Metabolome Modulation During Arsenic Stress in Plants

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1   Introduction

Progressively increasing contamination of arsenic (As) is a dreadful situation these days, which adversely influences all components of the environment. Arsenic is introduced in the environment through natural processes (volcanic eruptions, weathering of rocks, minerals, and fossil fuels) and anthropogenic activities (metal mining and smelting, agrochemicals, industrial effluents, and phosphate fertilizers), with its high level for the most part of India, Bangladesh, and China (Mondal et al. 2006, Tripathi et al. 2012a, b). The worst conditions of As contamination came across in Bangladesh and West Bengal (India) due to natural processes (Smith et al. 2000, Nordstrom 2002, Brammer and Ravenscroft 2009).

The subsurface mobilization of As is caused by the combination of chemical, physical and microbial factors, and various theories have been proposed to explain the mechanism of As mobilization (Mondal et al. 2006; Drewniak et al. 2012). Of these, the important theories are the pyrite oxidation and oxyhydroxide reduction (Hossain 2006). Flooding induces reducing (anaerobic) conditions in soils; hence, adsorbed arsenate [As(V)] is reduced to arsenite [As(III)] by anaerobic metal-reducing bacteria released by mediating reductive dissolution of As-rich Fe(III) oxyhydroxides (Islam et al. 2004). Besides natural processes, the global input of As

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to soil by human activities was estimated about 52,000 to 1,12,000 tons per year in earlier decade (Nriagu and Pacyna 1988).

Inorganic arsenic (iAs) forms such as As(V) and As(III) are class 1 carcinogens (IARC 2004). It is often implicit that organic forms, dimethylarsonic acid [DMA(V)] and monomethylarsonic acid [MMA(V)], are lesser toxic to humans than iAs (Schoof et al. 1999). However, reduced trivalent intermediate species of DMA and MMA are actually more cytotoxic and genotoxic than iAs (Thomas et al. 2001). There is a widespread chronic iAs poisoning in regions of South and Southeast Asia, South America, and elsewhere, due to the consumption of drinking water extracted from shallow underground aquifers with geogenically elevated iAs (Naujokas et al. 2013). According to the World Health Organization (WHO), the permissible limit of As in drinking water is 10 μg L^{-1}. Besides As-contaminated drinking water, plant-based foods are also recognized as an important source of iAs (Williams et al. 2005; Meharg et al. 2009).

Rice is specifically a problem regarding the entry of As into the food chain, owing to a combination of the anaerobic growing conditions and specific plant physiological characteristics. Nearly half the world's population relies on rice for subsistence diet. Rice contributes about 50% iAs intake to the populations exposed to high level of iAs in drinking water in South Asia (Meharg et al. 2009, Tripathi et al. 2012a, b). However, the excessive use of methylated arsenical pesticides in the USA has led to As buildup in soil as well as in rice grains (Williams et al. 2005). Besides, As stress also affects the productivity of the crop plants with high As accumulation.

In the view of past and recent researches, the studies of As uptake and metabolism have been taken into consideration in this review. Further, As toxicity and tolerance mechanisms are discussed for better understanding of the affected biological pathways and responsible metabolites insides the plants.

2   Arsenic Acquisition and Translocation in the Plant

To understand and control the As hazard from contaminated soils, it is crucial to know how various species of As are taken up by plants. Iron plays a pivotal role in the biogeochemical cycle of As, with Fe oxyhydroxides on soil particulate surfaces, or root surfaces of wetland plants as iron plaque, serving as strong adsorbent for As. Reductive dissolution of Fe oxyhydroxides under reducing environment releases the adsorbed As, leading to enhanced As availability to plants. There are significant varietal differences in the amount of iron plaque formation and, consequently, As sequestration in the iron plaque (Tripathi et al. 2014). Varieties that sequester more As in iron plaque translocate less As to aboveground tissues (Liu et al. 2004). Rice cultivars with high porosities tended to possess higher rates of radial oxygen loss and had higher capacities for limiting the transfer of As to aboveground tissues due to formation of more Fe plaques (Wu et al. 2012). Nonetheless, there are various factors other than the amount of iron plaque that may affect the uptake of As, e.g., the plant species, ionic species and their concentrations, pH of the rhizosphere, or the presence of other elements.

The mobility of As in the plants varies with As speciation as well as physicochemical characteristics of the soil (Marin et al. 1992). The most available and predominant forms of As are As(III), As(V), MMA, and DMA for plant uptake. The rate of uptake of different As species varies in the order of As(III) > As(V) > MMA(V) > DMA(V) in most of the plant, but exceptionally in rice the order is As(III) > MMA(V) > As(V) > DMA(V) (Raab et al. 2007). Differential uptake pathways responsible for the uptake of inorganic and organic species of As are described below.

2.1   Transport of Inorganic Arsenic

Arsenic mobility is dependent on the soil pH. Plant roots assimilate As(V) via phosphate transporters. The chemical analogs of inorganic phosphate (Pi) ions, such as $H_2AsO_4^{-1}$, gradually increase at pH range of 3–6; while, $H_2AsO_4^{-1}$ and $HAsO_4^{-2}$ species are significantly present at the pH between 7 and 8. Above pH 8 and below 11, the most predominant chemical form is $HAsO_4^{-2}$.

Plants have both low- and high-affinity Pi transport systems. High-affinity transport is mediated by PHT (phosphate transport) 1 proteins. Competition of As(V) with Pi for entry into the cell through both of these transport systems has been demonstrated in numerous plants, monocots and dicots, and both As-hyperaccumulators and non-hyperaccumulators (Ullrich-Eberius et al. 1989, Meharg and Macnair 1990, Tu and Ma 2003). Research validated that phosphate reduces As(V) uptake in plants (Ullrich-Eberius et al. 1989). The presence of minimum 13 and 9 members of phosphate transporter (*Pht1*) family was reported in rice and *Arabidopsis*, respectively (Zhao et al. 2010, Tripathi et al. 2012a, b). Due to sharing of same transport system, the toxicity of As(V) is lower under high P conditions (Tripathi et al. 2012a, b). Thus, Pi fertilization can protect plants, including the hyperaccumulator *P. vittata*, from As(V) toxicity (Tu and Ma 2003). In roots, the major part of As(V) is reduced to As(III), and the rest is further loaded into the xylem vessels by PHT proteins (Zhao et al. 2010). Therefore, some amount of As(V) is also present in shoots along with As(III) and other organic arsenicals (Zhao et al. 2010).

Recent studies have shown that a number of plant aquaporins belonging to the NIP (nodulin26-like intrinsic protein) subfamily are permeable to As(III), which is present predominantly as the undissociated neutral molecule (arsenous acid) at pH < 8 (Ma et al. 2008). NIP aquaporins also mediate transport of a range of small neutral molecules including ammonia, urea, boric acid, and silicic acid (Wallace et al. 2006). In yeast, a variety of proteins like hexose permeases are also involved majorly for As(III) transport (Finnegan and Chen 2012). While in plants, the role of yeast hexose permeases like proteins is still unknown if they provide a path for As(III) entry into plant cells. However, various plasma intrinsic proteins (PIPs) like OsPIP2;4, OsPIP2;6, and OsPIP2;7 are also suggested to transport As(III) in plants (Mosa et al. 2012). In rice, two silicon (Si) transporters, *Lsi1* (OsNIP2;1 aquaporin) an influx transporter and *Lsi2* (an efflux transporter), play a major role in the uptake of As(III) (Ma et al. 2008). The localization of *OsLsi2* to the proximal side of epidermal and endodermal cells (Ma et al. 2007) and OsLsi1 to the distal side of the

same cells (Ma et al. 2006) is an elegant example of the heterogeneous distribution of proteins in a membrane. Thus, usage of highly efficient Si uptake pathway for passage of As(III) might be explaining why rice is efficient in accumulation of As than other cereals (Williams et al. 2005). Thus, Si fertilization may be an effective strategy to decrease As accumulation in rice grown in As-contaminated soil (Tripathi et al. 2013b). *Lsi2* plays an important role in the root to shoot transport of As(III) and its accumulation in the rice grain. *Lsi2* has a low degree of homology (18%) with the As(III) efflux transporter *ArsB* in *Escherichia coli* (Ma et al. 2007). Mutants of rice defective in silicon efflux transporter (*Lsi2*) showed significantly decreased transportation of As(III) to the xylem and accumulation in shoots and grains transport (Zhao et al. 2010). In most plant species analyzed, As(III) dominates in the xylem sap, also even when As(V) is supplied to plant roots due to high capacity of roots for As(V) reduction.

2.2 Transport of Organic Arsenic

The mobility of organic As MMA(V) and DMA(V) within the plant appears to be substantially greater than that of As(III) or As(V) (Raab et al. 2007, Li et al. 2009). Interestingly, *Lsi1* also contributes to the uptake of undissociated pentavalent MMA and DMA (Li et al. 2009); the rice *lsi1* mutant has lost 80% and 50% of the uptake capacity for MMA and DMA, respectively, compared with the wild-type rice. MMA and DMA have lower dissociation constants than As(III). In contrast to As(III), the rice *Lsi2* is not involved in the efflux of MMA or DMA toward the stele, possibly because most MMA and DMA are dissociated at the cytoplasmic pH (Li et al. 2009). Inadequate information is available about phloem loading and unloading of As. Using rice panicles excised below the flag leaf node, Carey et al. (2010) found that DMA was transported to the immature grain approximately 30 times more efficiently than As(III). To ensure the As(III) and DMA loading in grain through phloem, stem girding was applied and further tenfold and 50% decreased was obtained for As(III) and DMA transport, respectively. The outcome implies that As(III) is transported to rice grain primarily through the phloem, while both the phloem and xylem are contributed equally for DMA transport to grain.

3 Arsenic Detoxification Mechanism in Plants

Plants are able to detoxify As by adopting a range of detoxification strategies. The first step involves As reduction, synthesis of metal binding peptides such as metallothioneins (MTs) and phytochelatins (PCs) to make complex with As and their compartmentalization/vacuolar sequestration to keep the free As concentration at its minimum level (Zhao et al. 2010, Tripathi et al. 2012a, b). The second step is the activation of antioxidant defense system to counteract the As-induced oxidative stress (Zhao et al. 2010).

Arsenic is mainly taken by terrestrial plants as As(V) or As(III) (Meharg and Hartley-Whitaker 2002), followed by rapid reduction of As(V) to As(III) through arsenate reductase (AR) enzyme or via reducing agents such as GSH and ascorbic acid (Meharg and Hartley-Whitaker 2002). Both roots and shoots of rice exhibit As(V) reduction activities (Duan et al. 2007), but roots may be quantitatively more important because As(III) is the main form found in the xylem sap of a number of plant species (Zhao et al. 2003). PCs have a high affinity for As(III), but not for As(V). PCs are thought to have a fundamental role in As detoxification in plants (Schmöger et al. 2000). Arsenic is a strong inducer of PC synthesis and combines rapidly with sulfhydryl-rich protective molecules like GSH and PC (Schmöger et al. 2000). They are synthesized posttranslationally from GSH by the transpeptidation of γ-glutamyl-cysteinyl dipeptides through the action of the constitutive enzyme PC synthase (Grill et al. 2006). The PC–As(III) complexes are likely to be stored in vacuoles. The yeast vacuolar transporter Ycf1p, a member of the ATP-binding cassette (ABC) superfamily, confers As(III) resistance by transporting the conjugated As(III)–(GS)$_3$ complex into the vacuole (Ghosh et al. 1999). In *Arabidopsis*, this transport occurs via the ABC transporters MRP1/ABCC1 and MRP2/ABCC2 (Song et al. 2010). Homologs of *Arabidopsis* ABCC1 and ABCC2 are found throughout the plant kingdom (Mendoza-Cózatl et al. 2011). A homolog of ABCC2 was among several ABC transporters to be upregulated at the transcript level in response to As in rice (Norton et al. 2008a, b). Thus, in many plants, As(III) present in root cells is rapidly complexed to PC and sequestered in the vacuole, severely restricting the transport of As from the root (Mendoza-Cózatl et al. 2011) and preventing its interaction with cellular metabolism.

Another probable constitutive mechanism of As detoxification is the efflux of As(III) to the external medium. Recently, Indriolo et al. (2010) characterized two genes from *P. vittata* (*ACR3* and *ACR3;1*), which encode proteins similar to the ACR3 As(III) effluxer of yeast. In another study, examining heterologous expression of the yeast As(III) efflux system, *ACR3* was cloned from yeast and transformed into wild-type and nip7;1 *Arabidopsis* (Ali et al. 2012). At the cellular level, all transgenic lines showed increase tolerance to As(III) and As(V) and a greater capacity for As(V) efflux. Expressing *Saccharomyces cerevisiae ScACR3* in rice enhanced As(III) efflux and also reduced As accumulation in rice grains (Duan et al. 2012). Rice transgenics overexpressing As(III)-S-adenosyl methyltransferase (*arsM*) has been found to methylate As and gave tenfold higher volatile arsenical, maintaining low As levels in rice seed along with MMA(V) and DMA(V) in the roots and shoots of transgenic rice (Meng et al. 2011).

4 Consequences of Arsenic Toxicity on Plant Metabolism

The mode of toxicity differs involving As species; As(V) interferes with phosphate metabolism such as phosphorylation and ATP synthesis, whereas As(III) binds to vicinal sulfhydryl (-SH) groups of proteins affecting their structures or catalytic functions (Hughes 2002). In plant tissue, As(V) is rapidly reduced to As(III) and

normally itself not have high enough cytoplasmic concentrations to exert toxicity (Bertolero et al. 1987). As chemical analog of phosphate, As(V) can disrupt several cellular biochemical reactions which used phosphate as a substrate (Gresser, 1981). Arsenate can be translocated through cellular membranes by PHT proteins, creating imbalances in phosphate supply. Possible As(V)-susceptible biological reactions include central to cellular metabolism, i.e., glycolysis, oxidative phosphorylation, biosynthesis of phospholipid, nucleic acids retrieval, and cellular signaling including protein phosphorylation/dephosphorylation.

The mode of action of As(III) differs substantially from that of As(V). As(III) is a thiol-reactive compound that can bind up to sulfhydryl groups such as glutathione (GSH) or phytochelatin (PC), thiol-containing proteins, and cofactors. The binding of As(III) to proteins can have profound effects on their folding (Cline et al. 2003). In various systems, proteins that are known to bind As(III) include transcription factors, signal transduction proteins, proteolytic proteins, metabolic enzymes, redox regulatory enzymes, and structural proteins. Compared to As(III), MMA(III) is a more potent inhibitor of various enzymes (Styblo et al. 1997). MMA(III) and DMA(III) both (low to mid micromolar concentration) were able to displace Zn^{2+} from a zinc-finger protein (Schwerdtle et al. 2003), an important class of proteins involved in gene expression and DNA repair.

A number of hydroponic studies infer that As phytotoxicity depends on the chemical species supplied to the plant, but the most phytotoxic form of As was still unidentified (Abbas and Meharg 2008). The inconsistent order of phytotoxicity of different As species might be a sign that As reacts in a different way with the offered nutrients or responds to plant species-specific manner.

At higher concentrations, As interferes with plant metabolic processes and can inhibit growth, often leading to death (Jiang and Singh 1994). Arsenic exposure could hamper normal growth of plants with toxicity symptoms such as biomass reduction, loss of yield and fruit production, and morphological changes (Mokgalaka-Matlala et al. 2008, Srivastava et al. 2009). Consequences of arsenic toxicity have been depicted in Fig. 1 which showed the alteration on growth, physiology, and plant metabolism. The severity of straight head disease (sterility of the florets/spikelets) was increased in rice grown in As-spiked soil solution (Zhao et al. 2010).

Interveinal necrotic (Singh et al. 2006) and whitish chlorotic symptoms (Shaibur et al. 2008) have been reported in As-stressed plants. Arsenic damages the chloroplast membrane and disorganizes the functions of integral photosynthetic process (Stoeva and Bineva 2003). Rate of CO_2 fixation and functional activity of photosystem II (PSII) were reduced in oat plants growing under As stress (Stoeva and Bineva 2003). As a consequence leaf gas exchange, the chlorophyll fluorescence ratio Fv/Fm, leaf photosynthesis activity, carotenoid content, and the functional activity of PSII decreased (Georgieva and Yordanov 1993, Stoeva and Bineva 2003).

High concentrations of As may interfere with plant metabolism and membrane stability index, impair macro- and micronutrient uptake, or may simply compete with essential plant nutrients (Meharg and Macnair 1990); for instance, As is known to compete with plant P uptake (Mokgalaka-Matlala et al. 2008).
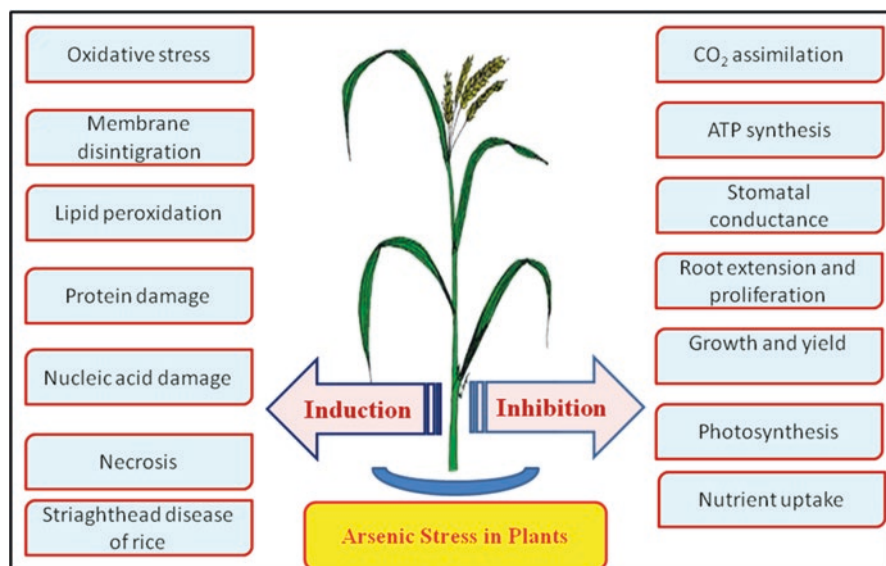
Fig. 1 Consequences of arsenic toxicity in plants

Arsenic exposure generates reactive oxygen species (ROS) in plant tissues and induces oxidative stress such as lipid peroxidation of biomolecules, ultimately resulting in death of the plants (Ahsan et al. 2008). Based on the chemical and physical properties, As causes toxicity in three different ways: (a) enhanced production of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\cdot$), (b) blocking of essential functional groups in biomolecules, and (c) displacement of essential metal ions from biomolecules. Arsenic stimulates generation of ROS, either by direct electron transfer or as a consequence of metal mediated inhibition of metabolic reactions (Zhao et al. 2010).

Under normal cellular conditions, ROS homeostasis is precisely balanced. At low level of environmental stresses such as temperature and light, minute ROS imbalances occur that can act as signals of cellular status and are easily managed by pre-existing antioxidant defense mechanisms (Van Breusegem and Dat 2006). However, under stronger stresses, such as As exposure, where ROS generation increases, these defense mechanisms may be harassed, leading to cellular damage and death (Van Breusegem and Dat 2006).

ROS are capable of causing considerable cellular damage through oxidation of lipids, proteins, amino acids, and nucleic acids (Møller et al. 2007). Exposure of protein to $OH^\cdot$ radical produces dimers, trimers, and tetramers by covalent cross-linking due to formation of intermolecular bityrosine. Exposure to $OH^\cdot$, $O_2^{\cdot-}$, and O_2 further leads to fragmentation or aggregation of protein. Arsenic may also affect the protein synthesizing machinery of the cells by their effect on enzymes of nitrogen metabolism (Schmidt et al. 2005). Lipid peroxidation not only compromises cellular function but leads to the production of lipid-derived radicals

(Van Breusegem and Dat 2006, Møller et al. 2007). The molecular targets that are most sensitive to the ROS produced by As exposure are not yet clear, although there are many candidates.

These insights provide the facts that As can affect growth and productivity due to excess of morphological, physiological, biochemical, and molecular alterations inside the plants. However, tolerant plant species adapt an array of detoxification strategies, viz., genomic, proteomic, and metabolomic approaches to overcome the As stress. A lot of reviews on As stress in plants reflect the genomic and proteomic modulation in detail. Thus, in the next section we summarize the role of various metabolites modulated during As stress in plants.

5   Metabolome Modulation During Arsenic Stress and Tolerance in Plants

Metabolomics refers to the identification and quantification of all low-molecular-weight metabolites required by the organisms during developmental stages (Arbona et al. 2013), and some metabolites have been reported to be involved under heavy metal stress tolerance strategies. In the following section, we discuss the role of metabolomics under arsenic stress. Despite the fact that transcriptomic approaches provide almost complete coverage and proteomics approaches are now capable of detecting most of the cellular protein complement, metabolomics is currently capable of determining only a small fraction of the metabolites found in any one cell. The more challenging aspect of metabolomic technologies is the refined analysis of quantitative dynamics in biological systems. For metabolomics, gas and liquid chromatographies coupled with mass spectrometry are well suited for analysis of high sample numbers in reliable measurement times with respect to both technical accuracy and the identification and quantification of small-molecular-weight metabolites. However, to the best of our knowledge, a very limited study has been performed to recognize the modulation of differential metabolomic pathway during As stress. Thus, an account about the metabolites involved in antioxidant systems, thiol metabolism, amino acid pool, carbohydrate metabolism, hormonal balance, and others has been particularly given below during As stress (Fig. 2).

5.1   Thiolic Content and Related Enzymes

The central role played by the binding of As(III) to sulfhydryl groups in GSH and PC in the detoxification of the metalloid indicates a critical importance for sulfur metabolism in determining plant survival in As-contaminated soils. The biosynthesis of GSH and PC that is typically induced by As exposure requires adequate supplies of the GSH building blocks glutamic acid (Glu), cysteine (Cys), and glycine (Gly). In both shoots and roots of wild-type *Arabidopsis*, the mass ratios of free Glu/Gly/Cys
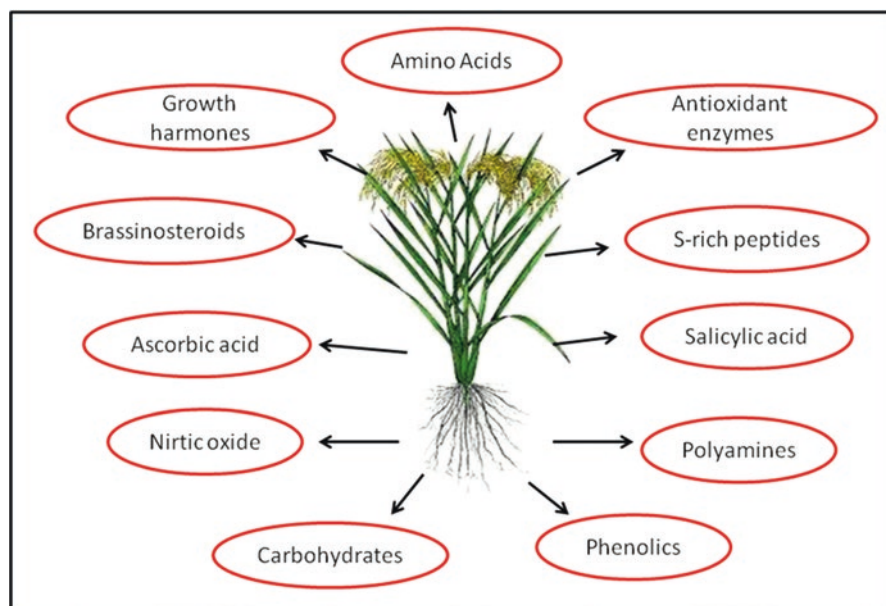
Fig. 2 Different metabolites modulated during As tolerance and detoxification

were about 20:3:1 (Muñoz-Bertomeu et al. 2009). Thus, at least in *Arabidopsis*, Cys is by far the limiting substrate for GSH biosynthesis. However, other studies indicate that As(V) exposure can decrease cellular Cys pools (Sung et al. 2009) and that under some growth conditions, it is possible that the synthesis of PC can deplete GSH pools, decreasing the antioxidant capacity of the cell (De Vos et al. 1992). The Cys content increased in some aquatic plants such as *H. verticillata*, rootless plant *C. demersum*, and crop plant *B. juncea* during As stress (Srivastava et al. 2007, 2009, Mishra et al. 2008, Tripathi et al. 2012a, b). Before sulfate acquired from the soil can be used for the biosynthesis of Cys, and thus the biosynthesis of GSH and PC, it must be reduced via sulfite to sulfide (Takahashi et al. 2011). In a sulfur metabolome study, high-resolution Fourier transform mass spectrometric approach in combination with stable isotope labeling was used in *Arabidopsis thaliana* (Gläser et al. 2014), and about approximately 140 sulfur metabolites that have not been assigned to the databases to date were identified. Further, unraveling of unidentified sulfur metabolite during As stress is of prime importance in plants.

Once sulfate is reduced to sulfide, the sulfide is combined with O-acetylserine to form Cys in a reaction catalyzed by O-acetylserine (thiol)-lyase (OAS-TL), also known as Cys synthase. The O-acetylserine is formed from Ser in a reaction catalyzed by Ser acetyltransferase. OAS-TL exists either as a free active homodimer or in association with Ser acetyltransferase as an inactive subunit of the Cys synthase complex (Takahashi et al. 2011). It appears that As(V) and As(III) exposure may cause a downregulation of OAS-TL in As-sensitive plants (Requejo and Tena 2006, Srivastava et al. 2009). Thus, it seems likely that Cys biosynthesis, and, therefore,

As detoxification through GSH and PC would be compromised. Indeed, the Cys and GSH levels in an As-sensitive variety of *B. juncea* were lower upon As exposure than in the untreated control (Srivastava et al. 2009). In contrast, a *B. juncea* variety with increased tolerance to As showed a more general induction of the sulfate assimilation and GSH biosynthetic pathways (Srivastava et al. 2009), similar to that seen in yeast (Thorsen et al. 2007). A list of As-modulated antioxidants are given in Table 1. In the As-tolerant variety, there was an induction in Cys synthase activity, as well as in the activities of Ser acetyltransferase and γ-ECS, the penultimate enzyme in the biosynthesis of GSH. These increases in enzyme activity were

Table 1 Arsenic affected thiolic enzymes in different plant species

Thiolic enzyme	Plant species	Remarks	References
5′-Adenylylsulfate reductase (APS) activity	*A. thaliana, H. verticillata*	The activity was found to be elevated	Abercrombie et al. (2008), Srivastava and D'Souza (2009)
Serine acetyltransferase (SAT) activity	*H. verticillata, C. demersum, B. juncea, O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)
Cysteine synthase (CS) activity	*H. verticillata, C. demersum, B. juncea, O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)
Glutathione-S-transferase	*H. verticillata, C. demersum, B. juncea, O. sativa*	Increased activity suggested role of GSH in As detoxification	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)
γ-Glutamyl cysteine synthase (γ-ECS) activity	*H. verticillata, C. demersum, B. juncea, A. thaliana, O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), Sung et al. (2009), and Tripathi et al. (2012a, b, 2013a)
Phytochelatin synthase (PCS) activity	*C. demersum, B. juncea, A. thaliana, tobacco, O. sativa*	As exposure increased the activity for more synthesis of PCs	Li et al. (2004), Gasic and Korban (2007), Mishra et al. (2008), Sung et al. (2009), and Tripathi et al. (2012a, b, 2013a)
γ-Glutamyl transpeptidase (γ-GT)	*C. demersum, B. juncea, O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)

accompanied by increased levels of both Cys and GSH, indicating that increased sulfur metabolism may be a viable mechanism for increasing As tolerance in plants. *Arabidopsis* may behave similarly, with transcripts for a γ-ECS, GSH synthetase, and PC synthase being induced during As exposure (Sung et al. 2009).

5.2   Antioxidant Enzymes

Arsenic intraconversion inside the plant may produce oxygen imbalance which causes oxidative damage after production of ROS (Tripathi et al. 2012a, b). To combat oxidative stress, various antioxidant enzymes have been increased their activities in the plants (Mishra et al. 2008, Tripathi et al. 2012a, b). The hyperaccumulator plants can accumulate a significantly higher concentration of As in their bodies, but they have evolved a variety of As tolerance mechanisms against higher levels of free radicals (Khan et al. 2009).

Upon exposure to As, the enzymes involved in the balancing of these free radicals include superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) (Souri et al. 2018). The antioxidative enzymes are generally electron donors and react with ROS to form a neutral and nontoxic end product. Under biotic or abiotic stress, the activation or the suppression of antioxidant enzymes to scavenge ROS takes place in conjugation with each other. Among these antioxidant enzymes, SOD is a key player that constitutes the first line of defense by dismutation of superoxide free radicals (O_2^-) into O_2 and H_2O_2 in plants (Tripathi et al. 2017). Arsenic-induced increase in the activity of SOD can be due to an enhanced level of O_2^- or the direct action of As on SOD gene overproduction.

Souri et al. (2018) observed a significantly higher increase (116%) in SOD activity in *Isatis cappadocica*, which was exposed to 800 μM As under hydroponic conditions. As-induced SOD activities have been observed in many other studies as well (Singh et al. 2006, Srivastava et al. 2007, Mishra et al. 2008, Tiwari and Sarangi 2017, Tripathi et al. 2017).

Catalase scavenges H_2O_2 level in peroxisomes in many stress conditions including As stress in an energy-efficient manner (Srivastava et al. 2017). Arsenic stress may modulate CAT activity depending upon the strength, extent, and kind of stress in various plants (Tripathi et al. 2012a, b, 2017). Accordingly, Souri et al. (2018) also observed that increased activity of CAT was responsible for H_2O_2 detoxification during lower level of SOD in the shoots of *I. cappadocica*.

Ascorbate peroxidase is a key enzyme of ascorbate–glutathione cycle and plays an important role for H_2O_2 detoxification (Singh et al. 2006, Tripathi et al. 2013a, b, 2017). The deviation of APX activity during As stress has been well-documented in ameliorating the harmful effects of H_2O_2 in various plants (Srivastava et al. 2007, Mishra et al. 2008, Souri et al. 2018, Tripathi et al. 2012a, b, 2017).

Glutathione reductase assists plants in the NADPH-dependent reduction of oxidized glutathione to maintain the ratio of GSH/GSSG for proper functioning of

the cells (Srivastava et al. 2009, Begum et al. 2016). The As-induced oxidative damage was partly mitigated by induced activity of GR, in order to sustain the balance of GSH (Souri et al. 2018). Similarly, GR overproduction has been noticed in many other plants under As stress (Mishra et al. 2008, Srivastava et al. 2007, 2009, Begum et al. 2016). It is established fact that As significantly affects the GSH level in plants by converting it into PCs. The higher demand for GSH is managed by higher GSH turnover through enhanced GR activities in As-stressed plants (Mishra et al. 2008).

In general, plants exhibit remarkable difference in their antioxidant potential during As stress (Singh et al. 2006, Srivastava et al. 2007, Mishra et al. 2008, Tiwari and Sarangi 2017, Tripathi et al. 2017). As-hyperaccumulators tolerate As stress by activation of antioxidative defense system in order to maintain lower ROS level, further leading to As detoxification and significant accumulation in tissue (Khan et al. 2009). Arsenic exposure also activates the antioxidant machinery in various plant species, such as *H. verticillata*, *C. demersum*, *B. juncea*, *O. Sativa*, and *Glycine max* L. (Srivastava et al. 2007, Mishra et al. 2008, Bustingorri et al. 2017, Hasanuzzaman et al. 2017).

5.3 Nonenzymatic Antioxidants (Ascorbate, Polyamines, and Phenols)

Ascorbate and dehydroascorbate (low-molecular antioxidants) act as nonenzymatic antioxidants in glutathione–ascorbate cycle for free radical scavenging. They are measured in various As-stressed plants, and increased ratio of AsA/DAsA was noticed in *P. vittata*, *P. ensiformis*, *H. verticillata*, and *O. sativa* (Singh et al. 2006, Srivastava et al. 2011, Tripathi et al. 2012a, b). These insights suggest the prominent role of ascorbate for removal of As stress.

The polyamines are also known to involve in ROS scavenging (Drolet et al. 1986) and the diminution of lipid peroxidation (Borrell et al. 1997) to protect the plant under As stress. Mascher et al. (2002) confirmed that levels of polyamines, viz., spermidine and spermine, are raised merely at lower As exposure; however, diamine is induced at higher doses during As(V) exposure in red clover plants (*Trifolium pratense*). The phenolic content was also showed their variation in rice during As stress (Chauhan et al. 2017). Out of eight, seven phenolic compounds have been identified from roots and shoots of the rice during As–Se interaction. Out of eight, all polyphenols were found to decrease during As stress in shoots and roots except rutin and ferulic acid in roots, which were increased about 25-fold and 87%, respectively. However, quercetin was detected only during As stress in both roots and shoots, while caffeic acid was not detected in shoots during As stress imparting its tissue-specific modulation (Chauhan et al. 2017). Consequently, differential metabolite profiling in plant species denote that plants may modulate their metabolome up to variable degree for As tolerance and toxicity mechanisms.

5.4 Amino Acids

Amino acids, the building block of protein, are known to chelate metals ion for metal tolerance inside the plants. The role of various essential and nonessential amino acids was explored for As tolerance. Dwivedi et al. (2010) analyzed the amino acid profile in grain of various rice genotypes during As stress. They concluded that most of the essential amino acid (EAA) metabolites such as valine, methionine, leucine, and alanine, and nonessential amino acids (NEAAs), viz., histidine, alanine, proline, glutamic acid, and cysteine, increased in most of the rice genotypes during As(V) exposure. Some other amino acids, viz., proline, glutamic acid, aspartic acid, and alanine, were also increased during As(V) stress in *Spinacia oleracea* (Pavlik et al. 2010). Among stress-responsive amino acid, proline is the most studied molecule and can function as an osmolyte and free radical scavenger and also can protect cell membrane damage. The level of proline was also elevated in *O. sativa* during As (III) stress (Mishra and Dubey 2006). The S-containing amino acid, cysteine, plays a central role in As detoxification, used as a primary metabolite for synthesis of GSH and PCs for effective complexation of As. The cysteine content increased in some aquatic plants such as *H. verticillata*, rootless plant *C. demersum*, and crop plants *B. juncea* and *O. sativa* (Srivastava et al. 2007, 2009, Mishra et al. 2008, Tripathi et al. 2012a, b) during As stress.

5.5 Carbohydrates

The carbohydrate metabolism is negatively affected during As stress in plants, and soluble sugars can be accumulated for As tolerance (Gramss 2012). In rice shoots, the relative amount of nonreducing sugars was decreased than reducing sugars during As stress (Jha and Dubey 2004) which imitates suppression of sucrose formation than accessible hexose monophosphate. The possible cause behind this variation is the reduced activities of starch-degrading enzymes, i.e., α- and β-amylase, and starch phosphorylase leading to As-induced plant toxicity (Jha and Dubey 2004). On the contrary, an increase of about 77–120% in starch phosphorylase activity was reported in *O. sativa* and *Phaseolus aureus* seedlings during As exposure, ensuing in a raised level of soluble sugars (Jha and Dubey 2004).

In addition, stimulation of sucrose-hydrolyzing enzymes, viz., acid invertase and sucrose synthase, was studied along with the inhibition of sucrose phosphate synthase activity during As toxicity (Jha and Dubey 2004). Acid invertases are responsible for the sucrose degradation for the formation of glucose and fructose (hexoses), which activity was directly correlated with hexose level (Kaur et al. 2012). The acid invertase and sucrose synthase are the key enzymes for the synthesis of hexoses, which might be shortly oxidized through the glycolytic pathway (Baud and Lepiniec 2009). The metabolic impairments as discussed above will lead to altered plant growth and development under As stress.

5.6   Nitric Oxide

Nitric oxide (NO) is a free radical signaling agent in plants which regulates several cellular functions under normal and stressed situations (Sahay and Gupta 2017). NO is considered as a reactive nitrogen species, and its useful and injurious effects are based upon its level and localization inside plant cells (Sahay and Gupta 2017).

The defensive role of NO against heavy metal stress occurs through unambiguous mechanisms: (i) prevention of Fenton reaction through reaction of NO with OH radicals to form HNO_2, thus defending cells against hazardous OH radical; (ii) avoidance of radical-mediated lipid peroxidation through reaction with lipid radicals; (iii) reaction with superoxide radical to form peroxynitrite ($ONOO^-$), which has lesser toxicity and lifespan than highly toxic H_2O_2; (iv) activation of antioxidants like SOD, CAT, APX, and POX; and (v) working as a signaling molecule for biological reactions leading to variation in gene expression (Saxena and Shekhawat 2013).

As a signaling molecule, NO mediates activation of antioxidants and the biosynthetic pathway of PCs in roots, which facilitate the induced uptake of sulfate by enhanced synthesis of amino acids and nonprotein thiols (Farnese et al. 2013). NO supply improves As tolerance in *Luffa acutangula* through dawdling As absorption and accumulation by increased cell wall thickness of the root epidermis (Singh et al. 2013). Hasanuzzaman and Fujita (2013) demonstrated that the exogenous NO promoted relative water content, chlorophyll pigment, antioxidant enzymes, and glyoxalase activities in As-treated wheat seedling. In *Vicia faba* seedlings, NO supplementation resulted in enhanced seed yield, photosynthetic content, plant hormones, and mineral nutrients to combat As toxicity (Mohamed et al. 2016). The internal concentration of NO was also increased during As(V) stress in *H. verticillata* and *O. sativa* (Srivastava et al. 2011, Tripathi et al. 2012a, b, 2015). Similarly, NO addition considerably lowered root As uptake and its transport to shoots in rice plants during As(III) treatment (Singh et al. 2017) and improved chlorophyll through increased Fe uptake.

Nitric oxide was effectively known to lessen adverse impact on growth and ROS-imposed injuries during As stress in *O. sativa* (Singh et al. 2015), *Vigna radiate* (Ismail 2012), and *Phaseolus vulgaris* L. (Talukdar 2013). NO pretreatment in plant scavenges ROS entirely for improvement of As tolerance (Farmer and Mueller 2013, Sahay and Gupta 2017).

5.7   Salicylic Acid

Salicylic acid (SA) is categorized in the group of phenolics and acts as endogenous phytohormone, which contributes in growth, photosynthesis, flowering, nitrate metabolism, and ethylene production (Vicente and Plasencia 2011). SA imparts its crucial job for signaling of protective act next to the pathogen attack and also responds to other undesirable surroundings to plants (Pandey et al. 2013).

SA also plays signaling role for abiotic as well as toxic metal stress like As (Wani et al. 2017). In hydroponics, SA application was capable for amelioration of As stress through increased internal concentrations of both SA and NO in rice plants during As exposure suggesting SA-mediated modulation of NO, correlated with increased activity of nitrate reductase (Singh et al. 2017). Furthermore, reduced expression of *OsLsi*2 gene during SA and NO interaction was accountable for lesser As uptake.

SA supplementation was reported to induce the level of proline, an antioxidant in plants, thus helpful against As and other metals toxicity (Yang et al. 2009). Similarly, exogenous SA imparts its role in pigment synthesis exhibiting better photosynthesis and dynamic growth in plants (Singh et al. 2017). The SA addition was supportive in extenuating chlorosis by enhancing Fe uptake. In another study, 1 mM SA addition showed induction in chlorophyll and protein content along with reduced lipid peroxidation to combat As toxicity in wheat seedlings (Zengin 2015).

SA also may indirectly involve in antioxidant stimulation through NO induction. Application of SA with As was more effective than SA pretreatment in lowering As uptake in rice. Enhanced level of enzymatic and nonenzymatic antioxidants and thiols plays an important role for As tolerance in plants (Singh et al. 2015). Similarly, Saidi et al. (2017) also reported that SA reduced the As-induced oxidative stress through increased synthesis of APX, CAT, and GPX in seedling of *Helianthus annuus*.

5.8   Growth Hormones and Brassinosteroids

Plant growth hormones are the bioactive molecules which are responsible for plant growth and development. Various studies reported that plant growth hormones have protective role against heavy metals toxicity such as Cu, Cd, Pb, As, etc. Initial research into plant hormones identified five major classes: auxin, cytokinins, gibberellins, ethylene, and abscisic acid (Thomas and Thomas 1979). This list was later expanded and brassinosteroids, jasmonates, and strigolactones are now considered as major plant hormones. An inadequate knowledge is available on the role of growth hormones in As stress amelioration, which needs to be elucidated through detailed analysis in plant.

The role of auxin hormone was identified during As(III) stress in *A. thaliana* (Krishnamurthy and Rathinasabapathi 2013). Auxin transporter mutants aux1, pin1, and pin2 showed its sensitive behavior to As(III) stress compared to the wild type (WT). Inhibitors of auxin transport considerably reduced As(III) tolerance in the WT, whereas addition of indole-3-acetic acid enhanced As(III) tolerance of aux1 but lesser than WT. Uptake assays of labeled H3-IAA exhibited As(III)-modulated auxin transport in WT roots. As(III) enhanced the H_2O_2 levels in WT although not in aux1, signifying a positive role for AUX1 for auxin transport through ROS-mediated signaling (Krishnamurthy and Rathinasabapathi 2013).

The role of cytokinins (CKs) was also investigated in *A. thaliana* for As tolerance (Mohan et al. 2016). CK signaling mutants and transgenic plants with reduced endogenous CK levels demonstrated tolerance to As(V) stress through induced level of thiol compounds and lowered uptake of As indicating regulatory role of CK for As tolerance (Mohan et al. 2016). The protective role of brassinosteroids (Brs) against As toxicity was also investigated in rice plants (Xu et al. 2018). The pooled effect of 24-epibrassinolide (Br24) or 28-homobrassinolide (Br28) on As uptake was studied in rice seedlings. The finding concluded that Br24 and Br28 may possibly hamper As accumulation and may ameliorate the As toxicity (Xu et al. 2018).

Methyl jasmonate (MJ) is also a significant plant growth regulator, concerned with plant protection against abiotic stresses; nevertheless, its potential function against metal stress is not very implicit. The impact of MJ was analyzed on physiological and biochemical parameters of As-treated (200 μM) *Brassica napus* (Farooq et al. 2016) showing considerable raise in leaf chlorophyll fluorescence and biomass than As-stressed plants. The findings revealed that MJ scavenge ROS through improved antioxidant defense system, secondary metabolite, and decreased As accumulation.

6   Conclusion

It is apparent from the above critical discussion that As exposure adversely affects plants at biochemical and molecular levels and influences a majority of physiological responses, such as inhibition in overall growth processes, photosynthetic efficiency, and biomass accumulation. Arsenic can induce oxidative stress via the enhanced production and/or inefficient elimination of ROS and consequently damage biomolecules and interferes with various metabolic pathways, either directly or indirectly, by intervening in activities of certain key enzymes.

Plants adapt several strategies to overcome As stress, such as complexation of As with S-peptides and compartmentalization and induction of stress-responsive amino acids and various enzymatic and nonenzymatic antioxidants (phenolics and ascorbate). Moreover, the signaling metabolites such as NO and SA are typically endowed with As tolerance involving different mechanisms. The fate and recognition of various unidentified sulfur metabolites must be studied during As stress in plants. Our knowledge on the role of growth hormones during As stress is scanty, which must be enriched through in-depth research. Besides, potential research is required to unravel the toxic effects of As on seed germination and plant development through metabolome modulation.

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