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Review

Metalloido-porins: Essentiality of Nodulin 26-like intrinsic proteins in metalloid transport

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ABSTRACT

Metalloids are a group of physiologically important elements ranging from the essential to the highly toxic. Arsenic, antimony, germanium, and tellurium are highly toxic to plants themselves and to consumers of metalloid-contaminated plants. Boron, silicon, and selenium fulfill essential or beneficial functions in plants. However, when present at high concentrations, boron and selenium cause toxicity symptoms that are detrimental to plant fitness and yield. Consequently, all plants require efficient membrane transport systems to control the uptake and extrusion of metalloids into or out of the plant and their distribution within the plant body. Several Nodulin 26-like intrinsic proteins (NIPs) that belong to the aquaporin plant water channel protein family facilitate the diffusion of uncharged metalloid species.

Genetic, physiological, and molecular evidence is that NIPs from primitive to higher plants not only transport all environmentally important metalloids, but that these proteins have a major role in the uptake, translocation, and extrusion of metalloids in plants. As most of the metalloid-permeable NIP aquaporins are impermeable or are poorly permeable to water, these NIP channel proteins should be considered as physiologically essential metalloido-porins.

Contents

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1. Introduction

1.1. Metalloids

Metalloids are defined as compounds that possess physical and chemical properties that are intermediate between metals and nonmetals. A rigorous assignment of the associated elements constituting the group of metalloids is very difficult. Arsenic (As), antimony (Sb), boron (B), germanium (Ge), silicon (Si), and tellurium (Te) are commonly considered as metalloids (Table 1). Certain other elements, such as selenium (Se), are sometimes added to the list of metalloids. Metalloids are generally present in the soil solution as either negatively charged ions or undissociated (uncharged) molecules depending on the pH and the redox potential of their environment (Fig. 1). It is likely that the only Si species that is available to organisms is orthosilicic acid (H_4SiO_4), which is uncharged at physiological pH ranges. Similar to Si, the most prominent and bio-available species of Ge in the soil solution is the uncharged germanic acid (H_2GeO_3/H_4GeO_4). As B does not undergo oxidation–reduction reactions at physiological conditions, its only bio-available forms are boric acid (H_3BO_3) and borate ($B(OH)_4^-$) which occur in a pH-dependent equilibrium. Due to the pK_{a1} value of 9.25 for boric acid (Fig. 1), the uncharged H_3BO_3 molecule quantitatively dominates over $B(OH)_4^-$ in physiological conditions. In comparison to B, the chemistry and speciation of As, Sb, and Se is more complex. The pH and redox potential are key factors controlling the oxidation and dissociation states of these elements, and thereby their availability and transport in the environment. Silicon, As, Sb, and Ge species can be chemically and/or biologically methylated yielding organic metalloid compounds. Plants have to deal with several chemical species of the same element depending on the chemical environment in which the metalloid occurs (Fig. 1). The speciation of the metalloid is determined either by the chemical environment of the soil solution or of the cell saps which might vary among plant organs or tissues, or even between the different compartments within a cell. Due to the high pK_a values (above 9, see Fig. 1) of most environmentally important metalloid acids, these metalloid species occur in soils or organisms dominantly as uncharged molecules.

While metalloids vary in their chemical properties such as atomic number and valence electrons, the chemical structures of their acids is highly similar (Table 1). Metalloids affect living organisms in different ways. Arsenic, Sb, Ge, and Te are highly toxic to consumers of metalloid-contaminated plants and to plants themselves, unless they are sequestered in vacuoles or complexed. In contrast, B, Si, and Se fulfill essential or beneficial functions in plants. When present at high concentrations, B and Se are also toxic to plants, and exposure causes a range of toxicity symptoms that are detrimental to fitness and yield. Consequently, metalloid homeostasis must be carefully regulated in plants. Evidently, the regulation of transport of these compounds into, out of, and within the plant represents a crucial control lever to adapt the plant metabolism to different levels of metalloids.

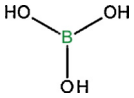
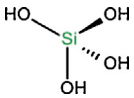
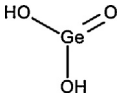
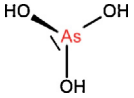
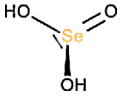
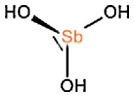
The transport of various metalloid species is regulated by active and/or passive transport mechanisms in plants [1,2]. The active and passive transport mechanisms are controlled by different transporter protein families, which together most efficiently regulate the uptake, translocation, and extrusion of various metalloid species (e.g. B, Si, and As) [1–3]. Knowledge about metalloid transport mechanisms on the molecular level was mainly gained in the last 10 years. Before then, it was assumed that the transmembrane transport of uncharged metalloid species, which include the biologically most important ones, was merely determined by the passive diffusion across the lipid bilayer, and not by proteins. Bit by bit, it was demonstrated that Nodulin 26-like intrinsic channel proteins (NIPs), which exhibit strict pore selectivity for uncharged molecules, are essential for the transport of environmentally important metalloids in plants. In the following, we highlight, synthesize, and extrapolate the current knowledge on the crucial functions of NIPs dominating the transport regulation of undissociated metalloid species in the plant kingdom.

1.2. Nodulin 26-like intrinsic proteins

NIP proteins belong to the major intrinsic proteins (MIPs), which form a family of essential membrane channel proteins facilitating the diffusion of water and small uncharged solutes in all domains of life [1]. MIPs are typical members of diffusion facilitater proteins. The process in which the flow of molecules across cell membranes is facilitated by special types of proteins is called facilitated diffusion (Fig. 2). Facilitated diffusion (protein-mediated) and simple diffusion (non-protein-mediated) are responsible for passive transport processes in biological systems [4]. The structure of MIP channels is highly conserved, although the amino acid sequences of the proteins differ substantially. MIPs form tetramers (Fig. 3A). Each monomer is composed of six transmembrane-spanning helices (TMHs) with five connecting loops (loops A–E) and two cytoplasmic termini (Fig. 3B). MIPs form a narrow path (ca. 0.2–0.5 nm in diameter) across various cellular membranes allowing the passage of just a single continuous file of substrate water molecules. The cavity forms the so-called aromatic/arginine (ar/R) selective filter toward the luminal side of the membrane. This filter is constructed of four amino acids, which are crucial for the substrate selectivity of MIPs [5].

GmNOD26 was the first described plant MIP [6]. Nodulins (abbreviated NODs) are proteins, which are involved in the symbiotic processes between legumes and rhizobia. Nodulin genes show a specific expression pattern in nodules, a specialized tissue in which the fixation of molecular nitrogen occurs. GmNOD26 is the major proteinaceous membrane constituent of soybean nodules representing 10–15% of the total membrane protein [6–8]. It belongs to the plant NIP family for which it became the eponym. GmNOD26 was the first plant MIP to be investigated on a biochemical level [6]. This was 3 years after the first biochemical description and identification of an MIP, from bovine lens fibers [9]. GmNOD26 was initially suggested to be permeable to malate as its phosphorylation status correlated closely with malate uptake across the symbiosome membrane [10]. While a functional evidence for a permeability to malate remains to be shown, GmNOD26 was found to be a functional water channel in 1997 [11], and in 2010 it was shown to facilitate the diffusion of ammonia when reconstituted

Table 1
Overview of chemical and physiological properties of metalloids.

Element	Atomic number	Oxidation number in physiological relevant bonds	Essential for plants	Essential for animals	NIP-transported chemical forms	Molar volume (cm^3/mol)	Molecular structure
Boron	5	III	+	–	Boric acid	43	
Silicon	14	IV	(+)	–	Silicic acid	54	
Germanium	32	IV	–	–	Germanic acid	n.d.	
Arsenic	33	III, V	–	–	Arsenous acid	59	
Selenium	34	IV, VI	–	+	Selenous acid	74	
Antimony	51	III, V	–	–	Antimonous acid	62	

Boric acid and germanic acid have planar structures, while silicic acid, arsenic acid, selenous acid, and antimonous acid have a tetrahedral molecular structure. Essentiality for plants and humans is indexed as followed: + = essential element for all species, (+) essential element for some species, – =non-essential element, (–) = essentiality unknown, (n.d.) = no data.

into proteoliposomes [12]. Subsequently, a physical interaction of the C-terminal domain of GmNOD26 with glutamine synthetase was demonstrated, strongly suggesting a role for this NIP in the transport of ammonia [13]. A physiological involvement of NIPs in the nitrogen transport metabolism of plants is supported by the fact that numerous NIPs are permeable to ammonia and/or urea both being nutritionally important nitrogenous molecules [14]. NIP proteins are also recognized as the major nodule constituents of other model legume species such as *Lotus japonicus* and *Medicago truncatula* [15,16]. In addition to the role in nitrogen transport metabolism, a role in metalloid transport may be speculated for legume NIPs other than GmNOD26 due to the fact that legumes require, e.g. B in high concentrations for nodule development [17]. Functional symbiosomes are only built up and retained in a functional capacity if plants have a sufficient B supply [18–21]. To date, it is not known how B is transported to the nodules and transported across various membranes therein. NIP channels might represent these putative transmembrane transport systems.

In eukaryotes, NIPs are uniquely found in plants. Compared to the other plant MIP subfamilies (i.e. the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the small basic intrinsic proteins (SIPs), and the uncharacterized X intrinsic proteins (XIPs)), NIPs are one of the member-richest MIP subfamilies from mosses to flowering plants. In *Selaginella moellendorffii*, 8 out of 19 MIP isoforms and in *Arabidopsis thaliana* 9 out of 35 MIP isoforms belong to the NIP subfamily [22]. They are also one of the most divergent plant MIP subfamilies with respect to their substrate specificities and amino acid sequences.

1.2.1. *Phylogenetic classification of NIPs*

All higher plant MIPs belong to the group of aquaporins. An ancient gene duplication in the evolution of MIPs was proposed as the source of the main functional division of MIPs into water-permeable aquaporins and glycerol-permeable aquaglyceroporins [23]. Despite this differential phylogenetic affiliation, NIPs and microbial and mammalian aquaglyceroporins are thought to be functional equivalents due to their identical substrate selectivities and physiological functions [24,25]. Bacterial, fungal, mammalian, and human aquaglyceroporins have key roles in the transport of glycerol and uncharged metalloid species [1,24]. The evolutionary origin of NIPs is still unresolved. NIPs cluster together with bacterial and archaeal NIP-like proteins in a reconstructed phylogeny as a very basal lineage within the aquaporins, but are separated from the widespread typical bacterial MIP groups: aquaporin Z or glycerol uptake facilitator protein [23,26]. This phylogenetic clustering may support the hypothesis that plant NIPs have been acquired through horizontal gene transfer from prokaryotic genomes [26]. A horizontal gene transfer suggests that the coding regions for NIP genes were transferred from ancient chloroplasts or from bacterial genomes into nuclei of plants [26]. However, the possibility that sequence similarities exist due to the convergent evolution of both channel types cannot be discarded. Therefore, a clustering of these proteins may only exist due to the phenomenon of longbranch attraction [23].

Phylogenetic analyses show that plant NIPs can be divided into five well-defined subgroups (specified as NIP1–NIP5), which are remarkably well conserved across species [23]. It is

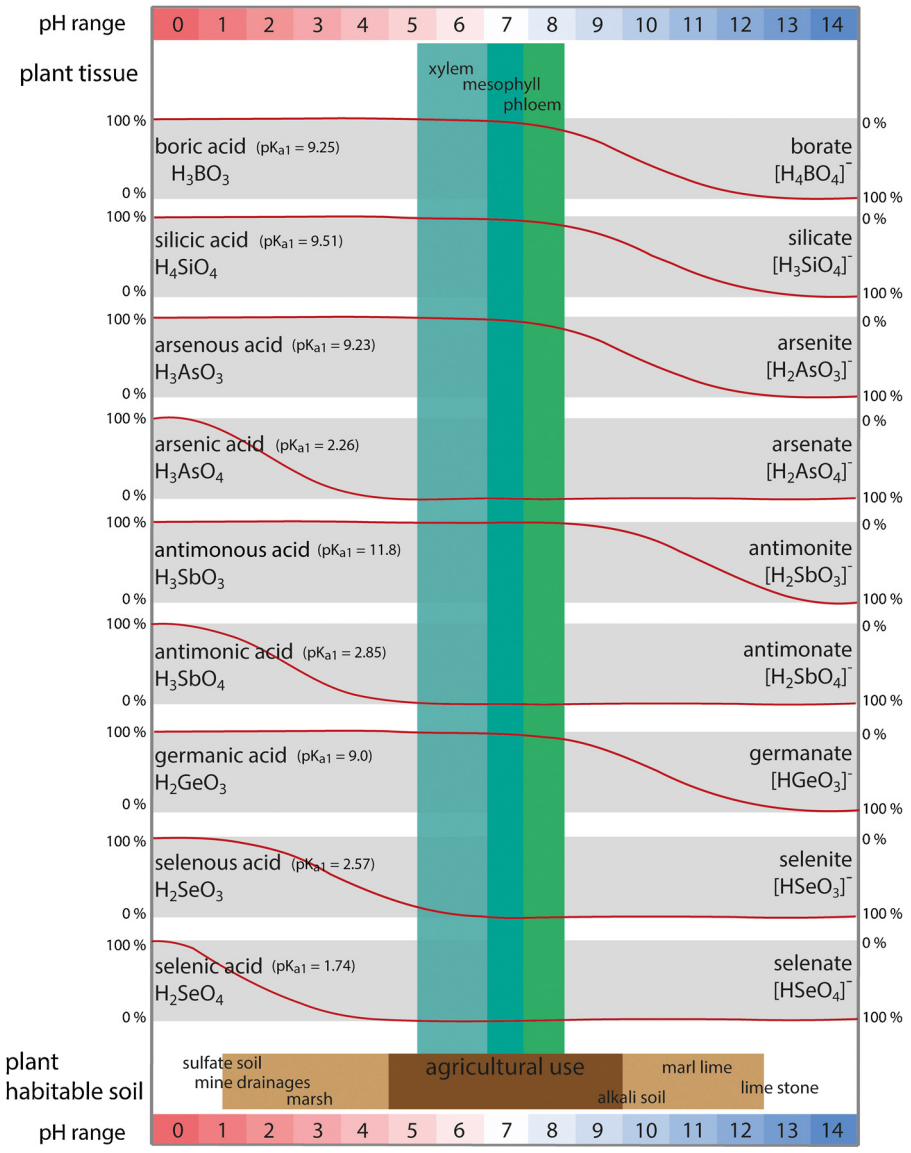


Fig. 1. pH-dependent dissociation equilibrium of hydroxylated metalloid acids. The red curves indicate the portion of the undissociated, neutral forms of the respective metalloids in a pH range from 0 to 14. Only these neutral forms are channeled by metalloido-porins of plants. The Hendersen–Hasselbalch equation was used for calculations. pK_a values are given at the structural formula. Boric, silicic, arsenous, antimonous, and germanic acid are weak acids occurring as uncharged molecules at physiologically relevant pH values. The deprotonated/charged forms are abundant only in alkali environments. In contrast, arsenic, antimonic, selenous, and selenic acid are strong acids with low abundance of their uncharged form in plant tissues and in agriculturally utilized soils.

important to mention that the identifiers given to the phylogenetic NIP subgroups (NIP1–NIP5) do not match the identifiers of single isoforms (e.g. AtNIP1–AtNIP7 in Arabidopsis). The NIP1 phylogeny subgroup of *Arabidopsis* includes: AtNIP1;1, AtNIP1;2, AtNIP2;1, AtNIP3;1, AtNIP4;1, and AtNIP4;2. The NIP3 phylogeny subgroup of *Arabidopsis* includes AtNIP5;1, AtNIP6;1, and AtNIP7;1. No NIP isoform of *Arabidopsis* belongs to the NIP2 phylogeny subgroup. In contrast to the study dividing NIPs into five phylogenetically distinct subgroups (NIP1–NIP5), two recent independent phylogenetic analyses classified NIPs in either four phylogenetically distinct subgroups (NIP-1–NIP-4) or six NIP ortholog clusters (NIPCL-I–NIPCL-VI) which only partly correspond to the designation of the above mentioned study [26,27]. Table S2 guides through the different functional and phylogenetic NIP subgroup clusterings and nomenclatures used in this report. The low level of node support and polytomies that can be observed in the different phylogenetic analyses of NIP genes illustrate that it is not clear how the NIP groups or single NIP isoforms within these groups are related to each other [23,26–28]. This partially unclear phylogenetic interrelationship among NIPs resulted in inconsistent nomenclatures of orthologous or paralogous isoforms in different species. Given the confusion that these inconsistent designations cause, a future priority of the research community should be the generation of a revised, consistent, and commonly accepted nomenclature not only for NIPs but also MIPs in general (e.g. as recently introduced for other transport protein families (ABC transporters) [29]). Based on the amino acid composition of the ar/R constriction region, NIPs have been additionally divided into three functional groups (NIP-I–NIP-III) [30,31]. These NIP subgroups are present in all higher plants, although the NIP-III group seem to be predominantly present in monocots [23,32]. The proliferation of the NIP-III genes in the *Poaceae* family is probably attributed to whole genome and segmental chromosomal duplication events, which occurred at the very beginning of the evolution of graminaceous plants [32]. From the physiological point of view, this proliferation of NIP channel proteins being permeable to silicic acid (see Section 2.3) might

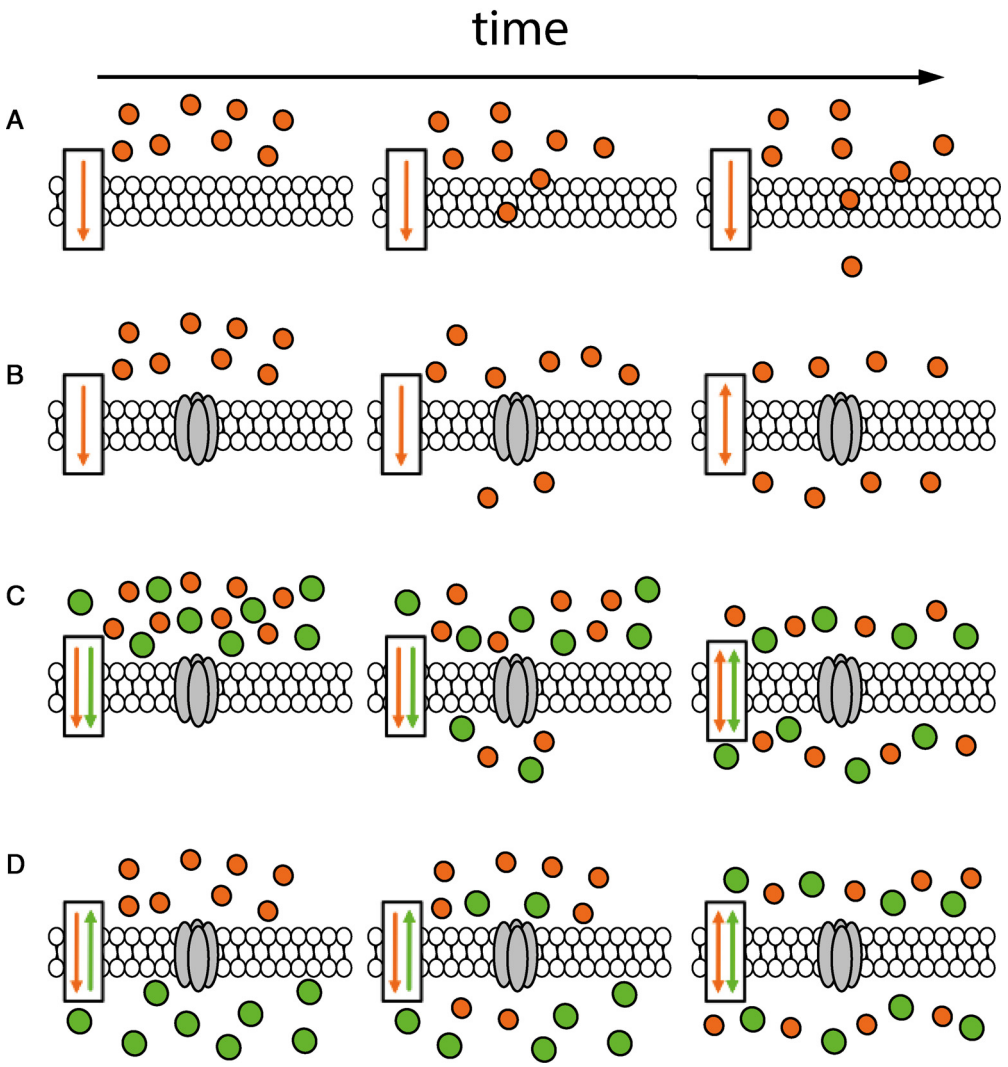


Fig. 2. Schematic depiction of different passive transport processes and transport rates across membranes. (A) Slow non-protein-mediated *simple diffusion* of a compound (orange bowls, e.g. boric acid) across a lipid bilayer at three successive time points. (B) Rapid aquaporin channel protein-mediated *facilitated diffusion* of a compound (orange bowls, e.g. boric acid) across a lipid bilayer at three successive time points. (C) Unidirectional aquaporin channel protein-mediated *facilitated diffusion* of two aquaporin substrates (orange and green bowls, e.g. boric acid and arsenite) across a lipid bilayer at three successive time points. (D) Aquaporin channel protein-mediated *facilitated diffusion* of two aquaporin substrates (orange and green bowls, e.g. boric acid and arsenite) across a lipid bilayer in opposite directions at three successive time points. Orange and/or green arrows indicate the direction of the chemical gradient for the same-color compound and therewith the driving force for the passive diffusion.

have paved the way for the efficient and beneficial use of Si in this particular plant group.

2. NIP-mediated metalloid transport in plants

For a long time, it was thought that uncharged metalloids such as the essential nutrient boric acid freely cross biological membranes only by passive non-protein-facilitated diffusion [33]. However, (i) gradients for uncharged metalloids (B and As) across plant membranes have been reported [34–36], (ii) B permeability coefficients of plant-derived vesicles were significantly higher than the permeability coefficients of artificial liposomes [35,36], (iii) fluxes of uncharged metalloid species such as B and As were inhibited by mercuric chloride, an MIP blocker [34,35], and (iv) glycerol, an MIP substrate, competitively inhibited As fluxes [34]. These data demonstrate that plant membranes generally prevent the free diffusion of undissociated metalloids and suggested that MIPs are the regulative transport proteins that adjust membrane permeability to these molecules. The NIP protein family seems to be predestined for the facilitated membrane diffusion of hydroxylated metalloid species, because their pore structure is selective for non-charged solutes with a certain molecular diameter providing hydrogen bond donors for substrate channel interactions.

2.1. Metalloido-porins for life: NIPs and aquaglyceroporins

As highlighted in this review, NIPs from primitive to higher plants channel certain chemical forms of all environmentally and biologically important metalloids. Moreover, NIPs not only facilitate the transmembrane diffusion of these metalloids, but are also essential for their transport into and within plants. So far, all obtained information on NIPs obviously argue for the enormous impact of this protein family on metalloid homeostasis in plants. MIPs were classified as *orthodox aquaporins*, *aquaglyceroporins*, *aquaammoniaporins*, or *peroxiporins* to highlight the identical molecular and physiological functions of MIPs of different organisms independent of their phylogenetic relationships [37,38]. We introduce the term *metalloido-porin* in accordance with these classifications and the fact that a large number of NIPs which transport metalloids are simultaneously impermeable or only

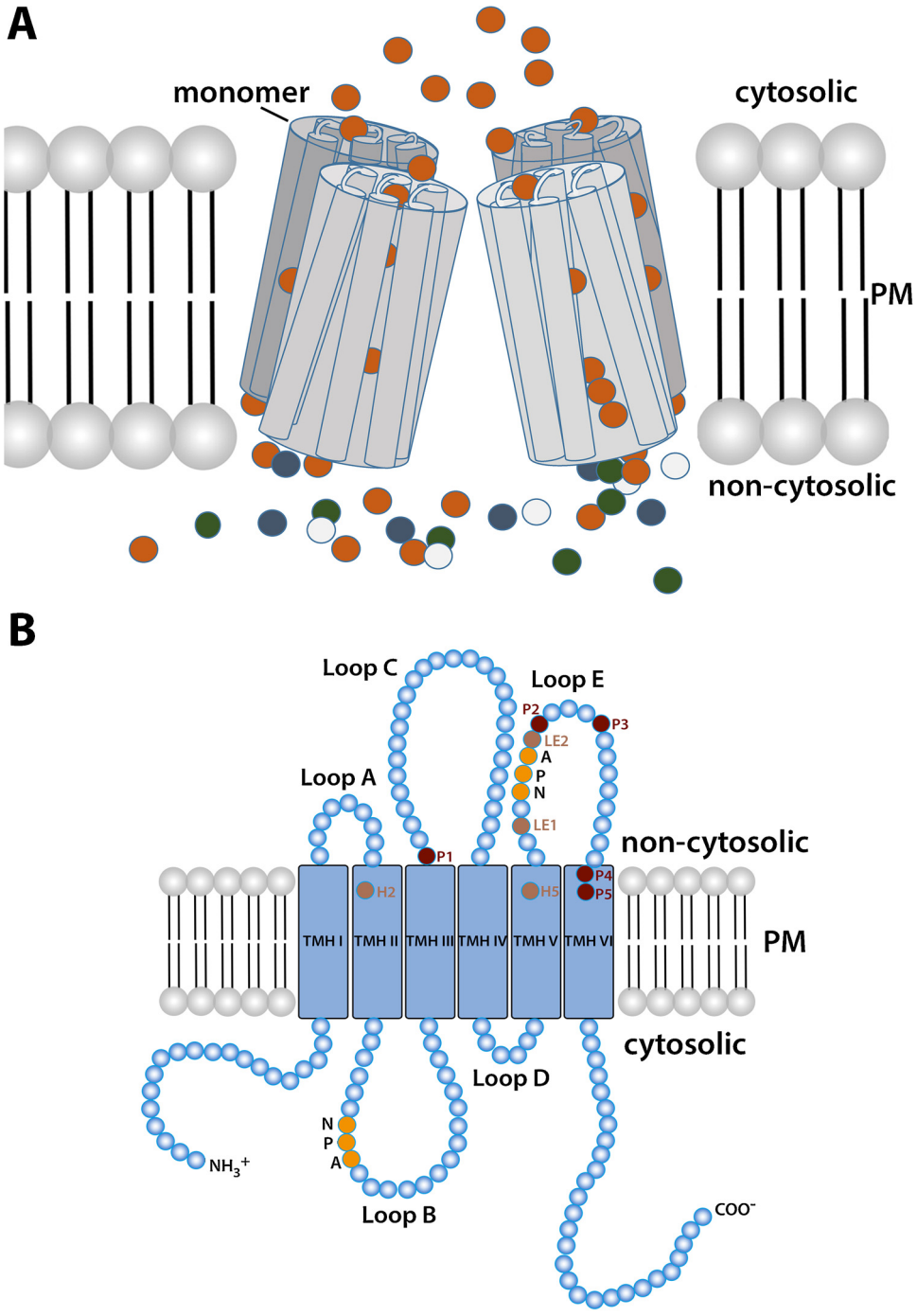


Fig. 3. Schematic representation of NIP channels. (A) Schematic of the tetrameric arrangement of four NIP monomers within the lipid bilayer, each facilitating the specific passage of substrate molecules, represented by different colored spherules. PM = plasma membrane. (B) 2D schematic of an NIP monomer residing in a lipid bilayer. Both N- and C-termini extend into the cytosolic side. Light-blue boxes represent transmembrane helix regions (TMHI to VI). Blue, orange, light- and dark-brown spherules represent individual amino acid residues. Orange spherules depict amino acids representing the NPA domains. Light-brown spherules mark the amino acid positions H2, H5, LE1, and LE2 positions forming the ar/R filter. Dark-brown spherules represent Froger's positions (P1 to P5) at which amino acid properties differ between aquaporins and aquaglyceroporins.

poorly permeable to water. NIPs are the sole known transporter protein class in the plant kingdom, which are essential for the uptake, translocation, or extrusion of various uncharged metalloid species. This, together with the fact that MIPs from all kingdoms of life fulfill essential functions as metalloid channels [1,39], suggests that the term *metalloido-porins* is a term best describing the essential role of certain isoforms of these channels in nature. Furthermore, this designation should overcome the false but widespread doctrine that plant aquaporins, alias water channels, are uniquely involved in plant water transport processes.

2.2. NIP-mediated boron transport

2.2.1. The role of NIPs in boron-deficient conditions

Today it is well established that B is indispensable for the growth of most plant species and that the amount of B which is required for plants is species-dependent [40,41]. Brassicaceae, sugar beet, and

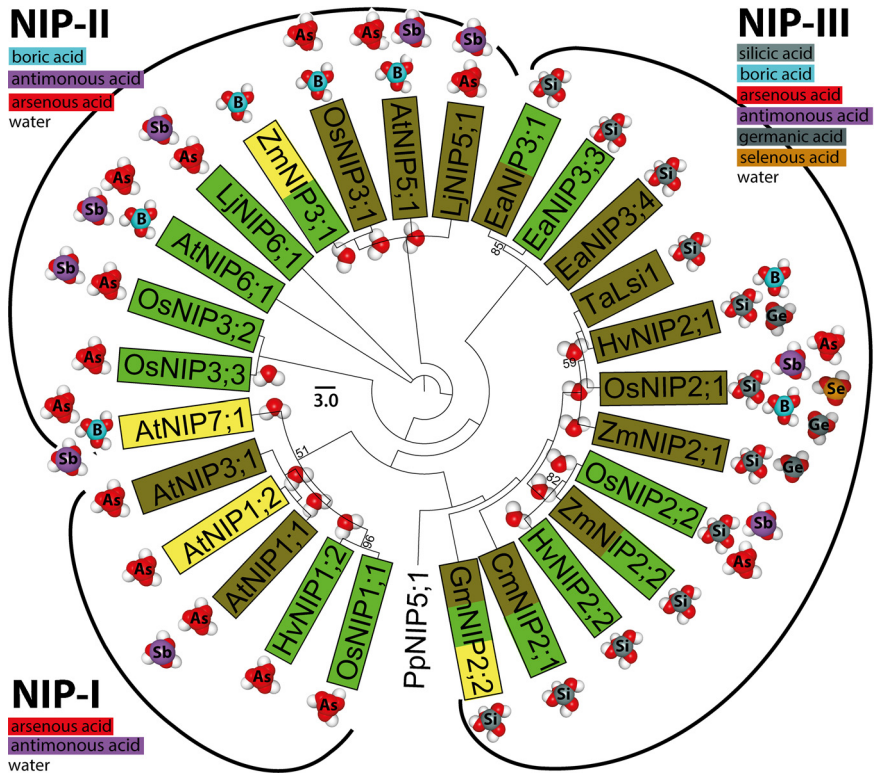


Fig. 4. Phylogenetic tree of metalloid permeable Nodulin 26-like intrinsic protein channels. In the NIP-I group only arsenous and antimonous acids are substrates for these channels, whereas the NIP-II and NIP-III groups show a less stringent selectivity. Background color of the displayed metalloido-porins indicates to the described predominant expression of their corresponding genes (brown: root, green: leaf/shoot, yellow: flower/reproductive organs). Molecular models next to the proteins show the described substrates (As: arsenous acid, B: boric acid, Ge: germanic acid, Sb: antimonous acid, Se: selenous acid, Si: silicic acid). The molecular water models next to the protein names and on top of the branches of the phylogenetic tree indicate a measured water permeability for these isoforms. Numbers next to branches indicate the percentage of node support for each branch. Only node support percentages less than 100 are shown. Scale bar: phylogenetic distance = 3.0.

cotton require large amounts of B throughout development. Grass species such as bread wheat and barley, and also legumes such as soybean and pea have very low B requirements or require B only at the onset of flowering and during seed development [42]. Apart from effects on root growth and root system architecture [42,43], B deficiency manifests itself in meristematic defects and in the lack of stem, leaf, and vascular tissue elongation [44,45]. Some of the symptoms of plants with imbalanced B nutrition can be explained by the biochemical role of B in the mechanical stability of cell walls [46]. Rhamnogalacturonan-II (RG-II) is a constituent of primary cell walls, and the connection of two RG-II monomers via borate esters determines the degree of packaging of the pectic polysaccharides. This in turn dictates the cell wall stability, flexibility, and available space for intercellular communication and signaling in a specific tissue [47,48]. The sites of B deficiency symptoms in meristems, nodes, and floral organs overlap with expression sites of genes coding for NIP channels permeable to B (Fig. 4).

Following the import of B into the root symplast and across cortex cells via NIPs, B is eventually exported into xylem vessels by active B transport proteins [3]. The first B transport protein, BOR1, was discovered in *A. thaliana* in 2002 [49]. AtBOR1 plays an essential role in active loading of B into the xylem of *Arabidopsis*. BOR transport proteins belong to the SLC4 anion-exchanger superfamily which also includes bicarbonate transporters [3]. BOR homologs are found in all plants and algae and are active borate anion efflux proteins. BOR function seems mainly to be implicated in the removal of B from cells to confer tolerance to high B or in the active allocation of B to neighboring cell types [3]. BORs and NIPs are both important transporter classes for efficient transport of B across plant membranes [3]. The primary uptake of B into the roots of Arabidopsis and rice, is facilitated by NIP-II class proteins AtNIP5;1 and OsNIP3;1, respectively (Fig. 4) [50,51]. The ortholog of maize, ZmNIP3;1, was demonstrated to be crucial for the transport of B within the plant and therewith critical for its vegetative and reproductive development [52]. In contrast to BORs, which transport the borate anion, NIPs facilitate the diffusion of uncharged boric acid, the dominant occurring B species at physiological pH values ($pK_a = 9.25$, Fig. 1). Compared to most other metalloid acids, boric acid has a planar structure (Table 1). With respect to this planar structure and its resulting lack of polarity boric acid represents an atypical aquaporin substrate in comparison to most other substrates, which are polar molecules. It will be interesting to unravel the chemical determinants of NIP pores allowing the selectivity for polar and non-polar molecules, as the polarity of an aquaporin substrate was assumed to be a critical parameter for its channel permeation. As an electron-deficient compound boric acid reacts as a weak Lewis acid by taking up one hydroxyl-ion forming borate. AtNIP5;1 and AtNIP6;1 were shown to transport B in yeast, oocytes, and plants demonstrating that they are functional B transporters [50,53]. Consistently, *Atnip5;1* and *Atnip6;1* knockout plants have typical B deficiency symptoms i.e. a reduced stability of the epidermis, abolished apical dominance, and perturbed cell differentiation even when optimal amounts of B are present in the growth medium [50,53]. While *AtNIP5;1* is expressed in the root epidermis, AtNIP6;1 is localized in phloem companion and phloem parenchyma cells within the nodes of young developing leaves where it probably facilitates the transit of boric acid from the xylem into the phloem [53]. The ability of AtNIP6;1 to efflux B out of the xylem at the nodes of Arabidopsis is essential for the allocation of B to developing and meristematic tissues. This can be concluded as *Atnip6;1* knockout plants show B-deficiency-dependent inhibition of the shoot growth. *AtNIP6;1* expression is induced upon B deficiency [53]. In rice, OsNIP3;1 is

responsible for the proper distribution of B in shoots. OsNIP3;1 facilitates boric acid uptake both into the root and into the phloem of mature leaves. Rice plants expressing a *OsNIP3;1* RNAi construct have a reduced shoot B content, resulting in a significantly reduced shoot biomass under low B conditions [51].

2.2.2. The role of NIPs in B-dependent fertility

The nutrient B is also crucial for the fertility of plants. Developing flowers are a sink for B, mainly because pollen development, maturation, and tube growth depends on B [42]. Pollen grains and developing seeds are also terminal B sinks [42]. NIP-mediated B transport activity plays an important role in the distribution and supply of B to the reproductive tissues in both monocotyledons and dicotyledons [42]. For example, B availability is essential for the development of tassels and inflorescences in maize. The maize mutant *tassel-less*, which has severe tassel defects during reproductive development, had a mutation in the *TLS1* gene which encodes the ZmNIP3;1 protein (Fig. 4) [52,54]. *Arabidopsis* mutants with reduced AtNIP5;1 and AtNIP6;1 activities are affected in the formation of their reproductive organs [50,53]. In particular, flower development is inhibited in *Atnip5;1* and *Atnip6;1* mutant plants resulting in sterility, at least under B-deficient conditions. The high B demand in the reproductive organs is underlined by the occurrence of flower-specific NIP channels and BOR transporters (e.g. *Vitis vinifera* VvBOR1: [55]; *O. sativa* OsBOR4: [56]). Developing pollen is symplasmically isolated from the mother tissue, and transport processes have to be postulated for the efficient transfer of B from the vascular tissue of the mother plant into the developing pollen grain. B transport into pollen is at least partly mediated by NIPs, but interplay between BOR transporters and NIP channels is most likely crucial for the coordinated B transport across the different cell layers and apoplastic barriers separating vegetative and male generative tissue. AtNIP7;1 transports B in heterologous uptake assays and is expressed in anthers (Fig. 4) [57]. Regulation of AtNIP7;1 might provide a mechanism to fine-tune B concentrations during pollen development. Analyses of *Atnip7;1* mutants will be useful for elucidating the role of AtNIP7;1 and of B in pollen development and germination. Still, the underlying mechanisms of B participation in the regulation of floral development and fertility are incomplete.

2.2.3. The role of NIPs in toxic boron conditions

Besides B deficiency, B excess also represents a detrimental stress for plants. Toxic effects of B have been known for more than 80 years [58]. In plant species in which B is hardly retranslocated in the phloem, e.g. *Arabidopsis*, symptoms of B toxicity are first visible as chloroses and necroses (*leaf burns*) starting from the margins and gradually spreading into the central parts of older leaves [59,60]. A QTL mapping approach in barley aiming at the identification of tolerance genes to high B concentrations in the soil revealed a locus that contained the *HvNIP2;1* gene, coding for an NIP-III class aquaporin. Plant lines of the mapping population carrying the *HvNIP2;1* allele of the tolerant cultivar exhibited a higher B tolerance and reduced B concentrations in leaves. This elevated tolerance was due to the reduced expression of the *HvNIP2;1* gene in this allelic variant of the locus [61]. Together, these data show that the regulation of NIP metalloido-porin activity and expression are important mechanisms for plants to adjust to either deficient or toxic B concentrations in their environment.

2.3. NIP-mediated silicon transport

Si is the second most abundant element in the earth crust after oxygen. The chemical behaviors of Si and B are similar. Both elements exclusively occur as oxides in nature and are present in their uncharged form at physiological pH levels (Fig. 1). Silicic acid (H_4SiO_4, $pK_{a1} = 9.51$) is the naturally occurring bioavailable form of Si (Fig. 1), and together with other silicates it accounts for 75% of the weight of the earth's crust. The tetrahedral character of silicic acid is similar to that of borate (Table 1), but different from boric acid. Si is not considered an essential element for plants in general, but it has highly beneficial effects for plant growth and yield in some species [62,63]. Silicic acid is most notably abundant in graminaceous plants (with up to 10% of the dry matter of rice plants). In graminaceous plants, silicates have a dominant role due to their structural and mechanical function in cell walls. Silicic acid plays an important role in stress responses, especially in disease resistance [64,65]. A higher Si content in plants reduces the success of some herbivore attacks [65] and the hypersensitive reaction of plants to pathogens is faster and more efficient under high Si levels [66–68]. This effect may result from free and non-bound silicic acid [69]. The efficient uptake and translocation of silicic acid by plants is mediated by NIPs in combination with active Lsi2 transport proteins [70].

The genes responsible for the *low silicon* content of rice *Lsi1* mutant lines have been identified and characterized [70]. Unexpectedly, the first identified plant Si transporter was an NIP aquaporin, OsNIP2;1 (OsLsi1) [71]. OsNIP2;1 is responsible for silicic acid uptake into the roots, and the subsequent allocation into the xylem stream [71]. NIP2;1 orthologs from other *Graminiae* species such as barley and maize are functional Si channels and were proposed as having major roles in the Si uptake and distribution within these species [72,73]. Shoot-localized NIP channels, such as OsNIP2;2, ZmNIP2;2, and HvNIP2;2 are responsible for the allocation of silicic acid out of the nodes and into the leaves of these crops [70]. Si is an essential element for the primitive vascular plant genus *Equisetum* (horsetails) [74,75]. Analysis of the root transcriptome of the Si hyperaccumulator *Equisetum arvense* revealed nine different NIP channels that form a multigene family (*EaNIP3;1–EaNIP3;9*) [76]. EaNIPs can probably be designated to the functional NIP-III subgroup due to a threonine residue in the H5 position of the ar/R selectivity filter (Fig. 3 and Table S1). Such a hydroxylated amino acid at the H5 position is common to all NIP-III class proteins (Table S1). Heterologous expression studies of *EaNIP* genes in oocytes and in *A. thaliana* demonstrate Si channeling capacity of the corresponding proteins. *EaNIPs* are differentially expressed in roots and shoots of horsetails, indicating that different members of the gene family have special organ specific functions in this primitive plant species [76]. Si uptake has also been shown for NIP-III class channels from dicotyledoneous plant species. Soybean GmNIP2;2 facilitates the transport of silicic acid when heterologously expressed in *Xenopus laevis* oocytes and its expression is upregulated when soybean plants face Si-deficient conditions [77]. Pumpkin CmNIP2;1 mediates Si uptake into roots, and the distribution of Si in the shoot of pumpkins, a Si accumulating species [78]. It is, therefore, likely that other dicotyledonous Si accumulators (e.g. from the orders of the Curcubitales, Urticales, and Commelinales) express NIP-III class channels that regulate and ensure Si transport processes. No silicic acid transport activity has been found in NIP-I and NIP-II class isoforms of *Arabidopsis* [79]. However, *Arabidopsis* plants, which express a wheat NIP-III channel, TaLsi1, under the control of the *AtNIP5;1* promoter allowed a 2.5–3-fold higher Si accumulation in the shoots of transgenic plants compared with wild-type when Si was supplied in the growth medium [80]. In summary, these data show that NIPs are essential for high-capacity transport of Si in various plant species. So far NIP-III class proteins permeable to Si have only been identified and characterized in *silicophile* plant species for which it is know that Si has beneficial nutritional effects. It will be interesting to investigate whether the expression of NIP-III channels regulating the uptake and allocation of Si are a prerequisite of land plants to physiologically benefit from this element.

2.4. NIP-mediated arsenic transport

Arsenic is one of the most dangerous poisons in nature. Arsenic is chemically similar to phosphorus (P) and can often replace it in organic molecules rendering them funtionally inactive. Arsenic can form bibasic and tribasic acids with a trivalent or pentavalent metalloid core (Table 1). Arsenic acid and phosphoric acid (and their salts arsenate (As(V)) and phosphate) have very similar chemical characteristics, which become manifest in the tetrahedral structure (Table 1) and their pK_{a1} values (phosphoric acid = 2.16, arsenic acid = 2.26). The replacement of P with As negatively interferes with phosphorylation-dependent processes, and with the formation of ADP and ATP as essential energy sources [81]. While As(V) is the major As species in aerated soils (oxidative environments) arsenous acid and its arsenite salts (As(III)) become predominant under reducing soil conditions (e.g. after strong rainfalls or in flooded paddy rice fields). As(III) toxicity is caused by the reaction of As(III) with functional thiol and sulfhydryl groups in various proteins, which leads to their deactivation or dysfunctioning [82]. In bacteria, yeasts, fish, mammals, and humans As(V) is transported via phosphate transporters and uncharged As(III) species are transported by specific aquaglyceroporins [24]. The evidence that As(III) fluxes are mediated also by plant aquaporins derived from kinetic uptake studies into rice roots. As(III) influx into roots is competitively inhibited by other aquaporin substrates such as glycerol and antimonous acid, and abolished by the aquaporin inhibitor mercury [34]. Independent studies using different heterologous expression systems demonstrated that uncharged As species are transported by isoforms of all three functional NIP subclasses. Uptake and toxicity growth assays in various As resistant *Saccharomyces cerevisiae* yeast mutants and strains showed that NIP proteins from *O. sativa* (OsNIP2;1, OsNIP2;2, and OsNIP3;2), *A. thaliana* (AtNIP5;1, AtNIP6;1, and AtNIP7;1) and *L. japonicus* (LjNIP5;1 and LjNIP6;1) significantly increased the sensitivity of yeasts toward As(III) [83], which was linked to an increased As uptake. When yeasts were cultured on As(V)-containing medium the expression of the same NIP isoforms enhanced the efflux of As(III) out of the cells which was intracellularly produced through the reduction of As(V). This clearly demonstrated the bidirectional permeability of NIPs to As(III) for the first time. An increased influx of As(III) into *X. laevis* oocytes expressing NIP isoforms from *O. sativa* (OsNIP1;1, OsNIP2;1, OsNIP2;2, and OsNIP3;1) and *A. thaliana* (AtNIP1;1, AtNIP1;2, AtNIP5;1, and AtNIP7;1), together with an increased sensitivity of yeast cells expressing AtNIP3;1, HvNIP1;2, HvNIP2;1, HvNIP2;2, and OsNIP3;3 provided additional evidence for the As(III) transport ability of NIPs [84–86]. The physiological relevance of NIP-mediated As(III) uptake, accumulation, and tolerance *in planta* was demonstrated using *nip* knockout mutants of *Arabidopsis* and rice. A forward genetic screen analyzing inhibition of root growth of mutagenized *Arabidopsis* lines on medium containing toxic concentrations of As(III) led to the identification of three tolerant lines [87]. All three independent lines carry a mutation in the same coding region, namely that of *AtNIP1;1*. All of the mutations resulted in a non-functional AtNIP1;1 channel [87]. The role of AtNIP1;1 in As(III) transport was further experimentally confirmed [87]. Mutated rice lines of *Osnip2;1* also had a reduced uptake capacity for As(III) [88]. OsNIP2;1 is the major As uptake transporter in paddies favoring As(V) reduction to As(III), while being simultaneously indispensable for Si accumulation (Fig. 4). These data indicate that the physiologically important Si uptake pathways formed by NIP channels are responsible for the high As contamination levels of various rice food products [84]. When grown in toxic As(V) conditions OsNIP2;1 promoted As detoxification by effluxing As(III) out of the roots along a concentration gradient, after its intracellular formation through the reduction of As(V). Heterologous expression of OsNIP2;1 in oocytes facilitates the uptake of uncharged pentavalent monomethylarsonic and dimethylarsinic acid [84]. A rice *Osnip2;1* mutant line took up only half of these organic As species in comparison to wild-type plants [84]. This further confirmed that OsNIP2;1 is the major transporter for diverse uncharged As species. In nature, methylated As species derive mostly from anthropogenic activities (waste, tube wells, pesticides) or from soil-borne microorganisms. A QTL mapping study aimed at the identification of As(V) tolerance genes using an As(V) tolerant (cv. Bala) and an As(V)-sensitive rice variety (cv. Azucena), postulated that there are three genomic tolerance loci that involve an epistatic interplay among them [89]. Any two of these three loci inherited from the tolerant parent led to an As(V) tolerant progeny. For one of these loci, there are two genes that are differentially regulated between the two rice cultivars: these are an *aminoacylase-1* and *OsNIP4;1* [89]. Both genes have higher expression levels in cv. Bala roots. Aminoacylases are enzymes that catalyze the chemical reaction of N-acyl-L-amino acid with water to carboxylate and L-amino acid. A function of such an enzyme in As tolerance is unknown while a protein belonging to a metalloid transporting group of channel proteins was ranked as of high interest. However, As transport by OsNIP4;1 has not yet been detected [85]. Another rice NIP isoform, OsNIP3;1, transported As(III) in the heterologous oocyte system [88]. *OsNIP3;1* expression was downregulated in response to As(III) but not As(V) stress in germinating rice plants exposed to As(III) or As(V) in the growth medium [90]. A downregulation might help to decrease the OsNIP3;1-mediated As root uptake under B deficient conditions, in which OsNIP3;1 plays a crucial role. Information on As transport mechanisms controlling As fluxes into and within plants, particularly rice, is highly valuable to develop strategies for breeding or engineering minimal-As-accumulating plants. Such food plants are necessary since As is not only acutely toxic at higher levels, but it is also classified as a group I human carcinogen by the International Agency for Research of Cancer. As-contaminated plant food products have increasingly caused public health issues [84]. Arsenic contamination of plants is especially high when plants are cultivated in agricultural regions with high bioavailable As concentrations, and in reducing soils where As(III) is the dominant As species. Additionally, if these plants depend on NIP-mediated uptake pathways to ensure the sufficient supply of the essential and beneficial metalloids, B and Si, the adventitious uptake of toxic As(III) molecules through these same channels cannot be prevented. Adventitious As(III) uptake in particular represents a problem for gramineous plants which highly express NIPs to ensure beneficial Si levels in the plant. Plant species which highly depend on NIP-mediated boric acid uptake such as *Brassica* crops are also prone to take up higher levels of As(III) when the soil is deficient for B but contains high levels of As(III). The identification of mechanisms that regulate NIP channel activity dependent on the metalloid availability or NIP proteins that have metalloid-specific pores, would allow developing plants that take up essential metalloids while excluding adventitious As uptake. As elaborated below in more detail, molecular mechanisms that regulate the pore selectivity of NIPs are still poorly understood. It will probably not be enough to uniquely modify the expression or properties of single NIPs to generate low-As-accumulating plants, but it will be necessary to include other As transporting proteins such as ABC transporters or Lsi2 proteins in a cooperative manner [84]. To date, all data on As(III) transport processes in plants show that NIPs constitute major transport facilitators for reduced and uncharged forms of this toxic metalloid in plants. It has to be resolved whether NIP-mediated As(III) transport is simply an adventitious side activity due to structural similarity of As(III) to metalloid nutrient substrates or whether NIP-mediated As(III) efflux is significantly implemented in physiological detoxification concepts of plants similar to aquaglyceroporin-involving As detoxification strategies in microbes.

2.5. NIP-mediated antimony transport

Similar to As, trivalent and pentavalent Sb species have no known physiologically important in vivo roles for plants but are toxic [91]. Various NIPs, like other mammalian and microbial aquaglyceroporins, facilitate the movement of trivalent uncharged Sb species (Sb(III)) in homologous and heterologous expression systems, as assayed by toxicity in growth assays [83,91]. The expression of NIPs from various plant species in a metalloid resistant yeast mutant reverted Sb resistance when grown in the presence of high potassium antimonyl tartrate concentrations [83]. *A. thaliana* T-DNA insertion mutants of *NIP1;1*, but not of *NIP1;2* and *NIP5;1*, exhibited reduced sensitivity to the presence of Sb(III), indicating that the passage of Sb(III) into Arabidopsis roots is selective, and at least in part mediated by AtNIP1;1 [91]. The question remains, whether NIP-mediated transport of Sb(III) is of physiological relevance in any condition, or whether it is just a promiscuity of NIPs in addition to its intrinsic metalloid nutrient transport function. Antimony is a very rare element in the Earth's crust. The toxic concentrations of Sb(III) in uptake assays [83,91] are normally not reached in reducing soils nor in oxidizing soils where ionic forms of antimonate (Sb(V)) are predominant (Fig. 1). Due to the increasing anthropogenic pollution of Sb (mainly antimony trioxide, Sb_2O_3) at certain sites, the knowledge of plant Sb(III) uptake and detoxification via NIPs might be of potential significant interest for phytoremediation approaches using Sb hyperaccumulating plants, or cultivars with limited translocation of Sb to edible plant parts. Many studies suggest that antimonous acid $Sb(OH)_3$ is the chemical Sb(III) species that is transported by NIPs and other aquaglyceroporins [24]. This is due to the fact that $Sb(OH)_3$ resembles arsenous acid ($As(OH)_3$), which is transported by MIPs, in its physicochemical properties (tetrahedral structure, molecular volume, electrostatic charge distribution, pK_a values, capacity to form hydrogen bonds, etc.) and meets all of the physicochemical requirements to be transported along an aquaporin channel pathway (Table 1). Interestingly, the actual existence of $Sb(OH)_3$ is still under debate by chemists. Some studies indicated that $Sb(OH)_3$ is metastable and, therefore, does not exist in nature in noteworthy amounts [92]. As a consequence NIP-mediated $Sb(OH)_3$ would be implausible. Salts of $Sb(OH)_3$ formally exist. In water, they form a gelatinous precipitate, which is formed by antimony trioxide ($Sb_2O_3 \cdot H_2O$). Therefore, experimental evidence is needed to ascertain which Sb species is actually channeled by NIPs or aquaglyceroporins. This question needs to be addressed to understand the mechanism of Sb uptake in organisms.

2.6. NIP-mediated selenium transport

Selenium has a special role among metalloids because it is an essential element for humans, but not for plants. Nutritional Se deficiency and toxicity symptoms are known for humans and animals. Se occurs in the 21st proteinogenic amino acid selenocysteine, which is encoded by the stop codon UGA in the mRNA of bacteria, archea, and some eukaryotes. Because of the higher reactivity of the Se atom compared to sulfur, selenocysteine has an important role in several catalytic sites of oxidoreductases involved in the defense against oxidative stress [93]. Vegetables and fruits are the most prominent sources of Se in human diets. The Se content of plants correlates directly with the content of bio-available Se in soils. Deficiency of Se in humans is frequent. It has been estimated that up to 1 billion people may have insufficient intake of Se [94]. A strategy to augment human intake of Se is to biofortify cereals, either through the usage of fertilizers (agronomic biofortification) or by breeding crops with an improved Se accumulation. Agronomic biofortification has been practiced in Finland since the mid-1980s with a mandatory supply of small amounts of Se (as potassium selenate) to all fertilizers [95]. This action taken has increased Se concentrations in food plants and more than doubled the Se intake by the Finnish population [95]. Since plants are a major source of Se to humans and Se occurs in agricultural soils in small amounts (typically ranging from 0.01 to 2 mg kg^{-1}), it is important to understand how plants take up and metabolize Se. Selenium occurs in soils mainly as selenite (IV) and selenate (VI). Se(VI), in its ionic forms $HSeO_4^-$ and SeO_4^{2-}, are transported via sulfate transporters [96], due to their similar tetrahedral structures and pK_a values ($pK_{a2\ selenate} = 1.74$ versus $pK_{a2\ sulfate} = 1.9$). Selenous acid (IV) is the Se species most probably transported by NIPs. Selenous acid is a weak acid with a pK_{a1} and pK_{a2} of 2.57 and of 6.6, respectively. Depending on the pH environment, selenous acid occurs uncharged (H_2SeO_3 in acidic pH conditions) or as the ionic $HSeO_3^-$ or SeO_3^{2-} forms in basic pH conditions (Fig. 1). It was shown that inhibitors ($HgCl_2$ and $AgNO_3$) of MIPs inhibit the uptake of Se into rice roots [97]. A selenite uptake kinetic study in maize roots [98] suggested that MIPs are involved in Se uptake. The first selenous acid transporter, OsNIP2;1 an aquaporin, was identified in rice [99]. Loss-of-function mutants of *Osnip2*;1 grown in the presence of selenite had significantly less Se content in shoots and xylem sap than the wild-type. When both plant types were grown on selenate containing growth medium they did not differ in their Se contents. These observations allowed the authors to postulate that OsNIP2;1 transports selenous acid, which was further supported by pH dependent uptake experiments. OsNIP2;1 is a selenous acid transporter when it is heterologously expressed in the yeast system [99]. Since Se is not essential for plants, this transport ability of NIPs is probably rooted in the chemical restrictions of the selectivity determining pore regions of the silicic acid channels and represents therewith a non-physiological side activity. Nevertheless, the ability of plant NIPs to transport Se might be of great interest in biofortification approaches to enrich stable food plants with this essential nutrient. Breeding or biotechnological approaches might result in the modified and coordinated expression of NIPs and sulfate transporters, aiming at the efficient translocation of Se to edible plant parts without any negative impacts on S or Si nutrition. Further studies are needed to address the question whether the permeability of NIPs to selenous acid is a feature of different NIP subgroups or whether it is restricted to the silicic acid permeable NIP-III isoforms of monocots and dicots.

2.7. NIP-mediated germanium transport

For a long time, it was observed that the uptake and translocation of Ge and Si in plants were similar and that plants with high Si contents are especially sensitive to Ge toxicity [100]. The knowledge about the chemical similarities between hydroxylated species of Si and Ge allowed for the usage of Ge as a selectable marker in toxicity screens and resulted in the successful selection of rice mutants defective in Si accumulation and the identification of the underlying responsible NIP aquaporin gene *OsNIP2;1* [63,71]. Moreover, the radioactive ^{68}Ge isotope and the non-radioactive isotopes in the form of germanic oxide (GeO_2) were validated as suitable tracers and chemical analogs for studying Si uptake in higher plants as well as in heterologous expression systems to investigate the transport ability of certain NIPs [71,79,100,101]. The chemical similarities between hydroxylated species of B and Ge were recognized as well and enabled Ge toxicity studies to be used to dissect B toxicity effects in barley cultivars differing in their NIP-mediated B transport capacity [102]. A B toxicity-tolerant cultivar had a low expression of *HvNIP2;1* with reduced toxicity symptoms upon the addition of GeO_2 to the nutrient solution. The dissociation of germanic acid with a pK_{a1} of 9 resembles that of boric and silicic acids (Fig. 1), but the molecular structure is different (Table 1). Germanic acid potentially occurs as a tetrahedral

ortho-acid (similar to silicic acid), or in the planar meta-acid form (similar to boric acid). It has to be resolved at what quantities each of these Ge species occur in plants and soil, and which form is transported by NIPs. The permeability of NIPs to germanic acid is likely a non-physiologic side activity and has probably evolved due to the structural similarity of germanic acid to metalloid nutrient substrates. It nevertheless represents a valuable tool to use Ge as an artificial and distinctly visualizable tracer, mimicking Si and B transport processes in plants.

2.8. Potential NIP-mediated transport of other metalloids

Whether uncharged species of other metalloids such as At, Po, or Te are transported via NIPs is unknown. Due to the extreme rarity of these elements and their biological insignificance for the majority of organisms, any potential NIP-mediated transport will be of limited biological importance.

2.9. Roles of NIPs in the transport of other substrates than metalloids

Most NIPs are highly permeable to glycerol, while only a few glycerol impermeable or poorly permeable NIPs have been described [71,103]. This transport ability suggested that NIPs are functional equivalents of mammalian and microbial aquaglyceroporins, which are essential regulators for transmembrane glycerol transport processes. Glycerol and its derivatives (e.g. glycerol-3-phosphate) are crucial metabolites for plants and play important roles as membrane constituents, in the energy metabolism and as osmolytes in abiotic stress situations. The question arises why neither a molecular nor a physiological involvement of NIPs in plant glycerol transport processes has been reported despite having the knowledge of glycerol permeable NIPs for more than 20 years? Should this lack of information really be due to a shortcoming of research initiatives or might NIPs eventually be irrelevant for glycerol transport processes in higher plants? With respect to the current knowledge about the physiological functions of NIPs and their evolution one can speculate that the acquisition of metalloid transport function, of originally important glycerol channels, i.e. NIPs, in non-vasculature plants and mosses, was a prerequisite for or at least went along with the evolution of higher plants. Independent of the necessity to retrace the functional history of NIPs it will be essential to finally resolve the question of whether certain NIPs of primitive and higher plants are physiologically important glycerol transporters or not. To answer this question should be a central aim of the plant aquaporin community, especially since almost all studies investigating the substrate spectrum of NIPs have made the effort to test glycerol permeability.

Uptake studies demonstrated that AtNIP2;1 is permeable to lactic acid ($pK_a = 3.9$), but only poorly permeable to water and glycerol [103]. Characterization of the transport ability in *Xenopus* oocytes revealed that neutral lactic acid, but not the anion passes the channel [103]. AtNIP2;1 is highly expressed in root tips under anaerobic conditions such as flooded soil conditions. In anoxic conditions plants accumulate lactic acid. Consequently, its concentration can reach damaging levels. A channel protein helping to efflux this toxic compound is of physiological relevance. So far, this is the only study revealing a potential role for NIPs in the transport of organic acids. There are examples of bacterial, trematode, and mammalian aquaporins, which are permeable to lactic acid or other small organic acids [104]. Future research will resolve whether specific MIPs including certain NIPs have a significant role in the facilitated transmembrane movement of undissociated carboxylates. Such a role is expected mainly at acidic pH conditions in which these carboxylates occur, due to their pK_a values of <4, as uncharged species.

Various transport assays analyzing the permeability of diverse NIPs have shown that also ammonia, urea, water, and hydrogen peroxide are channeled through numerous NIPs [5]. However, to date, no direct physiological evidence for any of these non-metalloid NIP transport abilities has been provided from *in planta* experiments (using, e.g. *nip* knockout mutants).

3. Factors influencing NIP function

3.1. Polar localization of NIPs ensures directed metalloid transport

Numerous NIPs that are essential for the uptake of boric acid and silicic acid are polar localized, primarily to the distal side of the cells in which they are expressed (AtNIP1;1 [87], AtNIP5;1 [50], HvNIP2;1 [72], OsNIP2;1 [71], OsNIP2;2 [105], ZmNIP2;1, ZmNIP2;2 [73]). The polar localization of NIPs seems to be a prerequisite for the participation of NIPs in physiologically important directed transport processes, especially in a cooperative manner with active metalloid efflux transporters (i.e. BORs and Lsi2 orthologs) which are themselves polar localized to the proximal sides of the same cell types [106]. We hypothesize that an oppositely localized active transport protein for the same metalloid species may be identified in cell types in which NIPs are likely to play a function in directed metalloid transport processes. Protein motifs or interaction partners, which ensure the polar localization of NIPs remain to be identified. In addition to the localization of NIPs in the plasma membrane, NIPs have been localized to intracellular membranes (i.e. vesicles and the ER) [107,108]. A localization of NIPs in vesicles of the biosynthetic-secretory anterograde pathway would be of biochemical interest regarding the molecular function of B to crosslink the cell wall component rhamnogalacturonan-II (RG-II). Polysaccharide synthesis of RG-II takes place in Golgi-derived secretion vesicles [109]. It has been proposed that the dimerization of RG-II monomers via borate esters occurs not after the secretion of RG-II into the cell wall, but rather during vesicle trafficking from the Golgi to the plasma membrane [110]. In light of these findings, it is likely that the intracellular in addition to the extracellular free B content is important for RG-II crosslinking. It is tempting to speculate on the involvement of NIPs in this process.

3.2. Metalloids impact on NIP expression

Another peculiarity of NIPs is that they seem to have lower transcript levels than isoforms of other plant aquaporin subfamilies (e.g. PIPs and TIPs) [5,111]. NIP expression is striking at specific developmental stages, cell types, or in response to external stimuli but not ubiquitous (Fig. 4). The expression of NIPs permeable to metalloids is linked to tissues and conditions in which a transmembrane metalloid transport is of physiological relevance (e.g. upregulation under B- or Si deficiency and expression in roots or nodal regions where B and Si are taken up from the soil or are distributed to plant leaves, respectively [50,70]). *AtNIP5;1* and *AtNIP6;1* genes are down-regulated under high B conditions and up-regulated upon B deficiency [3]. Rapid responses of metalloid transport processes are of biological importance because sudden changes in the availability of metalloids can occur naturally. For example, B solubility depends to a great extent on the soil water status, and soils in arid climate zones in particular can undergo very fast alternations between heavy rainfall and subsequent drought and evaporation. While the molecular mechanism which is significantly inducing *AtNIP5;1* gene expression already 3 h after B deficiency occurrence is unknown [50], an 18 bp region in its 5′UTR was demonstrated to be responsible for B-dependent degradation of the *AtNIP5;1* mRNA [112]. The half-life of the *AtNIP5;1* mRNA under high B conditions

is 10 min when the 18 bp region in the 5′UTR of the *AtNIP5;1* mRNA was present and 28 min when it was absent [112]. mRNA destabilization occurs as rapidly as 10 min after application of high B conditions. Whether the AtNIP5;1 protein is similarly destabilized is unknown. Interestingly, the 18 bp sequence of Arabidopsis also impacts B-dependent on the expression level of other downstream placed reporter mRNA sequences and an identical 18 bp sequence is found in further UTR sequences of AtNIP5;1 orthologs [112]. This suggests that the expression of various genes might be regulated via a B-dependent mRNA stabilization or translational efficiency mechanism. This would imply that the nutrient B itself is directly impacting on gene expression of organisms, which would qualify B as a yet unknown expression determining regulator. Boric acid can form ester bonds with ribose, the sugar component of RNA and can, therefore, chemically interact with RNA [113,114]. Such a direct interaction of B with mRNAs could explain the multiple yet non-understood effects of B-deficiency and -toxicity on expression patterns of various genes, which seem to be non-related to the function of B in cell walls. Whether a B-dependent destabilization of, e.g. *AtNIP5;1*-mRNA is caused by a direct interaction of B with ribose residues of the identified 18 bp long *AtNIP5;1* RNA sequence or via another yet unknown mechanism remains to be shown. Mechanisms inducing the expression of monocot NIP2;1 silicic acid channels under Si-deficient conditions are unknown. A study investigating the inducibility and mRNA expression levels of OsNIP2 Si transporters in six wild rice species with different genome types suggested that superior Si uptake, the important trait for rice growth, is basically conserved in wild and cultivated rice species [115].

3.3. Potential structural and regulatory features of NIPs affecting their selectivity

Members of the NIP subfamily have a number of features that clearly distinguish them from other plant aquaporins. On average, NIPs possess longer cytoplasmic terminal domains compared to other plant aquaporins [116]. No experimentally verified functional feature of these termini has yet been identified. They may serve as regulators of the activity or selectivity of NIPs by controlling the accessibility of the substrate to the pore or they might serve as post-translational modification or protein-protein interaction sites regulating the trafficking, the stability, the selectivity or activity of these channels. Soybean GmNOD26 has a calcium dependent protein kinase phosphorylation site at its C-terminus similar to numerous NIP-I group isoforms [116], which was shown to be phosphorylated in response to stress signals [117]. Phosphorylation of that site enhances water permeability of GmNOD26 in vitro and in vivo [117]. Members of the NIP-II group possess either predicted MAP kinase phosphorylation sites (conserved in almost all isoforms) at their N-terminus, or like AtNIP7;1, a MAP kinase site at the C-terminus [116]. Phosphorylation as a post-translational regulative modification of aquaporins that affects their gating or trafficking was demonstrated for various non-NIP aquaporins [111]. Another characteristic of NIPs are amino acid substitutions in the two NPA motifs of NIP-I and NIP-II subgroup isoforms (Table S1). MIPs are characterized by two highly conserved NPA motifs, which form one of the two constriction regions for solute passage (Fig. 3). AtNIP5;1 and AtNIP6;1 and all of their orthologs have an NPS-NPV or NPA-NPV instead of an NPA-NPA constriction region, respectively (Table S1). These amino acid exchanges result in a narrower and more polar NPA constriction region compared to aquaporins with a typical NPA motif pair and suggest, therefore, specific substrate selectivity features. It was calculated that the narrowest part of AtNIP5;1/6;1 and their orthologs is the "NPA constriction region" in contrast to other MIPs where the narrowest part is at the ar/R selectivity filter [30]. The four amino residues constituting the ar/R selectivity filter of NIPs are not found in these combinations in other plant MIPs (Table S1). The impact of these particular selectivity filter compositions as well as other yet unknown parameters potentially acting on NIP substrate specificity are not resolved yet.

As outlined above, members of all three functional NIP-I to -III subgroups channel metalloids both in heterologous expression systems and *in planta* (Fig. 4, Table S1). Therefore, the ability to transport uncharged metalloid species seems to be common to the different NIP subgroups, although differentially distinctive amongst them. To date, NIP1/NIP-I isoforms transport As(III) and Sb(III) species. NIP3/NIP-II isoforms are permeable to As(III), Sb(III), and boric acid, and NIP2/NIP-III isoforms are permeable to As(III), Sb(III), boric acid, selenous acid, germanic acid, and silicic acid (Fig. 4, Table S1). Transport of germanic acid, selenous acid, and silicic acid has only been shown for the NIP2/NIP-III isoforms present in monocots and some eudicots.

So far, only two studies systematically addressed the influence of the ar/R selectivity filter and the NPA region on the selectivity of NIPs for metalloids [79,102]. A mutational approach was used to determine whether a boric acid and As(III) permeable NIP-II subgroup isoform, namely AtNIP5;1, can be transformed into a silicic acid, boric acid, and As(III) permeable NIP-III subgroup channel (such as OsNIP2;1) by exchanging both NPA motifs and the ar/R selectivity filter [79]. When both NPA motifs and the ar/R selectivity filter of AtNIP5;1 were changed to those of the OsNIP2;1, the mutated channel did not acquire transport activity for silicic acid [79]. When the selectivity filter of AtNIP5;1 was phenocopied into OsNIP2;1, silicic acid, boric acid, and water permeabilities were unexpectedly lost. Assuming that the loss of channel functionality was not due to a mutation-related misfolding of the protein, these results indicate that the selectivity filter of a boric acid permeable NIP-II protein does not cause boric acid permeability in a NIP-III channel backbone. These results suggest that the metalloid selectivity is not just controlled by the known aquaporin selectivity filters, the NPA motifs and the ar/R constriction region. The overall transport activity of silicic- and boric acid appears to be strictly controlled, as the native proteins (OsNIP2;1 and AtNIP5;1) have the highest transport activity for either silicic- or boric acid compared with any tested mutant [79].

When amino acids of the ar/R selectivity filter of HvNIP2;1 (permeable to germanic acid, silicic acid, As(III), and boric acid) were substituted for residues with larger side chains to constrict the filter width, the ability of HvNIP2;1 to transport Ge and B was disrupted, whereas selectivity for As(III) was unchanged [102]. This implicates that mechanisms determining the pore selectivity for Ge, B and potentially Si do involve the ar/R selectivity filter and that such selectivity mechanisms allow discriminating between arsenite and other metalloids. Other mutations (i.e. Gly88Val, Gly88Ala/Ser207Val, and Ala132Thr) disrupted permeability to all three metalloids. As none of the mutated HvNIP2;1 proteins discriminated between boric acid and germanic acid, Ge seems to be a suitable analog for B in studies dealing with the impact of NIP-III type channels on the B metabolism. These results are consistent with the results obtained in the above-mentioned mutational study by Mitani-Ueno *et al.* [79]. In both studies, the permeability of NIPs to As(III) was less affected by changes in the ar/R filter when compared to the other tested metalloids [79,102]. These results suggest that it may be difficult to modify As(III) transport in plants without affecting permeability to silicic acid or boric acid by just focusing on NIP selectivity filter residues because of an apparently lower selectivity for arsenite than for the other metalloids. To ensure food safety of crops it would, however, be of central interest to exactly obtain such plants, which can take up essential or beneficial metalloids but are impermeable to toxic As species. As in addition to the pore size, other structural features potentially distributed along the pore pathway are also involved in controlling the metalloid

specificity of NIPs (indicated by the non-clustered substrate spectra of various phylogenetic and functional NIP subgroups in Fig. 4 and Table S1), the identification or generation of plants with such transport characteristics appears possible.

The analysis of the amino acid lengths of the six transmembrane helices (TMH1–TMH6, Fig. 3) and the five connecting loops (loops A–E, Fig. 3) of plant metalloido-porins showed that they are highly conserved. Only the length of loop C varies slightly: While for most NIP-II and NIP-III isoforms 21 amino acids are predicted, loop C of NIP-I isoforms seem to span 17 to 24 amino acids [28,32,118]. However, a correlation between the length of loop C and the metalloid substrate specificity is not supported. Froger *et al.* [119] identified five positions (P1–P5) in MIPs where the properties of the corresponding amino acids are differing between aquaporins and aquaglyceroporins. Positions P4 and P5 (two consecutive amino acids) are located in TMH6, while P1, P2, and P3 are located in loops C, E, and E respectively (Fig. 3) [119]. Amino acids constituting P2–P4 do not differ between NIP-I, NIP-II, and NIP-III isoforms (P2: threonine/serine, P3: alanine, P4: thyrosine) (Table S1). Si-permeable EaNIPs possess an aromatic phenylalanine residue in P1 alike NIP-I and NIP-II group isoforms (phenylalanine or thyrosine), while all other Si-permeable NIP-IIIs possess a leucine at this position. Therefore, this position seems not to correlate with metalloid selectivity. However, a phenylalanine residue in Froger's position P5 is common to all NIPs being permeable to Si, while all other NIPs have non-aromatic residues at this position (Table S1). We hypothesize that this residue might be important for Si selectivity. We also addressed the question whether specificity determining positions (SDPs) of NIPs can be correlated with their metalloid substrate selectivity (Table S1) [32,118] (this study). It seems that most of the SDPs are characteristic for specific phylogenetic NIP groups even allowing distinguishing between monocot and dicot NIP-III isoforms [118]. However, an unambiguous affiliation of most SDPs to certain metalloid permeabilities was not supported. Two SDPs, however, were distinctive for NIPs permeable to Si: the Froger's P5 phenylalanine in TMH6 and a polar serine/threonine residue in TMH5, which is aligned to hydrophobic isoleucine, alanine or valine residues in NIPs which are not permeable to Si (Fig. S1).

It might be speculated that amino acids of loops, termini, or even transmembrane helices of NIPs impact on the pore selectivity through an interaction with amino acid residues in juxtaposition to selectivity filter forming residues which then physicochemically impact on the selectivity filter residues. Such a change in electrostatic or conformational properties of the pore pathway would change the substrate selectivity or transport capacity of different NIPs despite possessing an identical selectivity filter. Molecular studies and crystal structures of diverse NIPs should be useful dissecting the transport selectivity of these proteins in greater detail.

4. When did NIPs became physiologically important metalloido-porins? Did NIPs pave the way from water to land life?

Knowledge on aquaporins in marine algae is scarce. Aquaporin sequences were identified in green and brown algae as well as in diatoms [26,120,121]. In phylogenetic analyses, algae aquaporins group together in five MIP subfamilies named MIP-A to MIP-E. PIP, SIP and GIP homologs are also present in algae while NIP-like aquaporins have not been identified yet [26,120,121]. Boron is essential for different forms of red, green and brown algae, diatoms, and cyanobacteria while for some green algae B is not essential [113]. Specific roles of B in marine algae remain unclear. The high concentration (0.4 mM) of B in seawater suggests that B deficient conditions are unlikely and that sufficient uptake into algae might be realized by passive non-protein-facilitated membrane diffusion. In diatoms, for which Si is an essential element, Si is taken up by active Si transport proteins (SITs) [122]. Orthologs of these Si transporters have not been identified in higher plants yet. Taken together, it seems that there is no need for additional channel-regulated metalloid transport mechanisms in algae, which might explain the absence of NIPs in these organisms.

The development of a vascular system was the prerequisite for the transition of plants from water to land 400 million years ago. The vasculature provided land plants with two advantages: a nutrient, metabolite, and water transport system that could function in the opposite direction of the gravitational force, and a rigid yet flexible skeleton for withstanding tension in both the aerial and subterranean environments [123]. It has been postulated that B usage in the cell wall together with lignification has an essential role in the evolution of the vascular system [45,124]. The role of B is not completely understood in non-vascular bryophytes. Mosses have roughly the same rhamnogalacturonan-II (RG-II) and B content as dicotyledons in their cell walls, but the amount of B cross-linked to RG-II is more than 200 times less [125]. Since B also seems beneficial for the growth of the moss *Physcomitrella patens* [126], it is possible that B might have a function apart from RG-II bridging. Phylogenetic NIP1, NIP2, NIP4, and NIP7 group isoforms are absent in *P. patens* [127], but the ar/R region of PpNIP5;1 is strikingly similar to that of AtNIP5;1 and OsNIP3;1, suggesting possible B channeling abilities. That implies that the NIP-II group had already evolved in an ancient ancestor of bryophytes and higher plants [26]. Thus, it is possible that this conserved NIP group that evolved before the evolution of vascular plants has retained the original function of NIPs in early terrestrial plants, namely transporting metalloids. Further characterization of moss NIPs should be extremely informative for the understanding of the functional evolution of NIP-mediated metalloid transport: Since when does the B selectivity of NIPs exist, and what purpose could NIPs have served in the ancestors of pre-vascular plants? Was NIP-mediated B uptake a prerequisite for the development of vascular plants? These questions show that research on non-vascular NIP channel proteins is a very relevant topic for understanding fundamental processes in the evolution of vascular plants. While B-permeable NIPs might have significantly contributed to the evolution of land plants the success story of NIPs as functional and physiologically important metalloido-porins has continued by allowing land plants to efficiently take up Si and use it as a beneficial nutrient. NIP-mediated Si transport mechanisms are probably most efficiently implemented in the plant metabolism of Poaceaes. Without the capacity to efficiently acquire Si from the soil and translocate it within the plant, Poaceaes would probably not have been what they are: the economically most important plant family. Cereals such as millet, oat, rye, maize, wheat, barley, and rice represent staple foods worldwide. One could argue that NIP-mediated Si influx into Poaceae plants is a physiological essentiality to ensure the feeding of the world's population.

5. Conclusions

The highly positive and negative impacts of metalloids on the environment, agriculture, and human health emphasize the importance of understanding metalloid transport mechanisms within crop plants. The application of this knowledge in food plant breeding programs should let to strategies actively and beneficially dictating metalloid transport processes. To date, NIPs represent the sole transporter protein class in the plant kingdom, which has been shown to be essential for the uptake, translocation, or extrusion of various uncharged metalloid compounds in many plant species.

The discovery that NIPs represent transmembrane transport pathways for uncharged metalloids revealed how required (B and Si) but also toxic (As) metalloids enter plants and therewith the

food chain. Food plants can be made safer when they would express NIPs, which exclude As as a substrate while still being permeable to B and Si. To improve plant food safety and to prevent health threats by As-contaminated diet the most urgent need will be to manipulate NIP functioning in rice [84]: To this aim, an adventitious uptake of the frequent paddy field pollutant arsenous acid by rice NIP channels should be minimized while a high uptake capacity for Si should be maintained. The plasticity of the pore layouts and overall amino acid sequences of NIPs suggest that such a favorable NIP pore selectivity can be achieved by targeted protein engineering strategies. In nature, such metalloid-specific NIP isoforms might have already been evolved in individual wild species or crop cultivars. A detailed analysis of the metalloid transport capacities of plants, which adapted to habitats in which an ability to discriminate between the uptake of different metalloids represents a selection advantage combined with next generating sequencing approaches might efficiently identify those metalloid-specific NIP protein layouts.

The attempt to optimize NIP-mediated B transport processes in breeding programs with the objective that crops can either deal with toxic or deficient soil B concentrations will demand opposing approaches: (1) For B deficiency-sensitive crops such as sugar beet or *Brassica* crops, the B-use-efficiency has to be improved in future to allow their cultivation in agricultural areas where B-deficient soils occur or temporal B deficiencies are faced due to environmental conditions. This implies that NIPs have to be regulated in a way that even traces of B can be taken up from the soil and that this B is most efficiently allocated to tissues, which have a high B demand such as meristems and flowers. (2) In contrast, in areas with potentially toxic B soil concentration the growth of B toxicity-sensitive crops such as barley or wheat demands that high B uptake has to be prevented, e.g. via a downregulation of root-localized NIP channels. However, despite a reduced uptake capacity, the delivery of B to the highly B demanding reproductive organs has to be maintained by these plants.

The ability of NIPs to transport toxic metalloids such as As, Se, Sb, or Ge makes these proteins candidates for phytoremediation strategies. Modified NIP expression might improve the capacity to take up toxic metalloids and allocate them to shoots of plant species, which are practicable for mechanical harvesting and subsequent contamination processes. NIPs might also be potential targets for biotechnological approaches aiming at the remediation of wastewaters or at the retrieval of metalloids from sewage sludge. To this aim NIPs could be heterologously expressed in microorganisms to increase their uptake of metalloids. This might be important as microorganisms, which have a potentially high facility to tolerate or fix toxic metalloids normally lack intrinsic uptake pathways to increase their resistance toward the toxic compound.

The uncovering of the dramatic physiological relevance of the few characterized NIP isoforms led us to suspect that the importance of these metalloido-porins for the metalloid transport metabolism of plants is much more far-reaching than presently known. Future research on NIP metalloido-porins at the molecular, genetic, and physiological levels is essential to fully unravel the regulation of metalloid transport processes, since our current knowledge is only the *NIP* of a newly discovered iceberg.

6. Materials and Methods

6.1. Phylogenetic analysis

Multiple sequence alignments for all NIP protein sequences were built using ClustalW as implemented in GENEIOUS PRO v6.1. Bayesian phylogenetic analyses were done in MrBayes version 3.2. For the amino acid alignment the best-fit model Cprev of amino acid substitution was selected in MrBayes. MrBayes was run by conducting two parallel Metropolis coupled Monte Carlo Markov chain analyses with four chains for two million generations. Trees were sampled every 500 generations. Convergence of the runs was assessed using the standard deviation of split frequencies being <0.01. Numbers beside the nodes indicate the posterior probability values in %. Accession numbers for the proteins which were used for the generation of the phylogenetic tree: EaNIP3;1: CCI55658, EaNIP3;3: CCI55660, EaNIP3;4: CCI55661, AtNIP1;1: NP_567572, AtNIP1;2: NP_193626, AtNIP3;1: NP_174472, AtNIP5;1: NP_192776, AtNIP6;1: NP_178191, AtNIP7;1: NP_566271, OsNIP3;1: BAP05658, OsNIP3;2: BAM09283, OsNIP3;3: BAM09284, OsNIP1;1: BAP05657, OsNIP2;1: BAE92561, OsNIP2;2: BAG54792, HvNIP2;1: B9X078-1, HvNIP1;2: D2KZ48-1, HvNIP2;2: C6KYS1-1, LjNIP5;1: A BY19373, LjNIP6;1: ABY19374, ZmNIP2;2: NP_001105020, ZmNIP2;1: NP_001105637, ZmNIP3;1: NP_001105021, PpNIP5;1: XP_001779449, GmNIP2;2: C6TKR9-1, TaLsi1: G0WXH5-1, CmNIP2;1: F1SX50-1.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.plantsci.2015.06.002

References

[1] T.P. Jahn, G.P. Bienert, MIPs and Their Role in the Exchange of Metalloids, 1st edition, Landes Bioscience Springer, New York, 2010.
[2] R. Zangi, M. Filella, Transport routes of metalloids into and out of the cell: a review of the current knowledge, Chem. Biol. Interact. 197 (2012) 47–57.
[3] K. Miwa, T. Fujiwara, Boron transport in plants: coordinated regulation of transporters, Ann. Bot. 105 (2010) 1103–1108.
[4] V. Raicu, A. Popescu, Integrated Molecular and Cellular Biophysics, 1st edition, Springer, Amsterdam, The Netherlands, 2008.
[5] G.P. Bienert, F. Chaumont, Plant aquaporins: roles in water homeostasis, nutrition, and signaling processes, in: M. Geisler, K. Venema (Eds.), Transporters and Pumps in Plant Signaling, Springer, New York, 2011, pp. 3–38.
[6] M.G. Fortin, N.A. Morrison, D.P. Verma, Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment, Nucleic Acids Res. 15 (1987) 813–824.
[7] C.D. Weaver, B. Crombie, G. Stacey, D.M. Roberts, Calcium-dependent phosphorylation of symbiosome membrane proteins from nitrogen-fixing soybean nodules: evidence for phosphorylation of nodulin-26, Plant Physiol. 95 (1991) 222–227.
[8] R.M. Dean, R.L. Rivers, M.L. Zeidel, D.M. Roberts, Purification and functional reconstitution of soybean nodulin 26: an aquaporin with water and glycerol transport properties, Biochemistry 38 (1999) 347–353.
[9] M.B. Gorin, S.B. Yancey, J. Cline, J.P. Revel, J. Horwitz, The major intrinsic protein (MIP) of the bovine lens fiber membrane: characterization and structure based on cDNA cloning, Cell 39 (1984) 49–59.
[10] L.J. Ouyang, J. Whelan, C.D. Weaver, D.M. Roberts, D.A. Day, Protein phosphorylation stimulates the rate of malate uptake across the peribacteroid membrane of soybean nodules, FEBS Lett. 293 (1991) 188–190.
[11] R.L. Rivers, et al., Functional analysis of nodulin 26, an aquaporin in soybean root nodule symbiosomes, J. Biol. Chem. 272 (1997) 16256–16261.
[12] J.H. Hwang, S.R. Ellingson, D.M. Roberts, Ammonia permeability of the soybean nodulin 26 channel, FEBS Lett. 584 (2010) 4339–4343.
[13] P. Masalkar, I.S. Wallace, J.H. Hwang, D.M. Roberts, Interaction of cytosolic glutamine synthetase of soybean root nodules with the C-terminal domain of the symbiosome membrane nodulin 26 aquaglyceroporin, J. Biol. Chem. 285 (2010) 23880–23888.
[14] M. Dynowski, M. Mayer, O. Moran, U. Ludewig, Molecular determinants of ammonia and urea conductance in plant AQP homologs, FEBS Lett. 582 (2008) 2458–2462.

[15] S. Wienkoop, G. Saalbach, Proteome analysis: novel proteins identified at the peribacteroid membrane from *Lotus japonicus* root nodules, Plant Physiol. 131 (2003) 1080–1090.
[16] C.M. Catalano, W.S. Lane, D.J. Sherrier, Biochemical characterization of symbiosome membrane proteins from *Medicago truncatula* root nodules, Electrophoresis 25 (2004) 519–531.
[17] R.O. Carpena, et al., Boron and calcium distribution in nitrogen-fixing pea plants, Plant Sci. 151 (2000) 163–170.
[18] M. Reguera, A. Espí, L. Bolaños, I. Bonilla, M. Redondo-Nieto, Endoreduplication before cell differentiation fails in boron-deficient legume nodules. Is boron involved in signalling during cell cycle regulation? New Phytol. 183 (2009) 8–12.
[19] M. Reguera, I. Bonilla, L. Bolaños, Boron deficiency results in induction of pathogenesis-related proteins from the PR-10 family during the legume-rhizobia interaction, J. Plant Physiol. 167 (2010) 625–632.
[20] L. Bolanos, et al., Essentiality of boron for symbiotic dinitrogen fixation in pea (*Pisum sativum*) rhizobium nodules, Plant Physiol. 104 (1994) 85–90.
[21] L. Bolanos, N.J. Brewin, I. Bonilla, Effects of boron on rhizobium-legume cell-surface interactions and nodule development, Plant Physiol. 110 (1996) 1249–1256.
[22] H.I. Anderberg, P. Kjellbom, U. Johanson, Annotation of *Selaginella moellendorffii* major intrinsic proteins and the evolution of the protein family in terrestrial plants, Front. Plant Sci. 3 (2012) 33.
[23] U. Johanson, J. Danielson, Phylogeny of major intrinsic proteins, plant aquaporins: roles in water homeostasis, nutrition, and signaling processes, in: G.P. Bienert, T.P. Jahn (Eds.), MIPs and Their Role in the Exchange of Metalloids, 1st edition, LandesBioscience Springer, New York, 2010, pp. 19–31.
[24] R. Mukhopadhyay, H. Bhattacharjee, B.P. Rosen, Aquaglyceroporins: generalized metalloid channels, Biochim. Biophys. Acta 1840 (2014) 1583–1591.
[25] Q. Liu, H. Wang, Z. Zhang, J. Wu, Y. Feng, Z. Zhu, Divergence in function and expression of the NOD26-like intrinsic proteins in plants, BMC Genomics 10 (2009) 313.
[26] F. Abascal, I. Irisarri, R. Zardoya, Diversity and evolution of membrane intrinsic proteins, Biochim. Biophys. Acta 1840 (2014) 1468–1481.
[27] G. Soto, K. Alleva, G. Amodeo, J. Muschietti, N.D. Ayub, New insight into the evolution of aquaporins from flowering plants and vertebrates: orthologous identification and functional transfer is possible, Gene 503 (2012) 165–176.
[28] T.A. Diehn, B. Pommerrenig, N. Bernhardt, A. Hartmann, G.P. Bienert, Genome-wide identification of aquaporin encoding genes in *Brassica oleracea* and their phylogenetic sequence comparison to *Brassica* crops and Arabidopsis, Front. Plant Sci. 6 (2015) 166.
[29] P.J. Verrier, et al., Plant ABC proteins—a unified nomenclature and updated inventory, Trends Plant Sci. 13 (2008) 151–159.
[30] I.S. Wallace, D.M. Roberts, Homology modeling of representative subfamilies of *Arabidopsis* major intrinsic proteins: classification based on the aromatic/arginine selectivity filter, Plant Physiol. 135 (2004) 1059–1068.
[31] N. Mitani, N. Yamaji, J.F. Ma, Characterization of substrate specificity of a rice silicon transporter, Lsi1, Pflugers Arch. 456 (2008) 679–686.
[32] Q. Liu, Z. Zhu, Functional divergence of the NIP III subgroup proteins involved altered selective constraints and positive selection, BMC Plant Biol. 10 (2010) 256.
[33] J.A. Raven, Short and long distance transport of boric acid in plants, New Phytol. 84 (1980) 231–249.
[34] A.A. Meharg, L. Jardine, Arsenite transport into paddy rice (*Oryza sativa*) roots, New Phytol. 157 (2003) 25–31.
[35] C. Dordas, M.J. Chrispeels, P.H. Brown, Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots, Plant Physiol. 124 (2000) 1349–1362.
[36] C. Dordas, P.H. Brown, Permeability of boric acid across lipid bilayers and factors affecting it, J. Membr. Biol. 175 (2000) 95–105.
[37] T. Henzler, E. Steudle, Transport and metabolic degradation of hydrogen peroxide in *Chara corallina*: model calculations and measurements with the pressure probe suggest transport of H(2)O(2) across water channels, J. Exp. Bot. (2000) 2053–2066.
[38] A. Almasalmeh, D. Krenc, B. Wu, E. Beitz, Structural determinants of the hydrogen peroxide permeability of aquaporins, FEBS J. 281 (2014) 647–656.
[39] G.P. Bienert, M.D. Schüssler, T.P. Jahn, Metalloids: essential, beneficial or toxic? Major intrinsic proteins sort it out, Trends Biochem. Sci. 33 (2008) 20–26.
[40] A. Asad, R.W. Bell, B. Dell, A critical comparison of the external and internal boron requirements for contrasting species in boron-buffered solution culture, Plant Soil 233 (2001) 31–45.
[41] K. Warrington, The effect of boric acid and borax on broad bean and certain other plants, Ann. Bot. 37 (1923) 629–672.
[42] H. Marschner, Marschner's Mineral Nutrition of Higher Plants, 3rd edition, Elsevier, London, 2012, pp. 233–243.
[43] B.D. Gruber, R.F. Giehl, S. Friedel, N. von Wirén, Plasticity of the *Arabidopsis* root system under nutrient deficiencies, Plant Physiol. 163 (2013) 161–179.
[44] T. Letho, T. Ruuhola, B. Dell, Boron in forest trees and forest ecosystems, Forest Ecol. Manage. 260 (2010) 2053–2069.
[45] M.A. Wimmer, T. Eichert, Mechanisms for boron deficiency-mediated changes in plant water relations, Plant Sci. 203–204 (2012) 25–32.
[46] M. Kobayashi, T. Matoh, J. Azuma, Two chains of rhamnogalacturonan II are cross-linked by borate–diol ester bonds in higher plant cell walls, Plant Physiol. 110 (1996) 1017–1020.
[47] A. Fleischer, M.A. O'Neill, R. Ehrwald, The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II, Plant Physiol. 121 (1999) 829–838.
[48] P. Ryden, et al., Tensile properties of *Arabidopsis* cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II-borate complexes, Plant Physiol. 132 (2003) 1033–1040.
[49] J. Takano, et al., Arabidopsis boron transporter for xylem loading, Nature 420 (2002) 337–340.
[50] J. Takano, et al., The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation, Plant Cell 18 (2006) 1498–1509.
[51] H. Hanaoka, S. Uraguchi, J. Takano, M. Tanaka, T. Fujiwara, OsNIP3;1, a rice boric acid channel, regulates boron distribution and is essential for growth under boron-deficient conditions, Plant J. 78 (2014) 890–902.
[52] A.R. Durbak, et al., Transport of boron by the *tassel-less1* aquaporin is critical for vegetative and reproductive development in maize, Plant Cell 26 (2014) 2978–2995.
[53] M. Tanaka, I.S. Wallace, J. Takano, D.M. Roberts, T. Fujiwara, NIP6;1 is a boric acid channel for preferential transport of boron to growing shoot tissues in *Arabidopsis*, Plant Cell 20 (2008) 2860–2875.
[54] A. Leonard, et al., Tassel-less1 encodes a boron channel protein required for inflorescence development in maize, Plant Cell Physiol. 55 (2014) 1044–1054.
[55] R. Pérez-Castro, et al., *VvBOR1*, the grapevine ortholog of *AtBOR1* encodes an efflux boron transporter that is differentially expressed throughout reproductive development of *Vitis vinifera* L, Plant Cell Physiol. 53 (2012) 485–494.
[56] N. Tanaka, et al., Roles of pollen-specific boron efflux transporter, OsBOR4, in the rice fertilization process, Plant Cell Physiol. 54 (2013) 2011–2019.
[57] T. Li, et al., *Arabidopsis thaliana* NIP7;1: An anther-specific boric acid transporter of the aquaporin superfamily regulated by an unusual tyrosine in helix 2 of the transport pore, Biochemistry 50 (2011) 6633–6641.
[58] S.V. Eaton, Effects of boron deficiency and excess on plant, Plant Physiol. 15 (1940) 95–107.
[59] R.O. Nable, G.S. Bañuelos, J.G. Paull, Boron toxicity, Plant Soil 193 (1997) 181–198.
[60] A. Shatil-Cohen, M. Moshelion, Smart pipes: the bundle sheath role as xylem-mesophyll barrier, Plant Signal. Behav. 7 (2012) 1088–1091.
[61] T. Schnurbusch, et al., Boron toxicity tolerance in barley through reduced expression of the multifunctional AQP HvNIP2;1, Plant Physiol. 153 (2010) 1706–1715.
[62] E. Epstein, Silicon, Annu. Rev. Plant Physiol. Plant. Mol. Biol 50 (1999) 641–664.
[63] J.F. Ma, E. Takahashi, Soil, Fertilizer, and Plant Silicon Research in Japan, Elsevier Science, Amsterdam, 2002.
[64] J. Van Bockhaven, D. De Vleesschauwer, M. Höfte, Towards establishing broad-spectrum disease resistance in plants: silicon leads the way, J. Exp. Bot. 64 (2013) 1281–1293.
[65] F. Fauteux, F. Chain, F. Belzile, J.G. Menzies, R.R. Bélanger, The protective role of silicon in the *Arabidopsis*-powdery mildew pathosystem, Proc. Natl. Acad. Sci. USA 103 (2006) 17554–17559.
[66] F. Fauteux, W. Rémus-Borel, J.G. Menzies, R.R. Bélanger, Silicon and plant disease resistance against pathogenic fungi, FEMS Microbiol. Lett. 249 (2004) 1–6.
[67] A. Fawe, M. Abou-Zaid, J.G. Menzies, R.R. Bélanger, Silicon-mediated accumulation of flavonoid phytoalexins in cucumber, Phytopathology 88 (1998) 396–401.
[68] R.R. Bélanger, N. Benhamou, J.G. Menzies, Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis f.sp. tritici*), Phytopathology 93 (2003) 402–412.
[69] P. Bauer, R. Elbaum, I.M. Weiss, Calcium and silicon mineralization in land plants: transport, structure and function, Plant Sci. 180 (2011) 746–756.
[70] J.F. Ma, Silicon transporters in higher plants, in: G.P. Bienert, T.P. Jahn (Eds.), MIPs and their role in the Exchange of Metalloids, 1st edition, LandesBioscience Springer, New York, 2010, pp. 99–106.
[71] J.F. Ma, et al., A silicon transporter in rice, Nature 440 (2006) 688–691.
[72] Y. Chiba, N. Mitani, N. Yamaji, J.F. Ma, HvLsi1 is a silicon influx transporter in barley, Plant J. 57 (2009) 810–818.
[73] N. Mitani, N. Yamaji, J.F. Ma, Identification of maize silicon influx transporters, Plant Cell Physiol. 50 (2009) 5–12.
[74] C.H. Chen, J. Lewin, Silicon as a nutrient element for *Equisetum arvense*, Can. J. Bot. 47 (1969) 125–131.
[75] M. Hodson, P. White, A. Mead, M. Broadley, Phylogenetic variation in the silicon composition of plants, Ann. Bot. 96 (2005) 1027–1046.
[76] C. Grégoire, et al., Discovery of a multigene family of aquaporin silicon transporters in the primitive plant *Equisetum arvense*, Plant J. 72 (2012) 320–330.
[77] R.K. Deshmukh, et al., Identification and functional characterization of silicon transporters in soybean using comparative genomics of major intrinsic proteins in *Arabidopsis* and rice, Plant Mol. Biol. 83 (2013) 303–315.
[78] N. Mitani, N. Yamaji, Y. Ago, K. Iwasaki, J.F. Ma, Isolation and functional characterization of an influx silicon transporter in two pumpkin cultivars contrasting in silicon accumulation, Plant J. 66 (2011) 231–240.
[79] N. Mitani-Ueno, N. Yamaji, F.J. Zhao, J.F. Ma, The aromatic/arginine selectivity filter of NIP aquaporins plays a critical role in substrate selectivity for silicon, boron, and arsenic, J. Exp. Bot. 62 (2011) 4391–4398.
[80] J. Montpetit, et al., Cloning, functional characterization and heterologous expression of *TaLsi1*, a wheat silicon transporter gene, Plant Mol. Biol. 79 (2012) 35–46.

[81] C.O. Abernathy, et al., Arsenic: Health effects, mechanisms of actions, and research issues, Environ, Health Perspect. 107 (1999) 593–597.
[82] J.M. Cobo, M. Castiñeira, Oxidative stress, mitochondrial respiration, and glycemic control: clues from chronic supplementation with Cr^{3+} or As^{3+} to male Wistar rats, Nutrition 13 (1997) 965–970.
[83] G.P. Bienert, et al., A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes, BMC Biol. 6 (2008) 26.
[84] A.A. Meharg, F.-J. Zhao, Arsenic and Rice, 1st edition, Springer, New York, 2012.
[85] M. Katsuhara, et al., Functional and molecular characteristics of rice and barley NIP aquaporins transporting water, hydrogen peroxide and arsenite, Plant Biotechnol. 31 (2014) 213–219.
[86] W. Xu et al., *Arabidopsis* NIP3;1 plays an important role in arsenic uptake and root-to-shoot translocation under arsenite stress conditions, Mol. Plant. doi:10.1016/j.molp.2015.01.005.
[87] T. Kamiya, et al., NIP1;1, an aquaporin homolog, determines the arsenite sensitivity of *Arabidopsis thaliana*, J. Biol. Chem. 284 (2009) 2114–2120.
[88] J.F. Ma, et al., Transporters of arsenite in rice and their role in arsenic accumulation in rice grain, Proc. Natl. Acad. Sci. USA 105 (2008) 9931–9935.
[89] G.J. Norton, et al., Rice-arsenate interactions in hydroponics: a three-gene model for tolerance, J. Exp. Bot. 59 (2008) 2277–2284.
[90] D. Chakrabarty, et al., Comparative transcriptome analysis of arsenate and arsenite stresses in rice seedlings, Chemosphere 74 (2009) 688–702.
[91] T. Kamiya, T. Fujiwara, *Arabidopsis* NIP1;1 transports antimonite and determines antimonite sensitivity, Plant Cell Physiol. 50 (2009) 1977–1981.
[92] B.W. Vink, Stability relations of antimony and arsenic compounds in the light of revised and extended Eh-pH diagrams, Chem. Geol. 130 (1996) 21–30.
[93] E.S.J. Arnér, Selenoproteins—what unique properties can arise with selenocysteine in place of cysteine, Exp. Cell Res. 316 (2010) 1296–1303.
[94] G.F. Combs, Selenium in global food systems, Br. J. Nutr. 85 (2001) 517–547.
[95] H. Hartikainen, Biogeochemistry of selenium and its impact on food chain quality and human health, J. Trace. Elem. Med. Biol. 18 (2005) 309–318.
[96] T.G. Sors, D.R. Allis, D.E. Salt, Selenium uptake, translocation, assimilation and metabolic fate in plants, Photosynth. Res. 86 (2005) 373–389.
[97] H. Zhang, et al., Selenium in soil inhibits mercury uptake and translocation in rice (*Oryza sativa* L.), Environ. Sci. Technol. 46 (2012) 10040–10046.
[98] L. Zhang, F. Yu, W. Shi, Y. Li, Y. Miao, Physiological characteristics of selenite uptake by maize roots in response to different pH levels, J. Soil Sci. Plant Nutr. 173 (2010) 417–422.
[99] X.Q. Zhao, N. Mitani, N. Yamaji, R.F. Shen, J.F. Ma, Involvement of silicon influx transporter OsNIP2;1 in selenite uptake in rice, Plant Physiol. 153 (2010) 1871–1877.
[100] E. Takahashi, S. Syo, Y. Miyake, Effect of germanium on the growth of plants with special reference to the silicon nutrition (parts 1–3), Sci. Soil Manure Jpn. 47 (1976) 183–190.
[101] G. Bárzana, R. Aroca, G.P. Bienert, F. Chaumont, J.M. Ruiz-Lozano, New insights into the regulation of aquaporins by the arbuscular mycorrhizal symbiosis in maize plants under drought stress and possible implications for plant performance, Mol. Plant Microbe. Interact. 27 (2014) 349–363.
[102] J. Hayes, et al., Germanium as a tool to dissect boron toxicity effects in barley and wheat, Funct. Plant Biol. 40 (2013) 618–627.
[103] W.G. Choi, D.M. Roberts, Arabidopsis NIP2;1, a major intrinsic protein transporter of lactic acid induced by anoxic stress, J. Biol. Chem. 282 (2007) 24209–24218.
[104] J. Rambow, B. Wu, D. Rönfeldt, E. Beitz, Aquaporins with anion/monocarboxylate permeability: mechanisms, relevance for pathogen–host interactions, Front. Pharmacol. 5 (2014) 199.
[105] N. Yamaji, J.F. Ma, A transporter at the node responsible for intravascular transfer of silicon in rice, Plant Cell 21 (2009) 2878–2883.
[106] J. Takano, K. Miwa, T. Fujiwara, Boron transport mechanisms: collaboration of channels and transporters, Trends Plant. Sci. 13 (2008) 451–457.
[107] M. Mizutani, S. Watanabe, T. Nakagawa, M. Maeshima, Aquaporin NIP2;1 is mainly localized to the ER membrane and shows root-specific accumulation in *Arabidopsis thaliana*, Plant Cell Physiol. 47 (2006) 1420–1426.
[108] M. Giovannetti, et al., Two putative-aquaporin genes are differentially expressed during arbuscular mycorrhizal symbiosis in *Lotus japonicus*, BMC Plant Biol. 12 (2012) 186.
[109] J. Egelund, et al., *Arabidopsis thaliana RGXT1* and *RGXT2* encode Golgi-localized (1,3)-a-D-Xylosyltransferases involved in the synthesis of pectic rhamnogalacturonan-II, Plant Cell 18 (2006) 2593–2607.
[110] D. Chormova, D.J. Messenger, S.C. Fry, Boron bridging of rhamnogalacturonan-II, monitored by gel electrophoresis, occurs during polysaccharide synthesis and secretion but not post-secretion, Plant J. 77 (2014) 534–546.
[111] F. Chaumont, S.D. Tyerman, Aquaporins: highly regulated channels controlling plant water relations, Plant Physiol. 164 (2014) 1600–1618.
[112] M. Tanaka, et al., Boron-dependent degradation of *NIP5;1* mRNA for acclimation to excess boron conditions in *Arabidopsis*, Plant Cell 23 (2011) 3547–3559.
[113] C.J. Carrano, S. Schellenberg, S.A. Amin, D.H. Green, F.C. Küpper, Boron and marine life: a new look at an enigmatic bioelement, Mar. Biotechnol. 11 (2009) 431–440.
[114] W.D. Loomis, R.W. Durst, Chemistry and biology of boron, BioFactors 3 (1992) 229–239.
[115] N. Mitani-Ueno, H. Ogai, N. Yamaji, J.F. Ma, Physiological and molecular characterization of Si uptake in wild rice species, Physiol. Plant. 151 (2014) 200–207.
[116] I.S. Wallace, W.G. Choi, D.M. Roberts, The structure, function and regulation of the nodulin 26-like intrinsic protein family of plant aquaglyceroporins, Biochim. Biophys. Acta 1758 (2006) 1165–1175.
[117] J.F. Guenther, et al., Phosphorylation of soybean nodulin 26 on serine 262 enhances water permeability and is regulated developmentally and by osmotic signals, Plant Cell 15 (2003) 981–991.
[118] R.M. Hove, M. Bhave, Plant aquaporins with non-aqua functions: deciphering the signature sequences, Plant Mol. Biol. 75 (2011) 413–430.
[119] A. Froger, B. Tallur, D. Thomas, C. Delamarche, Prediction of functional residues in water channels and related proteins, Protein Sci. 7 (1998) 1458–1468.
[120] H.I. Anderberg, J.Å. Danielson, U. Johanson, Algal MIPs, high diversity and conserved motifs, BMC Evol. Biol. 11 (2011) 110.
[121] K.V. Khabudaev, D.P. Petrova, M.A. Grachev, Y.V. Likhoshway, A new subfamily LIP of the major intrinsic proteins, BMC Genomics 15 (2014) 173.
[122] R.P. Shrestha, M. Hildebrand, Evidence for a regulatory role of diatom silicon transporters in cellular silicon responses, Eukaryot. Cell. 14 (2015) 29–40.
[123] Z.H. Ye, Vascular tissue differentiation and pattern formation in plants, Ann. Rev. Plant Biol. 53 (2002) 183–202.
[124] C.J. Lovatt, Evolution of xylem resulted in a requirement for boron in the apical meristems of vascular plants, New Phytol. 99 (1985) 509–522.
[125] T. Matsunaga, et al., Occurence of the primary cell wall polysaccharide rhamnogalacturonan II in pteridophytes, lycophytes, and bryophytes: implications for the evolution of vascular plants, Plant Physiol. 134 (2004) 339–351.
[126] E.L. Morgan, Y.C.A. Wu, J.P. Swiger, An aquatic toxicity test using the moss *Physcomitrella patens* (Hedw) B.S.G, in: W.G. Landis, J.S. Highes, M.A. Lewis (Eds.), Environmental Toxicology and Risk Assessment, American Society for Testing and Materials, Philadelphia, PA, 1993, pp. 340–352.
[127] J.A.H. Danielson, U. Johanson, Unexpected complexity of the Aquaporin gene family in the moss *Physcomitrella patens*, BMC Plant Biol. 8 (2008) 45.