Arsenic as a Food Chain Contaminant: Mechanisms of Plant Uptake and Metabolism and Mitigation Strategies

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Abstract

Arsenic (As) is an environmental and food chain contaminant. Excessive
accumulation of As, particularly inorganic arsenic (As_i), in rice (*Oryza
sativa*) poses a potential health risk to populations with high rice con-
sumption. Rice is efficient at As accumulation owing to flooded paddy
cultivation that leads to arsenite mobilization, and the inadvertent yet
efficient uptake of arsenite through the silicon transport pathway. Iron,
phosphorus, sulfur, and silicon interact strongly with As during its route
from soil to plants. Plants take up arsenate through the phosphate trans-
porters, and arsenite and undissociated methylated As species through
the nodulin 26-like intrinsic (NIP) aquaporin channels. Arsenate is read-
ily reduced to arsenite in planta, which is detoxified by complexation
with thiol-rich peptides such as phytochelatins and/or vacuolar seques-
tration. A range of mitigation methods, from agronomic measures and
plant breeding to genetic modification, may be employed to reduce As
uptake by food crops.

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INTRODUCTION

Inorganic arsenic (As_i) is a class 1 carcinogen (45). There is widespread chronic As_i poisoning in regions of Asia, South America, and elsewhere, due to the consumption of drinking water with geogenically elevated As_i, with the situation at its worst in the densely populated floodplains and deltas of South and Southeast Asia (13, 85). While As contamination in drinking water has attracted much attention, plant-based foods are also an important source of As_i. This issue has only been recognized in recent years (74, 76, 139, 140). Rice is

specifically a problem regarding the entry of As into the food chain, owing to a combination of anaerobic growing conditions and specific plant physiological characteristics. It is also the dietary staple for half the world's population. Intake of As_i from eating rice can be substantial; it is the dominant source for populations based on a rice diet and not exposed to high concentrations of As in drinking water (52, 76). Even for populations exposed to elevated As_i in drinking water, such as As-affected areas in South Asia, As_i intake from rice is significant, accounting for ∼50% (81, 90). There is an urgent need to understand how plants assimilate and metabolize As in order to develop mitigation strategies against this widespread contamination in the food chain.

Here, we review the biogeochemical aspects of As in the environment controlling its bioavailability to plants, the mechanisms of As uptake and metabolism in plants and potential mitigation strategies to reduce As uptake. We focus on rice, but also draw from recent advances in research on other plant species. Because it is a nonessential and toxic element, As is taken up inadvertently by plants through the pathways for essential or beneficial nutrients and detoxified via a variety of mechanisms. Along its route from soil to plants, As interacts with a number of elements, most noticeably iron (Fe), phosphorus (P), silicon (Si), and sulfur (S); these interactions are summarized in the sidebar, Four Elements that Interact Strongly with Arsenic, and discussed in more details in the following sections.

ARSENIC IN THE TERRESTRIAL ENVIRONMENT

Arsenic is ubiquitous in the environment; it is the twentieth most abundant element in the Earth's crust, with an average concentration of approximately 3 mg kg^{-1} (20). More than 200 As-containing minerals exist; frequently As is associated with S in minerals such as arsenopyrites (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3). Arsenic is released into the environment by both natural processes, such as

weathering of rocks, volcanic emissions and discharge from hot springs, and various anthropogenic activities such mining, smelting, and the use of As-containing pesticides, herbicides, wood preservatives, and feed additives.

Extensive chronic As poisoning is occurring in South Asia, potentially affecting tens of millions of people, due to As contamination in the drinking water extracted from shallow underground aquifers (13, 115). This problem is at its worst in the Bengal Delta region (encompassing Bangladesh and West Bengal, India), where tube-wells were installed to provide "safe" drinking water free from microorganisms causing gastrointestinal diseases, without prior knowledge of As contamination. In Bangladesh, nearly half of the shallow tube-wells produce water containing >10 μg L^{-1} As, which is the current As limit in drinking water recommended by the World Health Organization (WHO), with the exposed population estimated at 57 million (14). The geochemical and hydrological conditions causing this As contamination are still being debated, but it is clear that elevated As concentrations are linked to the reducing environment developed in Holocene alluvial and deltaic deposits (14). Anaerobic metal-reducing bacteria may play a key role in the release of As by mediating reductive dissolution of As-rich Fe(III) oxy-hydroxides (47). These groundwaters, some containing more than 1 mg L^{-1} As, are also used extensively for irrigating rice crops in the dry season, adding more than 1000 t of As per year to the agricultural soils in Bangladesh (3).

The global average concentration of As in soil is about 5 mg kg^{-1} (45). Uncontaminated soils typically contain <10 mg kg^{-1} total As, but the concentration can reach hundreds or thousands of mg kg^{-1} in contaminated environments (45, 48). The bioavailability of As to plants is governed by edaphic properties, environmental conditions and modification of the soil in the rhizosphere; these factors interact to influence As speciation in the soil. Arsenic has four oxidation states: −3, 0, +3, and +5, the last two being the most common in the terrestrial environment. Arsenate [As(V)] is the

FOUR ELEMENTS THAT INTERACT STRONGLY WITH ARSENIC

Iron: Fe plays a pivotal role in the biogeochemical cycle of As, with Fe oxyhydroxides on soil particulate surfaces, or root surfaces of wetland plants, serving as a strong adsorbent for As. Reductive dissolution of Fe oxyhydroxides under a reducing environment releases the adsorbed As, leading to enhanced As availability to plants. Fe-reducing bacteria are linked to the mobilization of As in aquifer of the river delta in Bangladesh.

Phosphorus: Arsenate is a phosphate analogue, entering plant cells via the phosphate transporters, and also interfering with phosphate metabolism. Use of phosphorus fertilizers to decrease As accumulation in plants has not always been successful, because phosphate competes with arsenate in both root uptake and adsorption on Fe oxides/hydroxides. Increasing cellular P status alleviates As toxicity.

Sulfur: S helps to detoxify As through complexation of arsenite with thiol-rich peptides. This complexation may also help to keep As in roots and restrict As translocation to shoots. Maintaining sufficient S nutrition may be particularly important in As-contaminated environments.

Silicon: The role of Si transporters in As uptake was realized only recently. Physiochemical similarities between silicic acid and arsenous acid allow the latter to be taken up via the pathway for the former. Si fertilization may be an effective strategy to decrease As accumulation in rice grown in As-contaminated soil.

predominant species in aerobic soils, whereas arsenite [As(III)] predominates in anaerobic environments such as submerged soils. Interconversion between these two As species is driven by both biotic and abiotic processes and strongly influenced by the redox potential and pH. Both arsenate-reducing and arsenite-oxidizing bacteria are present in soil. Microbial arsenate reduction occurs via two principal mechanisms: dissimilatory reduction in which arsenate serves as a terminal electron acceptor during anaerobic respiration, and detoxification in which arsenate is reduced to arsenite and then pumped out of the microbial cells (8, 44, 117). Abiotic oxidation of arsenite can occur rapidly through reaction with manganese oxide

Rhizosphere: the zone of soil surrounding plant roots that is modified chemically or biologically by the activities of roots

(e.g., birnessite) in soil (91), whereas abiotic arsenate reduction may take place in anaerobic and acidic environments with dissolved sulfide as the reductant (106). In aerobic soils, arsenate is usually present at very low concentrations (<1 μM) in soil solution because of the strong adsorption by oxides/hydroxides of iron and aluminum (27, 28); therefore, the bioavailability of arsenate is generally low. When soils are submerged, as in flooded paddy fields, As is mobilized into the soil solution mainly as arsenite (68, 148). This is a result of two processes: (*a*) the reductive dissolution of iron oxides/hydroxides and release of the associated As, and (*b*) reduction of strongly adsorbed arsenate to more weakly adsorbed arsenite leading to an enhanced partition of As from the solid to the solution phase (27, 121) (**Figure 1**).

Consequently, the bioavailability of As is more enhanced to rice plants grown under submerged conditions than to those grown under aerobic conditions (56, 148). Arsenite concentrations as high as 20 μM have been reported in the soil solutions from paddy fields contaminated by irrigation of As-laden groundwater, causing As toxicity and yield losses in rice (92).

Methylated As compounds, such as monomethylarsonic acid [MMA: $CH_3AsO(OH)_2$], dimethylarsinic acid [DMA: $(CH_3)_2AsOOH$] and trimethylarsine oxide [TMAO: $(CH_3)_3AsO$] (**Figure 2**), are found in some soils, sometimes as a minor component (41, 122), but can reach high concentrations (1). MMA and DMA can be produced from inorganic As through biomethylation by some soil microorganisms or algae (7). Both MMA and
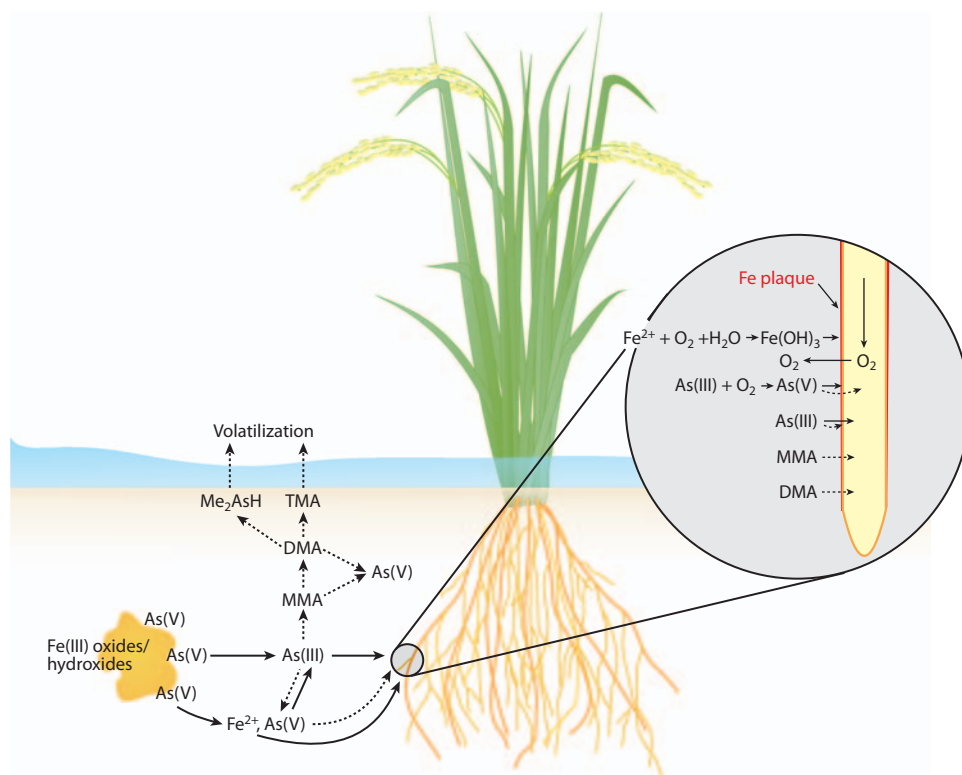
Figure 1

Arsenic mobilization and transformation in flooded paddy soil and interactions in the rice rhizosphere. Arrows with solid and broken lines indicate dominant and minor processes, respectively. For more details of the As methylation pathway, see **Figure 4b**.
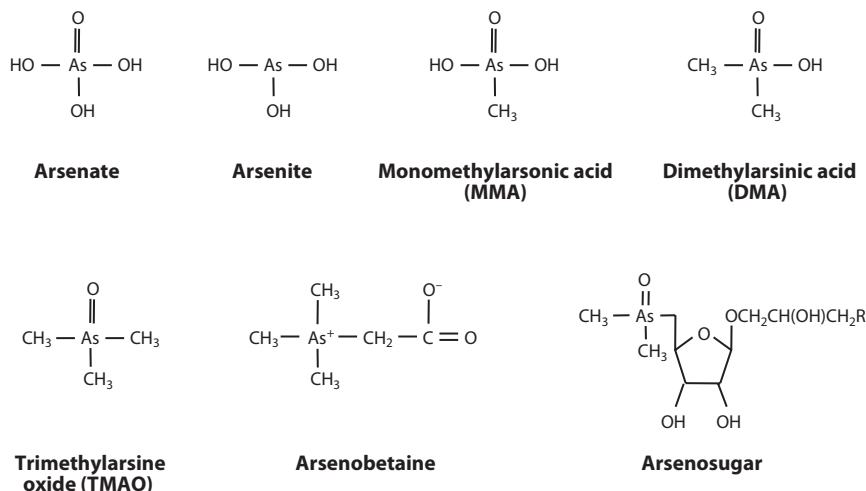
Figure 2

Arsenic compounds found in soils and terrestrial plants.

DMA (also known as cacodylic acid) have been widely used as pesticides and herbicides, the latter also as a cotton defoliant. DMA applied to soil can be transformed by microorganisms via two pathways: (*a*) reductive conversion to volatile organo-arsine species (e.g., dimethyl- or trimethylarsine) and emissions from the soil system, and (*b*) demethylation to produce the end products CO_2 and arsenate; the first pathway predominates under anaerobic conditions, whereas both pathways occur in aerobic soil (144) (**Figure 1**). The rate of degradation and the relative importance of the two pathways vary among different studies, probably due to different soil properties, microbial communities, and environmental conditions; for example, Gao & Burau (30) found that demethylation was quantitatively far more important than the evolution of gaseous arsines when DMA was added to an aerobic soil. In one study (143), the half-life for field-applied DMA and MMA was found to be about 20 days, although these compounds were still detectable in soil 1.5 years after applications. In field studies where inorganic or methylated As compounds were applied as pesticides or herbicides, As losses through volatilization, leaching, runoff, and crop removal were estimated to be approximately

0.03%/day (or 10%/year) (142). Volatilization of methylarsines, mainly trimethylarsine (TMA) and some dimethylarsine (Me_2AsH), was also found from an As-contaminated paddy soil treated with animal manure under flooded conditions, but the amount was relatively small, only 0.014% of the total soil As during a two-month incubation (78). Note that methylarsines are oxidized in the air rapidly during the day by UV irradiation and are then deposited on land.

Arsenobetaine $[(CH_3)_3As^+CH_2COOH]$ (**Figure 2**), the dominant As species in marine animals, was found to be present in an acidic fen soil with unclear origin (41). Arsenobetaine is rapidly demethylated in soil leading to the production of DMA and the end product arsenate (42). In some coastal regions, seaweeds rich in arsenosugars (**Figure 2**), which comprise a group of dimethylarsinoyl ribose derivatives and are the major As compounds in marine algae, are applied to land as fertilizers. This practice can increase soil As levels greatly in the long term (16). Arsenosugars in seaweed added to soil are degraded to DMA and As_i (16).

Several As-containing phenolic compounds, such as 3-nitro-4-hydroxyphenylarsonic acid (roxarsone), are widely used as feed additives to control parasites in poultry. These compounds

are degraded during composting of poultry litter and subsequent land application, releasing arsenate to the environment (31, 117).

ARSENIC ACCUMULATION AND SPECIATION IN PLANTS

Variation Among Plant Species in Arsenic Accumulation

Plants vary considerably in their ability to accumulate As. Arsenic concentrations in the above-ground part of plants growing in uncontaminated soils are typically <1 mg kg^{-1} dry weight (20, 48), circa less than one tenth of the soil As concentration [i.e., the As transfer factor (TF) <0.1)]. Plants with these low TFs are called excluders because of their restricted uptake and, more importantly, restricted translocation of As from roots to shoots.

At the other extreme, As hyperaccumulators are able to accumulate up to ~2% As in the above-ground parts, with the As TF usually exceeding 1 (66, 157). Following the first report of brake fern (*Pteris vittata*) as an As hyperaccumulator (66), 12 species of As hyperaccumulators have so far been identified, all fern species within the Pteridaceae family (mostly within the *Pteris* genus) (see Reference 157 for a review).

Between the excluders and hyperaccumulators, there are plant species with intermediate abilities to accumulate As. Examples include the Douglas-fir (*Pseudotsuga menziesie*) (40), several *Equisetum* species (71), and the Brassica *Isatis capadocica* (50). Some aquatic plants were found to contain high concentrations (>1000 mg kg^{-1}) of As, but this was a result of physicochemical adsorption to the plant's surface, facilitated by codeposition of Fe hydroxides (105). The rootless duckweed *Wolffia globosa* accumulates and tolerates 400 mg kg^{-1} As; its strong accumulation phenotype is likely a result of the absence of the root-to-shoot translocation barrier (155). Caution is needed when plant specimens collected from field-contaminated sites are analyzed for elemental concentrations, as surface contamination can lead to spurious interpretation.

Rice (*Oryza sativa*) is also an interesting case as it is much more efficient in As accumulation than other cereals such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (118, 141), with its As TF often approaching unity (141). The relatively high As accumulation in rice is due to two reasons: (*a*) enhanced As bioavailability under the anaerobic conditions of submerged paddy soils (discussed above), and (*b*) the inadvertent uptake and transport of arsenite through the Si pathway, which is highly efficient in rice (see below).

Arsenic Speciation in Plants

Determination of As (arsenic) speciation in plants is important for understanding As metabolism in plants and for assessing the toxicity of plant As to the consumers at the higher trophic levels. Research in this area has been greatly advanced in recent years with the aid of analytical techniques such as HPLC/ICP-MS/ES-MS and synchrotron radiation–based X-ray absorption spectroscopy (XAS). Analyses of a range of plant species collected from As-contaminated environments have shown that both terrestrial and freshwater aquatic plants contain predominantly As$_i$ (33, 53, 54). Both arsenate and arsenite are present, but their relative proportions vary among the plant species tested. However, sample preparation methods such as freeze-drying and chemical extraction used in these studies may cause oxidation of arsenite. More recent studies on plants grown under a controlled environment and fed with arsenate show that As is present predominantly as trivalent species [As(III)] (e.g., 22, 95, 101, 149). Arsenite is also the major species in the fronds of As-hyperaccumulating ferns (29, 66, 135). Furthermore, most of the arsenite in the root and shoot tissues of *Arabidopsis thaliana* and *Brassica juncea* is coordinated with the sulfydryl groups of thiol-rich peptides such as glutathione (GSH) and phytochelatins (PCs) (22, 95). Using HPLC coupled with ICP-MS and ES-MS, Raab et al. (101) showed that arsenite is complexed with a variety of thiol compounds in sunflower (*Helianthus annuus*);

here the most prominent complexes are GS-As(III)-PC$_2$, As(III)-PC$_3$, As(III)-(PC$_2$)$_2$, and As(III)GS$_3$. This complexation is not surprising because arsenite has a high affinity to sulfydryl groups, with the coordination in a 1:3 stoichiometry (21, 111). However, As hyperaccumulators such as *P. vittata* and *Pteris cretica* appear to be exceptions, containing arsenite mostly as uncomplexed species, due to low PC concentrations in tissues (100, 158).

Many plant species also contain small amounts of methylated As compounds, such as MMA, DMA, TMAO, and, occasionally, the tetramethylarsonium ion (33, 53, 54). In one study (33), methylated As species were reported to be the major As compounds in red clover (*Trifolium pratense*) growing on a site with high soil As, with MMA and DMA accounting for 35% and 24%, respectively, of the As extracted by methanol-water extract. Monomethylarsonous acid with As in the trivalent state [MMA(III): CH$_3$As(OH)$_2$]] can be complexed with thiol compounds, such as MMA(III)-PC$_2$ identified in the roots of sunflower exposed to inorganic As (101). Although pentavalent As does not form complexes with thiol groups directly, pentavalent As in DMA can bind to GSH when it is activated by sulfide, forming the dimethylarsinothioyl glutathione (DMAS-GS) complex in the sulfur-rich plant species *Brassica oleracea* (103).

Arsenosugars are found in some terrestrial plant species at low levels (53, 54); whether they are synthesized by plants is not known.

Arsenic in Food Plants and Implications for Human Health

For populations not exposed to elevated As in drinking water, foods represent the main sources of As intake for humans. Dietary intake of total As ranges from 10 to 200 μg per person per day in various countries (112, 145). Although seafood accounts for the majority (60%–90%) of the total dietary intake of As in countries such as the United States, Canada, and Japan, most of the As in seafood is present in organic forms (mainly

arsenobetaine in fish) that are relatively nontoxic (112). It is As$_i$ species that are of particular concern because they are chronic human carcinogens. The Joint FAO/WHO Expert Committee on Food Additives recommends a provisional tolerable weekly intake (PTWI) of As$_i$ of 15 μg kg^{-1} body weight, equivalent to 130 μg day^{-1} for a 60-kg person (145). No value has been set for organic As due to the lack of toxicological data. Arsenic in terrestrial food plants is dominated by As$_i$ (112). Dietary intake of As$_i$ from terrestrial food plants is generally low except for populations with rice as the staple diet (76, 112). In the Bengal Delta region, where As-contaminated water has been used for irrigation, relatively high concentrations of As have been reported in some vegetables and spices (107, 138), with As present only in inorganic forms (138). However, among all food categories, consumption of rice makes the largest contribution to the dietary intake of As$_i$ (76). In a global survey of 901 samples of polished rice, total As concentration varied from 10 to 820 μg kg^{-1} with a mean of 150 μg kg^{-1} (76). A global "normal range," i.e., not from an As-contaminated environment, of 80–200 μg kg^{-1} has been suggested (151). Arsenic contamination due to irrigation with As-tainted groundwater in South Asia or mining activities in China has resulted in further elevation of As levels in rice (74, 138, 160). For comparison, As concentrations in wheat grain or flour are generally <50 μg kg^{-1}, with a mean value approximately tenfold lower than that of rice (107, 112, 141).

The main As species in rice grain are As$_i$ and DMA, with MMA occasionally found at minor levels. The relative proportions of As$_i$ and DMA vary widely: The percentage of total As present as As$_i$ varies from 10% to 90% (76, 138, 139, 152). Rice produced in Asian countries contains predominantly As$_i$ (76), whereas rice produced in the southern-central states of the United States has considerably higher levels of total As but lower percentages of As$_i$ than in Californian or Asian rice (139, 140, 151, 152). The reasons for the high percentage of DMA in U.S. rice are unclear. Soil properties and

conditions, such as the composition of microbial community and the redox state, may influence As biomethylation in soil, and subsequently the uptake by rice. Some preliminary evidence suggests that a high content of soil organic matter and reducing conditions may be favorable to As biomethylation (41, 78). Genotypic differences in the percentage of DMA have been reported among rice cultivars (56, 59, 87); whether this reflects a difference in uptake/translocation of methylated As or the ability of As biomethylation in planta remains to be elucidated. The pathway of As methylation is discussed later, but clearly much remains unknown regarding the source of DMA, whether soil- or plant-derived, and how the uptake or in planta biomethylation is influenced by environmental factors. It is often assumed that DMA and MMA (both with As in the pentavalent oxidation state) are less toxic to humans than As_i (112). However, pentavalent MMA and DMA can be reduced to trivalent species as metabolic intermediates, and these trivalent intermediates are actually more cytotoxic and genotoxic than As_i (126).

Another factor to consider is the localization of As in rice grain. Elemental mapping using synchrotron X-ray fluorescence shows a striking accumulation of As on the periphery of rice grain in spots probably corresponding to the ovular vascular trace (**Figure 3a**) (61, 73), which is the maternal tissue containing xylem and phloem responsible for the transport of nutrients to the filial tissues of aleurone and endosperm. This distribution pattern explains why the rice bran (comprising mainly pericarp, vascular trace, aleurone, and embryo) is more enriched in As than in the polished white rice (endosperm) (120). Moreover, the bran fraction has a higher percentage of As_i than the endosperm; the latter has a higher percentage of DMA. Milling of rice to remove the bran fraction is a way to decrease As concentration, although this process also removes health-beneficial nutrients. On the other hand, rice bran and bran products have been marketed as
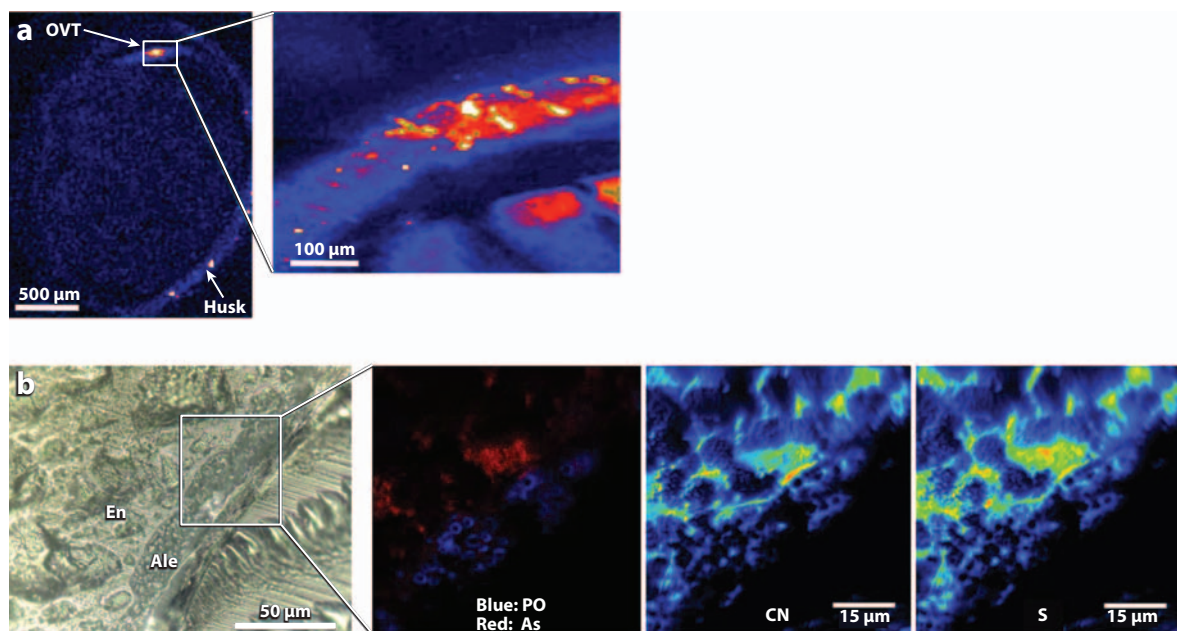
Figure 3

Arsenic distribution in rice grain. (*a*) Synchrotron X-Ray Fluorescence mapping of As in a cross section of rice grain with husk (61). OVT: ovular vascular trace. (*b*) Nano-SIMS subcellular localization showing the distribution of As with protein (CN) and S in the rice endosperm (En). Note the strong PO signal in the phytate granules in the aleurone (Ale) cell (82).

health foods; this may not be appropriate because of their high As_i concentrations (120). Within the endosperm, As is associated with the protein matrix especially in the subaleurone cells, and is possibly complexed with the sulfydryl groups (82) (**Figure 3b**).

With the information on As speciation in rice, the potential contribution of eating rice to the dietary intake of As_i can be estimated. For example, a typical Bangladeshi adult consuming 0.5 kg locally produced rice per day would have ingested As_i equivalent to 20%–150% of the PTWI, excluding As from other food sources and drinking water (138). Meharg et al. (76) further estimated that the potential excess cancer risk from rice As_i is significant for Asian populations, at a median of 22 and 15 per 10,000 people for Bangladeshi and Chinese, respectively, substantially higher than the U.S. EPA's upper risk target for carcinogens of 1 per 10,000. There is little risk for adult populations that consume only small amounts of rice (<50 g day^{-1}). Rice is commonly used in baby foods due to its low allergen potential. A range of rice-based baby foods were found to contain 60–160 µg kg^{-1} inorganic As (75). Arsenic exposure from these baby foods is lower than the PTWI, but considerably higher than that from drinking water at the current WHO guideline level of 10 ppb As.

ARSENIC TRANSPORT AND METABOLISM

The pathways of As uptake and metabolism in plants have recently been reviewed in depth (157). Below is a brief account of the key processes with emphasis on new findings and unresolved questions (**Figure 4**).

Arsenic Speciation in the Rhizosphere

The rhizosphere microenvironment may be substantially different from the bulk soil. The difference is particularly pronounced for wetland plants with roots growing in a generally anaerobic environment, where arsenite is the predominant As species in the bulk soil solu-

tion. However, arsenite may be partly oxidized to arsenate in the rhizosphere because of oxygen release through the arenchyma tissue of wetland plant roots. Moreover, ferrous iron is oxidized to form Fe hydroxide/oxyhydroxide precipitate [mostly ferrihydride: $Fe(OH)_3$], which is then coated onto the root surface forming an Fe plaque (11, 38, 59) (**Figure 1**). The Fe plaque has a strong affinity for the adsorption of arsenate, thus retaining arsenate on the root surfaces, although uptake of some arsenate into root cells is possible. The Fe plaque was found to have a significant effect on the absorption kinetics of As by rice roots, decreasing arsenate uptake but increasing arsenite uptake (18).

Although arsenate is the main species taken up by plants growing in aerobic soils, there is evidence of the presence of arsenite in the rhizosphere (131, 132). The occurrence of arsenite is likely a result of arsenite efflux from roots (60, 149). Arsenite extruded by roots may be reabsorbed by the roots or oxidized to arsenate in the rhizosphere.

Arsenic Transport

The most common forms of As in soil solution available for plant uptake are arsenate, arsenite, MMA, and DMA. Their uptake mechanisms are described below.

Arsenate uptake. With dissociation constants (pK_a) of 2.2, 6.97, and 11.5, most arsenic acid (H_3AsO_4) is dissociated as the oxyanions $H_2AsO_4^-$ or $HAsO_4^{2-}$ under normal pH conditions (pH 4–8), and they are the chemical analogs of corresponding phosphate ions. Arsenate is taken up by plant roots via phosphate transporters (**Table 1**). Evidence for this comes from physiological and electrophysiological studies showing a potent inhibition of phosphate on arsenate uptake (e.g., 1, 6, 130) and recent reports that *A. thaliana* mutants defective in phosphate transport are more tolerant to arsenate (17, 35, 114). In fact, some of these mutants were identified based on arsenate toxicity screening (17, 35). Furthermore, arsenate represses genes involved in the phosphate
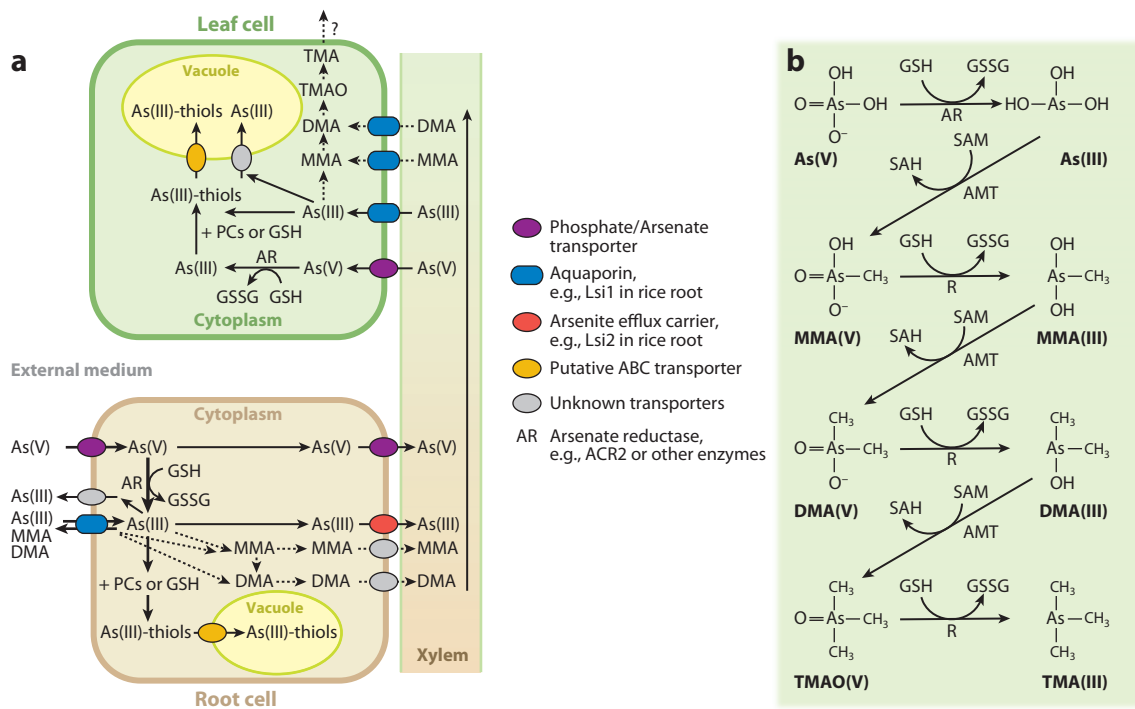
Figure 4

Arsenic uptake and metabolism in plants. (*a*) A simplified schematic diagram of arsenic transport and metabolism in plants. The thickness of arrow lines is indicative of the relative flux. Transporters for As uptake into leaf cells are assumed to be similar to those in roots, but there is little knowledge of their identities. (*b*) The Challenger pathway of arsenic methylation in microorganisms. AR: arsenate reductase; R: reductase; AMT: arsenic methyltransferase; GSH, reduced glutathione; GSSG, oxidized glutathione; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

starvation response, suggesting that arsenate may mislead the phosphate sensor and interfere with the phosphate signaling mechanism (17). Different phosphate transporters may vary in their affinity for arsenate. For example, the As hyperaccumulator *P. vittata* appears to have a higher affinity for arsenate than nonhyperaccumulator plants (96, 135). Presently the relative affinities of various phosphate transporters for arsenate and phosphate are poorly characterized. This information can be gained by assays of arsenate/phosphate transport activities in heterologous expression systems such as yeast or *Xenopus laevis* oocytes. Such information is needed for manipulation of plants for either decreased or enhanced arsenate uptake.

Arsenite uptake. In contrast to arsenate, arsenous acid (H_3AsO_3, pK_a = 9.2, 12.1, and 13.4) is mostly undissociated at normal pH conditions (>94% undissociated at pH <8.0). Therefore, plant roots take up arsenite mainly as the neutral molecule $As(OH)_3$. As in microorganisms and mammalian tissues (8), arsenite enters plant root cells via some aquaglyceroporin channels. In higher plants, the nodulin 26-like intrinsic proteins (NIPs) are the structural and functional equivalents of the microbial and mammalian aquaglyceroporins (133). NIPs are a subfamily of the plant major intrinsic proteins (MIPs), collectively known as aquaporins or water channels (69). Recent studies have shown that a number of NIPs are permeable to arsenite (9, 46, 49, 65) (**Table 1**). NIP aquaporins mediate transport of a range of small neutral molecules including ammonia, urea, boric acid, and silicic acid (69, 133). Whereas the permeability for boric and silicic acid is restricted to

Table 1 Plant membrane transporters for arsenic and other related substrates

Transporter group	Subgroup	Protein name	Functional assay system and transport substrates		References
			Plant assay	Heterologous assay (yeast, oocyte)	
Phosphate transporter		AtPht1;1	Phosphate, As(V)[a]		(35, 114)
		AtPht1;4	Phosphate, As(V)[a]		(114)
NIP aquaporin	I	AtNIP1;1	As(III)	As(III), glycerol, water	(49, 136)
		AtNIP1;2	As(III)	As(III), glycerol	(49, 136)
		OsNIP1;1		As(III)	(65)
	II	AtNIP5;1	B, As(III)	B, As(III), Sb(III), water	(9, 49, 124, 125)
		AtNIP6;1	B	B, As(III), Sb(III), glycerol, formamide, urea	(9, 125, 134)
		AtNIP7;1	As(III)	As(III), Sb(III)	(9, 46)
		LjNIP5;1		As(III)	(9)
		LjNIP6;1		As(III)	(9)
		OsNIP3;1		As(III)	(65)
		OsNIP3;2		As(III), Sb(III)	(9)
	III	OsNIP2;1 (Lsi1)	Si, Ge, As(III), MMA, DMA	Si, Ge, As(III), MMA, Sb(III), B, urea	(9, 55, 63, 65, 80)
		OsNIP2;2 (Lsi6)	Si, Ge	Si, As(III), B, Sb(III)	(9, 65, 80, 150)
Efflux carrier		OsLsi2	Si, Ge, As(III)	Ge	(64, 65)

[a]Based on tolerance to arsenate.

a few members of NIPs (80, 123), arsenite permeability is widespread in different subclasses of NIPs (157) (**Table 1**).

In rice roots, Lsi1 (OsNIP2;1), which is highly expressed in the distal side of the plasma membranes of the exodermis and endodermis cells where Casparian strips are formed, is a major entry route for silicic acid (63) and arsenite (65); mutation in this protein resulted in a 60% loss of the arsenite influx in the short term. However, the effect of Lsi1 mutation on As accumulation in rice shoots is relatively small over a longer growth period (65). This may be because aquaporin channels such as Lsi1 conduct solute transport in both directions depending on the concentration gradient. When supplied with arsenate, which is taken up by phosphate transporters and reduced to arsenite in root cells, the rice *lsi1* mutant effluxed smaller amounts of arsenite to the external medium than the wild-type plant (156). Similarly, heterologous expression of several *Arabidopsis* NIP

genes in yeast enhanced its tolerance to arsenate, possibly through increased arsenite efflux (9, 46). There are 9–13 NIP genes in the rice and *Arabidopsis* genomes. Some of the rice NIP genes are expressed mainly in the shoot and inflorescence tissues (108); their roles in As transport toward the grain remain to be investigated.

Although some NIP channels allow bidirectional transport of arsenite, efflux of arsenite from the exodermis and endodermis cells in rice roots toward the stele is mediated by the Si efflux carrier Lsi2 (65); mutation in Lsi2 had a dramatic effect on arsenite transport to the xylem and As accumulation in the shoots. Lsi2 is localized to the proximal side of the plasma membranes of the exodermis and endodermis cells, allowing solute efflux toward the stele for xylem loading (64). This process is a crucial step in the accumulation of As in rice shoot and grain; it is also the step in which Si exerts a strong inhibitory effect. Transport of Si mediated by Lsi2 is an active process driven by the

proton gradient (64). Lsi2 has a low degree of homology (18%) with the arsenite efflux transporter ArsB in *Escherichia coli* (64). Whether the substrates for Lsi2 are neutral molecules or anions of Si and arsenite is not known, although at the typical cytosolic pH of 7.5, only 2% of silicic acid and arsenous acid would be dissociated as anions.

Uptake of methylated As. The permeability of MMA and DMA across liposomes was estimated to be 1.4×10^{-13} and 4.5×10^{-11} cm s^{-1}, respectively (19). Therefore, simple diffusion of these molecules across the lipid layer of the plasma membranes would be too slow to account for their uptake into root cells. Recent studies have shown that the rice aquaporin Lsi1 also mediates the uptake of undissociated pentavalent MMA and DMA (55); 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 arsenite (p$K_a = 4.2$ and 6.1, respectively). This explains the sensitivity of their uptake to the external pH, increasing as the pH of the medium decreased, which is consistent with an increasing proportion of the undissociated species (55). At pH 5.5, uptake by rice roots decreases in the order of arsenite > MMA > DMA (1). The substrate properties that may explain this order are: (*a*) the extent of dissociation within the normal pH range, and (*b*) the number of hydroxyl groups; formation of the hydrogen bonds between the hydroxyl group of a substrate and the aquaporin protein along the pore structure greatly facilitates the flux through the channel (146). In contrast to arsenite, 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 (55). Despite its limited uptake (1, 102), for unknown reasons, DMA is more efficiently translocated from roots to shoots (55, 67, 102).

Long-distance transport. In most plant species analyzed, arsenite dominates in the xylem sap, suggesting that it is the main form loaded into the xylem (157). This is the case even when arsenate is supplied to plant roots, and is consistent with the fact that roots have a high capacity for arsenate reduction (see below). There is no evidence that arsenite in the xylem sap is complexed with thiol compounds (95, 101). In fact, complexation with thiols decreases arsenite mobility from roots to shoots (W.J. Liu & F.-J. Zhao, unpublished). Rice loads arsenite into xylem more efficiently than does wheat or barley, consistent with the highly expressed Si pathway in the former (118). *P. vittata* has an exceedingly efficient system to load arsenite into the xylem (119), but the underpinning mechanism has not been elucidated.

Little is known about phloem transport of As, such as the form of As transported and the transporters involved in phloem loading and unloading. In a recent study using rice panicles excised below the flag leaf node, Carey et al. (15) found that DMA was transported to the immature grain approximately 30 times more efficiently than arsenite. When the phloem flow was disrupted by stem girdling, transport of arsenite into the grain was decreased by tenfold, but that of DMA by only 50%. These results suggest that arsenite is delivered to rice grain mainly through the phloem, whereas both phloem and xylem pathways make an equal contribution to the transport of DMA to grain. Further evidence from a synchrotron μ-XRF study indicates that arsenite accumulates in the ovular vascular trace of the grain, whereas DMA permeates into the outer layer of the endosperm (15). Arsenic toxicity may interfere with As translocation to rice grain, resulting in decreased rather than increased grain concentration at higher As exposures (92).

Arsenic Metabolism

Two aspects of As metabolism are discussed here, whereas detoxification of As is considered later.

Arsenate reduction. The dominance of trivalent As in plant tissues when arsenate is the

form supplied to plants (22, 95, 149, 157) indicates a high capacity of arsenate reduction. Both roots and shoots of rice exhibit arsenate reduction activities (24), but roots may be quantitatively more important because arsenite is the main form found in the xylem sap of a number of plant species (157). The plant homologues of the yeast arsenate reductase Acr2p have recently been isolated from *A. thaliana* (23), *Holcus lanatus* (10), rice (24), and *P. vittata* (26). The plant ACR2 proteins are CDC25-like (cell division cycle) tyrosine phosphatases that have both phosphatase and arsenate reductase activities; PvACR2 from *P. vittata* appears to be an exception with only the activity of an arsenate reductase (26). Purified recombinant proteins of plant ACR2s are able to reduce arsenate in vitro using GSH and glutaredoxin as reductants. However, the in planta role of ACR2 remains unresolved, since there are conflicting reports on the phenotype of the ACR2 knockout or knockdown lines of *A. thaliana* with regard to arsenate tolerance and As translocation from roots to shoots (10, 23). Furthermore, the As speciation in the *Arabidopsis ACR2* knockout mutants is still dominated by As(III) (157), suggesting a functional redundancy of ACR2. The possible existence of other arsenate reductases or nonenzymatic reduction mechanisms warrants further investigation.

Arsenic methylation. An early study by Nissen & Benson (84) using paper chromatography suggested that P- and N-starved tomato plants, when supplied through roots with a radioactive [74]As (arsenate) solution for two days, were able to convert significant proportions of [74]As into methylated As species. In contrast, nutrient-sufficient plants showed little methylation of [74]As. In several recent studies where plants were fed only inorganic As in hydroponic culture, small amounts of methylated As were detected in plant tissues or xylem sap (79, 99, 101, 149). These reports provide circumstantial evidence for the existence of in planta biomethylation of As, albeit at a low level. Further unequivocal evidence should be sought

from experiments that employ axenic culture to rule out the possibility of microorganism-mediated methylation prior to plant uptake.

Little is known about the pathway and enzymology of As methylation in plants, although much can be inferred from the Challenger pathway (**Figure 4b**) established from studies on fungi (7). In this pathway, arsenite is the initial substrate for methylation catalyzed by *S*-adenosylmethyltransferase using the methyl donor *S*-adenosyl-ʟ-methionine (SAM). The *arsM* genes encoding As methyltransferases have been identified in the soil bacterium *Rhodopseudomonas palustris* (98) and in the thermoacidophilic eukaryotic alga *Cyanidioschyzon* sp. living in an As-rich geothermal environment (97). At a high temperature (60°–70°C), two *Cyanidioschyzon* ArsM proteins are able to methylate arsenite sequentially to mono-, di-, and trimethyl As compounds with the end product, TMA gas, being volatilized (97). SAM-dependent As methyltransferase activities were detected in the leaf extracts of bentgrass (*Agrostis capillaris*) (147). The rice genome contains methyltransferase genes with the same UbiE/Coq5 family protein motif as that of the microbial *arsM* genes (88), but the plant As methyltransferase(s) is yet to be identified. Arsenic methylation is accompanied by the oxidation of trivalent to pentavalent As; thus, a reduction step is also needed for further methylation to proceed. In humans this reduction is catalyzed by the glutathione transferase omega with reduced glutathione as the electron donor (4). Rice roots are able to reduce MMA(V) to trivalent MMA(III) in accordance with the Challenger pathway (55), but the enzyme(s) responsible for this reduction is unknown. Also not known is whether plants produce volatile species of methylated As such as TMA.

Arsenic Toxicity and Detoxification

The mode of toxicity differs between As species; arsenate interferes with phosphate metabolism such as phosphorylation and ATP synthesis, whereas arsenite binds to vicinal sulfydryl

groups of proteins affecting their structures or catalytic functions (43). Because arsenate is rapidly reduced to arsenite, the majority of the toxic effects of arsenate may actually be due to its reduction product, arsenite (43). Exposure to arsenate generates reactive oxygen species in plant tissues, and induces oxidative stress such as lipid peroxidation (e.g., 2, 39, 83, 104). Exposure to arsenite also upregulates a number of enzymes involved in the antioxidant responses (83, 104). Depletion of cellular reduced GSH may be the cause of As-induced oxidative stress. For nonhyperaccumulator plants, As toxicity often occurs at a shoot As concentration varying between 1 and 100 mg kg^{-1} (48), whereas the As hyperaccumulator *P. vittata* can withstand 5000–10,000 mg kg^{-1} of As in the frond tissue without suffering from toxicity (62, 128).

Although some plant species that colonize As-contaminated soils are able to restrict arsenate uptake through an adaptive suppression of high-affinity phosphate transporters (see Reference 72 for a review), As entering into cells has to be detoxified through complexation and/or vacuolar compartmentation. Another possible constitutive mechanism of detoxification in plants, suggested recently (60), is the efflux of arsenite to the external medium.

Complexation with thiol compounds. Arsenic is a strong inducer of PC synthesis (e.g., 111, 116). A number of genes or enzymes involved in glutathione synthesis, metabolism, and transport are upregulated in rice seedlings exposed to arsenate (2, 88), probably reflecting a higher demand for GSH under As stress. Blocking PC synthesis with L-buthionine-sulfoxime leads to hypersensitivity to both arsenate and arsenite (10, 110, 111). An *Arabidopsis* PC-deficient mutant is 10–20 times more sensitive to arsenate than is the wild type (37). Tolerance to arsenate is also enhanced by increased thiol synthesis in transgenic plants overexpressing a bacterial γ-glutamylcysteine synthetase gene (γ-ECS) (22, 58) or the *Arabidopsis* PC synthase gene (*AtPCS1*) (32, 57). These findings, and the fact that much of the arsenite in plant tissues is complexed with thiol-rich peptides (22, 95, 101), provide conclusive evidence that thiols, particularly PCs, play a crucial role in As detoxification in As nonhyperaccumulators. Note that the observed effect on arsenate tolerance is through the detoxification of arsenite, the product of arsenate reduction. In contrast, As hyperaccumulators such as *P. vittata* and *P. cretica* do not rely on a PC-based mechanism to detoxify As (100, 154, 158).

Vacuolar sequestration. The PC-arsenite complexes are likely to be stored in vacuoles. The yeast vacuolar transporter Ycf1p, a member of the ATP-binding cassette (ABC) superfamily, confers arsenite resistance by transporting the glutathione-*S*-conjugated arsenite [As(III)-(GS)$_3$] into the vacuole (34). The tonoplast vesicles prepared from *H. lanatus* roots are able to take up As(III)-(GS)$_3$ in a MgATP-dependent and charge-neutral fashion, consistent with ABC-mediated transport (10). The PC-arsenite complexes are also likely to be transported into vacuoles by an ABC protein, the identity of which is not yet known. In *P. vittata* fronds, As is stored in the vacuoles mainly as inorganic arsenite (62, 94). Because of the likely large concentration gradient from the cytoplasm to the vacuole, transport of arsenite across the tonoplast probably involves an energy-dependent active mechanism. A transporter(s) responsible for arsenite uptake into the vacuoles is not yet known but may be the key determinant of the hypertolerance phenotype in *P. vittata* and other hyperaccumulator plants.

POTENTIAL STRATEGIES FOR MITIGATING ARSENIC CONTAMINATION IN SOIL-PLANT SYSTEMS

Because of the complexity in the As transfer from soil to plants, not all strategies discussed below will be applicable in every situation. It is also recognized that social and economic conditions often dictate which strategy is feasible and applicable.

Agronomy

Because soil redox potential controls As mobility in paddy soil, water management can be used to minimize As toxicity to rice and As uptake and transfer into rice grain. Mid-season draining of water is an effective way to reduce the As-induced "straight-head" disease (spikelet sterility) in rice (137). Growing rice under aerobic soil conditions for the whole or part of the rice growing season markedly reduces As accumulation in the grain (56, 148). Aerobic rice is a new cultivation method to save water use, but yield is generally lower than for flooded rice (93); another potentially negative effect of aerobic rice is its tendency to accumulate more cadmium (5). Another way to maintain a higher redox potential is to grow rice in raised soil beds with water in the surrounding furrows; rice grown with this cultivation method contains significantly lower levels of As in the grain than does the conventional flooding method (25).

It has been reported that Si availability in soil has a large influence on As uptake by rice (12). This is not surprising because of the shared uptake pathway between Si and arsenite (65). It may be inferred that soils with a high Si availability, such as those developed from volcanic ash, are less likely to have the problem of excessive As accumulation in rice. In a greenhouse study, the addition of Si fertilizer markedly decreased As accumulation in rice shoots and, to a lesser extent, the concentration of As_i in the grain (56). Silicon fertilizers are commonly used in some rice-growing regions for yield benefits; their uses in As-contaminated paddy soils may prove to be an effective and practical way to mitigate the As accumulation problem.

Breeding

Significant genetic variations in the As concentration of rice grain have been reported (87), although there are also strong genotype by environment interactions (86). Rice cultivars with red bran are associated with higher As concentrations (87); whether there is a mechanistic link between bran color and As accumulation remains to be investigated. Quantitative trait loci (QTLs) have been reported for As concentrations in rice roots, shoots, and grain in a greenhouse study; the two QTLs associated with grain As explained 35% and 26% of the phenotypic variance (153). In the future, robust QTLs may be used in molecular marker-assisted breeding of low As-accumulating rice.

Enhanced tolerance to As is a useful trait for more contaminated environments that may result in toxicity, e.g., paddy fields contaminated with As from irrigation water (92). Genetic variation in arsenate tolerance has been reported in rice (89). In this study using a rice mapping population, arsenate tolerance is mapped onto three loci with epistatic interactions between them: progeny inheriting any two of the three genes from the tolerant parent exhibit tolerance (89). These loci have no apparent link with phosphate transporter genes.

Root-Induced Soil Manipulation

A recent greenhouse study showed that As accumulation in straw and grain correlated negatively with root porosity and the rate of radial O_2 release among 25 rice cultivars, presumably through the effect of O_2 release on Fe plaque formation, arsenite oxidation and subsequent arsenate retention on the Fe plaque (77). Breeding for rice cultivars with stronger O_2 release characteristic may have the potential for decreasing As accumulation. However, it should be emphasized that the rhizosphere effect on As uptake by wetland plants is complex, and Fe plaque may serve as a sink or a source of As at different growth stages of plants.

Phytoremediation

The exceptional ability of *P. vittata* and other hyperaccumulators to accumulate As may be explored in phytoremediation strategies. Although greenhouse studies demonstrate a considerable potential of As extraction from the soil by *P. vittata* (e.g., 129), results from two small-scale field trials are less promising owing to low biomass production (<1 t dry biomass ha^{-1}) (51, 109). Over the two-year growth

period, the total As removal by *P. vittata* fronds was only about 1% of the soil As in the top 30 cm of depth (51). The efficacy of phytoextraction is determined by biomass production and the TF; a combination of 10 t biomass ha^{-1} and an As TF of 20 could reduce soil As in the top 20 cm of depth by half after ten plant harvests (70). A number of factors should be taken into account when evaluating As phytoextraction strategies. First, the As TF of *P. vittata* can vary from below 1 to 100 depending on the As bioavailability in soil; As contamination from geogenic or mining sources is generally associated with a low bioavailability and hence is difficult to phytoextract from the soil (113). Second, even with an optimal combination of biomass and transfer factor, phytoextraction may be feasible only in low to moderately contaminated soils. Third, all known As hyperaccumulating ferns are from tropical or subtropical regions and do not thrive in cooler regions. Fourth, these ferns could be invasive plant species and their introduction to nonindigenous areas should be evaluated carefully with regard to potential ecological consequences.

Genetic Engineering

Different strategies of genetic manipulation may be pursued depending on the goal of modification, e.g., increased tolerance to better withstand an As-contaminated environment, increased uptake and tolerance for phytoextraction, decreased uptake, and/or increased methylation for improved food safety (127, 159).

A moderate increase in As tolerance has been demonstrated in transgenic plants overexpressing genes involved in the synthesis of PCs or their precursor GSH (32, 57). These studies show that enhanced PC synthesis in the transgenic plants alone does not lead to more As accumulation in the shoots. Overexpression of both *γ-ECS* and *PCS* in *Arabidopsis* produces a greater effect on As tolerance and accumulation than does overexpression of either gene alone (36). Dhankher et al. (22) demonstrated that dual overexpression in *Arabidopsis* of two

E. coli genes, the arsenate reductase gene *arsC* in the leaves driven by a light-induced soybean RuBisCo promoter and *γ-ECS* in both roots and shoots driven by a strong constitutive actin promoter, substantially enhances both the tolerance to and accumulation of As in the shoots. The leaf-specific expression of *arsC* presumably enhances arsenate reduction, even though the endogenous activity of arsenate reduction is already very high [>96% As present as As(III) in the untransformed plants], whereas *γ-ECS* overexpression boosts the biosynthesis of thiol-rich peptides for As(III) complexation. These results imply that enhanced shoot tolerance has the effect of driving more As accumulation in shoots. In future it may be possible to engineer high-biomass plants for As phytoextraction using genes from *P. vittata*, specifically those responsible for efficient xylem loading of As and detoxification in fronds, although the molecular mechanisms for As hyperaccumulation are obscure at present.

For crop plants, it is not possible to block the entry of arsenate or arsenite into plants entirely because of their shared transport systems with essential or beneficial elements. However, it may be possible to identify variants of phosphate transporters, NIP aquaporins, or Lsi2-like carrier proteins that are more discriminative against As. Enhanced PC production in roots may be a strategy to restrict As translocation to shoots through arsenite-PC complexation and vacuolar sequestration in roots. Another target would be to increase in planta As methylation to convert the more toxic As$_i$ to less toxic methylated As species, or even to volatilizable forms of As. This may be achieved by overexpression of plant genes coding for As methyltransferases, which have not yet been identified, or of microbial or algal *arsM* genes (97, 98).

CONCLUDING REMARKS

The nature of As being redox-active and highly toxic to organisms, and its propensity to be methylated, make As an interesting and complex element to study. Arsenic uptake and metabolism in plants need to be placed in a

wider context with regard to the biogeochemical cycling of As in the environment. Bioavailability and speciation of As in soil are strongly dependent on the environmental conditions; this knowledge is important as it determines the extent of As accumulation by plants and the consequences of As contamination in the food chain. The widespread As contamination in the environment, the mobilization of As into rice grain even in soils with baseline As concentrations, and the realization that excessive As accumulation in rice can present a health risk to humans have provided the recent impetus in research on this subject area.

Significant progress has been made in recent years in the understanding of As uptake, speciation, and detoxification in plants. There are, however, substantial knowledge gaps, especially with regard to the mechanisms of As sequestration in the vacuoles and of As loading and unloading in xylem and phloem, the regulation of As accumulation in grain, and the pathways and enzymes responsible for arsenate reduction and methylation. Recent advances in the analytical techniques for As speciation have been instrumental in enhancing our understanding of As biogeochemical cycling and plant As metabolism. Combining these analytical tools with molecular genetics and functional genomics should provide ample opportunities for unraveling the mechanisms of As transport, metabolism, and regulation.

SUMMARY POINTS

1. Environmental conditions influence As speciation in soil and its availability to plants. Flooding of paddy fields leads to mobilization of arsenite. Methylated As may be present in soil as a result of microbial and/or algal biomethylation, or from past uses of methylated As pesticides.

2. Arsenate is taken up by plant roots through phosphate transporters, whereas the uptake of undissociated arsenite and methylated As is mediated, at least partly, by NIP aquaporin channels.

3. Rice is efficient at As assimilation owing to arsenite mobilization in flooded paddy soil and arsenite uptake sharing the highly expressed Si pathway. Arsenic accumulation in rice grain represents a potential health risk to humans.

4. Arsenate is readily reduced to arsenite, which is detoxified by complexation with thiol-rich peptides and sequestrated in the vacuoles in As nonhyperaccumulating plants.

5. Excessive accumulation of As in rice can be mitigated through agronomic and crop-breeding strategies. Genetic modification may be employed to engineer plants more tolerant to As, or with reduced uptake for improved food safety.

FUTURE ISSUES

1. Genes and enzymes responsible for arsenate reduction in planta require further elucidation.

2. How is arsenite loaded into xylem, the bottleneck step in As accumulation in the shoots?

3. How do hyperaccumulators achieve exceedingly efficient root-to-shoot translocation of As?

4. What are the transport mechanisms for different As species through phloem, especially as to grain?

5. How are arsenite or arsenite-thiol complexes transported across the tonoplast for vacuolar sequestration?

6. Do plants methylate As, and if so, do they vary in this property, and what are the key genes involved?

7. Do plants volatilize As?

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Errata

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