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Insights into the Molecular Mechanism of Arsenic Phytoremediation

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Abstract
Arsenic (As) is a widespread carcinogenic pollutant. Phytoremediation is the most suited technology for alleviating the As contamination of soil. In this review, we have discussed the uptake mechanism and the associated transporters for different As species. Glutathione, phytochelatins, metallothionins, and secondary metabolites play important role in As detoxification and enhancing tolerance. The roles of MAPK signaling and calcium signaling are highlighted in the perception of As stress along with phytohormones signaling in stress tolerance. Furthermore, transcription factors involved in regulation of gene expression under As stress are discussed. High-throughput sequencing has reduced the time duration and enhanced the knowledge regarding understanding the molecular mechanism of phytoremediation. The role of CRISPR/Cas9 and synthetic genes in context to phytoremediation is discussed. We have provided a holistic understanding of the present knowledge about phytoremediation in the context of mechanisms of the As uptake and tolerance. A complete understanding of the phytoremediation process is essential for As-risk mitigation and will help in augmenting its efficiency and true potential.

Keywords Arsenic · Phytoremediation · Phosphate transporter · High-throughput sequencing

Introduction

Arsenic (As) is a ubiquitous toxic metalloid. The average concentration of As in earth's crust is estimated to be 2–5 mg/Kg (Moore 1991). Environmental sources of As are both anthropogenic and natural. The anthropogenic sources of As includes several insecticides, pesticides, and antifungal preservative for wood and leather. Furthermore, As is used in manufacturing textile dyes, alloy, paints and pharmaceutical products (Chung et al. 2014). Compounds of As are known to be mobile as well as persistent in the environment. The water-soluble forms of As leach to groundwater and create a major public health issue. A population of about 140 million in 50 countries has been thriving upon the water that is contaminated with As (As level > 10 µg/L) (Ravenscroft et al. 2009). Metalloid As can enter food chain via contaminated drinking water or consumption of As-accumulating crops.

The sites contaminated with As need immediate attention due to the associated severe health risks. The traditional methods for As removal are not economically viable due to the widespread nature of As pollution. Phytoremediation being a cost-effective and an ecofriendly method is the best available technology for As pollution mitigation in the present scenario. Phytoremediation can be defined as plant-based biological, chemical, and physical processes that help in removing, holding or rendering the contaminants harmless (Cunningham and Lee 1995). Although the idea of using plants for treating water and contaminated soil exists since ages, the search for cost-effective approach and advancement in the field have renewed interest in phytoremediation technology. The success of phytoremediation technology depends upon the selection of a plant species. Plants ranging from trees, shrubs, grasses, and aquatic plants have been proposed for phytoremediation purpose. Several studies have suggested As-hyperaccumulating ferns like *Pteris vittata* and *Pityrogramma calomelanos* for As phytoremediation (Francesconi et al. 2002; Ma et al. 2001; Shelmerdine et al. 2009). Various factors like physiological conditions of plant, uptake mechanism, and bioavailability of an element affect the degree of accumulation (Hasanuzzaman et al. 2015).

Plants can uptake toxic nonessential elements along with mineral nutrients (Clemens and Ma 2016). Metalloid

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As is accumulated through similar transport mechanism as phosphorus (P) which is key mineral nutrient for plant growth. Inside a plant cell, As strongly interacts with sulfhydryl groups (–SH) in proteins causing interference to cellular functions as well as physiological processes associated with normal growth and development (Hettick et al. 2015; Ullrich-Eberius et al. 1989). Several studies have shown metal(loid) stress modulates the expression pattern of various gene-networks, stress proteins, membrane transport proteins, signaling cascades, phytohormone synthesis, and signaling in plants (Kłodawska et al. 2018; Shukla et al. 2018; Thakur et al. 2019).

Harmful Effects of the Toxic Pollutant Arsenic

Owing to structural similarity, As interferes with and hampers P uptake in plants growing in As-contaminated soil. A study conducted on *P. vittata* exhibits the competition between As and P uptakes from soil (Han et al. 2016). The harmful effects of As toxicity on physiological parameters have been studied widely in different plant species. Damage to pigments is the first and easily observable effect of toxicity in plants. The fluorescence and reflectance of chlorophyll were estimated in two aquatic species: *Vallisneria gigantean* and *Azolla filiculoides*. These two species were found sensitive to As and exhibited damage in their photosystem (Iriel et al. 2015). The low level of chlorophyll content in plants exposed to As was attributed to the alteration in chlorophyll biosynthesis pathway in *Ceratophyllum demersum* (Mishra et al. 2016). Furthermore, exposure to As leads to decrease in leaf gas-exchange, water potential, seed germination, and biomass production (Shri et al. 2009; Stoeva et al. 2005). In a study conducted on mung bean (*Phaseolus aureus* Roxb.), As at 50.0 µM concentration enhanced lipid peroxidation and electrolyte leakage due to membrane damage (Talukdar 2013). Another study conducted on maize showed that activities of both enzymatic and nonenzymatic antioxidants increased due to As stress (Anjum et al. 2016). One of the most toxic biochemical effects of As stress is the production of reactive oxygen species (ROS) as a consequence of reduction of AsV to AsIII (Abbas et al. 2018; Kostecka-Gugała and Latowski 2018).

Phytoremediation

Various methods to clean up As-polluted soil and water include pre-oxidation, ion-exchange, activated alumina sorption, reverse-osmosis, lime softening, excavation of contaminated soil/material, stabilization or in situ fixation, capping, and soil acid extraction/washing (Talukder et al. 2015). Although it is of paramount importance to clean up the contaminated soil and drinking water, the widespread pollution of As makes it very costly and unviable with these available technologies. At this juncture, phytoremediation is the best available technology to alleviate As contamination. Phytoremediation can be defined as the use of plants to clean up toxic contaminants from soil and water by either removing them from or stabilizing them in plant tissue.

Plants can utilize two different kinds of approaches for phytoremediation: phytoextraction and phytostabilization (Fig. 1). While in phytostabilization the pollutants are immobilized in rhizosphere, phytoextraction involves concentration of pollutants in the shoot tissue. For phytostabilization, the indigenous plants are suitable, which can provide cover to the contaminated site and hence avoid chances of soil erosion or leakage of pollutants to water bodies. However, in case of phytoextraction, only plant species capable of root-to-shoot translocation of pollutant are suitable. It is a comparatively more challenging strategy and requires identification of suitable hyperaccumulators. *P. vittata* is a known hyperaccumulator of As. In a study, *P. vittata* was shown to phytoextract 20 times the soil As concentration in the shoots (Salido et al. 2003). *P. vittata* is also shown to extract As from groundwater (Tu et al. 2004), while, *Eucalyptus* species and *lupinus albus* are suggested as ideal candidates for phytostabilization of As (King et al. 2008; Vázquez et al. 2006). Recently, *Cyanoboletus pulverulentus* from Czech Republic was identified as As hyperaccumulator, and the maximum concentration of As in its fruit-bodies was recorded as 1300 mg/kg dry weight (Braeuer et al. 2018).

Mechanism of Arsenic Uptake

It is of utmost importance to understand the uptake mechanism of As in plants for improving the phytoremediation and to minimize the translocation and accumulation of As in edible parts of the crops. Understanding the uptake mechanism will not only help in phytoremediation but also in the production of safe crops that can be cultivated on the contaminated sites. Various forms of As available in soils, including arsenate (AsV), arsenite (AsIII), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA), have different mechanisms of uptake (Fig. 2). The most abundant form of As is AsV under oxidizing conditions, while AsIII predominates under reducing conditions, and these two forms are interconvertible.

Given the structural similarity, AsV is transported inside the plant cell via high affinity phosphate transporters (Meharg and Macnair 1992). First, high affinity phosphate transporter (PHO84) was identified in *Saccharomyces cerevisiae* (Bun-Ya et al. 1991). In plants, the phosphate transporters were first described in *A. thaliana*. The high affinity phosphate transporter1 family (PHT1) in *A. thaliana*

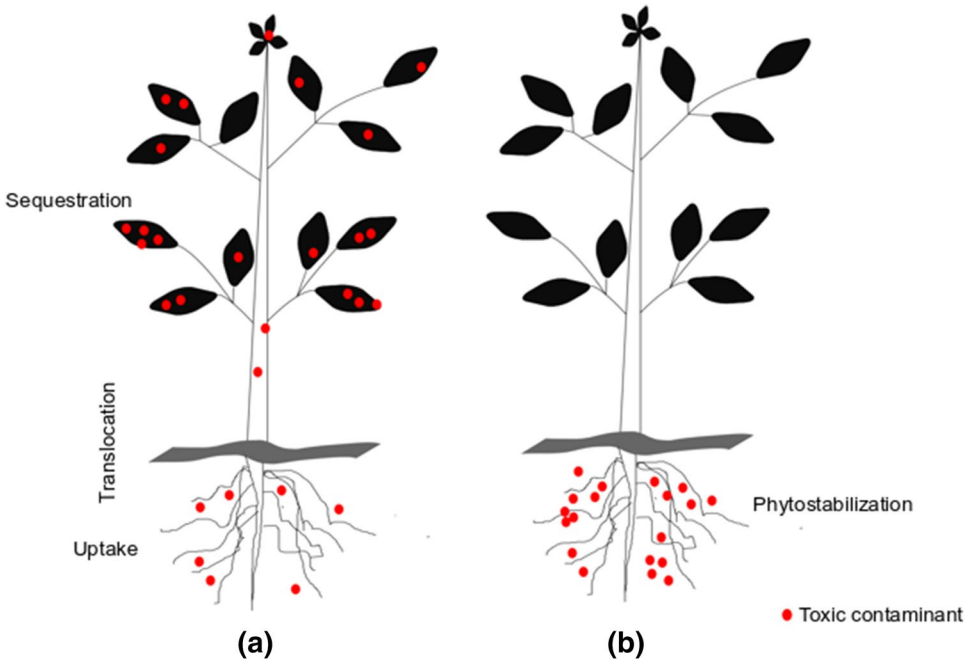

Fig. 1 A cartoon explaining different strategies of phytoremediation **a** phytoextraction **b** phytostabilization

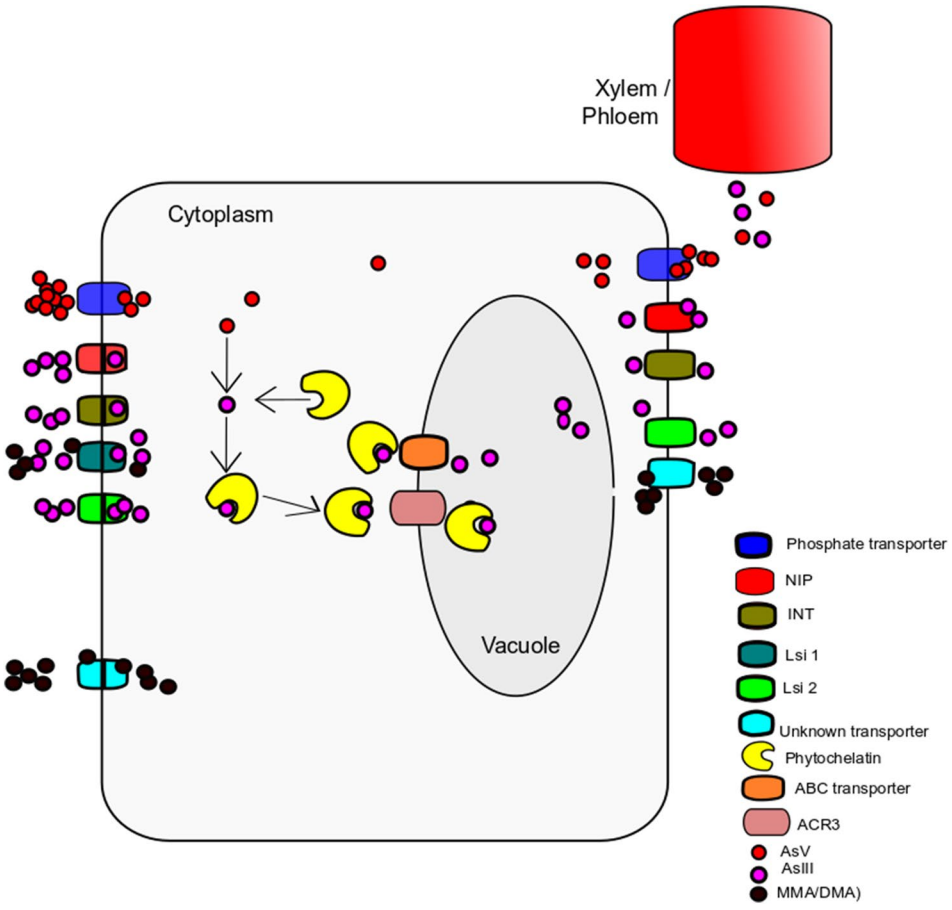

Fig. 2 General mechanisms of arsenic uptake and sequestration in plants

consists of nine members (PHT1;1 through PHT1;9). PHT1, a phosphate-H + symporter, contains 520–550 amino acids with an approximate molecular weight of 58 kDa and forms 12 transmembrane helices (Nussaume et al. 2011).

Since the discovery of PHT1 transporters, several studies have reported their homologs in different plant species (Rausch and Bucher 2002). A similar transport mechanism for both the elements has been supported by the observation that AsV hampers the Pi uptake (Meharg and Hartley-Whitaker 2002; Wang et al. 2002). Phosphate transporters, PHT1;1 and PHT1;4 contribute to Pi uptake in *A. thaliana* at both low and high Pi concentration. The double mutant *pht1;1Δ4Δ* which lack both PHT1;1 and PHT1;4, exhibited Pi deficiency even under high concentration of Pi in external medium (Shin et al. 2004). *A. thaliana* mutant having a semidominant allele for Pi transporter, PHT1;1, showed twofold increase in As accumulation in comparison to wild-type plants, and the overexpression of the same transporter in wild-type plants enhanced As accumulation (Catarecha et al. 2007). In As hyperaccumulator *P. vittata*, PvPht1;3 and PvPht1;5 exhibited similar affinity for phosphate, but PvPht1;3 showed higher affinity for AsV. The transcript's expression of PvPht1;3 was induced by low level of Pi and AsV, which further enhanced the rate of AsV uptake (DiTusa et al. 2016). Overexpression of Pi transporter gene *OsPT1* enhanced the As accumulation in *Oryza sativa* shoots (Kamiya et al. 2013).

AsIII is one of the most toxic and the predominant inorganic forms of As under reducing conditions like wetlands. It can bind to the sulfhydryl groups in proteins and interfere with general protein functioning (Abedin et al. 2002). Several transporters have been reported to take part in AsIII uptake including nodulin-26-like intrinsic proteins (NIPs), tonoplast intrinsic protein (TIP), inositol transporters (INT), and Si transporters (Ali et al. 2009; Bienert et al. 2008; Duan et al. 2016; He et al. 2016; Ma et al. 2008). Furthermore, the ABC-type transporters and arsenic compound resistance 3 (ACR3) transport the accumulated AsIII inside vacuole either directly or by complex formation (Indriolo et al. 2010; Song et al. 2014). NIPs are aquaporins permeable to metalloids like boric acid and arsenous acid (Li 2014). Various members (NIP1;1, NIP1;2, NIP5;1, NIP6;1, and NIP7;1) of NIP subfamily are reported to transport AsIII in *A. thaliana* (Bienert et al. 2008; Isayenkov and Maathuis 2008; Kamiya et al. 2008; Ma et al. 2008). Furthermore, NIP3;1 was shown to be involved in both uptake and translocation of AsIII in *Arabidopsis* (Xu et al. 2015). OsNIP3;2, an intrinsic membrane protein, was reported to be involved in AsIII uptake in rice, but has little role in root-to-shoot translocaion (Chen et al. 2017b). TIPs belong to subfamily of aquporins and localized in tonoplast and endosomal membranes. PvTIP4;1 was reported to transport AsIII in As hyperaccumulator *P. vittata* (He et al. 2016). INTs are localized on both vacuolar membrane and plasma membrane. Characterizations of AtINT2 and AtINT4 in *Saccharomyces cerevisiae* revealed their roles in long-distance transport of AsIII and hypothesized to function in phloem loading of AsIII (Duan et al. 2016). Lsi1 and Lsi2 are the two important silicon transporters involved in AsIII uptake in rice. While Lsi1 (OsNIP2;1) is an influx transporter that helps in AsIII uptake, Lsi2 is the efflux transporter and is involved in the transport of AsIII to xylum (Ma et al. 2008). As can be transported into the apoplast through passive uptake or with the help of Lsi2 transporter (Moore et al. 2011). Several studies have confirmed the presence of apoplastic As (Moore et al. 2011; Raab et al. 2007; Zhao et al. 2009).

The uptake mechanism of organic forms of As, like monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), is not fully understood yet. Various studies confirm that plants can uptake methylated-As through root and translocate it to shoot (Burlo et al. 1999; Carbonell-Barrachina et al. 1999). The Si transporter, Lsi1, known to take part in AsIII uptake was also found to uptake methylated-As species in rice (Li et al. 2009).

Mechanism of Arsenic Tolerance

The ability to survive in the presence of an element which is otherwise toxic is defined as tolerance of a plant species. The tolerance toward a specific toxic element in plants is governed by its interaction with the environment (Macnair et al. 1999). In the past, a number of As-tolerant plant species have been identified. These species including *Holcus lanatus* (Macnair and Cumbes 1987), *Andropogon scoparius Michx* (Rocovich and West 1975), *Deschampsia cespitosa, Agrostis capillaris* (Meharg and Macnair 1991), *Silene vulgaris* (Paliouris and Hutchinson 1991), *Agrostis castellana*, and *Agrostis delicatula* (De Koe and Jaques 1993). As-hyperaccumulating and As-tolerant plants over due course of time can accumulate As in significant amounts. To avoid the As toxicity, these plants utilize various strategies. The primary strategy of tolerance mostly involves reducing the concentration of toxic element at sensitive sites inside a plant cell. In case of toxic metalloid As, it can be achieved either via suppressing the As/Pi uptake system (Meharg and Macnair 1992) or activating the As efflux system which ultimately reduces the As content in the plant tissue. The exact mechanism of As efflux is not yet clear, but it is hypothesized to be similar to phosphate efflux (Mimura 1999; Zhao et al. 2009).

Inside the plant cell, there are a number of other strategies for As detoxification, which include chelation by glutathione (GSH), sequestration inside vacuoles, increased expression of heat-shock proteins (HSPs), and secondary metabolites like phenolics. GSH is a precursor of phytochelatins (PCs) known to participate in heavy metal detoxification (De Vos et al. 1992; Grill et al. 1989). PCs are thiol (-SH)-containing polymers of ɤ-glutamylcysteine of GSH. The formation of As-PC complexes is one of the key process in As detoxification (Hartley-Whitaker et al. 2001). In *Holcus lanatus*,

tolerance to high levels of As is attributed to As-PC complexes (Hartley-Whitaker et al. 2001). In a study conducted on *Nasturtium officinale,* PC as well as GSH contents were found to be increased with the accumulation of As in plant tissue (Namdjoyan and Kermanian 2016). Increases in PC and GSH syntheses in response to As stress have also been observed in other plants, viz., *H. lanatus*, *P. cretica* (Raab et al. 2004), *B. juncea* (Gasic and Korban 2007), and *Helianthus annuus* (Raab et al. 2005). Increased demands of GSH and PCs are met through enhanced GSH and PC biosyntheses. The enzyme arsenate reductase reduces AsV to AsIII and subsequently forms complexes with GSH and PC (Raab et al. 2004). The As-PC and As-GSH complexes then finally get sequestered in vacuoles through transporters like ABC-transporter (Bleeker et al. 2006). In *A. thaliana,* two ABCC-type transporters, viz., AtABCC1 and AtABCC2, were demonstrated to be involved in vacuolar sequestration (Song et al. 2010). AsIII can also be directly transported inside vacuole as well as loaded into xylem via ACR3 (Indriolo et al. 2010; Ma et al. 2008).

Apart from detoxification by sequestration in vacuoles, plants produce stress proteins (SPs) like HSPs and metallothionins (MTs). Several SPs are expressed constitutively but at basal level, and participate in proper folding and assembly of the newly synthesized proteins (Del Razo et al. 2001). MTs are cysteine-rich proteins and possess a highly conserved primary structure. MTs are classified under two types: type I and type II MTs. The type 1 MTs share similarity with equine renal MTs, while type II MTs do not share such homology (Steffens 1990). Similar to PCs, MTs are also involved in chelation of toxic elements through cysteine thiolate ligands. Moreover, MTs can also alleviate the oxidative stress by acting as ROS scavenger (Wang et al. 2004). The expression of HSPs and MTs are shown to increase with the increasing As stress (Rai et al. 2011). The transgenic tobacco plants overexpressing MsHSP23 showed increased tolerance to As stress in comparison to wild-type plants. It was hypothesized that the overexpression of MsHSP23 enhances stress tolerance by maintaining membrane stability and enhancing the scavenging of mitochondrial ROS (Lee et al. 2012). The HSPs act as molecular chaperons and are involved in protection and repair of proteins under stress conditions. Hsp90-1 protein gene was also found to be induced by As stress in tomato (Goupil et al. 2009).

Secondary metabolites like phenolics are shown to be upregulated in response to As stress as well as under various other biotic and abiotic stresses including lead stress in lupin (Izbiańska et al. 2014), *Medicago sativa* (Sima et al. 2012), copper uptake in *Matricaria chamomilla* (Kovacik et al. 2010), resistance to cold and heat stresses (Rivero et al. 2001). Induction of phenolics can protect a plant (1) by enhancing the endurance of cell wall and forming a physical barrier (Díaz et al. 2001; Michalak 2006); and (2) by acting as antioxidant (Rice-Evans et al. 1997). ROS production is a common consequence of abiotic stress in plants. The oxidative stress caused due to ROS inflicts damage to important biomolecules like DNAs, lipids, and proteins in plant cells. Phenolic compounds of plants, like lignin and flavonoids, scavenge ROS and behave as antioxidants by helping in reducing ROS, e.g., reduction of H_2O_2 by donating electrons to guaicol peroxidase (Sakihama et al. 2002). By abating, the oxidative stress phytophenols protect plants from abiotic stress. In vitro studies postulate flavonoids to be better antioxidants than ascorbate and α-tocopherol, but the supporting evidences in plants are limited (Sytar et al. 2013).

Heterologous expression is a useful transgenic tool which could be employed for augmenting the tolerance of crops in As-contaminated regions. In yeast, the efflux of As is mediated through ACR3 antiporter, and its heterologous expression in *A. thaliana* has improved As-stress tolerance (Ali et al. 2012). In another study, heterologous expression of phytochelatins synthase from C*eratophyllum demersum* (CdPCS1) in rice has enhanced the detoxification and sequestration of As in roots and shoots, while it decreased the accumulation in grains (Shri et al. 2014). Similarly, the expression of glutaredoxin from *P. vittata* (PvGRX5) is shown to increase As tolerance in *A. thaliana* (Sundaram et al. 2009). Heterologous expression of useful and well-characterized genes provides a useful transgenic approach for enhancing the As-stress tolerance as well as its phytoremediation efficiency.

Arsenic Stress Perception and Signaling

Perception is an important step of tolerance toward a particular stress. Protein kinases like mitogen-activated protein kinase (MAPK) play a significant role in perception of external stress signals and its transduction inside nucleus for cellular response. The MAPKs are part of MAPK signaling cascade that is involved in transducing the external signals to nucleus to activate appropriate response. MAPK signaling cascade is evolutionary conserved and has been reported to be active in animals and plants in response to various abiotic stresses including As stress. This cascade includes mainly three types of kinases, viz., MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK. These kinases act in sequential order by phosphorylating the specific kinases to transduce external signals and produce appropriate response by eventually phosphorylating the substrate protein (Widmann et al. 1999). The 42 and 44 kDa MAPKs were found to be activated according to concentration of As treatment in leaves and roots of rice. Significant increases in transcript levels of, *OsMPK3* in leaves, and *OsMPK3* and *OsMPK4* in roots, were observed, and further through docking analysis, interaction between *OsMPK3 and*

OsMPK4 was established in this study (Rao et al. 2011). In yet another study, AsIII-mediated stresses in two cultivars of *B. juncea* viz., Varuna and Pusa Bold, were reported to exhibit activation of 46 kDa MAPK at both 50 and 150 µM concentration of As (Gupta et al. 2009).

Calcium (Ca) signaling is also involved in perception of external stress. Ca^{2+} ions act as intracellular secondary messengers and are capable of initiating stress response. Ca^{2+} ions play significant role in alleviating oxidative stress (Price et al. 1994). The concentration of Ca^{2+} in cytosol changes under various abiotic and biotic stresses, which is sensed by Ca^{2+} sensors such as calmodulin. Several genes including CDPK, calmodulin (CALM), CBL, and CIPK involved in Ca signaling were found to be upregulated by As in rice roots (Huang et al. 2012). Ca was also shown to influence As accumulation and detoxification in hyperaccumulator species *Pteris vittata*. The distribution if Ca^{2+} ions were affected by the high As treatment. It was further observed that the Ca deposits increased in cytoplasm and decreased in vacuole due to As treatment (Li et al. 2006).

Induction of Phytohormone Signaling by Arsenic

Phytohormones or plant hormones are the organic substances produced by plants in small concentrations that act as signal molecules to control physiological processes (Davies 2010). Besides regulating the growth and development in plants, phytohormones play important role in As-stress tolerance. The gene expression studies and use of mutants have helped in elucidating their role in stress tolerance. The genes related to jasmonic acid (JA), abscisic acid (ABA), ethylene, cytokinin, and gibberellins (GA) synthesis and signaling were identified to be regulated by AsV treatment in rice roots (Huang et al. 2012). The upregulation of phytohormone synthesis and signaling genes indicates their involvement in managing the stress in plants. The genes related to JA biosynthesis viz., *OsDAD*, *OsLOX*, *OsAOS*, and *OsPCL* and signaling viz., *JAZ* and *JAR* were found to be upregulated by As treatment in rice (Yu et al. 2012). It was suggested that the phytohormones are also being accumulated in rice roots under As stress as the genes related to ABA synthesis (*OsNCED*), GA synthesis (*GA2ox3*), CK synthesis (*OsLOG*), ethylene synthesis (*OsACS*), and auxin synthesis (*OsASA2* and *OsASB1*) were found to be upregulated (Yu et al. 2012).

The level of phytohormones is regulated by several families of miRNAs. The phytohormones and miRNAs work in unison to regulate stress perception, signaling, growth and developmental processes in *B. juncea*. The miR159 accumulation in *B. juncea* was found to positively correlate with responsiveness to ABA the and results indicate the importance of ABA in As-stress signaling. Furthermore, GA and ethylene are also the targets of miR159 and it is deduced that the level of these hormones is regulated under As stress. Similarly, the miR319 was shown to regulate synthesis of JA which is a key hormone in As-stress perception (Srivastava et al. 2012). The *A. thaliana* mutants of auxin transporter aux1, pin1, and pin2 showed sensitivity toward AsIII and the external supply of indole acetic acid (IAA) enhanced the As tolerance of aux1 mutants. The study provided evidence that AsIII interferes with transport of auxin and hypothesized that auxin provide AsIII tolerance through ROS signaling (Krishnamurthy and Rathinasabapathi 2013).

Transcription Factors Involved in Arsenic-Stress Tolerance

The signaling cascades induced due to As stress act as adaptive response and impart tolerance to plants (Fig. 3). These signaling cascades control the expression of downstream

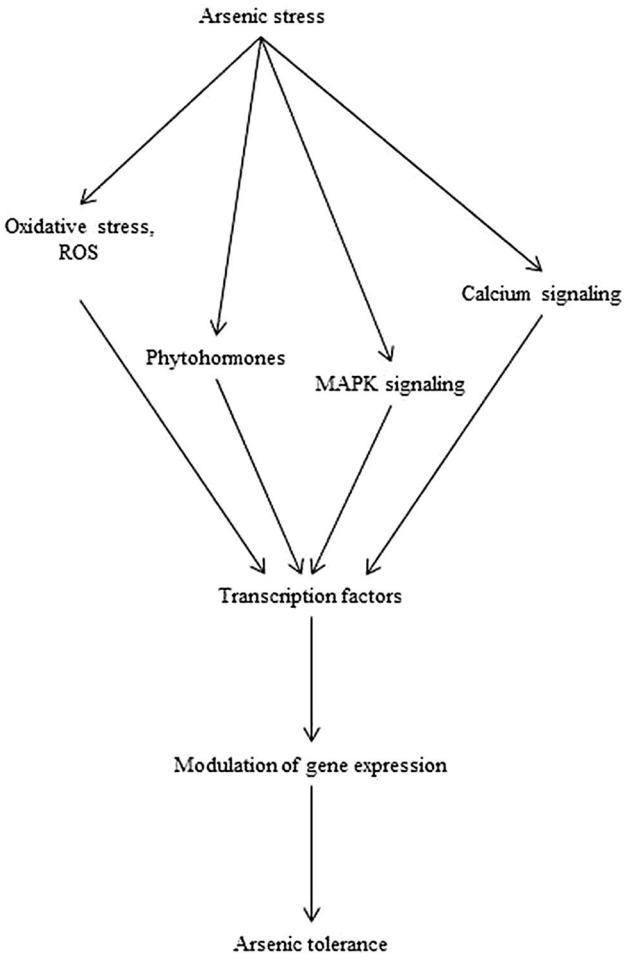

Fig. 3 Transcription factors play key role in imparting tolerance to arsenic stress via modulating gene expression

genes through transcription factors. WRKY family is one of the top ten largest transcription factor family that is present throughout the green plants (Ülker and Somssich 2004). Term WRKY was coined in 1996 while working on parsley (Rushton et al. 1996). They isolated three WRKY (WRKY1, WRKY2, and WRKY3) DNA binding proteins and suggested their role in signal transduction pathways. WRKY proteins can activate as well as repress transcription. Since then these transcription factors have been shown to regulate various signaling cascades under abiotic and biotic stress (Rushton et al. 2010). WRKY6 in *A. thaliana* was found responsive to AsV and further it reduced the AsV uptake via altering the gene expression of arsenate/phosphate transporter gene (Castrillo et al. 2013), while WRKY45 was reported to be involved in Pi uptake in Pi starved *A. thaliana* (Wang et al. 2014). In *A. thaliana*, a total of eight transcription factors (DRE-binding protein, AP2 domain-containing transcription factor, zinc finger (C2H2-type) protein, zinc finger (C2H2 type) protein, WRKY33, WRKY53, and WRKY40; NAC domain-containing protein) were found to be differentially expressed in AsV-treated plants (Abercrombie et al. 2008). Although earlier studies were conducted using model plant *A. thaliana*, crop specific studies have also established their role in understanding abiotic stresses (Rushton et al. 2010). In a study 231 transcription factors belonging to (APETALA2/ethylene response factor) AP2/ERF, Heat shock factor (HSF), Zinc-finger protein expressed in inflorescence meristem (ZIM), MYB, and WRKY families were identified in rice roots in response to As stress (Huang et al. 2012). Another study involving rice root and shoot under AsIII stress identified 468 differentially expressed transcription factors. Among these, transcription factors belonging to NAM, ATAF, and CUC (NAC), and WRKY families were found to be upregulated in rice roots suggesting their roles in AsIII-responsive gene regulation (Yu et al. 2012).

High-Throughput Sequencing and Other Advanced Techniques for Enhancing Phytoremediation Efficiency

Phytoremediation is a promising technology but it has not yet achieved its full potential. The main impediment for the success of this green technology is the uncertainty of its efficiency (Linacre et al. 2005). The use of transgenic plants with the desired traits like enhanced uptake and tolerance to toxic contaminant, ability to hyperaccumulate and high biomass production can provide viability to phytoremediation. For this, understanding the molecular mechanism associated with phytoremediation is important. Transcriptome profiling using high-throughput sequencing has provided leaps to the understanding of phytoremediation process at molecular level. The term transcriptome refers to the whole set of transcripts present in a cell or from a population of cells at a specific developmental stage or physiological condition (Wang et al. 2009). Transcriptome-profiling using RNA-seq is an actively developing technology for precise gene-expression measurements. It is increasingly gaining impetus for gene-expression-related studies due to the advantages like ability to detect novel transcripts and splice junctions, no hybridization related biasness and no prior knowledge of genomic sequence is required.

Although the technology of next-generation sequencing (NGS) is about a decade old, it has already contributed enormously in the health sector. The cost associated with NGS is decreasing gradually due to which it has become possible to make use of this technology to solve environmental problems like soil contamination. The high-throughput sequencing has been used successfully in As tolerance and hyperaccumulation studies in various plant species. High-throughput sequencing can provide a holistic and unbiased understanding of the underlying processes of phytoremediation (Fig. 4). Using RNA-seq,1725 differentially expressed genes involved in hormone metabolism and As accumulation along with transcription factors like HSF, and MYB and oxidative stress related proteins were identified in As-stressed *Panax notoginseng* (Liu et al. 2016).The phytoextraction efficiency of *Salix purpurea* was assessed using gene expression analysis of root, stem, and leaves using RNA-seq and transporters

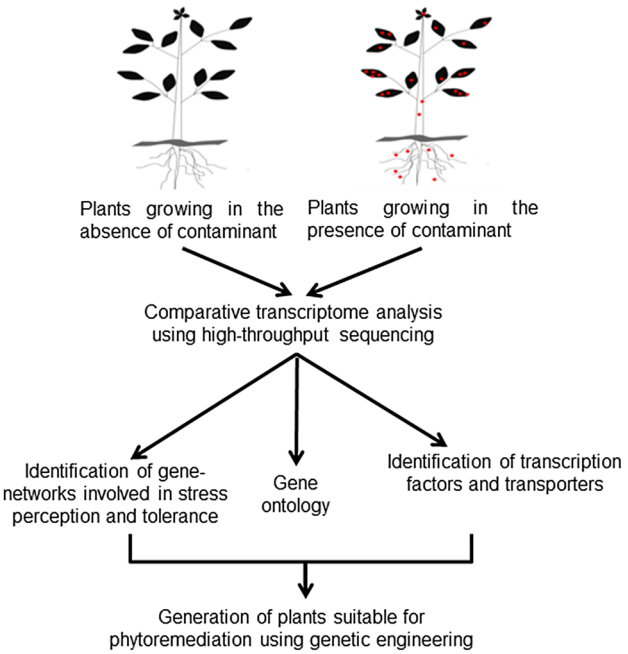

Fig. 4 General scheme of transcriptome analysis by high-throughput sequencing for understanding the process of phytoremediation

(PHO1, NIP1.1) responsible for As uptake were identified (Yanitch et al. 2017). Furthermore, Illumina sequencing was utilized for whole transcriptome profiling under AsIII stress. The genes related to lipid metabolism (59 genes) and plant hormones (324 genes) in response to AsIII treatment were identified (Yu et al. 2012). In a study conducted in our laboratory, a total of 340 affected gene-networks and 582 differentially expressed membrane transport proteins were identified using comparative transcriptome profiling in AsV-treated *B. juncea* seedlings. Glutathione and ABC transporters were found to be involved in sequestration of AsV. MAPK and Ca signaling pathways were found to be involved in AsV stress perception (unpublished data). miRNAs are involved in various processes associated with abtioc/biotic stress response, maturation, flowering and cell proliferation (Aukerman and Sakai 2003; Khraiwesh et al. 2012; Rodriguez et al. 2010). Several studies have reported the role of miRNAs in As-stress management. Using RNA-seq, 67 As-responsive miRNAs involved in regulation of cellular and metabolic processes as well As-stress responses were identified in Minghui 86, an *Indica rice* (Liu and Zhang 2012). A total of 69 miRNA of 14 different families involved in regulation of developmental processes, plant hormone biosynthesis, sulfur uptake, and assimilation were identified as As-responsive miRNA from *B. juncea* (Srivastava et al. 2012).The differential expression in leaves of maize was studied, and a total of 22 upregulated and 35 downregulated miRNA were identified. The identified miRNAs were found to influence metabolic processes involved in imparting adaptation to As stress, developmental processes, and hormone-signaling pathways (Ghosh et al. 2017).

The clustered regularly interspaced palindromic repeats (CRISPR) system is a genome-editing tool which can be used to enhance the phytoremediation efficiency (Basharat et al. 2018). The RNA-guided CRISPR/Cas9 system is a preferred tool as it is easy to design, highly specific and suitable for high throughput for gene-editing (Ma et al. 2015; Shan et al. 2013). Editing the genome of hyperaccumulators to fine tune their phytoremediation capabilities or production of safe crops growing in As contaminated regions, both can be achieved through CRISPR/Cas9 based system (Basharat et al. 2018; Chen et al. 2017a). To augment the phytoremediation efficiency, the genes involved in arsenic uptake (Pht1:8, Lsi1/2, ACR3, NIPs, and ABC transpoters) may be targeted for gene editing. Various microbes including bacteria and fungi are known to metabolize and detoxify As in nature (Bhattacharjee and Rosen 2007). Due to the ubiquitous nature of As, most of the microbes are known to possess arsenic-resistance (ars) operons which provided resistance toward AsIII and AsV (Rosen 1999). The overexpression of arsR, ars-regulating protein, results in increased accumulation of As by *Escherichia coli* (Kostal et al. 2004). The well-characterized genes responsible for As uptake and detoxification from microbes can be transferred to plants with the help of tools such as CRISPR/Cas9 and synthetic genes. The role of synthetic gene transfer becomes more significant in those cases where the natural gene transfer either fails, or the transferred gene could not express properly (Kunjapur et al. 2018). Using the synthetic gene triphenylmethane reductase (TMR) from the *Citrobacter* sp., *Arbidopsis* plants showed increased tolerance as well as detoxified crystal violet to less toxic leucocrystal violet (Fu et al. 2013). Similarly, the synthetic genes from the microbes can be transferred to plants for increasing the efficiency of As phytoremediation.

Conclusion

Phytoremediation can play an important role to mitigate the As pollution. A thorough understanding of the molecular mechanism involved in the uptake and tolerance of a toxic pollutant not only gives impetus to the phytoremediation technology but also help in producing the safe crops on contaminated sites. Various phosphate transporter and plant aquaporins have been identified which play important role in As uptake from the environment. Inside plant cell, strategies like chelation with glutathione, sequestration inside vacuoles, and production of various secondary metabolites help in detoxification of toxic metalloid. The stress transcriptome profiling using RNA-seq has helped in deciphering the underlying mechanism of As uptake and tolerance in plants. Genetic engineering can make use of the important genes identified through transcriptomics to develop ideal hyperaccumulator plants. Incorporation of advanced technologies such as CRISPR/Cas9 and synthetic gene will help in achieving the true potential of phytoremediation technology.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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