**New molecular mechanisms to reduce arsenic in crops**

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**Descriptive Words**

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**Abstract**

Arsenic is toxic to all life forms and is a potent carcinogen. Its accumulation in crop plants and subsequent consumption poses a serious threat to public health worldwide. Recent developments have enhanced our understanding of the molecular mechanisms governing arsenic uptake, detoxification and accumulation in plants. In particular, the identification of plant arsenate reductase enzymes and emerging details of the processes underlying arsenic distribution and deposition in the seed, will prove invaluable in the development of new strategies to mitigate this threat. Here we provide an outline of these recent developments and suggest new molecular mechanisms that could be employed to reduce arsenic in crops.

**Arsenic poisoning, a global menace**

Arsenic is a toxic metalloid that is found ubiquitously in the environment. Despite its use as a therapeutic agent for the treatment of acute promyelocytic leukaemia and trypanosomiasis, arsenic is toxic to all living organisms and has been categorised as a Class 1 carcinogen by the WHO. Acute arsenic poisoning is relatively rare but chronic poisoning is widespread. Indeed, it is estimated that over 200 million humans run the risk of arsenic poisoning, especially in areas such as South-east Asia where the local geology leads to substantial arsenic pollution of ground and surface waters [1]. Prolonged exposure to even very low concentrations of arsenic can lead to **arsenicosis** (see glossary) in the form of skin lesions, gastrointestinal, cardiovascular, neurological and respiratory diseases and malignancies [2]. Human exposure to arsenic predominantly occurs through intake of contaminated drinking water and via ingestion of crops that contain arsenic [3].

Though arsenic toxicity depresses plant growth, and hence crop yields [4, 5], it is the accumulation of arsenic in edible parts that poses risks of contaminating the food chain. The often unavoidable contamination of irrigation water sources inevitably leads to crop exposure to arsenic and therefore dietary intake can substantially contribute to chronic arsenic poisoning. The latter is particularly evident in the case of rice that is cultivated in flooded conditions [6, 7]; this species readily takes up arsenite, the prevalent form of arsenic found in paddies. When combined with arsenic rich geology, such as found in South East Asia, rice consumption is often a major contributor to chronic arsenic exposure.

These major public health risks can be alleviated by developing crops in which arsenic deposition in edible parts is low or even absent. Such an endeavour is becoming more feasible since considerable progress has been made in the past years regarding the molecular details of arsenic uptake and particularly the role of specific proteins in delivering arsenic to seeds and grains. How these new insights can be exploited to develop healthier crops will be discussed.

**New Insights into Arsenic Detoxification and Transport in Plants**

Plants need inorganic nutrients to grow and develop [8]. Nutrients are taken up and distributed throughout the plant via dedicated proteins that specialise in the transport of specific compounds. However, no protein is perfect and influx of unnecessary or even toxic compounds cannot be completely avoided. This notion is amply demonstrated where arsenic is concerned; Arsenic in soils occurs mostly in inorganic form as arsenate (AsV) and arsenite (AsIII) [7]. AsV is a chemical analogue of phosphate and this similarity hampers discrimination between these chemicals in various transport processes. In the cytosol, AsV interferes with essential cellular processes that rely on phosphate such as oxidative phosphorylation and ATP synthesis [5]. The size of AsIII, which in solution takes the form of neutral arsenous acid, is similar to that of essential nutrients such as boric acid and silicic acid and several mechanisms for the uptake and distribution of the latter also show high affinity for AsIII [9, 10]. AsIII has a strong propensity to bind sulfhydryl groups and consequently can negatively affect general aspects of protein functioning by interfering with secondary, tertiary and quaternary structure and with protein-protein interactions [5]. Besides AsV and AsIII, and depending on pH, redox potential and microbial activity, less abundant organic forms of arsenic are also found in the environment and within plant tissues and these include methylated arsenic species, arsenosugars and arsenobetaine [7, 11].

*AsV uptake.* It is now well established that AsV enters plants primarily via phosphate (Pht) transporters (Fig. 1). Studying loss of function mutants in Pht proteins generally showed improved tolerance to arsenic because less enters the root symplast [12-14]. Conversely, overexpression of *Arabidopsis thaliana* and rice Pht transporters resulted in increased sensitivity to AsV and increased arsenic uptake [14-17]. However, the properties of different Phts vary both within plants and between species. For example, AtPht1;1 and AtPht1;4 were identified as highly expressed in root tissues where they contribute to P uptake in both low and high external concentrations [12]. In rice, [17]and [18] found large differences in expression levels of various OsPht isoforms and isoform specific responses to arsenic. Differences in expression levels are also evident across cultivars and can impact on cultivar-specific arsenic tolerance [18]. Most Phts show a slightly higher affinity for P compared to arsenic but the recently identified PvPht1;3 from the arsenic **hyperaccumulator** *Pteris vittata* displayed a relatively high affinity for arsenic compared to other isoforms such as PtPht1;5 [19, 20]. This may be a hyperaccumulator-specific adaptation that contributes to the enhanced arsenic uptake and accumulation observed in this species.

*AsIII uptake.* In anaerobic conditions AsIII uptake predominates, as is exemplified by cultivation of rice in flooded paddy fields. AsIII uptake occurs via nodulin-26-like intrinsic proteins (NIPs) which constitute a sub-family of the **aquaporins** (Fig. 1) though some PIPs (plasma membrane intrinsic proteins) and TIPs (tonoplast intrinsic proteins) may play a minor role as well [9, 21]. The often large aquaporin gene families and overlapping expression patterns make it awkward to pinpoint which NIP isoform(s) is specifically involved in AsIII uptake but in *Arabidopsis* NIP1;1, 1;2, 3;1, 5;1 and 7;1 have all been implicated [22-24]. In rice the majority of AsIII uptake appears to take place through OsNIP2;1 [10, 25]. OsNIP2;1 is equivalent to Lsi1 (low silicon 1) which normally mediates silicon (Si) influx. OsNIP2;1 is strongly expressed in the roots where it localises to the plasma membrane on the distal side of exo- and endodermal cells [10, 25]. In addition, OsNIP3;2 is involved in As(III) uptake by rice lateral roots [26].

In rice, AsIII uptake is sustained by active efflux towards the stele through the efflux transporter Lsi2 (an anion permease from the ArsB/NhaD superfamily). Lsi2 is localised to the proximal side of the exo- and endodermal cells [10] in the root where lsi1 is located on the distal side. Loss of function of either Lsi1 or Lsi2 results in markedly reduced uptake and translocation of both silicon and AsIII to the shoot and, in the case of lsi2, the grain [10].

*Arsenic efflux.* In contrast to microbial organisms, AsIII specific efflux mechanisms have not as yet been discovered in plants. There is evidence of both AsIII and AsV efflux from plant roots; AsIII efflux appears to be an energy dependent process, while AsV efflux is likely passive [27]. Several NIP aquaporins confer enhanced AsV tolerance on yeast, indicating that they are bi-directional AsIII channels [9, 28] and some AsIII efflux activity has been demonstrated for OsNIP2;1 from rice roots (Fig. 1), with loss of function plants exuding 15-20% less than WT plants [29]. Transporters responsible for the remaining 80% of AsIII efflux in rice have yet to be revealed.

*Detoxification and sequestration.* Most organisms employ reduction of AsV to AsIII as part of their detoxification pathway (Fig. 1) and consequently cellular arsenic is predominantly found as AsIII in plants. Reduction is achieved by arsenate reductases that use glutathione as reductant. In *Arabidopsis* the reductase ATQ1/HAC1 is expressed primarily in the root hairs, epidermal cells and the stele [30]. Knockout mutations in *ATQ1/HAC1* result in increased sensitivity to AsV (but not AsIII), a significantly reduced AsIII/AsV ratio and reduced ability to efflux AsIII from the roots. Interestingly, AsV reduction greatly impacts on arsenic xylem loading and in *ATQ1/HAC1* loss of function mutants translocation of arsenic to the shoot is increased [30]. Knockout mutations of three HAC genes in rice had remarkably similar effects [31, 32].

Reduction of AsV to AsIII is a precondition for sequestration in root vacuoles, a process that requires AsIII **chelation** by phytochelatins and subsequent transport across the tonoplast. In *Arabidopsis*, the ABC transporters AtABCC1 and AtABCC2 are responsible for the vast majority of AsIII-PC sequestration in the vacuole [33]. In rice, OsABCC1 is expressed in the roots, leaves, nodes, peduncle and rachis where it localises to the tonoplast and it is thought to drive arsenic sequestration in this species [34].

In contrast, hyperaccumulators such as *Pteris* *vittata* do not appear to require phytochelatins for detoxification of AsIII [35] but instead sequester AsIII directly in the vacuoles by using an AsIII/H+ antiporter PvACR3. PvACR3 is related to the AsIII efflux transporter ACR3 found in the plasma membrane of yeast and bacteria and though ACR3 homologues have been found in mosses, ferns, lycophytes and gymnosperms, none have been identified in flowering plants [36].

*Long distance transport.* With the exception of hyperaccumulators, plants tend to prevent **xenobiotics** and toxic compounds from reaching photosynthesising tissues. Consequently, arsenic concentrations in root tissues are typically 5 to 20 times higher than those of shoots, e.g.[37]. Nevertheless, the relatively large mass of shoots compared to roots of most plants means there are substantial long distance fluxes with the net arsenic deposition depending on both delivery via the xylem and potential recirculation via the phloem. The majority of arsenic found in xylem sap is in the form of AsIII [38]. And while little is known about the exact proteins, it is generally assumed that the function of loading AsIII into the xylem is (partially) mediated by NIPs. Evidence for this notion comes from various sources: in *Arabidopsis*, loss of function in NIP7;1 led to a significant reduction in xylem sap arsenic and a lower shoot:root arsenic ratio [37]. A similar effect was recorded in NIP3;1 deletion mutants [23]. In rice, the AsIII-permeable NIP2;2 (Lsi6) is localised in the xylem parenchyma but its loss of function did not affect arsenic levels in the shoot [10]. Though confirmation of a role in arsenic transport *in vivo* is still lacking, work by [39] suggests that members of the NRAMP (natural resistance-associated macrophage proteins) family could also contribute to long distance arsenic transport. Xylem arsenic is largely in the form of AsIII but overall arsenic delivery to the shoot is also influenced by AsV transporters: Expression levels of OsPht1;1 [17] and OsPT8 [18] impacted on arsenic content in above ground tissues, most likely via their role in AsV uptake in roots.

 Net translocation to the shoot may also be influenced by recirculation; heavy metal flux from shoot to root via the phloem has been observed and may form part of tolerance mechanisms, e.g.[40]. Whether there is a role for the phloem in the translocation of arsenic from shoots to roots remains to be established but in *Arabidopsis* several transporters have been shown to impact on arsenic phloem levels [37, 41].

*Arsenic deposition in seeds.* A relatively small proportion of plant arsenic ends up in the seeds and grains. The past 3-4 years have yielded exiting new insights regarding the way arsenic reaches the seed. In general, seed development necessitates a large scale redirection of metabolites and minerals from vegetative to reproductive tissues and thus a major change in source-sink relationships within the plant. During seed maturation, the main sinks shift from initially being maternal to being filial in later stages. Seeds are typically fed by the phloem and this requires a loading step in the source tissue. But while in most sink tissues subsequent unloading remains symplastic, or requires crossing of one membrane at most, in seeds the minerals are unloaded across a membrane into the maternal apoplast and subsequently need to traverse at least one further membrane to reach the filial tissues such as the aleurone and endosperm. These three or more transmembrane events influence seed loading but also the partitioning of arsenic between inedible husks, the bran and the endosperm.

A detailed picture of the underlying mechanisms for phloem loading and unloading of arsenic remains to be established (see Outstanding Questions) and most of what we know derives from studies in rice and *Arabidopsis*. In rice, the first node has been identified as a critical hub for the distribution of Si between tissues with efflux transporters like Lsi2 and Lsi3 and NIP2;2/Lsi6 directing Si to either the seed husk or the flag leaf [42, 43]. As these transporters are also permeable to AsIII, they may prove equally important in the distribution of arsenic towards the grain. Indeed, excised panicles from Lsi2 knockout plants accumulated a greater proportion of arsenic in the flag leaf and a reduced amount in the grain [44].

Interestingly, there is evidence that organic arsenic forms such as dimethylarsinic acid (DMA) are translocated to the rice grain through both the xylem and phloem [45]. DMA is also more mobile within the grain compared to inorganic arsenic which accumulates primarily in the ovular vascular traces on the surface of the grain [46]. How DMA is deposited in the grain is not clear but it may directly, or indirectly, depend on the activity of NIPs and possibly that of peptide transporters such as OsPTR7 [47].

In *Arabidopsis* recent work shows that several transporters influence phloem arsenic levels which in turn impact on seed arsenic. Loss of function in NIP7;1 profoundly reduced seed arsenic content [37] and a similar phenotype was seen in NIP6;1 KO mutants (E. Lindsay, PhD thesis, University of York, 2016). NIP6;1 plays a role in B transport and is predominantly expressed in nodes where it is localised to the phloem region of vascular tissues. It is thought to mediate transfer of B from the xylem to the phloem, especially in B limiting conditions [48]. NIP7;1 is also involved in long distance transport of arsenic. Loss of function in NIP7;1 led to lower arsenic levels in both xylem and phloem and reduced seed arsenic by more than 50% compared to the WT [37].

A similar mechanism for arsenic loading of *Arabidopsis* seed was recently reported for the inositol transporters AtINT2 and AtINT4 [41]. Though not immediately obvious, many sugar transporters are capable of moving arsenic, particularly in its AsIII form [49]. The mechanism for this is unclear but is probably not based on substrate similarity. Evidence that sugar transporters are involved in plant arsenic distribution comes from INT knockout plants; loss of function in either generesulted in significantly reduced arsenic levels of the phloem and more importantly, a corresponding reduction in siliques and seeds [41]. The reduced level of arsenic in the silique suggests that INTs are primarily involved in phloem loading in the source tissue. In *Arabidopsis*, siliques of *nip7* mutants showed wildtype levels of arsenic (E. Lindsay, PhD thesis, University of York, 2016) but much lower seed arsenic [37], pointing to a role in arsenic unloading (Fig. 2)

**Developing Low Arsenic Crops**

To develop crops with low or negligible levels of arsenic in edible parts, three main strategies can be explored: reduced net arsenic intake, retention of arsenic in the root and for seed crops, minimising arsenic deposition in seeds (Fig. 3).

*Can we reduce arsenic uptake?* Reducing arsenic uptake in crops would provide an efficient way of mitigating the perils of diet based arsenic poisoning. However, reducing AsV uptake is likely to affect uptake of P, an essential, and often scarce nutrient. Consequently, such an approach easily creates P deficiency and hence is counterproductive. Engineering Pht type proteins that discriminate against AsV could potentially solve this conundrum but is not trivial given that the anion charge, atomic radius and pK values of PO43- and AsO43- are almost identical. The latter causes phosphate and arsenate to be coordinated within enzymes with similar chemistry and there is no precedent in nature of transporters that highly discriminate between these molecules. However, some bacterial strains isolated from environments where arsenic is rife, have a trick up their sleeve that might be exploited: in these organisms phosphate is bound by periplasmic binding proteins (PBPs) before it is taken up by ABC transporters. The 500-4000 fold higher affinity for phosphate of the PBPs is achieved via a unique mode of binding [50] due to subtle differences in proton bonding between the PBP Asp62 residue and one of the oxygen atoms of HPO42- or HAsO42-. When phosphate is complexed, the proton occupies a nearly central position between anion and protein whereas the proton is asymmetrically located when arsenate is bound. The latter leads to a weaker interaction between PBP and AsV. Plants produce a range of root exudates to optimise acquisition of scarce nutrients (for example organic acids to chelate phosphate or phytosiderophores to bind Fe3+). In analogy, bacterial PBPs can be expressed in planta using a root specific promoter. PBPs are relatively small proteins (30-40kD) encoded by a single gene making such an approach highly feasible. In parallel, bacterial ABC transporters could be engineered into crop roots (Fig. 4) to devise a phosphate uptake mechanism that discriminates strongly against AsV.

Net arsenic uptake is a function of influx and efflux and a large part of arsenic that enters the root is extruded fairly rapidly. The underlying mechanisms remain to be discovered but the use of heterologous efflux systems in rice and *Arabidopsis* [51, 52], such as the yeast Acr3 antiporter, has shown that efflux can be increased and, at least in *Arabidopsis*, lead to increased arsenic tolerance. Interestingly, in *Arabidopsis* the Acr3 activity promoted xylem loading and raised the shoot:root ratio for arsenic [52]. Identification of putative endogenous efflux mechanisms can be facilitated by association studies, such as GWAS, which not only point to relevant genomic loci but also help identify cultivars with high efflux capacity.

*How to protect rice from As?* Most rice is grown in paddies which means AsIII rather than AsV is the form in which arsenic enters the plant. AsIII enters rice roots by hijacking the highly efficient silicon (Si) uptake pathway consisting of the root expressed NIP2;1 (OsLsi1) and other NIPs. Si influx via NIP2;1 is passive and sustained by active efflux towards the stele through the efflux transporter OsLsi2 [10, 53]. Vectorial movement of AsIII toward the stele is most likely mediated by the same mechanism [53]. In the nip2;1 loss of function mutant, AsIII influx was approximately halved and levels of arsenic in xylem sap and shoots were also altered. However, because rice growth and stress resilience are inextricably linked to Si sufficiency, expression of both NIP2;1 and Lsi2 appear to be essential for rice cultivation. As with AsV and PO43-, the underlying problem is one of selectivity: essential nutrients such as boron (B) and Si are taken up as uncharged boric acid (H3BO3) and silicic acid (Si(OH)4) which not only have similar sizes to each other but also to AsIII which at neutral pH occurs as H3AsO3. A fair amount of variation has been observed in NIP selectivity filters but studies aimed at optimising transport of nutrients such as Si, while lowering arsenic transport rates, have so far not been successful [54].

 NIPs not only affect arsenic in rice but are probably instrumental in AsIII distribution throughout many plants and being able to alter NIP selectivity would be extremely useful. Most plant NIPs show AsIII permeability, while permeability of nutrients like Si and B is apparent in far fewer isoforms [28, 54] in spite of sometimes similar pore size [54]. This suggests that structural features other than the selectivity filter may also be important in determining substrate selectivity and especially other pore lining residues should be considered in this respect. Interestingly, a recent report demonstrated that the Killifish **aquaglyceroporin** KfAQP3a did not conduct AsIII whereas KfAQP3b does, in spite of 96% amino acid identity and a 100% identical selectivity filter [55]. Residues located in the C-terminus, rather than the selectivity filter (Fig. 3B), were responsible for this difference and KfAQP3a could be converted into an AsIII transporter by mutating three residues. The lack of AsIII permeability through KfAQP3a may help explain the low levels of arsenic accumulation in this fish. If KfAQP3a has significant transport capacity for beneficial nutrients such as Si and B, something that has yet to be determined, it would serve as an extremely interesting example to engineer improved arsenic tolerance in plants.

*Reducing arsenic translocation to the shoot.* The inherent tendency to move only small proportions of arsenic toward photosynthesising tissues can potentially be exploited to improve crop safety. Several mechanisms affect translocation of arsenic to the shoot: Work on arsenate reductases such as HAC1 [30, 31] and HAC4 [32] showed a strong correlation between the capacity to reduce AsV to AsIII and arsenic transport to above ground parts which suggests that enhancing reductase function could further limit arsenic deposition in edible parts. This is further borne out by work on glutaredoxins (GRxs): Ectopic expression of GRxs in *Arabidopsis* and rice increases arsenic tolerance and decreases arsenic accumulation in shoots, probably by augmenting the glutathione pool that sustains AsV reduction [56, 57]. OsCLT1, a plastid envelope transporter that may be involved in releasing plastidial glutathione and γ-glutamylcysteine into the cytoplasm [58] also augments reducing power. However, increased reduction is likely to require greater sequestration capacity into root vacuoles. Tissue specific overexpression of relevant root ABC transporters is an obvious approach to test this hypothesis and could be combined with strategies to increase root reductants. Manipulating expression of NIPs that are involved in xylem loading [23, 37] may also lower shoot arsenic but clearly this should not compromise nutritional aspects such as Si or B transport.

*Lowering arsenic deposition in seeds and grains:* Manipulation of transcript levels of many genes has been shown to impact on shoot arsenic and/or seed arsenic. This includes indirect effects from uptake systems such as OsPT1 and OsPt8 [17, 18], glutaredoxins [56,57] and reductases [30,31,32]. However, The identification of specific INTs [41] and NIPs [37] that individually lowered seed arsenic levels by more than 50% gives us an extremely promising proposition to reduce arsenic in edible plant parts and identifying their orthologs in relevant crop species is imperative. Testing combinations of INT plus NIP KO genotypes is a logical next step to potentially create additive phenotypes. The physiological role of AtNIP7 is unknown but its deletion did not affect plant growth in laboratory conditions [37]. Plants typically have large multigenic NIP families which suggests there may be enough functional redundancy to delete at least some without any adverse consequences. INTs are involved in the delivery of inositol to the reproductive tissues via the phloem (Fig. 2) and thus may have an indispensable role although single deletion mutants did not affect growth in standard conditions [41].

**Conclusions and future prospects**

Limiting the amount of arsenic that enters the plant and directing its deposition to tissues that have no dietary value would greatly reduce arsenic entry into the food chain. To achieve this, there is now a large number of targets that can be optimised and/or combined in model species but also in crops, either via engineering or via (molecular) breeding. Although our options to reduce uptake of AsIII and AsV may be limited, net uptake may be constrained by increasing arsenic efflux, either through heterologous systems or by manipulating as yet unknown endogenous extrusion mechanisms. Combining beneficial traits such as those that prevent translocation to the shoot can ensure more robust phenotypes. Once in the shoot, seed deposition can be decreased either at the remobilisation phase in source organs or when arsenic is deposited. In all these cases pyramiding of traits, which is increasingly made more feasible by modern genetic techniques such as genome editing, could be achieved on the basis of relatively few genes.

Genetic variability, either in enzyme turnover or in substrate specificity, has hardly been assessed with respect to arsenic related proteins but, as exemplified by work on HAC1 [30], can have significant impact on arsenic uptake and distribution. Genome wide association studies (GWAS) may be instrumental in catalysing progress in this regard. More insights into the regulation of arsenic transport is also urgently required: Which are the relevant transcription factors [59] and protein partners such as kinases [60] and ligases [61], and could there be a regulatory role for phospholipases [62]? Genetic diversity can also be mined to isolate genotypes with relatively low (or high) shoot arsenic levels, for example by reporters (e.g. luciferin or anthocyanin production) fused to promoters of arsenic sensitive genes. Screens of mutagenised populations and diversity panels would help tease out the underlying mechanisms and at the same time could identify promising crop cultivars.

**Outstanding Questions Box**

* It is well known that a large fraction (sometimes up to 80%) of the arsenic that is taken up by roots is rapidly extruded. What are the responsible transport proteins for this?
* For the model species Arabidopsis, we now have evidence of specific proteins impacting on levels of arsenic in seeds but what is the underlying mechanism? Do they mobilise arsenic from stores in the source tissue into the phloem? Do they unload the phloem in the seed and if so where? Do orthologous proteins exist in other species?
* Are there any structural elements in NIP proteins that would allow us to engineer NIPs with low AsIII permeability whilst maintaining their innate transport function? Expansive mutagenesis and structure function analyses would settle this.

**Trends Box**

* Considerable progress in determining the molecular details of arsenic uptake, detoxification and translocation within plants has been made in the past years.
* Recent developments concerning the role of specific proteins in delivering arsenic to seeds and grains are of particular importance regarding the threat posed by arsenic contamination to public health.
* These new insights can be utilised to inform new strategies for the development of arsenic tolerant crop varieties with reduced arsenic uptake and less accumulation in edible parts.

**Glossary:**

**Aquaporin**: a family of small membrane integral proteins originally identified as channels which specifically facilitate the osmotic gradient driven transport of water across plasma membranes.

**Aquaglyceroporin**: a subfamily of aquaporins characterised by a larger pore size and permeability to neutral molecules such as glycerol.

**Arsenicosis**: a health condition arising from chronic arsenic poisoning caused by prolonged exposure (through ingestion) of arsenic in contaminated food and drinking water.

**Chelation**: a process by which a metal atom or iron binds with a non-metallic ligand through at least two covalent bonds to form a heterocyclic ring

**Hyperaccumulator**: a plant species which accumulates an unusually large concentration of a contaminant in its above ground biomass. The threshold concentrations used to define hyperaccumulators varies between contaminants.

**Xenobiotics**: a foreign substance not a natural component of the environment or produced by the organism exposed to it, such as artificially created chemicals and environmental pollutants.

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**Figure legends**

**Fig. 1: Overview of arsenic transport and detoxification.**

A schematic representation of known and putative proteins and processes involved in arsenic uptake, efflux, distribution and detoxification in plants. In plant roots, arsenite (AsIII) and arsenate (AsV) are taken up by aquaporins (NIP) and phosphate transporters (PHT) respectively. AsV is reduced to AsIII by arsenate reductases (HAC) using glutathione (GSH) as a reductant. AsIII is complexed with phytochelatins (PC) prior to sequestration in the vacuole via ATP-binding cassette transporters (ABCC), or in the case of *Pteris* *vittata*, sequestered directly in the vacuoles via an AsIII/H+ antiporter ACR3. In rice, AsIII is effluxed towards the stele through Lsi2 (an AsIII/H+ antiporter). Long distance transport of AsIII through the xylem/phloem to the shoot and seed (see also Fig. 2) is at least partially mediated by NIP aquaporins and inositol transporters (INT). Abbreviations: M, maternal tissue (husk, bran); F, filial tissue (aleurone, embryo, endosperm); PHT, phosphate transporter; HAC, arsenate reductase; GSH and GSSG, glutathione in its reduced and oxidised form respectively.

**Fig. 2: Proteins involved in delivery and deposition of arsenic in seeds.**

Although the details are largely unknown, there is now evidence that NIP type aquaporins and INT type sugar symporters impact significantly on arsenic deposition in seeds. The current data suggest that INTs may be involved in mobilisation of arsenic from source tissue for example by pumping apoplastic arsenic into the phloem for subsequent transport to seed tissues. In contrast, NIPs are more likely to catalyse unloading of arsenic at the maternal seed tissues. Abbreviations: M, maternal tissue;

F, filial tissue.

**Fig. 3: Strategies to minimise net arsenic uptake.**

**(A)** Various strategies to reduce net arsenic uptake include: (1) replacement of non-selective phosphate transporters (PHT) with engineered transporters (see Fig. 4). Similarly, (2) replacement of endogenous NIPs with high AsIII permeability by isoforms that do not allow AsIII transport could lower AsIII influx in crops such as rice. Enhancing the AsIII efflux capacity (3) through either, as yet unknown, innate systems or by using heterologous transporters such as Acr3 from yeast or hyperaccumulator ferns is a further alternative. **(B)** Structural topology of an aquaporin monomer showing six transmembrane domains and five interconnecting loops (A-E). The positions of the conserved NPA motifs are shown. Blue asterisks denote the approximate location of the four selectivity filter residues which make up the Ar/R pore restriction site while red dots indicate the C-terminal three residues that define arsenite permeability in KfAQP3, possibly via interaction of the C-terminus with the D loop.

**Fig. 4: Using engineering to increase phosphate arsenate selectivity.**

**(A)** Bacterial periplasmic binding proteins (PBPs) from some gram negative bacteria have a remarkable capacity to bind phosphate (Pi) but discriminate strongly against arsenate (As) with a selectivity ratio of over 500. This PBP-generated enrichment of Pi ensures the uptake system, an ABC transporter with low selectivity that transports substrate after it dissociated from the PBP complex, is fed with Pi to As ratios of over 500. This level of selectivity is unprecedented in the rest of biology and could possibly be exploited to engineer phosphate specific uptake systems in plant roots **(B)**. For this, plants would heterologously express PBPs and their cognate ABC transporter. Like other root exudates, PBPs can be extruded into the rhizosphere where they chelate Pi but not AsV. As a result, the PBP-recognising ABC transporter would greatly discriminate against AsV uptake. Alternatively, plant phosphate (PHT) transporters can be used for the selective uptake of Pi from a zone where it is highly enriched.







