.5 µM dose of As, Chl levels were rapidly lost. Further, with the help of quantitative µXRF tomograms, they have reported that immediately after reaching the mesophyll, As start to affect the accumulation of Chl complexes and a sharp decline in all the Chl precursors was noticed except monovinylchlorophyllide *a* [26]. The increasing doses of As further aggravate the reduction in Chl precursors along with the reduction in pheophorbide *a* (an intermediate of Chl catabolism) [26]. Contrarily, Mahdieh et al. [27] noticed that the Chl content was increased in two wheat varieties under 2.5 mg As L^{-1} exposure. In *C. demersum* L., Mishra et al. [28] reported a reduction in the levels of b—carotene—like pigments showing an increasing trend at 5 µM As. Among the carotenoids, b—carotene facilitate non—photochemical quenching (*NPQ*) of excess energy [29]. Further, Chl to Car ratio was also reported to decline upon As exposure [28].

Impact of arsenic on photosynthesis and PS II photochemistry

The efficient photosynthesis is essential for survival and fitness of plant and Chl fluorescence offers the information about the status of photosynthetic apparatus and PS II, which is the most susceptible component. The concurrent loss of photosynthetic pigments is the earliest event of As toxicity, which decreases the photosynthetic performance of the cell [28]. However, higher As level inhibits photosynthetic electron transport rate and PS II reaction centre in later stage. According to Pandey et al. [29] and Rafiq et al. [30], As exposure causes injuries to chloroplast membrane and disorganizes the functions of crucial photosynthetic processes. Rahman et al. [31] while working on the effect of As on photosynthesis of five widely cultivated rice varieties reported that inhibition in photosynthetic performance was due to a loss in chloroplast ultrastructure and alterations in the Chls biosynthesis. Upon 2 and 5 mg As dm^{-3} exposure, a considerable reduction in the functionality of PS II and rate of carbon dioxide (CO_2) fixation was noticed in *Phaseolus vulgaris* L. cultivars by Stoeva and Bineva [32]. Under similar treatment, As decreased the net photosynthesis rate, transpiration rate (*E*), water potential (*Ψ*) and *PN/E* that decreased the

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rate of CO_2 fixation and consequently the functionality of PS II in bean plant [12]. The As—induced inhibition of *PN* concurrent with the inhibition of intracellular CO_2 concentration (*Ci*), *gs* and *E* is due to the abnormality of stomata. In maize cultivars, a pronounced decrease in the gas exchange attributes (photosynthesis, *gs*, *E* and *Ci*) under 200 µM As treatments were noticed by Anjum et al. [12]. The decrease in *PN*, *gs* and *E* under higher doses of As have also been reported in other studies too [13,33]. In contrast, the WUE in *Artemisia annua* was increased under 100 and 150 µM As stress [13]. In addition, the significant decrease in photosynthetic electron transport chain (ETC) activities was noticed; where PS II showed a minor decrease under both the doses of As, while PS I showed a significant increase under 100 µM dose of As. Besides whole chain electron transport rate, ATP and NADPH contents were increased under 100 µM As dose; while declined with 150 µM As in *A. annua* [13].

The changes in the photosynthetic pigments reflect the photochemical process of photosynthesis (PS II and I); where PS II is considered to be more sensitive under stress; therefore, this metalloid triggers the change in maximum quantum yield of primary photochemistry (F_v/F_m or ΦPS II) of the plants [26]. Wang et al. [34] reported significant increase in O_2 evolution upon As exposure, which suggests the inhibitory effect of As on donor side of PS II. In *Luffa* seedlings, Chl fluorescence parameters such as F_v/F_m, the activity of PS II (F_v/F_o) and photochemical quenching (*qP*) were decreased [21]; while *NPQ* values were increased under As stress. Singh et al. [4] and Mishra et al. [26] while working on eggplant and rice reported that As significantly affected the JIP—test parameters: Ψ_o, ΦE_o, PI_{ABS} and ratio of energy flux parameters (*ABS*, TR_o and DI_o per reaction centre; RC), *NPQ* and *QP* that decreased the number of active RCs thereby disturbing photosynthetic process. Besides this, carbon (C) reactions of photosynthesis are also the prime target of As [35].

Impact of arsenic on respiration

The respiratory oxygen uptake rate was increased in As treated *Pistia stratiotes* and *O. sativa* leaves [36], which probably is the result of chemical similarities in between As^V and iP, that struggle for the same active site in the mitochondrial ATP synthase [37]. This struggle leads to the synthesis of As—ADP, thereby causing a decrease in ATP levels. The lower levels of ATP trigger respiratory activity to generate more As—ADP; therefore,

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although respiration gets fasten to provide C skeleton through futile cycles of As—ADP generation but it generates more ROS in the cell [38].

Impact of arsenic on nitrogen metabolism status

Biological nitrogen fixation *i.e.* symbiotic relations of root nodules of legume; contributes a large amount of N in the biological systems. Porter and Sheridan [39] reported that roots of alfalfa (showing well—established N_2—fixation in symbiosis with *Rhizobium*) are very sensitive to As toxicity. Further, less than 50 % of the total number of root nodules were formed, when alfalfa was grown in As—contaminated areas [40] indicating that As strongly suppresses legume—*Rhizobium* symbiosis. Non—leguminous plants get N from soil nitrate (NO_3^-) or ammonium (NH_4^+). The NO_3^- through sequential reduction by nitrate reductase (NR) and nitrite reductase (NiR) activities is reduced in to NH_4^+, while NH_4^+ is incorporated into glutamine and glutamate through combined action of glutamine synthetase—glutamate synthase (GS—GOGAT) pathway [41], which then is assimilated into amino acids, proteins, nucleic acids and other metabolites [42]. A considerable decline in the transcripts for different NO_3^- and NH_4^+ transporters in the roots of As—stressed rice plants was reported by Norton et al. [43] and Chakrabarty et al. [44]. The NR activity in the root, rhizome and frond has been reported to decrease under 150 and 300 mM of sodium arsenate treatment in *Pteris spp.* [45]. Similar repression in NR activity was also reported by Chakrabarty et al. [44] in rice seedlings; whereas an enhancing effect was recorded in *Arabidopsis* [46]. In *P. vittata* and *P. ensiformis*, NO_3^- and NO_2^- contents and NiR activity were decreased in the root, rhizome and frond under As stress [45]. Singh et al. [22] while working with rice seedlings reported an increasing trend for NR activity, while NO_2^- content showed a decreasing trend under 25 and 50 µM As in both root and leaves. In another study, Ahsan et al. [47] found the lower amount of GS protein in As treated rice roots.

Impact of arsenic on reactive oxygen species (ROS) and oxidative stress biomarkers

Accumulation of As in cell creates disturbance in the cellular homeostasis, which witnessed over—production of ROS: superoxide radical (SOR, $O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) resulting into oxidative injuries to lipids, protein and nucleic acids [8,10,37]. In plants, ROS formation takes place due to

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leakage of electrons form overloaded ETC and get renders to molecular oxygen (O_2) [48] or when As^V reduces into As^{III} during As detoxification process [49]. When electron carriers of photosynthetic machinery over—reduced, triplet excited Chls pass the excitation energy to O_2, generating $^1O_2$ [50]. These cytotoxic ROS due to As exposure, causes lipid peroxidation and membrane leakage, which ultimately collapse the system and reduces crop productivity [15]). The ROS provoked membrane damage is considered a main reason of cellular toxicity by As in diverse crop plants. In a recent study, a higher dose of As (267 µM) for 10 days, declined the membrane stability index (MSI), where it was 78.8 % in *P. vittata* and 22.3 % in *P. ensiformis* as compared to control [51].

Impact of arsenic on the antioxidant defense system

Since, over—production of ROS beyond the threshold limit disturbs various cellular metabolism, thus reducing crop productivity. In this concern, keeping the ROS levels under the threshold limit, plants have articulated sound responses in terms of antioxidants against over—produced ROS to avoid oxidative burst at the cellular level. Antioxidant defense mechanisms is highly studied aspect of plant metabolism [51,52], which directly is linked with As stress tolerance mechanism in plants. In *L. acutangula*, As treatment improved the activities of antioxidants [21]. Similarly, the activities of SOD and CAT were increased under 50 µM As treatment, while that of APX, dehydroascorbate reductase (DHAR) and glutathione reductase (GR) were found to decrease in *Phaseolus vulgaris* [15]. In *B. juncea*, the activities of SOD and GPX showed strong antioxidative response under As^{III} exposure (50 and 150 µM), while decrease at 300 µM As exposure [51]. Further, the isoenzyme profiling showed five and two bands of SOD and GPX, respectively [51]. In garlic plant, upon 200 mM As exposure, CAT activity was declined in root, while remained unaffected in shoots; the native—PAGE analysis for SOD isoenzymes showed single Mn—SOD and two Cu/ Zn—SOD bands in root, while in shoots only Cu/ Zn—SOD bands were noticed [10]. In *Arabidopsis*, genes encoding transcripts for SOD (Fe—SOD; chloroplast, Mn—SOD; mitochondria and Cu/ Zn—SOD; cytoplasmic) revealed that transcripts for Cu/ Zn—SOD were up—regulated more than two—folds, while transcripts of Fe—SOD were down—regulated about five—folds [48].

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Impact of arsenic on non—enzymatic antioxidants

The non—enzymatic antioxidant defense system consists of a wide range of non—enzymatic antioxidants (proline: Pro, Cys, ascorbate: AsA and components of thiol pools *i.e.* GSH, non—protein thiols: NPTs and PCs), which directly scavenge the ROS by ensuring the plant protection. Ruíz—Torres et al. [10] evidenced that GSH and GSSG contents in root and shoots and GSH/GSSG in shoots were reduced, while the phytochelatins (PC2 and PC3) were sharply raised upon As^{V} exposure. Similarly, As^{V} also causes hindrance in thiol metabolism, reduced GSH content and GSH/GSSG; however, enhanced the levels of NPTs and GSSG [22]. The GSH, PCs, AsA, Car and anthocyanin contents were generally found to accumulate during As exposure [53,54,55]. Singh et al. [4] reported that Pro content and the activity of Pro biosynthesis enzyme *i.e.* Δ^{1}—pyrroline—5—carboxylate synthetase (P5CS) was increased; while the activity of Pro degrading enzyme *i.e.* proline dehydrogenase (ProDH) was declined.

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