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Arsenic accumulation in rice (*Oryza sativa* L.) is influenced by environment and genetic factors

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ABSTRACT

Arsenic (As) elevation in soil will have a negative impact on both the yield and grain quality of rice (*Oryza sativa* L.). The mechanistic understanding of As uptake, translocation, and grain filling is an important aspect to produce rice grains with low As concentrations through agronomical, physico-chemical, and breeding approaches. In the paddy soil-water system, a range of physico-chemical, biological, and environmental factors governs the speciation of As and their concentrations. Major As transporters such as phosphate and aquaglyceroporins assimilate both inorganic (As(III) and As(V)) and organic As (DMA(V) and MMA(V)) species from the soil solution. A number of metabolic pathways (i.e., As (V) reduction, As(III) efflux, and As(III)-thiol complexation and sequestration) are likely to play a key role in determining the translocation and substantial accumulation of As species. The order of translocation efficiency (caryopsis-to-root) for As species in rice plants is as follows: DMA(V) > MMA(V) > inorganic As species. The pattern of inorganic and organic As species loading into the grain is largely determined by the genetic makeup of the rice plants, maturity stage, and its environmental interaction. Therefore, the knowledge of As metabolism in rice plants and how it is affected by plant genetics, the environment, and its interactions would pave the way to developing adaptive strategies to mitigate the accumulation of As in rice grains.

**Keywords:** Arsenic speciation, Arsenic transporters, Arsenic metabolism, Detoxification, Grain filling, Arsenic toxicity
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1. Introduction

Rice (*Oryza sativa*) is the dominant dietary exposure route to arsenic (As), a non-threshold carcinogen. Rice grains can contain approximately 10-times as much as the baseline total of As when compared to other cereal grains (Williams et al., 2007). Rice and rice-based products can lead to an intake of excessive amount of inorganic As, particularly, in the populations in South and South-East Asia (Kile et al., 2007; Meharg et al., 2009). Rice is the primary source of As in a non-seafood diet in Europe and the United States (Fu et al., 2011). Ohno et al. (2007) have demonstrated that rice contributed 56% of the total As intake; the corresponding figure for drinking water, solid food, liquid food, and cereals were 13, 11, 4.4, and 16%, respectively. Contrasting results have also been reported elsewhere suggesting that drinking water has contributed to 93.5% of total As uptake, and rice and vegetables have only contributed to 6.2% and 0.3% of total As intake, respectively, in a groundwater contaminated Bangladeshi setting (Rahman et al., 2013).

Arsenic in the geological environment is mainly associated with sulfur (S) in minerals such as arsenopyrites (FeAsS), realgar (As4S4), and orpiment (As2S3) (Majzlan et al., 2014). Natural processes such as rock weathering, alluvial deposits may contribute As release into the paddy environment (Bundschuh and Maity, 2015; Herath et al., 2016). Anthropogenic activities (i.e., mining and use of As-contaminated groundwater in the form of irrigation water) promote the accumulation of natural As in paddy ecosystems. Moreover, As-containing insecticides, herbicides, feed additives, and wood preservatives are the potential anthropogenic sources of As in rice ecosystems (Chen et al., 2016b; Zhao et al., 2009).

Natural soils typically contain 0.1 to 10 mg kg⁻¹ of total As (Zhao et al., 2010a). European Community (EU) recommends that soils to be used for agricultural purposes should be
contained less than 20 mg kg$^{-1}$ of total As (Bhattacharya et al., 2009; Shrivastava et al., 2017). Arsenite (As(III)) and arsenate (As(V)) are the most commonly found inorganic As species whereas monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) are the frequently reported organic As species in paddy soil-water systems (Honma et al., 2016; Jia et al., 2012). Both inorganic and organic As species are acquired by rice roots through various nutrient assimilatory pathways and they are translocated via a variety of mechanisms (Ma et al., 2008; Wang et al., 2016b).

Attention is required to understand how rice plants take up and metabolize As species so as to develop mitigation measures against this global contamination in the food chain. Taking these demands into account, this review presents recent progress in As dynamics in the rice ecosystem, and its uptake by rice roots and translocation to the rice grains. Further, mechanisms (i.e., metabolism/detoxification) that have evolved to mitigate the accumulation of As in rice tissues are discussed highlighting the major knowledge gaps that need to be addressed in future research.

2. Arsenic phytoavailability in paddy soil-water system

Paddy soils under conventional paddy management practices are subjected to flooded and non-flooded conditions, during initial and final stages of the growth of rice, respectively. The changes in water management regimes may alter redox potential (Eh) of the paddy soil-water systems (Pan et al., 2014). During flooded conditions, water replaces the gaseous phase in the soil matrix. A number of redox reactions (from high Eh ($\sim +700$ mV) to low Eh ($\sim -300$ mV): reduction of Mn(IV), Fe(III), and SO$_4^{2-}$, and methanogenesis) take place either sequentially or simultaneously during this phase (Sahrawat, 2015). Arsenate in the soil matrix is converted to As(III), which is highly mobile than As(V), when Eh turns more negative. The
As(III)/As(V) ratio is high in the soil solution during the flooded conditions and reverses for non-flooded conditions (Yamaguchi et al., 2014).

Iron (III) plaque, consisting of amorphous or crystalline Fe hydro(oxides) (FeOOH), has found to have a significant effect on the sequestration of both As(V) and As(III) (Eq. 1 and Eq. 2) (Liu et al., 2004). A higher ratio of As/Fe in Fe(III) plaque around rice roots compared to that of Fe minerals in soil matrix indicates the high capacity of Fe(III) plaque for the retention of As in the rhizosphere (Yamaguchi et al., 2014). Radial oxygen loss (ROL), the process that diffuses O₂ into the rhizosphere through root aerenchyma, promotes the formation of Fe(III) plaque under flooded conditions (Mei et al., 2009). Root anatomy (i.e., root porosity) in rice genotypes strongly correlates with ROL and substantial formation of Fe(III) plaque (Mei et al., 2009; Wu et al., 2011a). In addition, growth stage of rice plants and background Fe concentration in paddy soils have an effect on the quantity of Fe(III) plaque in rice roots (Li et al., 2015). Therefore, As retention by Fe(III) plaque and subsequent accumulation of As in plant tissues (i.e., root, shoot, husk, and grain) of different rice genotypes are remarkably varied (Lee et al., 2013; Liu et al., 2006).

\[
\text{FeOH}^- + \text{AsO}_3^{3-} + 3\text{H}^+ \rightarrow \text{FeH}_2\text{AsO}_4^{2-} + \text{H}_2\text{O} \\
\text{FeOH}^- + \text{AsO}_3^{3-} + 3\text{H}^+ \rightarrow \text{FeH}_2\text{AsO}_3^{2+} + \text{H}_2\text{O} \quad \text{(Eq. 1)}
\]

\[
\text{FeOH}^- + \text{AsO}_3^{3-} + 3\text{H}^+ \rightarrow \text{FeH}_2\text{AsO}_3^{2+} + \text{H}_2\text{O} \quad \text{(Eq. 2)}
\]

Paddy soil consists of indigenous FeOOH including: ferrihydrite (Fe₅HO₈.4H₂O), goethite (α-FeOOH), lepidocrocite (γ-FeOOH), and hematite (α-F₂O₃). Iron redox cycling (Fe(II) oxidation and Fe(III) reduction) has a significant impact on the mobility and bioavailability of As in paddy ecosystems (Yu et al., 2016b). Iron-reducing bacteria can convert Fe(III) to
Fe(II) (Eq. 3) through two different pathways; dissimilatory reduction and assimilation reduction (Das et al., 2016; Qiao et al., 2017).

\[
FeOAs(OH)_{2(s)} \rightarrow Fe^{2+}_{(aq)} + AsO_{3(aq)}^- + 2H^+_{(aq)} \quad \text{(Eq. 3)}
\]

In contrast, Fe(II)-oxidizing bacteria may re-oxidize Fe(II) to Fe(III), particularly, under non-flooded conditions. Since FeOOH has a strong affinity with both As(V) and As(III), it significantly reduces the mobility and bioavailability of As in paddy soil-water, and the subsequent uptake and accumulation of As in rice tissues (Yu et al., 2016b).

Recent studies have demonstrated that amorphous Fe oxide-bound As has played an important role in the sinking of As (Liu et al., 2015; Yu et al., 2016a). The conversion of poorly crystalline ferrihydrite (surface area: 100 - 700 m² g⁻¹) to other crystalline Fe oxides phases such as goethite (surface area: 8 - 200 m² g⁻¹) and hematite (2 - 115 m² g⁻¹) may decrease the sorption of As due to the reduction of adsorption sites. External supplementation of Fe (i.e., Fe oxides, mixed Fe sources, and Fe-rich industrial byproducts) can lead to reducing As burden in rice ecosystems for different reasons (Farrow et al., 2015; Yu et al., 2017). Firstly, Fe amendments may increase the percentage of amorphous Fe fraction in paddy soil. Secondly, Fe supplementation could increase the quantity of Fe(III) plaque deposited on root apoplast. Yu et al. (2017) have assessed the effect of Fe compound supplementation on As accumulation in rice tissues throughout the whole growth stages of rice plant and have found that Fe amendments significantly reduced the accumulation of As in rice tissues at the grain-filling stage.
Nitrogen is one of the key nutrients, enhancing the growth of rice, and is supplied to paddy fields as N fertilizers (Ata-Ul-Karim et al., 2017). The process of Fe-redox cycling could be affected by nitrogen (N) cycling (i.e., nitrification, denitrification, and ammonification) (Yu et al., 2016b). The coupled NO$_3^-$ reduction and Fe (II) oxidation may reduce the fate of As in the paddy environment (Li et al., 2012). Nitrate (NO$_3^-$)-dependent Fe(II)-oxidizing bacteria can promote the oxidation of Fe(II) under flooded conditions as shown in Eq. 4 (Sun et al., 2009b). As described above, the higher the level of Fe(III), the lower the concentration of bioavailable As in paddy soil-water. The reduction of Fe(III) could be inhibited by NO$_3^-$ reduction because of their competition for electrons (Yu et al., 2016b). Therefore, high level of NO$_3^-$ in paddy soil may decrease Fe(III) reduction and subsequent uptake and accumulation of As in rice tissues.

\[
\text{Fe}^{2+}_{(s)} + 5\text{H}_2\text{O}_{(l)} + \text{NO}_3^-_{(aq)} \rightarrow 2\text{Fe(OH)}_{3(s)} + 4\text{H}^+_{(aq)} + \text{NO}_2^-_{(aq)}
\]  
(Eq. 4)

Anammox-bacteria can drive the process of ammonia (NH$_3$) oxidation under anaerobic conditions. Ammonia oxidation is coupled with Fe(III) reduction as shown in Eq. 5 (Shrestha et al., 2009). However, the impact of NH$_3$ oxidation on Fe-redox cycling in paddy ecosystem remains unclear.

\[
\text{NH}_4^+_{(aq)} + 6\text{FeOOH}^-_{(s)} + 10\text{H}^+_{(aq)} \rightarrow \text{NO}_2^-_{(aq)} + 6\text{Fe}^{2+}_{(s)} + 10\text{H}_2\text{O}_{(l)}
\]
(Eq. 5)

Organic fertilizer, added to keep paddy soil fertility, may influence As dynamics. Humic substances (i.e., humin, humic acid, and fulvic acid), which are key components of organic matter, have a positive and negative effect on the mobility and bioavailability of As (Jiang et al., 2009; Yu et al., 2016b). Arsenic species can be adsorbed to humic substances through
organo-As complexes (Williams et al., 2011). In contrast, the process of adsorption of humic substances to sorption sites in soil matrix may increase the bioavailability of As in soil solution due to the site and electrostatic competitions (Mladenov et al., 2015). Humic substances could stimulate microbes-driven Fe(III) reduction by facilitating electron shuttling (Newman and Kolter, 2000). As a result, As(V), sequestrated in FeOOH, may reduce to highly mobile As(III).

Sulfate ($SO_4^{2-}$) in soil matrix is reduced to sulfide ($S^{2-}$) when decreasing redox potential from -120 to -180 mV (Sahrawat, 2015). Sulfate-reducing bacteria can promote the reduction of $SO_4^{2-}$ in paddy environment (Das et al., 2016). The reduction of $SO_4^{2-}$ to $S^{2-}$, particularly, under flooded conditions, leads to immobilization of As(III) due to precipitation of As(III), as arsenic sulfide, and iron sulfide minerals (Burton et al., 2014). External sulfur (S) supplementation may promote the formation of low molecular weight thiol-rich peptides in rice tissues, which have a high affinity with As(III), and therefore, may reduce As translocation from root to grain (Zhang et al., 2011).

Manganese oxides (MnOH) play an important role in immobilizing As through oxidation of As(III) to As(V) and substantial complexation of As(V) (Eq. 6 and Eq. 7) (Lafferty et al., 2011). Xu et al. (2017b) have demonstrated that MnOH, as an amendment (1200 mg kg$^{-1}$), has decreased the accumulation of As in rice straw and grains by 30 - 40%.

\[
MnO_{2(s)} + H_3AsO_3(aq) + 2H^+_{(aq)} \rightarrow Mn^{2+}_{(aq)} + H_3AsO_4(aq) + H_2O(l) \]  
(Eq. 6)

\[
2Mn – OH_{(s)} + H_3AsO_4(aq) \rightarrow (MnO)_2 AsOOH_{(s)} + 2H_2O(l) \]  
(Eq. 7)
Bacteria, fungi, eukaryotic algae, and archaea, inhabiting in the rhizosphere, can influence on the speciation of As through different biotransformation pathways: oxidation, reduction, methylation, and volatilization (Jia et al., 2012). A variety of genes in microorganisms encode for As-transformation enzymes and transporters. The process of microbial oxidation of As(III), coded for by \textit{aioA} gene, can lead to producing As(V) (Zhang et al., 2015). In contrast, microbial As(V) reduction, coded for by \textit{arsC} and \textit{arrA} genes, may increase the mobility of As, particularly, under flooded conditions (Malasarn et al., 2004; Qiao et al., 2017; Villegas-Torres et al., 2011). Inorganic As species present in paddy ecosystems can be transformed into organic As species such as MMA(V), monomethylarsonous acid (MMA(III)), DMA(V), and dimethylarsinous acid (DMA(III)), and the process is coded for by \textit{arsM} gene. For this reason, organic As species found in paddy soil-water are likely to be microbe-mediated products. Microorganisms can volatilize organic As to arsines, primarily as trimethylarsines ((CH$_3$)$_3$As), with a smaller quantity of arsine (AsH$_3$), monomethylarsine (CH$_3$AsH$_2$) and dimethylarsine ((CH$_3$)$_2$AsH) (Hayat et al., 2017; Jia et al., 2012). As a result, microbes-driven As transformation may decrease the phytoavailability of As in paddy soil-water systems. Moreover, microorganisms can indirectly impact on the speciation and mobility of As by influencing Fe, N, and S cycling, as discussed above. Recent findings have further revealed that soil amendments such as biochar may significantly affect the abundance and diversity of microorganisms, and the consequent transformation of As (Qiao et al., 2017; Qiao et al., 2018).

3. Arsenic metabolism in rice plants

Arsenic metabolism in different rice tissues is crucial for understanding uptake of inorganic and organic As species, their translocation, and grain filling. There are different genes involving in As metabolism including reduction, efflux, transformation, complexation
and sequestration (Table 1). Fig. 1 illustrates genes associated with the uptake, translocation, and grain filling of inorganic and organic As species in the rice plant.

3.1. Uptake of inorganic Arsenic species

3.1.1. Arsenite

Arsenite, the main As species under flooded conditions, is acquired dominantly through nodulin 26-like intrinsic proteins (NIPs), collectively termed as aquaporin channels. In rice roots, OsNIP2;1, also called Lsi1, highly expressed in the distal side of the plasma membranes of the exodermis and endodermis cells where Casparian strips exist, is the major pathway for the influx of silicic acid (Si(OH)₄) as well as As(III) (as arsenous acid, As(OH)₃⁺) into rice roots (Fleck et al., 2013; Ma et al., 2008). This could be attributed to the similar sizes of both Si(OH)₄ and As(OH)₃⁺ with tetrahedral orientation along with more or less similar dissociation constants (pKₐ) (9.2 and 9.3, respectively). Ma et al. (2008) demonstrated that NIP genes (OsNIP1;1 and OsNIP3;1) in oocytes mediate As(III) uptake but the Si(OH)₄ pathway was unaffected. Rice OsNIP3;3 and OsNIP3;2 are highly expressed in the lateral roots and stele region of the primary roots suggesting that As(III) is taken up by the highly active part of the root system (Chen et al., 2017b; Katsuhara et al., 2014). In addition, plasma membrane intrinsic proteins (PIPs) such as OsPIP2;4, OsPIP2;6 and OsPIP2;7 are associated with the uptake of As(III) (Mosa et al., 2012) but their functional role is not well studied.

Aquaporin channel, OsNIP2;2 (Lsi2) being a Si(OH)₄ efflux transporter, localized on the plasma membrane of cells in both the exodermis and endodermis of rice roots; however, contrary to Lsi1, Lsi2 is localized on the proximal side of the same cells. Preloading of the *Xenopus oocytes* with Si resulted in a release of Si back into the external environment, demonstrating that Lsi2 is a Si efflux transporter (Ma et al., 2007). Having an influx
transporter on one side and an efflux transporter on the other side of the cell, As(III) cannot pass freely towards the stele (Zhao et al., 2010b). In addition to the Lsi2, As(III) efflux transporters are found in other organisms. For instance, Arsenic Compounds Resistance protein 3 (ACR3) in yeast extrudes As(III), however, ACR3 does not exist in flowering plants like rice. Meng et al. (2011) found that the introduction of ACR3 (ScACR3) into rice decreased total As concentration in rice grains by nearly 20% suggesting that the transgenic rice plant extruded As(III) back into the external environment. In this aspect, As(III) efflux is a key mechanism which releases a portion of As(III) into the external environment minimizing cellular As accumulation.

In some plant species, uptake of As(III) is a bi-directional process; the direction is determined by the concentration gradient. For instance, aquaporins from different plant species such as Pteris vittata (PvTIP4;1), Lotus japonicas (LjNIP5;1 and LjNIP6;1), and Arabidopsis thaliana (AtNIP1;1, AtNIP1;2, AtNIP3;1, AtNIP5;1, AtNIP6;1, and AtNIP7;1) are known to be associated with As(III) efflux into the external environment (Bienert et al., 2008; He et al., 2016; Kamiya and Fujiwara, 2009; Xu et al., 2015). In this sense, a transgenic approach, overexpressing As(III) efflux transporters is likely to mitigate the accumulation of As in rice tissues.

Following the uptake, besides the As(III) efflux, As(III) has a high affinity for thiol-rich peptides such as glutathione (GSH) and phytochelatins (PCs) that are derived from GSH with the general structure (γ-Glu-Cys)n-Gly (n=2 - 11) in the rice roots. Overexpression of the PCs leads to As resistance in transgenic plants, increasing the level of As in roots, but a remarkably low level of As in rice grains (Shri et al., 2014). After being formed, As(III)-PC complexes are transported into vacuoles for the sequestration by C-type ATP-binding cassette
transporter (OsABCC1) which is expressed in the exodermis and pericle. Similarly, it has been reported that AtABCC1 and AtABCC2 in Arabidopsis thaliana mediate the transportation of As(III)-PC complexes into the vacuole (Song et al., 2010). The favorable pH (approximately 5.5) in vacuoles may provide an ideal condition for the stability of As(III)-PC complexes (Zhao et al., 2009). Therefore, vacuole sequestration of As(III)-PC complexes plays an important role in alleviating the translocation of As(III) into the rice grain.

Gene expressions associated with As(III) assimilation (As(III) influx and efflux) are largely varied with the developmental stages of the rice plant and cultivar type. A recent study by Wu et al. (2017) demonstrated that expression of Lsi1 and Lsi2 genes were significantly down-regulated in non-flooded conditions compared to flooded conditions. These genes are strongly associated with As assimilation and it might be one of the major factors that suppress the accumulation of As under non-flooded conditions. It has been found that a mutation in Lsi1 remarkably reduced the uptake and accumulation of As(III) in rice shoots and grains (Ma et al., 2008). Similarly, Guo et al. (2009) demonstrated that a Si-defective mutant in rice plant showed a decrease in As(III) uptake (Guo et al., 2009). Therefore, understanding the temporal distribution of As species and expression of genes across As efficient and inefficient cultivars are paramount for developing mitigation measures of As in rice.

Rice plants are an efficient Si accumulator and can contain Si in shoots about 10% of shoot dry weight (Meharg and Meharg, 2015). In this regard, supplementation of Si fertilizers to As-contaminated paddy soils may provide an effective strategy to minimize As(III) uptake (Guo et al., 2007; Seyfferth and Fendorf, 2012). It has been reported that Si application to the paddy soil, decreased the total As concentration by 33% in rice grains (Fleck et al., 2013).
similar study by Wang et al. (2016a) showed that high Si application rates (9000 kg ha\(^{-1}\)) decreased the total As concentration in rice grains by 20%. Thus, Si application to As-contaminated paddy soils is beneficial for rice plants, partly because of the decreased uptake of As(III) by rice roots, and partly because of the increased plant resistance to biotic and abiotic stresses.

3.1.2. Arsenate

Arsenate is the most prevalent As species under non-flooded paddy soils. Physiological and electrophysiological studies have revealed that As(V) and PO\(_4^{3-}\) share the same uptake pathway (Muehe et al., 2014). In the rice genome, 13 PO\(_4^{3-}\) transporter genes (OsPHTs) have been identified; however, their response to As(V) and contribution to As(V) uptake in rice have not been studied in detail yet.

Phosphate transporter, OsPHT1;8 (OsPT8), was found to have a high affinity for both PO\(_4^{3-}\) and As(V) uptake in rice. The overexpression of the OsPT8 has increased the maximum influx of As(V) by 3 - 5 times in rice plants (Wang et al., 2016b; Wu et al., 2011b). The PO\(_4^{3-}\) transporter gene, OsPHT1;1, also mediates the uptake of As(V) into the rice roots (Kamiya et al., 2013). It has been found that other plant species may contain PO\(_4^{3-}\) transporters which are mediating As(V) uptake. For example, PO\(_4^{3-}\) transporters, such as PvPht1;3 in *Pteris vittata* and AtPHT1;1, AtPHT1;4, AtPHT1;5, AtPHT1;7, AtPHT1;8, and AtPHT1;9 from *Arabidopsis thaliana* acquire As(V) from the rhizosphere (DiTusa et al., 2016; Fontenot et al., 2015; Shin et al., 2004).

Following uptake, As(V) is readily reduced to As(III) in rice roots. As(V) reductase enzymes play a key role in controlling the conversion of As(V) to As(III) (Xu et al., 2007).
Efficient As(V) reduction takes place in all tissues of the root apex which was supported by fluorescence-X-ray absorption near-edge spectroscopy (XANES) analysis (Batista et al., 2014). Recently, Shi et al. (2016b) identified two As(V) reductases, OsHAC1;1 (abundant in the epidermis, root hairs, and pericycle cells) and OsHAC1;2 (abundant in the epidermis, outer layers of cortex, and endodermis cells) which play an important role in the reduction of As(V) to As(III) in rice roots.

Interestingly, As(III) is found in considerable concentrations in plant roots exposed to As(V). However, after the reduction of As(V), As(III) can be released into the external environment via As(III) efflux system or complexed with thiol compounds as mentioned earlier. A new As(V) reductase, OsHAC4, has been identified and it is highly expressed in the epidermis and exodermis of roots, the location which is ideal for the efflux of As(III) to the external environment (Xu et al., 2017a). Similar As(V) reductases, namely As(V) tolerance QTL1 (ATQ1) and High Arsenic Content 1 (HAC1) were identified in Arabidopsis thaliana (Chao et al., 2014). Therefore, the reduction of As(V) to As(III) and subsequent As(III) efflux or As(III)-thiol complexation in rice roots are likely to play a major role in determining the level of total As in rice grains.

Since As(V) is an analogue for PO₄³⁻ in plant uptake process, the application of external PO₄³⁻ to the As-contaminated paddy soil could alleviate the accumulation of As(V) in rice tissues (Lihong and Guilan, 2009). Studies have clearly demonstrated that the supplementation of PO₄³⁻ fertilizers to the As-contaminated paddy soils has greatly decreased the concentration of total As in rice grains (Geng et al., 2005). Excess supplementation of PO₄³⁻ fertilizers may lead to eutrophication in surface water bodies. Therefore, a close monitoring must be carried out under excess usage of PO₄³⁻ fertilizers.
3.2. Uptake of organic As species

Rice plants are not capable of methylating inorganic As \textit{in vivo}, therefore, organic As species (DMA(V) and MMA(V)) are probably come from the rhizosphere through microbial-mediated methylation processes (Jia et al., 2012; Zhao et al., 2013a). The exact mechanisms that drive the uptake of organic As species in rice are largely unknown. It has been suggested that aquaporin Lsi1 mediates the uptake of undissociated MMA(V) and DMA(V) in rice plants (Li et al., 2009). The pH of the medium has a great effect on the uptake of MMA(V) and DMA(V). More precisely, pH can alter the equilibrium between protonation and dissociation that eventually influence the availability of the MMA(V) and DMA(V) for the membrane transporter, Lsi1 (Li et al., 2009). Therefore, Lsi1 plays a major role in acquiring both inorganic As(III) and organic As species from the rhizosphere.

There have been not many reports on the metabolism of organic As species in rice roots. A recent study by Mishra et al. (2017) revealed that MMA(V) is readily reduced into MMA(III) which is then bound with thiols in rice roots. In addition, MMA(V) itself can complex with various thiol groups in rice plants. Mishra et al. (2017) found that 16 different MMA(V)-thiol complexes were present in the rice root and shoot. However, DMA(V) is known to neither complex with thiols or convert into the reduced forms. The direct determination of organic As species - thiol complexation in various rice genotypes is an important step for understanding As metabolism in rice plants.

3.3. Arsenic species translocation from root to shoot

It is likely that both active and passive transporters are associated with the uptake and translocation of As in rice plants. Active transportation is the process of passing materials against a concentration gradient by using the energy of metabolism (Liang et al., 2006).
Passive transport is the movement of water and materials without using the energy of metabolism and mainly transport through transpiration flow. For example, a large number of nutrients such as potassium (K), nitrogen (N), Si, is transported through both active and passive processes in rice plants (Bao et al., 2015; Yang et al., 2014). Following the uptake by rice root, various transporters and compounds have involved the translocation of As species from root to shoot.

The translocation of inorganic As species from root to shoot is determined by various processes. Lsi2 mediates the efflux of uncomplexed As(III) in the direction of xylem. In addition, the Natural Resistance-Associated Macrophage Protein (NRAMP) transporter, OsNRAMP1, may also facilitate the xylem loading of As(III) (Tiwari et al., 2014). Different metabolic activities such as As(III) efflux back into the external environment, and formation of As(III)-thiol complexes and consequent sequestration in vacuoles, limit the translocation of As(III) from root to shoot. Studies have revealed that rice cultivars with low levels of total As in their grains had remarkably higher concentrations of PCs in roots compared to the cultivars with high total As levels in their grains (Duan et al., 2011). In contrast, Batista et al. (2014) found that PCs produced in rice cultivars in response to elevated levels of As exposure did not necessarily reduce the total As concentrations in rice grains.

The translocation efficiency of organic As species from root to shoot is much higher than that of inorganic As species (Raab et al., 2007). It could be attributed to the favorable dissociation of MMA(V) and DMA(V) at cytoplasmic pH. Since MMA(V) is partly reduced to MMA(III), only untransformed MMA(V) is transported to the shoots (Li et al., 2009). In addition, the lack of DMA(V)-PC complexation enhances the DMA(V) to be readily moved between root and shoots (Zhao et al., 2013b). Putative peptide transporter in rice, OsPTR7,
involve in the long-distance (root to grain) translocation of organic As species in rice. The OsPTR7 transcript is more prevalent in the leaves, nodes, and roots at the flowering and grain filling stage; however, during the seedling stage, OsPTR7 is more abundant in the shoots than in the roots (Tang et al., 2017).

There have been no reports of volatile As species released by rice plants under typical growing conditions. However, a large amount of volatile As is released from paddy soil due to microbial activities. The volatilization of (CH$_3$)$_3$As from rice plants grown in an axenic system was detected when rice plants were treated with trimethylarsine oxide ((CH$_3$)$_3$AsO) but not with other inorganic or organic As species (Jia et al., 2012). A soil bacterium, *Rhodopseudomonas palustris*, is found to have a direct influence on As volatilization. When an *arsM* gene from *Rhodopseudomonas palustris* was expressed in rice, transgenic rice produced volatile arsenicals (i.e., (CH$_3$)$_3$As) which were 10 times greater than that of the control (Meng et al., 2011). Therefore, producing transgenic rice plants with *arsM* gene could be adapted to mitigate the As burden in rice plants by achieving As volatilization out of the rice crop.

3.4. Phloem and xylem - derived pathways of As species transport

A recent study by Ye et al. (2017) revealed that As(III) was the prevalent As species (69%) in the phloem exudates of As(III)-exposed rice, while As(V) dominated (58%) in As(V)-exposed rice and the remainder was As(III). When rice plants were treated with MMA(V) and DMA(V), the major As species in phloem exudates were 55 and 59% of MMA(V) and DMA(V), respectively. Interestingly, a large proportion of As(V) (45 and 41%, respectively) was detected in the phloem exudates when rice was treated with MMA(V) and...
DMA(V). This observation could correspond to the demethylation process; however, the mechanisms underlying demethylation remain unclear.

Phloem transportation is likely to be responsible for 54, 56, 100, and 89% of As(III), As(V), MMA(V), and DMA(V) transport into the rice grain, respectively. Organic As species are more mobile than inorganic As species in the phloem as described by Ye et al. (2017). This finding is similar to those of the study reported by Carey et al. (2010). However, Carey et al. (2010) accounted the phloem transport to be responsible for 90 and 55% of transportation of As(III) and DMA(V) to the grain, respectively. The difference in the above two studies could be attributed to the experimental methods used. Stem girding, which blocks phloem transportation, has reduced grain $^{73}$As radioactivity by 97% compared to the ungirdled control (Zhao et al., 2012). Therefore, it can be suggested that phloem acts as the primary route of transport to rice grains for both inorganic and organic As species. Transporters responsible for As species loading into the vascular tissues in rice plant are need to be investigated in detail. Inositol transporters (INT) such as AtINT2 and AtINT4 are associated with phloem loading of As(III) in Arabidopsis thaliana (Duan et al., 2016) and similar transporters might responsible for phloem loading of As(III) transport in rice.

Synchrotron µX-ray fluorescence (µ-XRF) mapping has indicated that As(III) was sequestrated in the phloem, top node, and internode, limiting As(III) loading into the rice grain. The OsABCC transporter, which is expressed in the tonoplast of phloem in nodes, enhances the transportation of As(III)-PC complexes to vacuoles (Song et al., 2014). The knockout mutant of OsABCC has decreased the level of total As in nodes but has increased the level of total As in grains. Therefore, it is likely that rice plants have evolved a mechanism to minimize As loading into their grains.
There are only few reports discussing the transportation of As species through xylem in rice plants. Arsenic(III) is the prevalent form of As present in the xylem of *Pteris vittata* and *Cucumis sativus* (Mihucz et al., 2005; Su et al., 2008). The predominant As species in the xylem sap of rice is vary with water management practices. Ye et al. (2015) found that As(V), accounting for 64 - 88%, was the dominant form of As in the xylem sap of rice under aerobic treatment. In contrast, in the flooded treatment, the prevalent species in the xylem sap in rice was As(III), accounting for 26 - 77%, followed by As(V) (12 - 54 %) and DMA(V) (11 - 20 %) (Ye et al., 2015). Highly expressed Si pathway may load more As(III) into the xylem, in particular, in the flooded cultivation of rice.

3.5. Arsenic species loading into the grain

Arsenic species loading into rice grain is particularly important since it leads to the primary pathway of As exposure in human beings. From the available data, it is clear that there are significant site and genotype interactions on the distribution and concentration of As species in rice grains (Norton et al., 2009; Sommella et al., 2013). DMA(V) translocates from root to rice grain more efficiently (approximately 10-fold greater) compared to inorganic As species (Carey et al., 2010). As mentioned earlier, lack of complexation of DMA(V) may promote the efficient movement of DMA(V) in rice plants. The re-translocation of DMA(V) and MMA(V) from the flag leaf into the filling grain is more efficient than that of As(V) and As(III) which are not detectable even leaves were fed with As(III). The formation and subsequent sequestration of As(III)-PC complexes in rice leaves may restrict the As(III) from loading into the phloem and subsequent translocation to the rice grain (Zheng et al., 2011).

Synchrotron analysis has revealed a marked difference in the pattern of inorganic and organic As species loading into the grain. As(III) is mostly retained in the ovular vascular
trace (OVT) region, the vascular entry point into the rice grain and comprises of phloem and xylem cells, whereas DMA(V) disperses throughout the outer layers and into the endosperm (Carey et al., 2010). It is suggested that DMA(V) in the rice grain is derived from the re-translocation of DMA(V) accumulated before flowering. In contrast, inorganic As species mainly transport to the rice grain during flowering. In this respect, DMA(V) would accumulate at considerable concentrations in the initial stage of grain filling and later is diluted by carbohydrate filling to the grain (Zheng et al., 2011). This is exemplified by Carey et al. (2012) that the direct shoot-to-grain translocation is during grain filling and not from vegetative stores that is responsible for inorganic As in rice grains. Relatively higher As concentrations in bran than in polished grain could be due to following reasons: firstly, the bran acts as a physiological barrier for As translocation into the rice grain, secondly, As is likely to be concentrated in the protein-rich tissues including embryo and aleurone layer. So far, none of the studies has shown any effort towards the quantitative determination of As species in different layers of the rice grain under different growing stages, water management regimes, and with supplementation of nutrients and amendments in As-contaminated paddy environment.

3.6. Translocation efficiency of arsenic species

The highest translocation efficiency (caryopsis-to-root) in rice was reported for DMA(V) followed by MMA(V) and inorganic As species (Geng et al., 2017; Jia et al., 2012). The order of translocation efficiency differs in various parts of the rice tissues. Dittmar et al. (2010) showed that the straw to grain transfer factor declined exponentially with increasing the concentration of total As in straw and eventually reached a constant value (~ 0.04). The high yielding rice cultivars tend to have a high efficiency of translocation than the rice cultivars with low yielding capacity (Bhattacharya et al., 2010; Zheng et al., 2011).
Regardless of the location and season, total As concentrations in rice tissues decreased in the order: root >> shoot > straw > husk > grain (Table 2 and 3).

Agronomic practices greatly influence the speciation and accumulation and speciation of As in rice grains. The dynamics of total As in rice grain under both flooded and non-flooded conditions revealed that total As concentrations in rice grains were 10 - 15 times greater in rice grown under flooded conditions than in rice grown under non-flooded conditions (Xu et al., 2008). This is consistent with the study by Spanu et al. (2012) who showed that intermittent irrigation has produced rice grains with 2.8 µg kg\(^{-1}\) of total As whereas the corresponding figure for continuous flooding irrigation was 163 µg kg\(^{-1}\). Correlation analysis revealed that a significant positive correlation existed in total As concentrations between soil pore water and rice grains. Similarly, studies have indicated that an increased level of soil As lead to high level of total As in rice straw and grains (Dittmar et al., 2010; Hossain et al., 2008). However, contradicting results have also been reported and these could be due to various factors including irrigation intensity, underlying geology, weather conditions, fertilizer applications, and diseases.

4. Arsenic risk to rice plant and humans

4.1. Impact on yield

Straighthead disease, in which the panicles remain upright due to lack of grain filling and sterility, is most common in rice due to a high level of As exposure (Li et al., 2016). Both chlorophyll a and b levels in rice leaves tend to decrease under As exposure and this may adversely affect the photosynthesis (Rahman et al., 2007a). Increased level of As(V) in paddy soil pore water negatively affects plant growth parameters including plant height, grain yield, number of filled grains, and root biomass. Further, accumulation of As(V) and As(III)
enhances the production of reactive oxygen species (ROS) which damage macromolecules and cell membranes (Finnegan and Chen, 2012). A recent study by Xu et al. (2017a) demonstrated that As(III) binds with reduced cysteine residues in proteins, hence, influence the catalytic functions. Moreover, the preferential translocation of DMA(V) into filial tissues such as embryo, endosperm, and aleurone causes marked reduction in seed setting rate (i.e., spikelet sterility) and loss of yield (Wang et al., 2015). Rice cultivation in an environment with minimal As level or As-tolerance rice cultivars are, therefore, an important aspect of achieving the goal of sustainable production of rice in terms of quality and quantity.

4.2. Contribution of rice to arsenic intake in humans

Rice and rice-based products with high As concentrations may increase health risk for the humans (Sandhi et al., 2017; Signes-Pastor et al., 2016). The level of total As and As species in rice grains are depended on the rice genotype, physico-chemical, and environmental interactions as summarized in Table 4. Arsenic-containing rice husks are used as cattle feed in several countries (i.e., Bangladesh and China) and may act as an indirect pathway of As exposure to humans through the food chain (Abedin et al., 2002). In addition, As in rice grains leads to the loss of trace mineral nutrition such as Zn, Se, and Ni and amino acid content (Dwivedi et al., 2010; Williams et al., 2009a). High levels of As in rice bran increases the risk, particularly for children, and people on gluten-free products (Abedin et al., 2002). Removing the bran layer during rice processing is an effective way to elimination of inorganic As. However, from a nutritional point of view, removal of the bran results in a substantial loss of nutrients in rice grains.

There have been many studies focusing on the risk assessment of human exposure to As. For example, the lifetime cancer risk in West Bengal, India was found to be $7.62 \times 10^{-4}$ which
exceed the United States Environmental Protection Agency (USEPA) threshold value of $10^{-4}$
- $10^{-6}$ (Mondal and Polya, 2008). Dittmar et al. (2010) predicted that under current irrigation
practices in Bangladesh, total As concentration in rice grains would increase from ~ 0.15 to
0.25 - 0.58 mg kg$^{-1}$ by 2050. This figure increases the human As intake by 1.5 - 3.8 fold
through rice. Therefore, precautionary measures must be undertaken to control the intake of
As from rice considering special attention to regions prone to As contamination.

5. Concluding remarks

Arsenic dynamics in paddy soil-water and rice grains are a function of geochemical,
microbial and plant genetic factors. The knowledge gained with respect to speciation,
mobility, and bioavailability of As in paddy soil-water, and subsequent uptake, efflux,
translocation, and sequestration of As is important to develop mitigation measures. Rice
production is required to be increased to meet the demand for ever-growing populations.
Therefore, there is an urgent need to adapt As mitigation measures in As-prone areas in the
world to reduce As burden. Firstly, the mobility and bioavailability of As in paddy-soil water
system could be decreased by using agronomical, physico-chemical, and biological
approaches. Alternative water management practices may play an important role in reducing
As mobility and bioavailability in paddy environment due to changes in redox chemistry.
Supplementation of fertilizer and soil amendments can lead to reducing the bioavailability of
As for rice plants. Optimum supplementation rates of fertilizer and soil amendments are
required to be investigated before introducing them to As-contaminated paddy lands.

Microorganisms are a key factor influencing As mobility and bioavailability in paddy soil-
water system through direct and indirect pathways. The kinetics of microbes-driven As
biotransformation and related rate limiting factors are needed be examined with respect to
different As-contaminated localities. Secondly, uptake and translocation of As species in rice
plant could be decreased by altering the expression of transporters associated with the uptake, efflux, translocation, and sequestration of As. It is important to study in detail whether changes in expression of transporters related to As metabolism are impact on the uptake and translocation of other essential elements and compounds in rice plants.

Questions regarding the uptake and translocation of As species in rice plants remain unanswered and must be the aim of future studies. These include the following: (1) the extent of active and passive As uptake and translocation in rice, (2) a detailed survey of possible As demethylation and volatilization mechanisms and identify related genes involving in As demethylation and volatilization rice tissues, (3) the spatial distribution of As species and temporal variations of their concentrations in rice grain at development stages in various rice genotypes under different water management regimes and soil amendments, and (4) determination of As-thiol complexes qualitatively and quantitatively in different rice cultivars at different growing stages.

As-containing rice grains are a major source of As, particularly, for those consuming a large amount of rice and rice-based products. The development of advanced technologies to an in-situ measurement of As species and associated elements and genes at cellular and sub-cellular levels in rice plants is significantly important to identify rice grains for safe consumption. Therefore, scientific-based studies in As dynamics safeguard the paddy rice ecosystems and human from the As burden.
References


Table captions

Table 1
Key representative genes involved in As uptake, transport, and metabolism in rice plants.

Table 2
Arsenic concentration in irrigation water, soil and different plant tissues worldwide from field experiments.

Table 3
Arsenic speciation in different rice tissues from glasshouse experiments.

Table 4
Arsenic speciation in market-based rice in different countries.
Table 1
Key representative genes involved in As uptake, transport, and metabolism in rice plants.

<table>
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<tr>
<th>Function</th>
<th>Gene category</th>
<th>Gene name</th>
<th>Reference</th>
</tr>
</thead>
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<td>As(V) uptake by root, loading into the xylem,</td>
<td>Phosphate transporters</td>
<td>OsPHT1;8 (OsPT8)</td>
<td>Wang et al. (2016b)</td>
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<td>phloem, and grain</td>
<td>OsPHT1;1</td>
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<td>Kamiya et al. (2013)</td>
</tr>
<tr>
<td>As(III) uptake by root</td>
<td>Nodulin 26-like intrinsic proteins (NIPs)</td>
<td>OsNIP2;1 (Lsi1)</td>
<td>Ma et al. (2008)</td>
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<td>OsNIP2;2 (Lsi2)</td>
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<tr>
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<td></td>
<td>OsNIP3;3</td>
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<td>Plasma membrane intrinsic proteins (PIPs)</td>
<td>OsPIP2;4</td>
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<td></td>
<td>OsPIP2;6</td>
<td>Katsuhara et al. (2014)</td>
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### Arsenic Uptake and Metabolism in Rice

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<td>DMA(V) and MMA(V) uptake by root</td>
<td>Nodulin 26-like intrinsic proteins (NIPs)</td>
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<td>As(V) reduction to As(III) in root</td>
<td>As(V) reductase</td>
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<td>OsHAC4</td>
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<td>As(III) efflux from root to the external environment</td>
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<td>As(III) complexation in root and phloem</td>
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<td>DMA(V) loading into the xylem, phloem, leaves, and grain</td>
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<td>As(III) loading into the xylem</td>
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Table 2

Arsenic concentration in irrigation water, soil and different plant tissues worldwide from field experiments.

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<th>Country</th>
<th>Rice variety</th>
<th>Irrigation water (µg L⁻¹)</th>
<th>Total As (mg kg⁻¹)</th>
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<td></td>
<td></td>
<td>68 - 136</td>
<td>11.41 - 61.04</td>
<td>0.571 - 1.916 0.380 - 0.182 0.995 0.436</td>
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<td>Boro and Aman</td>
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<td>2.64 - 12.52 1.20 - 2.48 0.22 - 0.81</td>
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<td>(0.08 - 0.18)</td>
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a - As concentration in soil solution

The range of total As concentrations is shown in parenthesis
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<th>Rice genotype</th>
<th>Type of plant tissue</th>
<th>Total As (mg kg(^{-1}))</th>
<th>As species (mg kg(^{-1}))</th>
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<td>29 Japonica subspecies and 8 indica subspecies</td>
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<td>T116</td>
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<td>45.6 - 80.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> - values are as percentages

<sup>b</sup> - sum of the As(III) and As(V)
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<sup>a</sup> - sum of As(III) and As(V)

<sup>b</sup> - sum of DMA(V) and MMA(V)

dl - below the detection limit

The range of concentration for respective As species is shown in parenthesis.
Figure captions

**Fig. 1.** Genes involving in inorganic and methylated As species uptake, transformation, and localization in rice plant.
Fig. 1. Genes involving in inorganic and methylated As species uptake, transformation, and localization in rice plant.