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Arsenic Transport, Metabolism and Toxicity in Plants

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Abstract

Arsenic is a toxic metalloid present in large areas in some parts of world including densely populated areas of Bangladesh and West Bengal, India. Being a carcinogenic metalloid, it affects the health of millions of people of affected areas through drinking water and food. Nonetheless, the spread of arsenic contamination reaches to non-affected areas also. Rice is the major crop of the affected areas and is thus the most important carrier of arsenic in grains and in various rice based products throughout the world. Arsenic exists in various inorganic and organic forms with arsenite and arsenate being the major inorganic forms of concern. This global issue has got attraction of a number of studies to understand the details of arsenic uptake, transport, metabolism and toxicity in plants. The transporters responsible for the uptake and root-to-shoot transport of inorganic arsenic have been identified. In addition, transporters responsible for sequestration of arsenic in vacuoles are also discovered. Inside the plants, arsenic induces the production of reactive oxygen species and causes oxidative stress leading to damage to proteins, carbohydrates, lipids and DNA and ultimately cell death. Various antioxidant enzymes and molecules are increased to counteract the oxidative stress. In addition, specific arsenic-binding ligands like phytochelatins are synthesized to chelate and sequester arsenic in vacuoles. This is achieved througth concerted modulation of synthesis and degration of thiols. A number of molecular changes including altered expression of microRNAs and transcription factors take place. The available knowledge about the arsenic metabolism and its toxicity paves the way to tackle the issue. This update discusses not only the present knowledge on this issue but also the lacunae, which need to be filled.

1. Introduction

Arsenic (As) is widely distributed toxic metalloid in the environment due to natural biogeochemical processes and anthropogenic impacts. It has no known physiological function in plants or animals; instead it has been ubiquitously used as poison for long time in human history. There are various forms of As, however, not all of them are not toxic. Inorganic arsenic (Asi) has been classified as a class 1 carcinogen (Leemakers *et al.,* 2006). Heavily As enriched ground water has been reported in many regions of the world affecting health of millions of people due to chronic As poisoning. The situation is at the worst in South and Southeast Asia where As concentrations up to 2000 μg l^{-1} has been reported. The contaminated water is being used for drinking and irrigation purposes (Hossain, 2006). Crops and vegetables growing in As loaded soil, through irrigation water or through other agricultural use of arsenicals, may accumulate significant amount of As causing food chain contamination (Tripathi *et al.,* 2007). Thus, besides drinking water, food chain contamination has been recognized as major source of As intake with rice being the major food source (Meharg and Zhao, 2012). Furthermore, As induced yield loss has recently been recognized as other side of As calamity threatening sustainable food production particularly in the highly populated areas of the world (Brammer and Ravenscroft, 2009; Panaullah *et al.,* 2009). In light of this, understanding the mechanistic details of As uptake, transport and detoxification is crucial to counter the problem of As contamination.

Several studies on As hyperaccumulators and As tolerant plants have provided important leads about As metabolism in plants (Bleeker *et al.,* 2006; Lei *et al.,* 2008; Xie *et al.,* 2009; Karimi *et al.,* 2009; Drava *et al.,* 2012). However, these plants have evolved specific mechanisms to withstand higher levels of As and thus, may not simulate the toxicity mechanism in the plants which are not adapted to high level of As. Therefore, other studies have focused on non-hyperaccumulator and non-tolerant plants including aquatic and crop plants and have shown that As may inhibit plant

metabolism (Srivastava *et al.,* 2011; Dwivedi *et al.,* 2012; Tripathi *et al.,* 2012; Dave *et al.,* 2013) and that too at much lower concentrations than previously thought (Mishra *et al*., 2014). This update presents recent knowledge about As transport, metabolism and toxicity in plants.

2. As Transport, Accumulation and Metabolism

Arsenic exists in various inorganic and organic forms. The prevalent forms include AsIII, AsV, MMA and DMA. The specific distribution patterns of different As species make toxicity differences to different plant organs, cells, and organelles and that might in turn influence plants' ability to take up more As. AsV and AsIII are the two inorganic forms of which AsV is prevalent in aerobic environments, while the AsIII is the predominant form in anaerobic environments (Zhao *et al.,* 2010a). The major organic species include monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAO).

Among the integrated mechanisms of As tolerance/detoxification in plants, a few important ones include low rate of AsV uptake, efflux of accumulated As, and sequestration to non-reactive locations specially vacuole. The execution of these mechanisms and hence the eventual regulation of As accumulation is mainly dependent on the function of several transporters. AsV, being an analog of inorganic phosphate (Pi), gains entry into the plants through phosphate transporters (Wu *et al.,* 2011). This creates a situation of competition that might go in favour of AsV when its concentrations and/or availability are more than that of Pi subsequently leading to Pi deficiency. This is one of the major modes of AsV toxicity to plants. Analysis of the rice and *Arabidopsis* genomes revealed presence of at least 13 and 9 members of the phosphate transporter (Pht1) family in these species, respectively (Mudge *et al.,* 2002; Paszkowski *et al.,* 2002). Shin *et al.* (2004) indicated that Pht1;1 and Pht1;4 mediate a significant proportion of the As(V) uptake in *Arabidopsis*. Modifying the phosphate concentration through external application or through genetic approaches affects the toxicity of AsV (Shin *et al.,* 2004; González *et al.,* 2005; Catarecha *et al.,* 2007; Wu *et al.,* 2011). In the *Arabidopsis pht1–3* mutant, which has a compromised Pi uptake system, As accumulates without causing toxicity (Catarecha *et al.,* 2007) because of slower rate of As accumulation. The root-to-shoot AsV movement occurs through xylem after loading by PHT proteins (Catarecha *et al.,* 2007; Zhao *et al.,* 2010a; Mendoza-Cózatl *et al.,* 2011; Wu *et al.,* 2011). AtPht1;9, a root-expressed transporter, has role in AsV uptake at root-soil interface (Remy *et al.,* 2012). Other transporters like AtPht1;7 and AtPht1;8 have also been found to have roles in AsV uptake and transport (Remy *et al.,* 2012; LeBlanc *et al.,* 2013). In rice, OsPht1;8 plays role in AsV uptake and transport (Wu *et al.,* 2011). In the study of Wu *et al.* (2011), mutation of OsPHF1 (phosphate transporter traffic facilitator 1) and its overexpression had negative and positive impact on Pi uptake and transport but no similar effects were observed with respect to AsV under flooded conditions. In contrast, in hydroponic conditions, PHF1, and also Pht1;8 and PHR2 (phosphate starvation response 2) had similar effects on Pi and AsV uptake and transport. Thus, Pi transporters and associated regulators do affect Pi and AsV uptake but eventual As accumulation in plants is governed by geochemical reaction in soil. Internal cell-to-cell movement of AsV occurs through several classes of phosphate transporters viz., mitochondrial dicarboxylate transporters, which are localized to the inner mitochondrial membrane and responsible for dicarboxylate exchange with co-substrates such as Pi, between the cytosol and the organelle matrix (Palmieri *et al.,* 2008).

Arsenite exists as a neutral molecule [$As(OH)_3$] at the prevailing pH and Eh of the environment and hence, its uptake occurs via aquaglyceroporin channels of nodulin 26-like intrinsic protein (NIP) family, which can also transport other neutral molecules viz., silicic acid and boric acid (Isayenkov and Maathuis, 2008). Arsenite uptake in plants occurs through aquaporin family of major intrinsic proteins. Out of four different classes of MIPs, two have been reported to mediate AsIII transport. The major ones are members of nodulin26-like intrinsic proteins (NIPs, Meharg and Jardine, 2003; Bienert *et al.,* 2008; Isayenkov and Maathuis, 2008; Ma *et al.,* 2008). Bienert *et al.* (2008) showed that the OsNIP2;1 and OsNIP3;2 from rice are bi-directional As(III) channels. In rice, the OsNIP2;1/OsLsi1 silicon transporter has been implicated as the major AsIII uptake protein, while AsIII efflux from rice root cells to the xylem is through the OsLsi2 silicon transporter (Ma *et al.,* 2008). OsNIP3;3 has also been found to be involved in AsIII transport in yeast (Katsuhara *et al.,* 2014). Mosa *et al.* (2012) suggested a role of plasma membrane intrinsic proteins (PIPs) in AsIII bi-directional transport in rice. They cloned five PIPs of rice, OsPIP1;2, OsPIP1;3, OsPIP2;4, OsPIP2;6, and OsPIP2;7. Out of these, overexpression of OsPIP2;4, OsPIP2;6, and OsPIP2;7 in *Arabidopsis* increased AsIII tolerance and biomass, while these plants did not show significant As accumulation in long duration treatments. In short duration exposures, both active influx and

efflux of AsIII was observed. Tiwari *et al.* (2014) have demonstrated an involvement of NRAMP1 (Natural Resistance-Associated Macrophage Protein 1) in AsIII transport in rice. OsNRAMP1 was found to reside on plasma membrane of endodermis and pericycle cells and was proposed to mediate the xylem loading of As. Recently, Lim *et al.* (2014) identified a heavy metal induced RING E3 ligase 1 (OsHIR1) gene from rice, which was significantly upregulated with As and Cd treatments. Authors showed that HIR1 interacts with tonoplast intrinsic protein 4;1 (OsTIP4;1) in the plasma membrane and degrades the TIP4;1 protein via the ubiquitin 26S proteasome system. Heterogeneous overexpression of OsHIR1 in *Arabidopsis* exhibited As- and Cd-insensitive phenotypes and resulted in decreased As and Cd accumulation in the shoots and roots, relative to the control.

Inside the plant, most of As, if it is taken up as AsV, is rapidly transformed into AsIII and gets transported as AsIII through the xylem to the fronds (Pickering *et al.,* 2006; Su *et al.,* 2008). In the fronds, AsIII is sequestered as free AsIII in the vacuole (Pickering *et al.,* 2006). It has been shown that PvACR3 is involved in the vacuolar sequestration of AsIII (Indriolo *et al.,* 2010). Duan *et al.* (2012) introduced ScACR3 into rice and observed that in the transgenic lines, As concentrations in both shoots and roots were about 30% lower than in the wild type, while the As translocation factors were similar between transgenic lines and the wild type. The roots of transgenic plants exhibited significantly higher As efflux activities than those of the wild type. Importantly, ScACR3 expression significantly reduced As accumulation in rice straws and grains. When grown in flooded soil irrigated with As(III)-containing water, the As concentration in husk and brown rice of the transgenic lines was reduced by 30 and 20%, respectively, compared with the wild type. Chen *et al.* (2013) utilized transgenic approach and expressed PvACR3 in *Arabidopsis* and found that PvACR3 localized to the plasma membrane in *Arabidopsis* and increased AsIII efflux into external medium in short-term experiments. In long duration experiments, PvACR3 substantially reduced As concentrations in roots and simultaneously increased shoot As. LeBlanc *et al.* (2013) *Arabidopsis thaliana* plants overexpressing the high-affinity Pi transporter family members, AtPht1;1 or AtPht1;7, were found to be hypersensitive to AsV due to increased AsV uptake. However, co-overexpression of the yeast ABC transporter YCF1 in combination with AtPht1;1 or AtPht1;7 suppressed the AsV-sensitive phenotype while further enhancing AsV uptake (LeBlanc *et al.,* 2013). Thus, positive influence of vacuolar sequestration further increases plants' ability to take up more As as is the case with hyperaccumulators.

In case of angiosperms, ACR3 homologs are missing and transport of uncomplexed free AsIII/AsV may possibly be mediated by some other transporters. For example, TIP4;1 as discussed above. Nonetheless, the transporters responsible for mediating the transport of phytochelatin complexed AsIII has been found. These are the members of ABC transporter family, ABCC1 and ABCC2 (Song *et al.,* 2010). They demonstrated that in the absence of two members of a subclass of ATP binding cassette (ABC) transporters, ABCC1 and ABCC2, *A. thaliana* becomes extremely sensitive to As and As-based herbicides. PC-producing *Saccharomyces cerevisiae* heterologously expressing these two ABCC transporters (*AtABCC1* and *AtABCC2*) showed enhanced As tolerance and accumulation and their membrane vesicles exhibited a pronounced [As(III)]–PC_2 transport activity. Vacuoles isolated from *atabcc1 atabcc2* double knockout plants exhibited a very low residual As(III)–PC_2 transport activity. In rice also, *OsABCC1* has been demonstrated to be involved in As transport to vacuole of phloem companion cells of nodes and hence to regulate As accumulation in grains (Song *et al.,* 2014). AsThe efflux of As from the roots to the medium is demonstrated in plants like *Lycopersicon esculentum*, *Azolla* spp. and *H. lanatus* (Zhang *et al.,* 2008; Logoteta *et al.,* 2009). Zhao *et al.* (2010b) found that rice silicon transporter, Lsi1 (Low silicon rice 1; OsNIP2;1), which is an aquaporin and a major route for AsIII entry into rice roots, is also responsible for the efflux of As in the form of AsIII.

The complexation of As in the form of AsIII with S-containing ligands, glutathione (GSH) and phytochelatins (PCs) is an important mechanism which does contribute towards As detoxification in all plants studied to date although to a different extent in nonhyperaccumulator, e.g. *Helianthus annuus,* and *H. verticillata* (Raab *et al.,* 2005; Srivastava *et al.,* 2007), hyperaccumulator (Raab *et al.,* 2004) and hypertolerant (Karimi *et al.,* 2009) plants. The process of As complexation not only leads to immediate detoxification of As (in terms of loss of reactivity in chelated form) but also affects the translocation of As either from root to shoot or from root to the medium (Liu *et al.,* 2010). Duan *et al.* (2011) investigated whether PCs influence As accumulation in rice grain by using six rice cultivars varying in grain As accumulation. They did found that shoot PCs concentration correlated negatively with grain As accumulation. Further, when leaves were sprayed with buthionine-sulphoxime (BSO)

at grain filling stage, GSH and PC levels as well as As concentration in shoots declined, while As concentrations in husk and brown rice increased significantly. Thus, PC complexation of AsIII in rice leaves also reduces As translocation from leaves to grains. In an transgenic approach too, when Shri *et al.* (2014) heterologously expressed phytochelatin synthase gene from *Ceratophyllum demersum* (*CdPCS1*) in rice, it resulted in significant increase in As in root and shoot but a significant decline occurred in grains and husk. Chen *et al.* (2015) demonstrated that nodes in rice act as filters and restrict As from getting transported to the grains and they further indicated that PCs play important role in this function of nodes of As storage. Very recently, a different class of transporters responsible for inositol uptake in phloem was found to transport As also in *Arabidopsis* (Duan *et al.,* 2016). The disruption of *AtINT2* or *AtINT4* was found to decrease As concentration in phloem, silique as well as seeds in *A. thaliana* which was attributable to reduced phloem loading of As.

In addition to inorganic As species, various organic methylated As species have also been detected in plants and it was initially suggested that plants may methylate As (Raab *et al.,* 2005, 2007). However, further studies could not find methylated forms of As in several plant species grown under axenic conditions (Jia *et al.,* 2012; Lomax *et al.,* 2012). Hence, the authors suggested that methylated derivatives of As were likely taken up by the plants from soil where they are formed due to microbial reactions (Lomax *et al.,* 2012). The protonated, uncharged forms of the methylated As species, MMAV and DMAV enter rice roots through the aquaporin channel OsLsi1 (Li *et al.,* 2009). Li *et al.* (2009) showed that the rice lsi1 mutant defective in the silicon/arsenite transporter Lsi1 lost about 80% and 50% of the uptake capacity for MMA(V) and DMA(V), respectively, compared with the wild-type rice. Silicic acid and arsenite have a high pKa (9.2) and, therefore, are present mostly as neutral molecules under neutral or acidic conditions, MMA and DMA have relatively low pKas (4.2 and 6.1, respectively). As a result, MMA and DMA will dissociate significantly in the acidic to neutral pH range. Because aquaporin channels are permeable only to uncharged molecules, pH can influence the uptake of MMA and DMA by shifting the equilibrium between protonation and dissociation (Li *et al.,* 2009). Although, the rate of uptake for MMAV and DMAV is much slower than that of AsIII or AsV (Abedin *et al.,* 2002; Raab *et al.,* 2007; Abbas and Meharg, 2008) and uptake rate decreased with increasing number of the methyl groups (Raab *et al.,* 2007; Jia *et al.,* 2012), their mobility within the plant is substantially more than that of AsIII or AsV (Raab *et al.,* 2007; Li *et al.,* 2009; Carey *et al.,* 2010, 2011). Zhao *et al.* (2012) confirmed that Asi has a relatively low mobility by using radioactive 73As tracer. Therefore, Asi accumulates to a greater extent in the vegetative tissues which have higher transpiration rate. DMA can be transported by the xylem (Ye *et al*., 2010), which was demonstrated by the observation of DMA in guttations which are from the xylem sap (Yamaji *et al*., 2008). Furthermore, DMA is extremely efficiently translocated to rice grain through phloem from the flag leaves to the rice grain (Carey *et al*., 2010, 2011). The transporters responsible for the efflux of MMAV and DMAV are still unknown.

Owing to differential efficiencies for uptake and translocation of Asi and organic species, the relative proportions of Asi and DMA in rice grain vary widely. Norton *et al.* (2009) showed 4–5 fold variations in grain As concentration among 76 rice cultivars grown in two paddy fields in Bangladesh, and there was significant correlation between two different As contamination sites among the common genotypes. In addition to total grain As concentration, there was significant genotypic difference of As speciation in rice grain (Liu *et al.,* 2006; Li *et al.,* 2009; Norton *et al.,* 2009). The proportion of DMA ranges from 10% to 90 %, depending on the rice cultivars (Norton *et al*., 2009), the soil environments in which rice was grown (Williams *et al*., 2006; Zavala *et al*., 2008; Meharg *et al*., 2009) and water management (Xu *et al*., 2008; Arao *et al*., 2009; Li *et al*., 2009). Different studies by Williams *et al.* (2005), Zavala *et al.* (2008) and Zhu *et al.* (2008) have analyzed the As speciation in rice samples from Bangladesh, India, China, Europe and USA and they all have reported a higher percentage of iAs (~75-80%) in Bangladeshi, Indian and Chinese rice. In comparison, European and US rice had lower percentages of iAs and corresponding high percentages of DMA. Norton *et al.* (2009) grew a common panel of rice cultivars at six field sites in Bangladesh, India and China and analyzed As speciation in the grain. They noticed significant effects of site, genotype and genotype x site interactions on As speciation and importantly the effect of site outweighed that of genotype substantially. Pillai *et al.* (2010) grew ten rice cultivars belonging to the *indica* or *japonica* subtype and originating from the US or Asia in a paddy field in Arkensas, USA. They found high percentages of DMA in the grain from all cultivars (48-77%, mean 63%). Hence, environmental and site specific factors appear to have more strong influence on the percentage of inorganic and organic As in rice grains compared to the genotype. The uptake and transport efficiencies also

vary depending on the age and growing stage of plants due to variable need of plants for nutrients during different life stages and hence owing to variations in transporters expressions. Arao *et al.* (2009) found that an aerobic treatment for 3 weeks before and after heading was most effective for reducing the As concentration in grain. Lomax *et al.* (2012) also noticed that exposure to As (Asi and DMA) for 1 week at flowering stage caused more As accumulation in rice plant as compared to that during tillering, stem extension and heading stages. Inorganic As, especially AsIII, shows a strong accumulation in the ovular vascular traces, which are located on the surface of the grain and are the conducting tissues that transport water and minerals into the grain. In contrast, DMA permeates readily into the endosperm (Carey *et al.,* 2010, 2011; Zheng *et al.,* 2013).

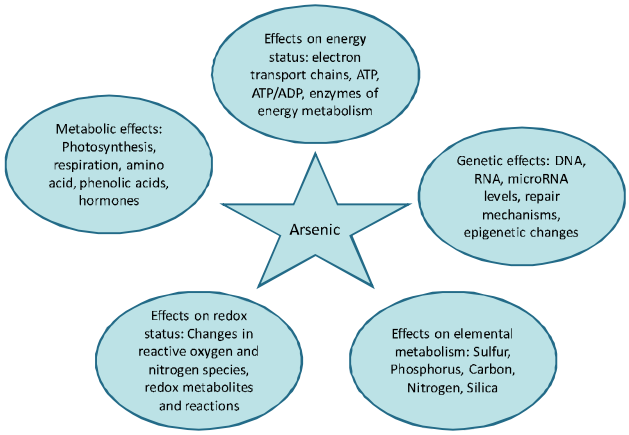

Fig. 1: A summary of effects of arsenic on plants at various levels

3. Arsenic Toxicity Mechanism

In the last decade, numerous morphological, physiological and biochemical analyses have been conducted to investigate the responsive behavior of rice to arsenic (Abedin and Meharg, 2002; Ahsan *et al*., 2008; Azad *et al*., 2009; Shri *et al*., 2009) (Fig. 1). Excess As can inhibit seed germination (Abedin and Meharg, 2002; Shri *et al*., 2009) and early seedling growth (Srivastava *et al.,* 2013a), interfere with photosynthesis (Srivastava *et al.,* 2013b; Mishra *et al.,* 2014), suppress growth, modulate nutrient and amino acid profile of plants and grains (Dwivedi *et al.,* 2010, 2012; Pathare *et al.,* 2013), modify hormonal balance of plants (Srivastava *et al.,* 2013c), impact carbon, nitrogen and sulfur metabolisms (Singh *et al.,* 2009; Finnegan and Chen, 2012; Pathare *et al.,* 2013) and eventually reduce grain yield in rice. By using infrared (FTIR) and near infrared (FTNIR) spectroscopy, Boccia *et al.* (2013) demonstrated molecular modifications predominantly associated with chemical interactions of iAs with biomolecules such as nucleic acids, carbohydrates, lipids, and proteins in *Vicia faba*. The modified shapes of the intramolecular hydrogen bond suggested changes in the secondary protein structure of *Vicia faba* involving the α helix to β secondary (i.e., sheet and turn) structure ratios. The effects of As on the anatomy of plants, including ultrastructural changes in chloroplasts of *Pteris vittata* leaves, an As hyperaccumulator species (Li *et al.,* 2006); and changes in mesophyll cells of the cortex root and cellular differentiation of vessel elements of *Phaseolus aureus* (Singh *et al.,* 2007) have been described. Recently, in *Leucaena leucocephala* (Scheneider *et al.,* 2013), it was observed that with increasing concentration of As, the amount of starch grains and amyloplasts to decreased and the amount of lipids increased. There was reduction in intracellular spaces, degradation of organelles and vesicles and cortical and epidermal structures were collapsed due to the loss of turgor. A large number of genetic modifications (Abercombie *et al.,* 2008, Norton *et al.,* 2008; Chakraborti *et al.,* 2009; Yu *et al.,* 2012; Sharma *et al*., 2015) and protein profile modulations (Requejo and Tena, 2005, 2006; Ahsan *et al.,* 2008, 2010; Liu *et al.,* 2013) have also come into with the advancement in As related studies. Zheng *et al.* (2013) reported DMA induced abnormal florets before flowering and a sharp decline in the seed setting rate after flowering compared to Asi in rice. It was found that DMA tended to accumulate in caryopsis and induced higher toxicity to the reproductive tissues resulting in markedly reduced grain yield, whereas Asi mainly remained in the vegetative tissues and had no significant effect on yield.

Arsenic toxicity to plants is governed by its concentration in the medium, its eventual accumulation in different tissues and cellular compartments and also by its speciation chemistry. A lot of research has been conducted to understand the As toxicity mechanisms to date and consequently, three important scenarios have emerged, which most likely play a cumulative role in regulating As toxicity. These three scenarios include (1) induced production of reactive oxygen species (ROS) leading to oxidative stress, (2) reduced photosynthetic efficiency and (3) direct interaction of As species with metabolites and biomolecules including proteins, carbohydrates, lipids and nucleic acid. Out of these three major scenarios, the matter of debate is that which is the major player, and more importantly, which is the primary one. But it should be very clear that each can influence the other one and therefore the need is to

understand the cumulative nature of the progression of these mechanisms during the course of As build up in plants so as to know about the process of As induced reduction in germination and early seedling growth, inhibition of root and shoot growth, delaying of flowering and seed setting, reduction of grain yield and death of plants.

There are differences in toxicity of various inorganic and organic forms of As due to mode of action. So, plants show variable order of toxicity of different species (Finnegan and Chen, 2012). It is however considered that methylated forms of As can disrupt plant metabolism more strongly even at lower concentrations than inorganic forms. Finnegan and Chen (2012) further pointed that inconsistency of phytotoxicity results may also be attributed to the fact that complete information of As species in the plants is still not known. For example, in beetroot, up to 75% of the As could not be extracted As from tissues with high As accumulation (Száková *et al.,* 2010).

AsV and Pi are chemically analogous and this causes both of them to compete for the same binding site and biochemical reactions when AsV gains entry in plants. Since, Pi is an important component of a number of biochemical reactions through ATP and biomolecules like RNA and DNA, replacement of Pi with AsV disrupts vital cellular processes. The most important reaction requiring Pi is the phosphorylation of ADP to ATP catalyzed by the ATP synthases of respiratory and photosynthetic ETC. It was found that mitochondrial enzyme can use AsV instead of Pi and produce ADP-AsV (Gresser, 1981). The AsV-esters are highly unstable in water and undergo spontaneous and rapid hydrolysis (Rosen *et al.,* 2011; Tawfik and Viola, 2011). The unstable nature of the AsV-esters hence creates futile reaction cycles that uncouple respiratory and photosynthetic ETC (Finnegan and Chen, 2012). Other important enzymes are also reported to be able to use AsV instead of Pi viz., GAPDH (Orsit and Cleland, 1972), aspartate-β-semialdehyde dehydrogenase (Kish and Viola, 1999) and PNP (Park and Agrawal, 1972).

AsIII and also its organic derivaties, MMAIII and DMAIII are highly reactive toward thiol groups and can bind up to three sulfhydryl groups. Therefore, AsIII binds to thiol containing GSH and its polymers, PCs, which is in fact the mode of its detoxification. AsIII also binds with thiol groups of proteins and cofactors and either reduces or inhibits their activity. Dihydrolipoamide, which in plants is a co-factor associated the mitochondrial and plastid pyruvate dehydrogenase complexes (mtPDC, ptPDC), the 2-oxoglutarate dehydrogenase complex (OGDC), the Gly decarboxylase complex (GDC) and the branched-chain 2-oxoacid decarboxylase complex (BCOADC), has been recognized as an important cellular target for AsIII binding (Bergquist *et al.,* 2009). The stability of AsIII-monothiol, AsIII-dithiol and AsIII-trithiol complexes is about 1–2 s, 1.3 min and 155 min, respectively (Kitchin and Wallace, 2006b). Hence, AsIII preferentially binds with proteins having three or more cysteine residues viz., zinc-finger proteins (Zhou *et al.,* 2011). Further, binding stability of AsIII is dependent on the proximity of cysteine residues to one other and the optimal spacing is CX0–14C (Kitchin and Wallace, 2006a) for dithiols. A large number of proteins in *Arabidopsis* are predicted to have cysteine residues in optimal spacing for dithiol or trithiol AsIII binding (Kitchin and Wallace, 2006a; Finnegan and Chen, 2012).

One of the most common observations and the mostly supported mechanism of As toxicity has been the induced production of ROS, including superoxide (O2−), the hydroxyl radical (OH), and H_2O_2 (Mylona *et al.,* 1998; Requejo and Tena, 2005; Srivastava *et al.,* 2007; Ahsan *et al.,* 2008; Srivastava *et al.,* 2011). ROS can damage proteins, amino acids, purine nucleotides and nucleic acids and cause peroxidation of membrane lipids (Møller *et al.,* 2007). As induced lipid peroxidation (Srivastava *et al.,* 2007; Mishra *et al.,* 2008), carbonyl production due to protein damage and DNA damage (Srivastava *et al.,* 2011) have been observed. These changes ultimately lead to increased cell death (Srivastava *et al.,* 2011). Hence, the prime mechanism of As toxicity has been proposed to be the disturbed redox state of the plants and it was suggested that magnitude of redox disturbance is positively correlated to the extent of damage (Srivastava *et al.,* 2011). Nonetheless, there is still lack of knowledge regarding the mechanisms of As-induced ROS production. It has been proposed that As detoxification processes, including the reduction of AsV to AsIII (Mylona *et al.,* 1998) and the induction of PC synthesis and consequent shortage of GSH for normal cellular functioning (Mylona *et al.,* 1998; Srivastava *et al.,* 2007) are important paths for induced ROS production. Another way can be indirect pathways viz., through negative impact on photosynthetic efficiency (Mishra *et al.,* 2014) and due to decreased activities of antioxidant enzymes like superoxide dismutase (SOD) and increase in the activity of prooxidant enzymes viz., NADPH oxidase and ascorbate oxidase (Srivastava *et al.,* 2011) and glycolate oxidase (Gupta *et al.,* 2013). The shift in redox state happens at two levels (Foyer and Noctor, 2011). Superoxide and the hydroxyl radical can directly oxidize both GSH and ascorbate. Alternatively, H_2O_2 can oxidize GSH and ascorbate through the action of specific peroxidases, or in the case of GSH, also through the action GRXs and GSH-S-transferases (GST). The reliance

of the ascorbate-GSH cycle on the diversion of carbon to ascorbate biosynthesis, plus the diversion of carbon, nitrogen, and sulfur in the form of Glu, Cys, and Gly to support the biosynthesis of GSH and PC, requires a remodeling of metabolism to focus on the production of the precursors for these compounds (Srivastava and D'Souza, 2009). Pathare *et al.* (2013) made an attempt to evaluate the impact of As on carbon, nitrogen and sulfur metabolism to understand how they are balanced and remodeled to tackle the As load by comparing contrasting varieties of *Brassica juncea* and found that large changes occur in these metabolisms in varietal and time-dependent manner.

In studies of Srivastava *et al.* (2011, 2013a) disturbance to energetic equilibrium has been proposed as another important mechanism of As toxicity plants in combination with changes in redox equilibrium. The As induced negative effects in *Hydrilla verticillata* could be linked to an altered energetic and redox equilibrium [analyzed in terms of ATP/ADP, NADH/NAD, NADPH/NADP, reduced glutathione/oxidized glutathione, and ascorbate/dehydroascobate ratios] (Srivastava *et al.,* 2011). Authors calculated redox potentials of important redox couples and found that ASC/DHA and GSH/GSSG demonstrated significant decline in comparison to control and hence their redox potentials shifted towards significantly oxidizing directions (ca. 30 to 54 mV for ASC/DHA and −298 to −285 mV for GSH/GSSG). In contrast, the ratio of NADPH/NADP increased due to the accumulation of NADPH (redox potential of NADPH/NADP shifting to reducing side: −287 to −318 mV), which indicated lack of coordination among three redox couples was disturbed. The generation and consumption of energy molecules is one of the most important criteria in the regulation of whole cellular functioning (Dobrota, 2006). Authors found that the ratio of NADH/NAD and ATP/ADP decreased significantly due to the utilization of NADH and ATP. The consumption of NADH probably occurred for the synthesis of ATP in respiratory ETC and that of ATP for various cellular processes to allow cells to tolerate the As stress implied. Photosynthetic dark cycle needs an input of ATP and NADPH from photosynthetic ETC. However, if NADPH gets accumulated, it would lead to an over-reduction of the ETC, misdirection of electrons to oxygen and hence an increased generation of ROS. Srivastava *et al.* (2013a) further studied the response of adenine and pyridine metabolism during germination and early seedling growth (ESG) of *Brassica juncea*. The study found that the activity of enzymes of NAD metabolism, viz. NAD kinase, NADP phosphatase, nicotinamidase and poly(ADP-ribose) polymerases as well as that of adenine metabolism like adenylate kinase and apyrase was significantly altered which affected the effective mobilization of NAD during germination and ESG and disturbed the equilibrium of NAD to NADP and ATP to ADP. A very important observation was significant improvement in germination percentage and germination strength of the seeds supplied with 1 mM ATP along with As. The authors proposed that redox and energetic equilibrium act as prime determinant of As toxicity to plants. However, it is still intriguing whether changes in ATP levels were due to As impact on the associated metabolic enzymes or due to Pi replacement with AsV or due to primarily negative impact on the photosynthetic efficiency.

A recent report by Mishra *et al.* (2014) suggested that effects of As on photosynthetic efficiency act as prime reason of As toxicity ahead of earlier supposed oxidative stress events. They suggested that with the increase in intracellular weakly bound As, a decline in photosynthetic pigments was noticed, leading to moderate growth inhibition through decreased light harvesting. However, the core photosynthesis (e-transport and PSIIRC) were affected only when the level of As crossed a specific threshold. The reduced photosynthetic performance then led to enhanced generation of superoxide and eventually the appearance of severe toxic symptoms. Liu *et al.* (2013) conducted proteomic analysis of rice shoots after exposure to AsV and found a total of 38 differentially displayed proteins. The important proteins related to photosynthesis were ribose-5-phosphate isomerase type A subfamily, triosephosphate isomerase, the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, chloroplastic aldolase; oxygen-evolving complex protein 1, the adenosine triphosphate (ATP) synthase CF1 alpha subunit, the ATPase a subunit, and the chloroplast 23 kDa polypeptide of photosystem II. They also observed deterioration and distortion on grana thylakoid structure upon As exposure.

4. Future Directions

As research has advanced greatly in the recent past however a lot more information is yet to be revealed. One important lack is the understanding of dynamics of As toxicology and detoxification. It still needs to unravel how toxicity initiates and how it proceeds. Further, how plant system balances its metabolism to cope up with incoming As requires to be delineated in dynamic manner. Recently novel transporters for As uptake and transport have been discovered, and it appears very likely that other transporters might be present in plants. Molecular tools using these efflux transporters involved in xylem and phloem loading of As may lead to development of plants having enhanced phytoremediation potential on one hand and reduced

grain arsenic load on the other hand. It therefore demands more studies to understand dynamic nature of As accumulation, toxicology, and detoxification.

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