Chapter

Arsenic Phytoremediation: Finally a Feasible Approach in the Near Future

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Abstract

Arsenic, a class-1 carcinogenic, is a ubiquitous metalloid found in the atmosphere, soils, natural waters, and organisms. The World Health Organization (WHO) estimates that hundred million people worldwide might be chronically exposed to arsenic in drinking water at concentrations above the safety standard. Conventionally applied techniques to remove arsenic species show low removal efficiency, high operational costs, and high-energy requirements. The biological methods, especially phytoremediation, could be cost-effective for protecting human health and the environment from toxic metal contamination. Plants, as sessile organisms, have developed an extraordinary capacity to tolerate arsenic through three main strategies: uptake repression, sequestration into the vacuole, or extrusion. Therefore, arsenic perception and tolerance require a coordinated response that involves arsenic transporters, extrusion pumps, vacuole transporters, and the activation of the phytochelatin biosynthetic pathway. For phytoremediation to become a feasible strategy for arsenic removal from contaminated sites, it is essential to completely understand the molecular mechanisms of arsenic uptake, extrusion, and sequestration, as well as how this response is coordinated. The new genome-wide technologies provide a unique opportunity to understand the molecular mechanisms underlying arsenic perception and accumulation in plants that will open up new possibilities for phytoremediation of arsenic-contaminated waters and soils.

Keywords: arsenic, phytoremediation, hyperaccumulator, biotechnological approaches, *Arabidopsis thaliana*, *Pteris vittata*, rice

1. Introduction

Arsenic is a poison naturally present in the Earth's crust, where it constitutes the 20th most abundant element. Contamination with this element is derived from natural activities, such as volcanism, erosion, or leaching into aquifers, as well as from anthropogenic activities, like mining, smelting ores, or industry. The most abundant forms of arsenic in the environment are the inorganic oxyanions of arsenite (As(III)) and arsenate (As(V)), both of them highly soluble in water [1–3]. As(III) is more toxic and relatively mobile in contaminated soils, whereas As(V) is considered relatively less toxic.

Arsenic from contaminated soil and water resources poses an environmental threat for all living beings, since it is bioavailable for crops and animals, eventually

entering the food chain. Once inside the cell, As(V) interferes with phosphate metabolism, due to its structural analogy to this compound, while As(III) binds to sulfhydryl groups of proteins affecting their functions. Arsenic toxicity is, thus, mostly derived from its interference with enzymes involved in DNA synthesis and repair and cellular energy homeostasis, among others [4]. This metalloid is a well-documented genotoxic agent and class I carcinogen affecting the skin and internal organs. Arsenic effects on human health have been a matter of comprehensive reviews [5–10]. For these reasons, chronic arsenic exposure, reported in many regions of the world, constitutes an environmental and public health problem of devastating proportions, particularly in Bangladesh [11, 12]. Besides the exposure to contaminated water, another major source of chronic arsenic exposure is derived from dietary consumption of contaminated rice. This crop accumulates relatively high amounts of arsenic and constitutes the main staple food for over 3 billion people across the world, mostly in Asia, being also extensively used for infant feeding [13–17].

For all these reasons, it is imperative to develop strategies to efficiently limit the effects of arsenic contamination and its entry in the food chain. Physicochemical treatments have been assayed for arsenic-contaminated waters, and recently nanomaterials and metal-organic frameworks (MOFs) are emerging as new promising adsorbent materials. In general, physicochemical treatments are effective at high arsenic concentrations but fail to remove it when contamination levels are low. Another drawback of these techniques is their economic cost, hardly affordable in economically poor rural areas [18, 19].

An alternative approach is the use of living organisms to mitigate arsenic contamination, a strategy named bioremediation. This strategy takes advantage of the ability of microorganisms and plants to tolerate and accumulate arsenic present in nature [20]. The use of plants to clean up contaminated environments is an environmentally friendly green technology, well-accepted sociologically, relatively easy to implement, and potentially cost-effective that can be used to treat both contaminated soils and waters [21–23]. The main drawbacks of this strategy include the requirement of prolonged periods of time to be effective, lack of reproducibility due to environmental constraints, and the handling of the plant-fixed arsenic waste disposal. Another limitation is that high concentrations of arsenic may inhibit plant growth and, therefore, phytoremediation performance. A recent report has shown that plants accumulating arsenic could efficiently extract and convert it into valuable compounds, such as arsenic nanoparticles, with potential medical applications [24]. In addition, recent advances in the understanding of arsenic toxicity and the pathways of arsenic uptake, accumulation, and tolerance in different plant species, mostly in *Arabidopsis thaliana* (*A. thaliana*) and rice, have enabled the identification of potential biotechnological targets to overcome these limitations and will improve efficiency of arsenic phytoremediation in the near future [25–28].

In this review, we focus on the phytoremediation strategy to combat arsenic poisoning: we provide a brief overview on the mechanisms of arsenic perception and signaling in different organisms, as well as a survey of plants with proven or potential use for arsenic phytoremediation. Finally, we address several biotechnological approaches that could potentially improve efficiency of arsenic phytoextraction and enable the development of safer edible crops.

2. Arsenic perception and signaling

In this section, we present an overview of the known molecular mechanisms involved in the arsenic response particularly in yeast and plants that will be the basis to design new applied strategies in the near future.

2.1 Arsenic response studies in prokaryotes: the pioneer studies of ars operon in *E. coli*

The identification of arsenic resistance genes in prokaryotes was first reported over 50 years ago [29]. This laboratory identified one strain of *Staphylococcus aureus* (*S. aureus*) resistant to antibiotic, arsenic and several heavy metal compounds, due to the presence of a resistance factor (R-factor) that was located at the plasmid, pI258. Later on, another R-factor, responsible for arsenic resistance, was found in *Escherichia coli* (*E. coli*), the R773 plasmid [30]. This was the very beginning of arsenic detoxification gene discoveries. Few years later, arsenic resistance genes were cloned from the *E. coli* R773 plasmid, confirming that this plasmid is responsible for arsenic tolerance and contains all the genes involved in the arsenic response, named the *ars* operon [31]. Since then, many works have been done to elucidate the complexity of the R-factors and the genes that constitute the *ars* operon in several species [32].

In this review, we will describe the main genes composing the *ars* operon and their function; we will not focus on the distribution of this operon in prokaryotes, comprehensively reviewed previously [32].

The *ars* operon is widely variable, comprising a group of genes represented in different species with a different range of complexity [32] responsible for *ars*enic tolerance in prokaryotes. For instance, the *S. aureus ars* operon, named *arsRBC*, is composed of three genes, *arsR*, *arsB*, and *arsC*. However, other species such *E. coli* contain additional genes in the operon, like *arsD* and *arsA*.

One of the most important components of the *ars* operon is *arsR*, the key regulator of arsenic response in prokaryotes. This gene encodes a metalloregulatory protein (ArsR) that acts as a repressor of the whole operon [33, 34]. ArsR is a member of the SmtB/ArsR family [35], a transcriptional repressor in its homodimeric form that contains a helix-turn-helix DNA-binding domain and metal-binding sites [35–38]. Under non-arsenic conditions, *arsR* directly binds to the *ars* operon repressing the expression of the downstream genes. In the presence of arsenic, even at low concentrations, this metalloid interacts with the ArsR homodimer, inducing its dissociation from the DNA [35]. Therefore, ArsR is considered the main sensor of the system through direct interaction with arsenic.

Another component of the operon, *arsC*, encodes an arsenate reductase [39] involved in the reduction of As(V) into As(III) using glutathione (GSH) and glutaredoxin (GRX) as electron donors [40]. On the other hand, different variants of ArsC from other species (*S. aureus*) use thioredoxin as an alternative electron donor [40]. The third gene of the operon, *arsB*, encodes a membrane protein which functions as a proton antiporter, involved in As(III) extrusion [41]. The versatility of ArsB is extraordinary. In many species, ArsB is present as a single component in which the As(III) extrusion is coupled to electrochemical energy, in an ATP-independent way [41]. In contrast, in many other species, such as *E. coli*, ArsB appears complexed with *arsA*. ArsA encodes an ATPase that forms a complex with ArsB that functions as an efficient ATPase-dependent arsenic pump, named ArsAB. ArsA displays a specific metal-binding domain in which As(III) is coupled. Several studies confirm that the presence of both genes gives an extraordinary bacterial survival advantage under arsenic stress conditions compared to the bacterial species holding only the ArsB protein.

The last component is *arsD*. Initially, ArsD was described as a metalloregulatory protein [42] with a proposed role in the transcriptional regulation of the ars operon, similar to ArsR. Few years later, it was shown that ArsD is a metallochaperone and not a regulatory protein [43]. This was an important contribution due to the fact that very little was known about metallochaperones by that time. The authors suggested that, under non-arsenic conditions, ArsD weakly interacts with the complex

ArsAB (the energetic arsenic pump). However in the presence of As(III), this chemical species is able to bind to ArsD [44], forming the complex ArsD-As(III), which can subsequently strongly interact with ArsAB, and then, As(III) is extruded. Hence, ArsD provides an efficient fine-tune mechanism of As(III) extrusion tightly modulated by As(III) concentration.

In conclusion, in non-arsenic conditions, ArsR stays attached to the operator of the operon and halts the transcription and expression *of the ars-resistant genes*. Conversely, when arsenic is incorporated into the cells through aquaporins or high-affinity phosphate transporters (for As(III) and As(V), respectively), ArsR directly interacts with the metalloid, triggering the operon transcription. ArsC reduces As(V) into As(III), which directly binds to ArsD, which in turn brings As(III) together with ArsAB, leading to the extrusion of the metalloid in an ATP-dependent manner. The detailed knowledge of all these mechanisms involved in arsenic tolerance paved the way to understand how eukaryotes are able to cope with the presence of arsenic.

2.2 *Saccharomyces cerevisiae*: a suitable model to study arsenic response in eukaryotes

Saccharomyces cerevisiae (*S. cerevisiae*) is an excellent model for the study of biological processes in eukaryotic organism, including arsenic stress responses. In this model, a single locus comprising three genes, *ACR1*, *ACR2*, and *ACR3*, is mainly in charge of the arsenic response and tolerance [45].

ACR1 encodes an AP-1-like transcription factor that has been recently characterized [46]. *ACR1*, also named Yap8, is able to interact directly with As(III) due to the presence of three cysteines, similar to ArsR in prokaryotes. When Yap8 interacts with the metalloid, it suffers a conformational change that avoids its ubiquitin-proteasome degradation. As a result, Yap8 protein remains stabilized bonded to the *ACR2/3* promoter (both genes are controlled by the same promoter, but they are transcribed in opposite directions). In the presence of As(III), Yap8 facilitates the recruitment of RNA polymerase II and triggers the transcription of *ACR2* and *ACR3*. *ACR2* encodes an arsenate reductase enzyme that reduces As(V) to As(III) [47]. However, previous experiments have demonstrated that ACR2 alone is not capable of increasing arsenic tolerance in yeast, since it requires the expression of an additional member of the cluster, ACR3. *ACR3* encodes a transmembrane protein directly involved in As(III) transport [48]. It has been proved that *acr3* mutants are sensitive to both As(V) and As(III), leading to the conclusion that this transporter is not only able to extrude As(III) to the media but also to incorporate it into cell compartments.

Similarly to ArsR in prokaryotes, Yap8 is considered the arsenic sensor in yeast. There is only one difference between the mechanisms of action of both transcription factors: while ArsR is a repressor, Yap8 acts as an activator. The identification of Yap8 implied an important contribution, since this protein was the first arsenic sensor described in eukaryotes, giving rise to a promising model on how other arsenic sensors may act in higher organisms including plants.

2.3 Arsenic response in eukaryotes: *Arabidopsis thaliana*

We will focus this section on the mechanisms of arsenic uptake and detoxification in the plant model *A. thaliana*. This basic knowledge is crucial to develop novel strategies for phytoremediation of contaminated soils and waters and to obtain staple crops able to produce safe food when grown in contaminated lands. In recent years, huge efforts have been made to understand the underlying molecular

mechanisms of accumulation and tolerance of arsenic in plants [25–28]. Although much knowledge has been acquired over the last years, several aspects of the signal transduction pathway are still completely unknown; in particular, the desired arsenic sensor still needs to be identified in plants.

The mechanisms of incorporation and extrusion of As(V) and As(III) have been extensively described in *A. thaliana*. The prevalent chemical form of arsenic in soils is As(V); As(V) uptake is mediated by high-affinity phosphate transporters (PHT) from the *PHT1* family, mainly *PHT1;1* and *PHT1;4*, as a consequence of the structural similarity between these two anions [49]. As(III), present in paddy soils and anoxic environments, is incorporated by aquaporins from the nodulin 26-like intrinsic protein (NIP) subfamily, mainly *nip1;1* [50]. This family has been shown to be in charge of As(III) uptake and extrusion and in root-to-shoot translocation. Consistently, several studies confirm that *nip1;1* mutants are more tolerant to As(III) [50–52].

Once inside the cell, As(V) is rapidly transformed into As(III) through an As(V) reductase enzyme. Initially, AtACR2 was thought to be the main arsenate reductase in plants [53], which was identified by sequence homology with the yeast ACR2. A few years later, whether AtACR2 was the main As(V) reductase raised many questions among the scientific community [54], and a major role of AtACR2 remains to be confirmed. Later on, our group identified the gene (At2g21045) which encodes the major As(V) reductase in *A. thaliana*. The name of the gene refers to the QTL1, which confers the As(V) tolerance in *A. thaliana* as we reported by natural variation studies [55]. Afterwards the relevance of this protein in the arsenic response were corroborated by an independent group, which named it HAC1 from high arsenic content [56].

Once As(V) is reduced to As(III), it can be extruded by the previously mentioned NIP transporters. Even though As(V) tolerance in *nip1;1* mutants has not been tested yet, they are likely to display an As(V) hypersensitive phenotype, since they are not capable of extruding As(III) to the media. An alternative pathway for As(III) detoxification involves its sequestration by phytochelatins (PCs), forming PC-As(III) conjugates. PCs are peptides synthesized from glutathione and hold the basic structure (ɤ-Glu-Cys)n-Gly where n is a number ranging from 2 to 11 [57]. Indeed, PCs could be considered functional analogues of ArsD in terms of directly binding to As(III) [58]. ABCC transporters are ATP-dependent transporters responsible for the incorporation of PC-As(III) conjugates into the vacuole and thus essential for arsenic detoxification in *A. thaliana* [59].

In conclusion, arsenic in plants is incorporated into the cells either through the high-affinity phosphate transporters PHT (As(V)) or through NIPs (As(III)). Once inside the cell, it is reduced by ARQ1/HAC1 and subsequently gets sequestered by PCs. The resulting PCs-As(III) complexes interact with ABCC transporters mediating PCs-As(III) transport into the vacuoles. Alternatively, a fraction of As(III) can be extruded into the media through the NIPs transporters (**Figure 1**).

Overall, the detoxification mechanisms have been designed with the aim of protecting living organisms from the most dangerous chemical form of arsenic, As(III). Nevertheless, a novel phosphate vacuolar transporter has been recently identified [60]. Those mutants show an As(V) resistant phenotype since they cannot accumulate phosphate inside the vacuoles, and as a consequence it is likely that phosphate present in the cytoplasm provoked the repression of the As(V)/phosphate transporter *PHT1;1* preventing the entry of As(V). This novel mechanism provides the first insights into the crosstalk between As(V) and phosphate signal transduction pathways.

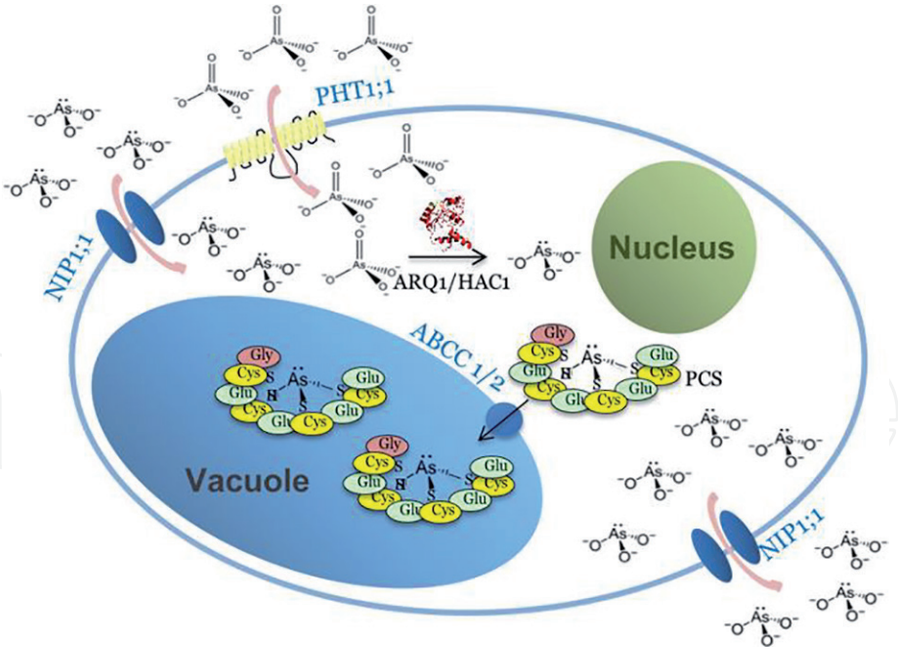

Figure 1.
Mechanisms of arsenic uptake and detoxification in A. thaliana.

3. Plant species with potential use for arsenic phytoremediation

The mechanisms of arsenic tolerance in plants—discussed above—confer adaptive responses that enable plant growth in the presence of this metalloid. There is an immense natural diversity in the arsenic response among different plant species. Some of them have developed an extraordinary capacity to grow in the presence of arsenic and hyperaccumulate this metalloid, holding a great potential to be used for phytoremediation strategies. The most critical parameter in plant species required for phytoremediation is a high extraction capacity. This can be achieved in different ways: via hyperaccumulation of the contaminant inside the cells or via high growth potential or high biomass production in the presence of the toxic compound. They should also be robust and adapted to a variety of biogeochemical environments, seasonal fluctuations, and climatic conditions, in order to obtain good extraction rates in different locations over the years. In addition, the ability to detoxify a variety of xenobiotics in complex degraded environments would be desirable.

A plant species is considered arsenic hyperaccumulator if it is able to accumulate more than 1000 μg g^{-1} of dry weight [61, 62]. The brake fern *Pteris vittata* (*P. vittata*) is the arsenic hyperaccumulator species par excellence, being the most extensively reported and the best characterized until the moment [63–69]. The arsenic phytoremediation potential of *P. vittata* was first described in 2001. It was reported to tolerate as much as 1500 μg g^{-1} arsenic in soil, accumulating up to 15,861 μg g^{-1} in 2 weeks and up to 22,630 μg g^{-1} in 6 weeks, under these conditions [70]. Since then, many reports have confirmed its arsenic hyperaccumulator capacity in greenhouse experiments and field conditions [63–69]. Some of them showed its utility to reduce arsenic contamination in crops grown intercropped with this fern [71–73]. Several studies have been conducted in order to elucidate the molecular mechanisms underlying the exceptional arsenic accumulation capacity and tolerance of *P. vittata*. Different aspects of arsenic uptake, detoxification, and tolerance have been characterized, showing differential strategies in relation to non-hyperaccumulator plants [74–82]. For example, the existence of an arsenic-inducible As(V)/phosphate transporter with high affinity for As(V) that could account for its great arsenic

accumulation capacity has been recently described [74, 83]. Likewise, a differential antioxidant response could be the mechanism underlying the different degrees of tolerance observed among different species from the *Pteris* genus [80]. Therefore, most probably, the extraordinary extraction capacity and tolerance of *P. vittata* result from a specific combination of all these new mechanisms. Genome-wide studies are providing vast information that will help to have a more complete picture of these mechanisms in the near future [81–83]. Among them, a relevant recent report has shown the utility of transcriptomic and tonoplast-proteomic strategies to uncover a regulatory system mediated by alternative splicing and long noncoding RNAs in response to As(III) and As(V) treatments, as well as the identification of different transporter families and other genetic components underlying arsenic response and tolerance in this hyperaccumulator fern [82]. Besides its intrinsic role in phytoremediation as hyperaccumulator, the characterization of the molecular mechanisms operating in *P. vittata* will provide new regulatory elements that may confer increased arsenic accumulation capabilities in other species by genetic engineering.

Other species have been evaluated for their arsenic phytoremediation potential. Many of them are endemic species that have been selected because of their ability to grow in arsenic-contaminated areas, and thus they are good candidates for their use as arsenic phytoremediators [61, 63, 84–87]. As mentioned above, the remediation of arsenic-contaminated waters and paddy soils deserves special attention due to safety concerns. Several wetland plants and aquatic macrophytes have been shown to hyperaccumulate arsenic when growing in contaminated environments and could contribute to solving this devastating trouble [66, 88–94].

4. Potential transgenic/biotechnological approaches for phytoremediation and arsenic-free crop development

As mentioned above, huge efforts have been made in recent years in order to understand the underlying molecular mechanisms of accumulation and tolerance of arsenic in plants. These initiatives provide a basic knowledge that is crucial to develop novel phytoremediation strategies for environmental cleanup and for producing safe staple crops grown in contaminated lands. The key elements of the arsenic response in the model plant *A. thaliana* and other plant species will make possible to tailor the arsenic uptake and accumulation in crops for different purposes [28, 95, 96].

Phytoremediation of arsenic-contaminated areas requires the development of new plant varieties with enhanced uptake and accumulation of this metalloid in order to remove as much arsenic as possible from the environment. On the contrary, inhibiting the arsenic uptake and translocation to the edible parts and promoting its extrusion outside the cells will be imperative for the development of safe and productive crops grown on arsenic-contaminated lands [25, 96].

4.1 Generation of arsenic transgenic plants for phytoremediation purposes

One of the drawbacks of the phytoremediation approach is that it may have low efficiency as a consequence of phytotoxicity when plants are exposed to high arsenic levels. Moreover, some natural hyperaccumulators do not produce enough biomass, which is crucial for successful phytoremediation, and are also restricted to very specific climatic conditions [96]. Next-generation "omic" approaches are paving the way to increase plant tolerance and extraction of arsenic, holding promising results for phytoremediation [97]. Arsenic uptake, accumulation, and tolerance can be augmented through the modulation of influx/efflux plasma membrane transporters, the regulation of the arsenate reductase activity, and the increase of the amount

of PCs and glutaredoxins [98]. The coordination of this element in the arsenic perception and tolerance is a key aspect to sequester arsenic from the cytosol into the vacuoles or its translocation from root to shoot via xylem loading [96, 99].

4.1.1 Arsenic uptake enhancement: phytoextraction

The knowledge acquired in studying the mechanisms of arsenic uptake and translocation by hyperaccumulators such as *P. vittata* (see above) provides important information to generate promising phytoremediation tools. Root-to-shoot translocation studies will contribute to obtain plants with increased shoot arsenic content, which could greatly benefit phytoremediation applications [98].

P. vittata displays an enhanced As(V) uptake derived from both increased *PvPHT1;3* expression and high affinity of this Pi/As(V) transporter for As(V). This transporter has greater affinity for As(V) than its counterpart from *A. thaliana*, *AtPht1;5* [74]. Therefore, we propose the heterologous expression of the transporter *PvPHT1;3*, which is more specific for As(V) uptake over Pi, in a high biomass plant as a potential strategy for extracting large amount of arsenic from contaminated lands or waters.

Another interesting example of a biotechnological application has been provided by the overexpression of the *PvACR3* transporter from *P. vittata* in *A. thaliana* [78]. This transporter mediates the translocation and storage of As(III) into the vacuolar system in *P. vittata*. *A. thaliana*-overexpressing plants display a significant increase of As(III) export from root to shoot, as well as an increment of arsenic tolerance. The strong expression of this transporter makes it to localize in the plasma membrane of the transgenic plants. This was expected to increase As(III) extrusion to the external medium or its sequestration in the root vacuoles, but there was an enhanced translocation of As(III) into the aerial parts instead [100]. Thus, it seems to be useful to constitutively express *PvACR3* transporter in a vigorous crop to facilitate root-to-shoot translocation for phytoremediation purposes.

Importantly, the next-generation gene-editing CRISPR/Cas9 technology is nowadays an emerging tool to obtain improved crops [101]. This technology is target-specific and allows targeting multiple genes in the genome with high efficiency and specificity. Thereby, this system opens up the possibility to obtain precisely edited crops with enhanced arsenic extraction and accumulation. For example, engineering the aquatic plant *Lemna* with CRISPR/Cas9 for point mutations in the As(V)/phosphate transporters and As(III)-PCs vacuolar transporters at the same time would be a feasible strategy for cleaning arsenic-contaminated waters.

4.1.2 Arsenic plant tolerance improvement: increasing thiol-rich compound production

As(III) chelated with sulfhydryl-rich proteins forms complexes that get sequestered into the vacuoles through vacuolar transporters. Therefore, arsenic tolerance in plants can be enhanced by modifying GSH and PCs [97]. The constitutive expression of the PC biosynthetic gene *AtPCS1* under the CaMV 35S promoter in the non-accumulator plant *Nicotiana tabacum* enhanced PC levels. This resulted in an increased capacity of vacuolar arsenic and cadmium accumulation in roots and improved plant tolerance to these toxic elements [102]. The level of GSH, a precursor of PC, is a limiting factor for high PC production [103], so we suggest to combine *PCS1* overexpression along with the GSH biosynthetic genes *glutamate-cysteine ligase 1* (*GSH1*) or *glutathione synthetase 2* (*GSH2*) overexpression to maximize arsenic accumulation and tolerance.

In plants, arsenic exposure increases GSH content, which has been correlated with the feedback induction and increased expression of glutathione S-transferases

(GSTs) [104]. GSTs quench reactive molecules with the addition of GSH and protect the cell from oxidative damage. Indeed, overexpression of a rice *glutathione s-transferase* in *A. thaliana* led to increased tolerance toward arsenic and other abiotic stresses [105]. This may be attributed to the lower accumulation of reactive oxygen species and enhanced GST activity. Thus, gene expression of *GST* gene family could help to evolve strategies for developing arsenic-tolerant crops through biotechnological tools in the future.

4.1.3 Rhizoremediation: a beneficial plant-microbe interaction for arsenic phytoextraction

Rhizoremediation takes advantage of the interaction between plants and bacteria living in the rhizosphere for phytoremediation purposes [96, 106]. Arsenic-resistant bacteria associated to the rhizosphere have been demonstrated to play an important role in promoting plant growth and arsenic phytoextraction capacities from contaminated soils [107, 108]. Some bacteria and fungi increase the capability of plants to cope with arsenic by the release of phytohormones such as indole-3-acetic acid and/or essential vitamins and iron. These nutrients promote plant growth and reduce arsenic cytotoxicity. In addition, some microorganisms play an important role in arsenic bioavailability by catalyzing redox reactions that enhance the efficiency of arsenic uptake by the plant roots [109].

Bacterial strains isolated from the rhizosphere of autochthonous plants grown on arsenic-contaminated industrial lands have been used to enhance phytoremediation in *P. vittata* [108]. These arsenic-tolerant bacteria promoted plant growth and tolerance, as well as arsenic phytoextraction. This is possible due to plant growth elicitors released by microorganisms in particular siderophore-arsenic complexes, which are easily available for the plants promoting arsenic translocation to the shoots. Furthermore, arsenic can be volatilized by reducing As(V) and As(III) to the organic compound arsine, which can be easily taken up by the plant and released to the air through the leaves.

4.2 Development of arsenic-free food crops

Overall, the generation of arsenic-free crops in the aboveground organs involves increasing root sequestration or extrusion in order to reduce root-to-shoot translocation of the metalloid. Basic breakthroughs have been made in *A. thaliana* and other crop models, particularly in rice [25]. As already mentioned, rice naturally accumulates high amounts of arsenic in the form of As(III) when it is cultivated in paddy low-oxygen-containing soils, where As(III) is more prevalent [28]. For this reason, during the past decade, much research has been done to understand the mechanism of arsenic uptake, translocation, and grain filling. These studies are extremely useful for the production of rice with low concentrations of arsenic [25, 95].

4.2.1 Arsenate reductases and inositol transporters: from Arabidopsis to rice

AtARQ1/HAC1 constitutes the main As(V) reductase in the roots of *A. thaliana*. Knockout mutants of this gene show hypersensitivity to As(V) and increased translocation of arsenic to the shoots mediated by the phosphate transport system, leading to the hyperaccumulation of As(V) in aboveground tissues [55, 56]. Therefore, this As(V) reductase is essential to avoid root-to-shoot arsenic translocation. *OsHAC1;1*, *OsHAC1;2*, and *OsHAC4* are close rice homologues of *AtARQ1*/*HAC1*, highly expressed in roots. These genes are responsible for mitigating arsenic accumulation in rice shoots and grain. The overexpression of these rice As(V) reductases

in roots results in grains exhibiting low concentrations of arsenic compared to wild-type grains [110, 111].

In *A. thaliana*, inositol transporters *AtINT2* and *AtINT4* are responsible for As(III) loading into the phloem and are key transporters regulating arsenic accumulation in plant seeds [112]. Thus, mutations in these transporters could potentially avoid arsenic accumulation in rice kernel. However, orthologues of inositol transporters in rice still need to be identified.

4.2.2 Arsenic extrusion strategies

In yeast, ACR3 from *S. cerevisiae* is the main As(III) efflux pump [48], and orthologues of this protein have not been found in *A. thaliana* or in rice [78]. In order to enhance arsenic efflux to the medium, yeast *ScACR3* was expressed in *A. thaliana*, leading to improved arsenic tolerance [113]. Importantly, *ScACR3* was also expressed in rice under the control of the CaMV 35S promoter, leading to a greater increase of As(III) efflux and mitigating arsenic accumulation in rice grain compared to wild-type plants [114]. Similarly, the heterologous expression in plants of the As(V) efflux transporter *ArsB* from *E. coli* could result in less arsenic accumulation. Hence, heterologous expression of well-characterized arsenic transporters from bacteria and fungi in plants could be a useful approach for crop improvement, since they allow the development of plants capable of growing in the presence of toxic levels of arsenic. Nevertheless, plants have a remarkable capacity to extrude As(III) from roots. Several transporters of the NIP aquaporin family are involved in a bidirectional transport of As(III) in plants [51]. We suggest to enhance As(III) efflux by coordinating an increased As(V) reduction through *AtARQ1* overexpression along with *NIP1;1* overexpression.

4.2.3 Decreasing arsenic accumulation in rice kernel: Lsi1 and Lsi2 knockout approach

In rice roots, the aquaporin OsNIP2;1/Lsi1 is a major entry route for Si(IV) [28]. This carrier also mediates As(III) uptake and methylated arsenic compounds, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) [115]. Knockouts of this gene show significant lower levels of these arsenic species than wild-type rice plants at short times of exposure [116]. However at longer expositions, these knockout rice plants start to accumulate arsenic since there are bidirectional solute transporters driven by concentration gradient. Lsi2 is an additional aquaporin found in rice. Lsi2 mediates Si(IV) efflux and As(III) transport into the symplast avoiding the Casparian strip and then being easily loaded into the xylem [117]. A knockout mutation of this gene in rice leads to a dramatic reduction on As(III) transport to the xylem and its accumulation in the shoots and grains [117].

4.2.4 Arsenic sequestration in root vacuoles to avoid translocation

An *ACR3* orthologue that mediates As(III) transport into the vacuole was recently identified in *P. vittata* [78]. When ectopically overexpressed in *A. thaliana*, it significantly favors arsenic translocation to the aerial parts instead of the accumulation in the root vacuoles [118]. Therefore, it seems to be a good option to highly express this vacuolar transporter specifically in the root tissues in order to sequester arsenic in the root vacuoles and prevent As(III) from being translocated to the shoot and grains [97]. Additionally, the rice vacuolar transporter OsABCC1, an *A. thaliana* ABCC1 orthologue, is involved in As(III)-PC sequestration inside the vacuoles as well; thus the overexpression of this transporter in roots could help to limit arsenic content in the grains [119]. Moreover, it has been recently shown that transgenic rice

overexpressing *OsPCS1* showed significantly lower arsenic content in grains [120]. These results provide a promising strategy to decrease arsenic translocation by making use of vascular transporters and PC overproducers to obtain low-arsenic crops.

4.2.5 Conversion of arsenic to less toxic volatile forms

Microorganisms are able to volatilize metals and metalloids into the atmosphere through methylation [40]. Several phytoremediation approaches for mercury and arsenic volatilization have been proposed as a suitable strategy to produce safe crops grown in contaminated crops. However, plants do not have in their genome genes encoding As(III) methyltransferases [25]. Therefore, the methylated species of arsenic (MMAs and DMAs) found in plants are the result of microbial activity in the rhizosphere. The volatile arsenic species produced by these microorganisms are then taken up, translocated, and released to the atmosphere by the plant [25]. The reduction of arsenic accumulation through volatilization in rice has been achieved by overexpression of As(III) methyltransferases from bacteria and fungi [121, 122]. Although this approach is very efficient, the release of contaminants into the atmosphere is controversial.

4.2.6 Controlling the AsV/Pi transporter specificity

It has been recently shown that the phosphate transporter PvPHT1;2 from *P. vittata* displays a significantly lower As(V) uptake than other phosphate transporters [123]. The heterologous expression of this transporter in tobacco plants resulted in plants with increased shoot biomass in the presence of As(V) due to lower arsenic accumulation. Thus, it is tempting to speculate that genome-wide analysis of phosphate transporters from several species with different accumulation rates will allow the identification of new alleles that favor As(V) or phosphate uptake for phytoremediation or food safety purposes, respectively. Importantly, discovering the mechanisms that modulate substrate specificity of the arsenic transporters would be essential to develop safer crops or hyperaccumulators, which would specifically release or accumulate arsenic, respectively, without interfering with the homeostasis of essential metals or phosphate [97].

4.2.7 Phytochelatin and GSH pathway to decrease As accumulation

As mentioned above, one of the most important peptides involved in arsenic accumulation and detoxification of arsenic is the PC, forming As(III)-PC complexes that can be sequestered in the vacuoles. Endosperm-specific intron-containing hairpin RNA-mediated gene silencing of *OsPCS1* and *OsPCS2* resulted in lower arsenic sequestration in the seed vacuoles by PCs in the transgenic rice [124]. This led to the accumulation of significantly less arsenic in the grains than those in the wild type. GRXs are cysteine-rich proteins and GSH-dependent redox enzymes in charge of protection against oxidative stress, maintaining the intracellular GSH pool [125]. Recently, two arsenic-responsive rice GRX families of proteins from rice, OsGrx_C7 and OsGrx_C2.1, have been reported [126]. The heterologous overexpression of these proteins in *A. thaliana* increased plant tolerance to arsenic and reduced As(III) shoot and seed accumulation. This low As(III) accumulation in rice can be due to the fact that GRXs regulate intracellular As(III) levels by an indirect or direct modulation of the NIP transporters involved in As(III) uptake and extrusion [126]. Understanding arsenic-induced stress pathways is of central importance for the design of rice crops displaying less arsenic content in their edible parts either through the upregulation of *OsGrxs* or the seed-specific downregulation of *OsPCS*.

4.2.8 Iron plaques on rice roots restrict arsenic availability

The oxygenation of roots as a consequence of oxygen diffusion from aerenchyma to the rhizosphere and microbial growth leads to the oxidation of iron and the subsequent formation of iron plaques on the surface of roots particularly in rice [127]. The formation of these plaques is highly variable among different rice genotypes [128]. These iron plaques promote arsenic adsorption and sequestration on the root surface through the formation of Fe-As complexes, thus restricting arsenic bioavailability for plants. Hence, there is a direct relationship between iron plaque formation and reduced arsenic accumulation in the aboveground biomass in rice [128]. Elucidating the genetic mechanisms that affect iron plaque formation would encourage the generation of genetically modified rice with low arsenic accumulation.

4.2.9 Transcription factors: the key coordinators of the arsenic response

Transcriptional regulation is a major factor in the regulation of the capacity of plants to tolerate and accumulate arsenic [99]. Therefore a suitable approach to enhance plant phytoremediation performance will be the identification of key transcriptional regulators of the arsenic response. In *A. thaliana* two WRKY transcription factors have been identified as regulators of As(V) uptake. On the one hand, WRKY45 activates *PHT1;1* expression in response to phosphate starvation, mediating the As(V) influx as a phosphate analogue [129]. On the other hand, WRKY6, an As(V)-responsive transcription factor restricts As(V) uptake by the downregulation of *PHT1;1* [130]. Recently, *OsARM1*, a MYB transcription factor, has been identified in rice. This gene is strongly induced by As(III) and negatively regulates arsenic-associated transporters genes, namely, *OsLsi1*, *OsLsi2*, and *OsLsi6*, having a potential role in the transcriptional regulation of arsenic response in rice [131].

A coordinated network of arsenic, transport, chelation, trafficking, and sequestration mechanisms is crucial to uptake, translocate, and detoxify arsenic. To achieve this, there must be a strong transcriptional regulation following arsenic exposure [99]. However, sensing of arsenic and the signal transduction pathway remains completely unknown. As a matter of fact, a master regulator controlling the expression of other key transcription factor molecules in response to arsenic has not been identified yet. Discovering such transcription factor would be fundamental for developing genetically modified crops that trigger the expression of arsenic detoxification genes to adapt plants to the stress in a synchronized manner.

4.2.10 Relevance of transcriptomic approaches for phytoremediation strategies

The basic discoveries of the arsenic signaling pathway drawn from model plants such as *A. thaliana* hold great potential for the development of new phytoremediation strategies using crop plants. Recent studies are taking advantage of "omic" technologies to elucidate the genetic regulators and pathways responsible for arsenic response in plants. Cadmium and arsenic are toxic elements in rice that often appear together in contaminated paddy field soils [25]. To address whether rice has a common molecular response mechanism against both cadmium and arsenic toxicity, the identification of key genes—expressed in response to these contaminant elements—was recently aimed through a transcriptomic analysis by RNA-sequencing [132]. They found that the vast majority of the genes that responded to both arsenic and cadmium fall into five categories: redox-, glutathione metabolism-, cell wall biogenesis-, expression regulation-, and

transmembrane transport-related genes. From these, they selected the most differentially expressed genes and confirmed 27 common responsive genes to both As and Cd stress by RT-qPCR [132]. Additionally, our group performed an RNA-seq of *A. thaliana* seedlings exposed to As(V) (unpublished results), in order to provide a deeper understanding of the molecular mechanisms responsible for the arsenic stress response. **Table 1** shows the 27 arsenic- and cadmium-responsive genes in rice and their corresponding homologous arsenic-responsive genes in *A. thaliana*. The conclusion to be drawn from this is that most of the arsenic- and cadmium-responsive genes found in both *A. thaliana* and rice belong to redox and glutathione metabolism, as well as transmembrane transport, suggesting a conservation of these processes between these species; thus, carrying out research in both species is extremely useful for crop development and improvement.

	Arsenic- and cadmium-responsive gene in rice	**Arsenic-responsive orthologue in *A. thaliana***
Redox-related genes	Os06g0216000	
	Os07g0638300	
	Os07g0418500	AT2G46950
	Os03g0227700	AT3G50660
	Os01g0294500	AT2G38380
Glutathione metabolism-related genes	Os09g0367700	AT2G29490, AT2G29450
	Os03g0283200	AT5G02780
	Os10g0530900	AT2G29440
	Os12g0263000	AT5G27380
Cell wall biogenesis-related genes	Os11g0592000	
	Os05g0247800	
	Os03g0155700	AT2G03090
	Os03g0416200	AT5G15630
Expression regulation-related genes	Os02g0168200	AT1G14600
	Os07g0129200	
	Os07g0597200	AT1G74360
	Os08g0203300	
	Os09g0423200	
	Os02g0557800	AT1G59940
	Os06g0692500	
Transmembrane transport-related genes	Os01g0695800	AT2G36910
	Os04g0524500	AT1G65730
	Os01g0939100	AT2G41560
	Os01g0307500	AT4G10310
	Os01g0247700	AT1G62262
	Os01g0623200	AT4G27970
	Os03g0107300	

Table 1.
Cadmium-responsive genes in rice and their corresponding homologous arsenic-responsive genes in A. thaliana.

5. Conclusions

Arsenic contamination poses a global threat for all living organisms; for this reason, different strategies have been developed to cope with this serious challenge. Among them, phytoremediation is a promising approach. In this chapter, we have provided a brief overview on the status of arsenic phytoremediation from polluted soils and waters, with special focus on the mechanisms of arsenic perception and tolerance in several organisms, plant species used for arsenic phytoremediation, and biotechnological approaches that are driven to increase phytoremediation efficiency as well as crop protection. Although much knowledge and experience have been gained over the last years, there are still many aspects to be discovered and improved. We have exciting times ahead: the exploitation of natural variation, the use of "omic" technologies and biotechnological approaches, a holistic perspective of plant-soil-microbiota interactions, and valorization of plant-fixed arsenic will provide unique opportunities to boost this green strategy.

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Conflict of interest

The authors declare no conflict of interest.

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