Mirza Hasanuzzaman · Kamrun Nahar
Masayuki Fujita *Editors*

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ISBN 978-981-13-1291-5 ISBN 978-981-13-1292-2 (eBook)
https://doi.org/10.1007/978-981-13-1292-2

Library of Congress Control Number: 2018958709

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Arsenic (As) is the most talked about metalloid in the modern world. It is a poisonous metalloid and historically known as "king of poisons," and its toxic potential has been known for millennia. It is the 20th most common element in the earth's crust, and it is present in the terrestrial, marine, and freshwater environments in various chemical forms, usually combined with other metals, sulfur, or oxygen. Arsenic may cause substantial damages to plant and animal kingdom. Therefore, As has become a great concern because of its chronic and epidemic effects on human, plant, and animal health. Naturally occurring As in the water has impacted the lives of millions of people; the situation has been called the "largest mass poisoning of a population in history". For example, in Bangladesh and West Bengal, India, more than 50 million people are drinking water containing As.

Since the last three decades, the toxic effects of As in plants have been investigated widely. Because of its occurrence in all soils and natural waters plants have obviously evolved in the presence of As ions. The complexity of As chemistry and biochemistry confound many efforts to understand the mechanism of toxicity. Different forms of As showed different mechanisms of toxicity. The rate of uptake or accumulation of As also greatly depends on several factors like soil type, plant species, and mechanisms of uptake. Among the cultivated crop plants, rice is the most affected crop from As threats because of the fact that rice is the only major crop grown in waterlogged condition for most of the time, and that rice is particularly efficient at assimilating some forms of As, particularly those generated under anaerobic conditions, and exporting them to grain. In line with the abundance and toxic effects of As in plants the tolerance mechanisms in the plant are being investigated widely. Molecular approaches in revealing the As stress-responsive genes provide effective clues in developing tolerance in plants. Recently, bioremediation technologies using plants and microbes are drawing special attention due to its effective and eco-friendly perspectives. Numerous research works have been carried on different aspects of As chemistry and the mechanisms of toxicity and tolerance in plants. This book presents a collection of 19 chapters written by 57 experts in the field of plant physiology, environmental sciences, and plant biochemistry.

We the editors would like to give special thanks to the authors for their outstanding and timely work in producing comprehensive chapters. We are highly thankful to Dr. Fumiko Yamaguchi, Senior Editor (Editor, Ecology and Animal Science), Springer, Japan, for her prompt responses during the acquisition. We are also thankful to Sivachandran Ravanan, Production Editor of this book and all other editorial staffs for precious help in formatting and incorporating editorial changes in the manuscripts. Special thanks to Dr. Md. Mahabub Alam, Noakhali Science and Technology University, Bangladesh, and Sayed Mohammad Mohsin, Sher-e-Bangla Agricultural University, Bangladesh, for their generous help in formatting the manuscripts. The editors and contributing authors hope that this book will include a practical update on our knowledge of the role of plant nutrients in abiotic stress tolerance.

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Dr. Hasanuzzaman's current work is focused on the physiological and molecular mechanisms of environmental stress tolerance (salinity, drought, flood, and heavy metals/metalloids). Dr. Hasanuzzaman has published over 100 articles in peer-reviewed journals. He has edited six books and written 30 book chapters on important aspects of plant physiology, plant stress tolerance, and crop production. According to Scopus®, Dr. Hasanuzzaman's publications have received over 2700

citations with an h-index of 26. He is editor and reviewer for more than 70 peer-reviewed international journals and was a recipient of the "Publons Peer Review Award 2017 and 2018." He has been honored by different authorities for his outstanding performance in different fields like research and education, and he has received the World Academy of Science Young Scientist Award (2014). He has presented 25 papers, abstracts, and posters at international conferences in several countries (USA, UK, Germany, Australia, Japan, Austria, Sweden, Russia, etc.). Dr. Hasanuzzaman is a member of 40 professional societies and is the acting Publication Secretary of the Bangladesh Society of Agronomy and the Bangladesh JSPS Alumni Association. He is a fellow of The Linnean Society of London.

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is a reviewer of international journals. She is active member of about 20 professional societies. Dr. Nahar attended different international conferences and presented 10 papers and posters in national and international conferences in different countries (USA, Australia, Japan, Austria, Russia, China, etc.).

Dr. Masayuki Fujita is Professor in the Laboratory of Plant Stress Responses, Faculty of Agriculture, Kagawa University, Kagawa, Japan. He received his B.Sc. in Chemistry from Shizuoka University, Shizuoka, and M.Agr. and Ph.D. in Plant Biochemistry from Nagoya University, Nagoya, Japan. His research interests include physiological, biochemical, and molecular biological responses based on secondary metabolism in plants under various abiotic and biotic stresses; phytoalexin, cytochrome P450, glutathione *S*-transferase, and phytochelatin; and redox reaction and antioxidants. In the last decade, his works were focused on oxidative stress and antioxidant defense in plants under environmental stress. His group investigates the role of different exogenous protectants in enhancing antioxidant defense and methylglyoxal detoxification systems in plants. He has supervised 4 M.S. students and 13 Ph.D. students as main supervisor. He has about 150 publications in journals and books and has edited 8 books.

Chapter 1
Arsenic Uptake and Transportation in Plants

Dariusz Latowski, Anna Kowalczyk, Kamila Nawieśniak, and Stanisław Listwan

Abstract The arsenic uptake and translocation systems in plants are dependent on As species. Uptake of inorganic arsenate [As_{in}(V)] is conducted via specified group of high-affinity phosphate (P_i) transporters belonging to the PHS family, called P_i transporter 1. Recently identified transcription factors involved in the regulation of As_{in}(V) intake in plants are also described in this chapter. The role of other proteins such as mitochondrial proteins localized to the inner mitochondrial membrane and responsible for dicarboxylate exchange between the mitochondrial matrix and the cytosol or P_i transporter traffic facilitator 1 located in the endoplasmic reticulum (ER) of *A. thaliana* is not omitted. Uptake of inorganic arsenite [As_{in}(III)], as well as the organic derivatives of As from environment and distribution in plants, is conducted by channels created by proteins belonging to three of the five plant aquaporin subfamilies called nodulin 26-like intrinsic protein (NIP), membrane (PIP), and tonoplast intrinsic proteins (TIP). The significance of ABC (ATP-binding cassette) transporters which are responsible for transferring of As_{in}(III)-phytochelatin complexes across the tonoplast to the vacuole as well as the role of transporters responsible for inositol uptake in As translocation from the xylem into the phloem is explained. Additionally, the meaning of some elements like S, Si, and Fe in As influx in plants is considered.

Keywords Arsenic species · Ion flux · Metalloids · Phytochelatins · Soil pollution

1.1 Introduction: Uptake and Transport of Arsenic Depend on Soil Properties and As Species

Although no specific As uptake systems have evolved (Stolz et al. 2006), the uptake of this metalloid from As-contaminated soils or water by plants including plant crops such as rice, brussels sprout, or other vegetables is commonly observed

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M. Hasanuzzaman et al. (eds.), *Mechanisms of Arsenic Toxicity and Tolerance in Plants*, https://doi.org/10.1007/978-981-13-1292-2_1

(Larsen et al. 1992; Cottingham et al. 2013). In the human body, about 40% of As content comes with the food, and plants are the primary route by which As enters in the food chain (BIAM 2002; Colangelo and Guerinot 2006). So, As accumulation in plants and its introduction to the food chain by plants are serious issue. Therefore, identification of As transporters and channels, as well as understanding As transport mechanisms in plants, can be applied in safe cropping and phytoremediation of As-contaminated areas in the future (Zhao et al. 2010a; Zhu et al. 2008). For safe cropping, resistant plants able to prevent accumulation in the harvested plant product are required, whereas for phytoremediation, the resistant plants capable of growing at high As concentrations and accumulate As in harvestable biomass are needed.

Among the three allotropes and nine oxidation states, either organic As (As_{org}) or inorganic As (As_{in}) is available to plants in four main forms of As: inorganic arsenate [As_{in}(V)], arsenite [As_{in}(III)], and their organic derivatives, i.e., monomethylarsonic acid (MMA) and dimethylarsinic acid (DDA) (Fig. 1.1) (Kläning et al. 1989; Norman 1998; Ellis and MacDonald 2004; Janiak et al. 2012). Generally, in the environment, the content of As_{org} is lower than As_{in} (Abedin et al. 2002). Moreover, the concentration of As_{org}(III) is lower than As_{org}(V) because of high volatility of As_{org}(III) (Mestrot et al. 2011).

As availability to plants depends on soil composition, texture, and other physicochemical properties of the soil, whereas As uptake and transportation systems in the plant are strictly connected with As species. In fine-textured soil, low content of As is observed, while coarse-textured soils with little ion exchange capacity and less colloidal material contained more As. Under oxidative conditions, As(V), the oxidized form, dominates As(III), whereas under reducing conditions occurring in such environment as flooded rice paddy fields, more mobile As(III) dominates As(V) (Punshon et al. 2017). Moreover, when anoxic conditions develop, redox potential (E_H), responding to the extent of aeration of the soil drops, electron acceptors are depleted causing reduction and dissolve of iron oxides and oxyhydroxides, thus increasing mobility of As previously strongly bound with these molecules (Fendorf and Kocar 2009; Meharg and Zhao 2012). This reductive dissolution of iron-bearing minerals under anaerobic conditions is the dominant biogeochemical process in the transition from As_{in}(V) to As_{in}(III). Forming of the Fe plaques in the rhizosphere of rice and other plants growing on flooded areas (e.g., water species) is a common mechanism of As uptake limitation (Chen et al. 2005; Seyfferth et al. 2010). Fe plaque consists of ferrihydrite, a widespread on the Earth's surface hydrous ferric

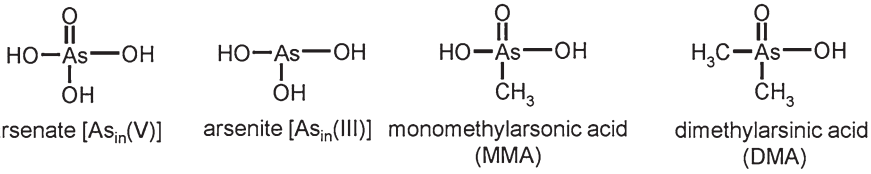

Fig. 1.1 Chemical structures of four main forms of inorganic and organic arsenic species occurring in the environment

oxyhydroxide mineral. It is formed on the root surface as a result of Fe^{2+} to Fe^{3+}oxidation by the oxygen released through aerenchyma of the roots of the plants growing in anaerobic soils. Oxidized iron strongly adsorbs arsenates. Iron plaques on the roots of tested plants contain 70–80% $As_{in}(V)$ and 20–30% $As_{in}(III)$. Additionally, bacteria colonizing the root iron plaque are also able to oxidize As(III) (Hu et al. 2015). It was shown that the concentrations of $As_{in}(V)$ within iron plaques of rice roots were five times higher than in root tissues of rice. Thus, the iron plaque formed on roots surface is natural barrier protecting from the migration of As to the plant. The properties of iron plaque depend on the genotype of plants (Dwivedi et al. 2010) and microbial composition, but also on silica which, as a next factor affecting the formation of iron plaque, controls the As concentration in iron plaque as well as in plants. Results of short-term experiments on As uptake by excised rice roots demonstrated that iron plaques limit $As_{in}(V)$ uptake but increase this process in case of $As_{in}(III)$ (Hansel et al. 2002; Blute et al. 2004; Liu et al. 2004a, b; Chen et al. 2005; Zhao et al. 2009). It was supposed that this relation is associated with $As_{in}(III)$ transporter systems efficiently operating within rice cell membranes. These systems shift the balance of $As_{in}(III)$ binding reaction to the form not associated with iron plaques but quickly absorbed by the roots of rice. $As_{in}(V)$ is stronger bound with soil particles such as aluminosilicates or aluminum hydroxides than $As_{in}(III)$, and thus reduction of $As_{in}(V)$ to $As_{in}(III)$ is one of the most significant factors increasing As bioavailability.

Additionally, silicic acid and silicates as well as phosphate being structural analogues of $As_{in}(III)$ or $As_{in}(V)$, respectively, can facilitate release of As adsorbed on soil particles into the soil solution at their high concentrations (Luxton et al. 2006). Values of pH below 4 and above 9 are another factor releasing As from its strong bonds with soil particles (Meharg and Zhao 2012). At the physiological pH range, predominating forms of $As_{in}(V)$ are deprotonated arsenates ($[H_2AsO_4]^-$), whereas $As_{in}(III)$ up to pH 8.28 exists mainly as protonated arsenous acid (H_3AsO_3). Protonated form of $As_{in}(V)$, i.e., arsenic acid (H_3AsO_4), dominates only below pH 1.31 (Bienert and Bienert 2017). The local alterations in E_H, pH, and the other physicochemical soil properties including the content of organic matter occurring in the rhizosphere and caused by plants and microbes also strongly influence concentrations and bioavailability of As (Acosta et al. 2015; Seyfferth 2015; Andres and Bertin 2016; Xiao et al. 2016). The decrease of E_H and an increase in the level of organic matter foster As methylation in soils (Frohne et al. 2011). Of the four main As forms available for plants, the organic, methylated derivatives are absorbed with the slowest rate and much slower than inorganic forms of As. On the other hand, the mobility of organic As derivatives in plants is greater than inorganic (Carey et al. 2010, 2011; Ye et al. 2010). Besides, DDA is generally better absorbed than MMA. Among of inorganic forms of As, $As_{in}(III)$ is better assimilable by plants than $As_{in}(V)$ (Raab et al. 2007a; Finnegan and Chen 2012).

Methylated As forms, independently on their state of oxidation, as well as $As_{in}(III)$, due to their physicochemical similarities to silicic acid, can be absorbed and translocated in plants via silicon (Si) influx-efflux systems but also by other channels dedicated to transport of small neutral molecules such as glycerol or anti-

monite. Thus the presence of silicic acid, as well as some other small neutral molecules, can competitively inhibit uptake of these As forms from the environment (Bienert and Bienert 2017).

$As_{in}(V)$ as structural analogue of inorganic phosphate (P_i) is absorbed by plants through phosphate transporters (the Phosphate Transporter 1 family of proteins, PHT1). Since the P_i affinity of PHT is higher than for $As_{in}(V)$, it is known that phosphate-supplemented soil usually reduces the uptake of $As_{in}(V)$ by plants. Additionally, increasing or decreasing PHT1 or appropriate Si transporters content in plant plasma membrane, by genetic engineering techniques, can also increase or decrease rate and amount of all main forms of As absorbed by plants (González et al. 2005; Ma et al. 2008; Zhao et al. 2009; Chen et al. 2011; Wu et al. 2011; Cao et al. 2017).

1.2 Arsenite and Arsenic Methylated Derivatives

1.2.1 Uptake and Translocation Systems

Plants use several systems to uptake arsenite [$As_{in}(III)$] and As methylated derivatives independently on their oxidation states from the environment but also to transport them into xylem or phloem and subsequently to particular plant cells and inside them between their subcellular compartments. Moreover, some of these systems can also be used to efflux As. The most researched transport systems of these As species belong to aquaporins (AQPs) (Bienert and Bienert 2017).

AQPs are integral membrane proteins in almost all living organisms excluding only some intracellular bacteria or thermophilic Archaea (Abascal et al. 2014). They exist in the various cellular membranes, including the plasma membrane, the endoplasmic reticulum, the mitochondria, the chloroplast, the vacuole, and even the vesicles involved in the trafficking pathway (Maurel et al. 2015; Bienert and Bienert 2017).

In these membranes, AQPs form pores and thus efficiently facilitate or enable the uptake, translocation, sequestration, or extrusion water and small mainly uncharged solutes. Although AQPs function as homo- or heterotetramers, each monomer can also work as a channel on its own. Additional central pore formed by four monomers closely associates together as tetramer, probably serves as another transport path (Fig. 1.2c) (Yool et al. 1996; Fu et al. 2000). AQP monomers are highly conserved, and two structural segments in each monomer can be distinguished. Each segment consists of three long membrane-spanning α-helices (marked as H1–H3 in the first and H4–H6 in the second segment), one reentrant short α-helix (marked as HB in the first and HE in the second segment), and two interconnecting loops (marked as LA, LB in first and LD, LE in the second segment). The parts of LB and LE, with the conserved and functionally important Asn-Pro-Ala (NPA) motifs, form HB and HE, respectively. Two structural segments of each monomers are connected together by additional, the fourth loop (LC), linking directly helix H3 with H4

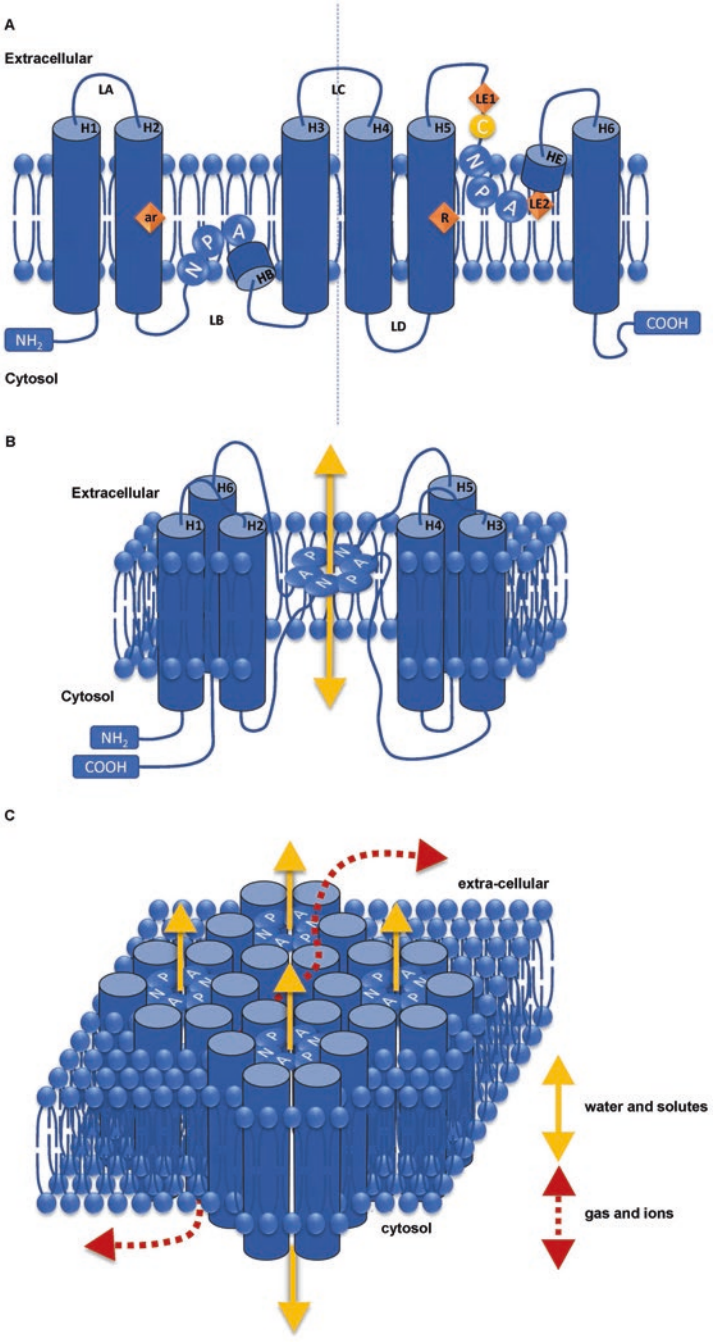

Fig. 1.2 The structures of AQP monomer (**a**, **b**) and homotetramer (**c**). H1–H6, α-helical domains; HB, HL, short α-helices with functionally important NPA (Asn-Pro-Ala) motifs; LA–LE, interconnecting loops; ar, R, LE1, LE2 (in diamonds), four amino acid residues created the functionally important aromatic selectivity filter, termed commonly as ar/R or more precisely as ar/R/LE1–LE2; **c** (in a circle), a cysteine residue (Cys 189) that can bind mercury blocking the AQP function

(Fig. 1.2a, b). α-Helices of AQPs create a solute-conducting channel with length between 20 and 28 Å and diameter from 4 to 6 Å. Although both ends of the channel on the membrane surfaces are widen funnel-shaped, the pore diameter can be constant over its length of the channel or narrow down around the narrowest region of the pore termed as the aromatic selectivity filter (Fu et al. 2000; Sui et al. 2001). The selectivity filter is located about 8–9 Å away from the first NPA motif and together with NPA motifs is the most important and narrowest region of AQPs that underlies their specificity (Törnroth-Horsefield et al. 2006; Frick et al. 2013a, b; Kirscht et al. 2016). The two NPA (Asn-Pro-Ala) motifs form the pore in which Asn residues are responsible for interaction with ligands by hydrogen bonds and probably create effective electrostatic proton barrier in AQPs (Fig. 1.2) (Tajkhorshid et al. 2002; Ilan et al. 2004; Forrest and Bhave 2007). The aromatic selectivity filter, termed commonly as ar/R or more precisely as ar/R/LE1–LE2, is formed with four amino acid residues – two located in second and fifth α-helices at position also termed as H2 and H5 and two others in second NPA box located in loop E at position signed as LE1 and LE2 (Fig. 1.2a) (Fu et al. 2000; Sui et al. 2001; Savage et al. 2003). These four residues include a conserved arginine residue (R) in loop E and less conservative aromatic residues (ar) at H2 α-helice (de Groot and Grubmüller 2001). The selective filter determines the rate of molecules transport by steric blocking too large molecules. Besides, the selective filter and NPA motifs create key interactions, such as van der Waals forces or hydrogen bonds, with transported molecules (Fu et al. 2000; Sui et al. 2001). Moreover, it was concluded that ar/R/LE1–LE2 together with NPA motifs are responsible for disruption of the hydrogen bonds between water molecules in the AQP pore and thus exclusion of the transport of protons via the Grotthuss mechanism (Kosinska-Eriksson et al. 2013; Zeuthen et al. 2013; Kreida and Tornroth-Horsefield 2015). N- and C-termini of each monomer of AQPs are oriented toward cytoplasm, and in some AQPs, these termini are posttranslationally modified and can possess sulfhydryl residues such as Cys, what explains AQP inhibition by mercury and other heavy metals (Fig. 1.2a) (Maurel et al. 2015; Bienert and Bienert 2017). Therefore, also As penetration can be partially inhibited not only by alternative AQP substrates such as mentioned above (glycerol, antimonite, or silicic acid), but also by mercury and other heavy metals which interact with sulfhydryl residues of Cys (Meharg and Jardine 2003).

The selectivity and kinetic parameters of aquaporins are commonly tested in four systems: (i) in isolated organelles, protoplasts, or tissues of living organisms (Ramahaleo et al. 1996; Uehlein et al. 2003; Besserer et al. 2012; Noronha et al. 2014); (ii) in vesicles isolated from cells or their membranes including transgenic cells with expressed aquaporins (Jung et al. 1994; Fang et al. 2002; Niemietz and Tyerman 2002; Schnurbusch et al. 2010); (iii) in liposomes or planar lipid bilayers with purified and reconstituted aquaporin proteins (Ye and Verkman 1989; Zeidel et al. 1992; Weaver et al. 1994; Verdoucq et al. 2008) and; (iv) in heterologous expression systems such as *Xenopus laevis* oocytes and yeast (Preston et al. 1992; Dordas et al. 2000).

Plant aquaporins belong to the ancient superfamily of major intrinsic proteins (MIPs) (Saier et al. 2016). Based on subcellular location and sequence homology, they are separated into five subfamilies:

(i) Plasma membrane intrinsic proteins (PIPs) (Kammerloher et al. 1994)
(ii) Tonoplast intrinsic proteins (TIPs) (Maeshima 2001)
(iii) Nodulin 26-like intrinsic proteins (NIPs) (Wallace et al. 2006)
(iv) Small basic intrinsic proteins (SIPs) (Johanson and Gustavsson 2002)
(v) X intrinsic proteins (XIPs) (Kaldenhoff et al. 2007)

It was documented that As_{in}(III) and/or As methylated derivatives are transported by aquaporins belonging to PIPs, TIPs, and all three functional NIP groups (NIP-I, NIP-II, NIP-III).

Based on permeation function, plant AQPs are divided to three major groups:

(i) Aquaporins that transport water
(ii) Aquaglyceroporins that permeate water and other neutral solutes (Borgnia et al. 1999)
(iii) Aquaporins that conduct ionic species, based on the evidence of human aquaporins (Yool et al. 1996; Fu et al. 2000; Yu et al. 2006)

Arsenous acid [$As(OH)_3$] and methylated As derivatives (Fig. 1.1) like many other molecules including boric acid [$B(OH)_3$] (Takano et al. 2006; Tanaka et al. 2008; Hanaoka et al. 2014), germanic acid (Ma et al. 2006; Hayes et al. 2013), selenous acid (Zhao et al. 2010b, c), and silicic acid [$Ge(OH)_4$ and $Si(OH)_4$] (Ma et al. 2006) are transported by aquaglyceroporins (Bienert et al. 2008a, b; Ma et al. 2008; Kamiya et al. 2009; Xu et al. 2015; Li et al. 2016). Transport of these acids was explained by similarity in the structure and atomic radii of their molecules [$As(OH)_3$: 3.57 Å; $B(OH)_3$: 3.43 Å; $Si(OH)_4$: 4.19 Å; $Ge(OH)_4$: 4.48 Å] as well as several physicochemical and structural characteristics with glycerol which is the canonical NIP substrate (Porquet and Filella 2007).

However, recent studies on NIP2;1 mutants with the changes in specific amino acid residues within the ar/R/LE1–LE2 selectivity filter of rice, *A. thaliana*, and barley suggested that metalloid permeation seemed to be controlled not only by atomic radii of molecules but also by some differences in interactions of metalloids with AQPs preceding interactions with ar/R/LE1–LE2 residues (Ma et al. 2008; Mitani-Ueno et al. 2011; Hayes et al. 2013).

1.2.1.1 NIPs in Arsenic Transport

In 2008, three independent studies for the first time demonstrated that uncharged H_3AsO_3 molecules permeate certain plant NIPs (Bienert et al. 2008a; Isayenkov and Maathuis 2008; Ma et al. 2008). Moreover, applying the heterologous expression of plant NIPs in frog oocytes and yeast cells clearly revealed NIPs as important bidirectional channels both in influx and efflux of As_{in}(III) and organic form of As

(MMA and DDA) (Bienert et al. 2008a, Ma et al. 2008; Li et al. 2009a). Today, six of ten identified *O. sativa* NIPs (OsNIPs:) work as As_{in}(III) channels, similarly to the *A. thaliana* NIPs (AtNIPs) where six of nine identified are permeable to As_{in}(III). Additionally, two NIPs of *Lotus japonica* (LtNIPs) and one of *Hordeum vulgare* (HvNIP) make transport of As_{in}(III) possible.

The major channel for influx and efflux of As_{in}(III), MMA, and DDA in *O. sativa* is OsNIP2;1 which is also the first identified silicon (Si) channel for Si uptake from the environment in higher plants. In spite of OsNIP2;1 as a channel – not a transporter – it is also called the Si transporter (OsLsi1).

The ar/R/LE1–LE2 motif of OsNIP2;1(OsLsi1) comprises Ser at the H5 position, two residues of Gly at positions H2 and LE1, and conserved Arg residue at L2 position. This composition of ar/R/LE1–LE2 is specific for NIP-III subfamily of AQPs. The small-size amino acid residues of ar/R/LE1–LE2 motif form a selective filter with diameter larger than pore diameter of NIP-I and NIP-II (Ma and Takahashi 2002). The differences of the amino acid composition at ar/R/LE1–LE2 are the basis for the division all NIPs into three subgroups: NIP-I which are permeable to glycerol, lactic acid, and water; NIP-II which are less permeable to water than NIP-I, but due to larger pore diameter than pore of NIP-I, they are permeable to larger solutes like boric acid, formamide, and urea; and last, NIP-III subgroup, with the largest pore diameter which apply to the transport of the silicic acid (Abbas et al. 2018).

OsNIPs2;1 (OsLsi1) were indentified in the distal side of epidermal and endodermal membrane cells of rice root. Therefore, they participate in an uptake of uncharged As species (As_{in}(III), MMA, DDA) from environment into the root cells of *O. sativa* as well as cooperate with other channels and transporters facilitating migration of this metalloid species within the plant.

One of these transporters is OsLsi2 – silicic transporter located in the membranes of the same cells as OsNIPs2;1 (OsLsi1) but on the proximal side and is responsible for distribution of As_{in}(III), but not organic As species, from root cells to xylem or stele tissues and as a consequence to accumulation of As_{in}(III) in rice grains. Thus the cooperation of OsLsi2 with OsNIPs2;1 (OsLsi1) and other NIPIII channels is considered to be the main mechanism enhancing accumulation of As_{in}(III) in rice grains (Ma et al. 2006, 2007, 2008). In this cooperation, Si or As penetrate into the cell by NIP-III channels, which are located at the exodermis side of cellular membranes, and leaking from the cells by Lsi2-type transporters located at endodermis side of cellular membranes (Ma and Yamaji 2015). NIP channels and Lsi2-type transporters are located in the membrane of the same cell but with opposite polarity. It is also possible, that these two transporter types are not present in one cell, but in adjacent cell layers (Sakurai et al. 2015).

On the other hand, it is worth remembering that the NIP channels such as OsNIP2;1 (OsLsi1) operate as bidirectional channels. As it was shown in frog oocytes, expression of OsNIP2;1 (OsLsi1) facilitates both the influx and efflux of As_{in}(III) and two tested organic derivatives of As, i.e., MMA and DDA (Ma et al. 2008; Li et al. 2009b). It shows that these As species permeate OsNIP2;1 (OsLsi1) bidirectionally between soil and plant root cells (Khalid et al. 2017). Thus, OsNIP2;1

(OsLsi1) is postulated to be responsible to approximately 20% efflux of As(III) in rice plants (Zhao et al. 2010a).

Lsi2 proteins are not NIP family members, and unlike OsLsi1 (OsNIPs2;1), they do not form the channels but operate as transporters. They are found in many *Liliopsida* and *Magnoliopsida* species including *A. thaliana* (Ma and Yamaji 2015).

The other Si channel, which was reported to strongly cooperate with OsNIPs2;1 (OsLsi1) in Si and probably As distribution in rice, is OsNIP2;2 (OsLsi6). In quantitative trait locus (QTL) analysis OsNIP2;2 (OsLsi6) was identified as contributing to increase of the methylated As level in the grain (Kuramata et al. 2013). The evidence that OsNIP2;2 (OsLsi6) can transport MDA and DDA to grain is still missing; however, it seems very likely that, as on the one side, OsNIP2;2 (OsLsi6) is expressed in the node below the rice panicle after the onset of grain filling (Yamaji and Ma 2009) and, on the other, DDA is translocated into the grain with high mobile in the panicle vascular system (Carey et al. 2010, 2011). Moreover, it was evidenced that OsNIP2;2 (OsLsi6) is polar-localized to the adaxial side of xylem parenchyma cells in the blade and the leaf sheath, and in the shoot, this protein is responsible for the unloading of the silicic acid from the xylem sap into the cytoplasmic leaf space (Yamaji and Ma 2009).

OsNIP3;2, which is expressed mainly in the lateral roots and the stele region of the primary roots, in anthers and suspension cells is another channel which can cooperate with OsNIPs2;1 (OsLsi1) in the distribution of As throughout plant organs (Li et al. 2016). Recently, it was presented that although this protein is involved in As_{in}(III) uptake by lateral roots, its contribution to As accumulation in the shoots is limited (Chen et al. 2017a). The importance of OsNIP2;1 aquaporin both in As_{in}(III) and the organic derivatives of As uptake was presented in *Osnip2;1* knockout rice line. The level of As in shoot of this rice mutant was reduced by 71% and in roots by 53% compared to wild-type plants when these two plant types were exposing an As_{in}(III) (Ma et al. 2008). When plants were treated with MMA and DDA (Fig. 1.1), the level of As in *Osnip2;1* mutant plants was about 50% lower than in wild-type plants (Li et al. 2009a).

The recently identified NIPs member engaged in As_{in}(III) uptake from the environment to rice root cells is OsNIP3;3 (Ali et al. 2012; Katsuhara et al. 2014; Li et al. 2016). Two other channels, i.e., OsNIP1;1 and OsNIP3;1, are also shown to be able to mediate in As_{in}(III) transport in rice, but they probably only support OsNIPs2;1 (OsLsi1) because their expression levels in rice roots are very low (Meharg and Zhao 2012).

In *A. thaliana* as the most important for As_{in}(III) uptake from the environment to the root, AtNIPs were identified: AtNIP1;1, AtNIP3;1, AtNIP5;1, and AtNIP6;1. Additionally, AtNIP3;1, AtNIP5;1, and AtNIP6;1 are involved in transmembrane As_{in}(III) transport and facilitate As_{in}(III) translocation from the root to the stem. Studies with frog oocyte heterologous expression systems demonstrated two additional NIP channels permeable to As_{in}(III), i.e., AtNIP1;2 and AtNIP7;1. In *A. thaliana*, NIP1;2 is strongly expressed in seeds, whereas AtNIP7;1 is selectively expressed in anthers and pollen tissues (Bienert et al. 2008a; Isayenkov and Maathuis 2008;

Kamiya et al. 2009; Xu et al. 2015; Li et al. 2016). Additionally, it was found that regulator of AtNIP1;1 is a calcium-dependent protein kinase (CPK31). The *A. thaliana* mutant of *cpk31* similar to *nip1;1* mutant and the double mutant cpk31 nip1;1 had a higher tolerance to As_{in} (III) than wild-type and cpk31 mutant (Ji et al. 2017).

Applying of yeast heterologous expression systems allowed to identify the other representatives of NIPs subfamily which were shown as permeable to As_{in}(III). There were two proteins of *Lotus japonicus*, i.e., LjNIP5;1 and LjNIP6;1, functioning as bidirectional As(III) channels and three of *Hordeum vulgare*, i.e., HvNIP1;2, HvNIP2;1, and HvNIP2;2 (Katsuhara et al. 2014; Li et al. 2016).

1.2.1.2 PIPs in Arsenic Transport

PIPs are the most abundant, homogenous subfamily of plant AQPs. They form water intrinsic channels in the plasma membrane, and thus they are extremely significant for plants water balance (Maurel et al. 2015; Chaumont and Tyerman 2014). For a few of them, it was evidenced that they are able to transport molecules other than water such as urea, H_2O_2, and CO_2 but also several types of uncharged metalloids including As (Mosa et al. 2012). They are divided into two subgroups, i.e., PIPs1 and PIPs2, with more than 50% sequence identity (Chaumont et al. 2001).

The level of 5 rice PIPs, i.e., OsPIP1;2, OsPIP1;3, OsPIP2;4, OsPIP2;6, and OsPIP2;7, and 13 of *Brassica juncea*, i.e., five PIPs1 and eight PIPs2, was reduced by As_{in}(III) (Mosa et al. 2012; Srivastava et al. 2013). The reduced expression of the mentioned above PIPs genes is in line with a decrease of water content in plants under As_{in}(III) stress, finally resulting in inhibition of seedling growth (Srivastava et al. 2013). On the other hand, at the same time, the increase of the level of reactive oxygen species (ROS) in root plant is observed, and it was shown that ROS also drive to repress of PIP2 genes expression in the root (Wudick et al. 2015). Therefore, it needs to be explained if alterations in level of PIP are the effect of direct As_{in}(III) stress or rather oxidative stress generated by As. Another unresolved question of As_{in}(III) transport by PIPs is molecular mechanism of this transport. It is unclear why orthologous PIP isoforms easily transporting of As_{in}(III) are impermeable to As, in spite of showing 100% similarity in the selective filter and NAP regions and a high degree of overall sequence homology.

1.2.1.3 TIPs in Arsenic Transport

TIPs are subfamily of AQPs commonly located in the plant vacuolar membrane called tonoplast. Among of the other AQPs, they are characterized by highly variable sequences, particularly in selected filter region. The vacuolar subtypes in plants are distinguished on the basis of specific TIP isoforms in the tonoplast. Moreover, the cell differentiation status and the developmental stage of the plant are also related to specific isoforms of TIPs (Jauh et al. 1999).

The high variability in ar/R/ LE1-LE2 sequences results additionally in a broad spectrum of their substrates. It is known that TIPs are permeable to water, and thus they play key role in turgor and widely understood of cellular osmoregulation of plant cells, but besides water, TIPs were as well shown to be able to transport urea (Liu et al. 2003; Soto et al. 2008), NH_3 (Jahn et al. 2004; Loqué et al. 2005), glycerol (Gerbeau et al. 1999; Li et al. 2008), H_2O_2 (Bienert et al. 2007), and various metalloids including As_{in}(III) (Maurel et al. 2015). Up to now, only one TIP was evidenced to be permeable for As_{in}(III). It was identified as TIP4;1 in fern – *Pteris vittata* – the best known As hyperaccumulator. However, although PvTIP4;1 belongs to TIPs subfamily, it is located rather in plasma membranes than in tonoplast. Additionally, it is important to notice that transcription of PvTIP4;1 gene is strongly limited to roots (He et al. 2015). The permeability to As_{in}(III) uptake and translocation were confirmed for PvTIP4;1 in *A. thaliana* where it was constitutively expressed. Additionally, expression of *PvTIP4;1* in yeast cells allowed to show that Arg-Cys substitution in ar/R selectivity filter of PvTIP4;1 made it impermeable to As (He et al. 2015).

1.2.1.4 Transport Systems for Arsenite and Methylated Derivatives of As Other than AQPs

AQPs are supported in arsenite and organic derivatives of As transport by other systems including proteins, glutathione, and its oligomers – phytochelatins. Besides the abovementioned silicic transporter OsLsi2 among other proteins, we can indicate the proteins identified in *P. vittata* which are similar to yeast Arsenical Compounds Resistance 3 (ScACR3) permeases active in As_{in}(III) efflux, and therefore called PvACR3 and PvACR3;1 (Indriolo et al. 2010).

ACR3 are included in the family which is one of the bile/arsenite/riboflavin transporter (BART) superfamily (Mansour et al. 2007). Based on operon analyses, it is postulated that these proteins may operate either as primary active transporters, similarly to the ArsB and ArsAB families with ATP hydrolysis, or secondary carriers. Up to now, four of these proteins were functionally characterized, i.e., ACR3 protein of *S. cerevisiae*, also called the ARR3 protein (Wysocki et al. 1997), ArsB protein of *Bacillus subtilis* (Sato and Kobayashi 1998), and PvACR3 and PvACR3;1 (Indriolo et al. 2010). ArsB protein of *B. subtilis* is not related to ArsB of *Escherichia coli* despite the same terminology. ScACR3 and ArsB of *B. subtilis* are plasma membrane carriers which use a proton antiport mechanism to export both arsenite and antimonite however with low affinity (Maciaszczyk-Dziubinska et al. 2011).

PvACR3 and PvACR3;1 are not located in plasma membrane but in tonoplast. Similarly to other known members of ACR3 family, PvACR3 and PvACR3;1 also decrease As_{in}(III) level in the cytosol, but instead of efflux of As(III) from cell to environment, they transfer it into the vacuole. ACR3 seems to be more significant than PvACR3;1. The essential role in As resistance of *P. vittata* was shown by knocking down the expression of ACR3 and ACR3;1 in the gametophyte of this fern species. Only ACR3 mutant results in an arsenite-sensitive phenotype. Moreover,

both in gametophytes and in sporophyte roots, expression of *acr3* was shown to be upregulated by As. Inversely, expression of *acr3;1* is unaffected by As (Indriolo et al. 2010). Recently, PvACR3;1 gene was cloned and expressed in *A. thaliana, Nicotiana tabacum*, and *S. cerevisiae*. In roots of both transgenic plants, increased As retention was observed. The level of As in shoots of transgenic plants was 55–61% lower than in wild-type control under laboratory conditions and in soil experiments with transgenic tobacco of about 22% lower than in control. Additionally, it was shown that PvACR3;1 in transgenic *A. thaliana* is also located in the tonoplast indicating that in plant roots, As_{in}(III) retention is conducted by the same detoxification mechanism as in As hyperaccumulator, i.e., by As_{in}(III) sequestration into vacuoles (Chen et al. 2017b).

It is worth to note that *P. vittata* as As hyperaccumulator contains two copies of ACR3 genes; single copies were identified also in other fern species as well as in moss, lycophytes, and gymnosperms. However, up to now, no ACR3 genes have been detected in angiosperms. Angiosperms are unable to As hyperaccumulation and they usually do not even show the tolerance to As (Indriolo et al. 2010).

Whereas in As hyperaccumulating fern, As_{in}(III) is rapidly transported from roots to fronds where it is stored in vacuoles mainly due to ACR3 proteins, in non-hyperaccumulators most of the arsenite is bound with thiol groups of glutathione or phytochelatins and retained in root cell vacuoles by the action of ABC transporters. Contrary to As nonaccumulator plants, in hyperaccumulators only few thiol complexes with As_{in}(III) are observed (Chakrabarty 2015). ABC transporters are active transporters which hydrolyze ATP to release energy to transport substrates across membranes. They consist of two distinct types of domains. One of them is the nucleotide-binding domain (NBD) also called ATP-binding cassette domain (ABC) from which the name of the whole ABC transporters family comes from. This family belongs to one of the largest and probably one of the oldest superfamily engaged in molecule transport. Besides the NBD (ABC) domain, the transmembrane domain (TMD) is present in ABC transporter structure. Each molecule of ABC transporters consists of at least two TMDs and two NBDs. NBDs (ABC domains) are located in the cytoplasm, they show highly conserved sequence, and they are responsible for ATP binding and hydrolysis. On the contrary, sequences and architecture of TMDs are variable in order to identify and interact with ABC ligands. Besides, TMDs due to energy coming from ATP hydrolysis can undergo conformational changes which make possible transport of ABC ligands across the membrane. In tonoplast of *A. thaliana* and rice cells, two members of ABC transporters family, i.e., ABCC1 and ABCC2, were shown to be involved in the transport of As_{in}(III) complexed with thiol groups of peptides and proteins into vacuoles (Song et al. 2010, 2014).

The rice ABCC1 transporters, localized in tonoplast of phloem and phloem companion cells of nodes, were presented to be responsible for the inhibition of the translocation of As_{in}(III) into grains by transporting thiol-As complexes into vacuoles of phloem cells in node cells. In 2015, it was confirmed that the As_{in}(III) distribution into the grain in rice is limited by nodes which act as As_{in}(III) filter (Chen et al. 2015). It is widely accepted that in nonaccumulator plants, As transport from

the roots to the shoots is highly restricted by As_{in}(III) complexation with thiol groups. In *Brassica juncea* root cells, whole pool of As_{in}(III) was found to be complexed with thiol components, whereas major As transported species within the xylem and phloem was uncomplexed As_{in}(III) (Kopittke et al. 2014). In rice, during 2–4 days of experiment, only 10% of total As_{in}(III) absorbed by plants was detected in shoots and slightly more than 3% in the grains (Zhao et al. 2012). Thus, As_{in}(III) is suggested to be poorly transported by either xylem or phloem, although phloem was considered as the primary route of transport to grains for As_{in}(III). On the other hand, it is postulated that organic As species are transported very efficiently by phloem and xylem (Awasthi et al. 2017).

The most efficient As_{in}(III) loading mechanism into the xylem was detected in As hyperaccumulator, *P. vittata* (Su et al. 2008). Rice was shown to load arsenite into xylem sap more efficiently than other crop plants, e.g., barley or wheat (Su et al. 2010), although As uptake and transfer into rice grains were proven to be strongly dependent on rice cultivar and As bioavailability in soil (Batista et al. 2014).

On the other hand, higher phytochelatin level and reduction of As translocation in the plant are observed in rice exposed to higher As concentrations (Duan et al. 2011).

It is worth noting that ABC proteins can serve as As_{in}(III) transporters but only when the metalloid is complexed with thiol groups. The glutathione and phytochelatin synthesis are induced by cytokinin depletion, and thus cytokinins can influence As transport in plants. Moreover, in 2016 chloroquine-resistance transporter-like transporter (OsCLT1) was identified in plastids of rice as a regulator of glutathione homeostasis and phytochelatin biosynthesis and thus affecting As uptake and distribution in plants. The lack of this transporter in *Osclt1* mutants showed lower level of phytochelatin 2 and As than wild-type plants under exposition both to As_{in}(III) and As_{in}(V) (Yang et al. 2016). Additionally, phytochelatin synthase genes in rice (*Ospcs*1, *Ospcs*3, and *Ospcs*13), as well as ABC transporter genes (Osabcg5, Osabci7_2, and Osabc6), were shown to be upregulated by sulfur. On the other hand, sulfur decreases the expression of other tonoplast transporter gene, i.e., *tip*4;2 especially important in As transport in *P. vittata* (Zhang et al. 2016). Besides, when transport of As_{in}(III) complexes with thiol components in plants is studied, it should also be considered that these complexes are stable within the low pH range 1.5–7.5. At higher pH values, such as pH of phloem sap, they dissociate. Therefore, it is clear that although a high level of thiol components was detected in the phloem sap of *Ricinus communis* or *Brassica napus*, As_{in}(III)-thiol complexes were not identified (Ye et al. 2010).

Another factor affecting As translocation from root to shoot in *P. vittata* is transpiration. It was evidenced that plants with higher transpiration also had a higher level of As in their shoots and, inversely, plants with lower transpiration by 28–67% showed also a lower level of As in shoot by 19–56% (Wan et al. 2015).

Another protein suggested to facilitate As_{in} (III) uptake and its translocation from root to shoot is rice Natural Resistance-Associated Macrophage Protein 1 (NRAMP1). OsNRAMP1 located in the plasma membrane of endodermis and peri-

cycle cells may facilitate As_{in}(III) transfer into xylem and thus xylem movement of As_{in}(III) from root to shoot. OsNRAMP1 gene expression in yeast as well as in *A. thaliana* resulted in enhance of As and cadmium accumulation. In plants, the higher level of As and Cd was detected both in root and shoot (Tiwari et al. 2014). Thus, OsNRAMP1 cooperates with another non-AQP protein, i.e., OsLsi2, and they both help in xylem loading of As_{in}(III) and in root to shoot transportation. As_{in}(III) from xylem sap can also be transported to phloem by inositol transporters (INTs). It was shown that INTs of *A. thaliana* (AtINT2 and AtINT4) which are responsible for inositol uptake from phloem were also involved in the translocation of As_{in}(III) from xylem to phloem and finally into seeds (Duan et al. 2016).

Recently, a putative peptide transporter (PTR7) as a new DMA long-distance transporter from roots to grains was postulated in rice, based on significant expression of *ptr7* in rice roots, leaves and 1st node during ripening of the grain and lack of DMA in grain of rice OsPTR7 mutant, despite grains of wild-type control plant contain 35% As as DMA (Tang et al. 2017).

1.3 Arsenate Uptake and Its Translocation Systems

Arsenate [As_{in}(V)] and phosphate (P_{in}) as structural chemical analogues with similar electrochemical profiles share the same transport pathways in plants. Protein transporters of P_{in} in plants belong to three families. Two of them are members of inorganic phosphate transporter (PiT) family and known as P_{in} transporters 1 (PHT1) and 2 (PHT2). The third group belongs to the ion transporter (IT) superfamily and is termed as phosphate permease family (PHO1). The proteins of PHT1 family are H^+/P_{in} symporters, and they transport P_{in} from the environment into the plant (Bucher 2007; Javot et al. 2007).

The proteins of PHT2 family, in spite of their high similarity to the mammalian phosphate/Na^+ symporter (PNaS) family, in plants, function as H^+/P_{in} symporters and therefore belong to PiT family. Members of PHT2 family occur in plastid membranes of plants (Versaw and Harrison 2002; Bucher 2007).

Proteins belonging to PHO1 family probably transport P_{in} both to the xylem and phloem tissue as well as into cells, such as root epidermal cells, cells of the cortex, or pollen (Wang et al. 2004). Furthermore, on the base of P_{in} uptake kinetics studies, P_{in} transporters are divided in two groups, one with high and another one with low affinity for P_{in} (Dunlop et al. 1997; Misson et al. 2004; Miller et al. 2009). The high-affinity P_{in} transporters, with K_M values in the range of 2.5–12.3 μM, play an important role in the uptake of P_{in}, whereas the low-affinity transporters, with K_M values between 50 and 100 μM (Nussaume et al. 2011), are responsible for translocation of acquired P_{in} (Smith et al. 2001).

Studies with a number of plant species including *P. vittata* (Wang et al. 2002), duckweed (*Lemna gibba*) (Ullrich-Eberius et al. 1989), *A. thaliana* (Clark et al. 2003), velvet grass (*Holcus lanatus*) (Macnair and Cumbes 1987; Meharg and Macnair 1990), and also crop plants such as barley (*Hordeum vulgare*) (Asher and

Keay 1979) or wheat (*Triticum aestivum*) (Zhu et al. 2006) show that $As_{in}(V)$ and P_{in} are absorbed by the roots and transported in plants by the same transporters, which belong to PHT1 family. Furthermore, it was proved that $As_{in}(V)$ competes with P_{in} while ingestion process into cell via PHT1 in many monocots and dicots species, both in As-hyperaccumulators and non-hyperaccumulators plants (Ullrich-Eberius et al. 1989; Meharg and Macnair 1992; Wang et al. 2002; Abedin et al. 2002; Clark et al. 2003; Esteban et al. 2003; Tu and Ma 2003; Bleeker et al., 2003).

PHT1 family was identified in 1996 as a specific family of plant plasma membrane proteins (Muchhal et al. 1996). Up to now, more than 100 PHT1 proteins have been characterized in plants. They are expressed mainly in roots. However, some members of the PHT1 family were also detected in leaves and flowers (Nussaume et al. 2011). Proteins belonging to this family contain conserved amino acid residues sequence, i.e., GGDYPLSATIxSE, although single modifications in amino acid residues in the range of this signature are also observed (Karandashov and Bucher 2005). Proteins of PHT1 family show from 60% to 95% similarity of amino acid sequence between various plant species including *A. thaliana*, rice (*O. sativa*), wheat (*T. aestivum*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), tobacco (*Nicotiana tabacum*), *Medicago truncatula*, *Catharanthus roseus*, or *P. vittata* (Ma et al. 2001; Rausch and Bucher 2002; Di Tusa et al. 2016). Moreover, the amino acid sequence of *A. thaliana* PHT1 shares 34% identity and around 50% similarity with yeast PHO84 proteins (Raghothama 1999). One of the two bacterial clusters of phosphate transporters (PiTs) is also close to the PHT1 (Saier et al. 1999).

On the base of hydrophobicity analysis, it was revealed that PHT1 members have 12 hydrophobic membrane-spanning domains (MSDs) each composed of 17–25 amino acid residues. The hydrophobic domains are separated by six extracellular and five intracellular hydrophilic loops. Additionally, as it results from computer analyses, MSDs are divided into two groups of six domains by the longest, hydrophilic loop which is located centrally in the protein molecule (Raghothama 1999). The central loop and the C-terminal and N-terminal of PHT1 members are predicted to be located inside the cell (Fig. 1.3).

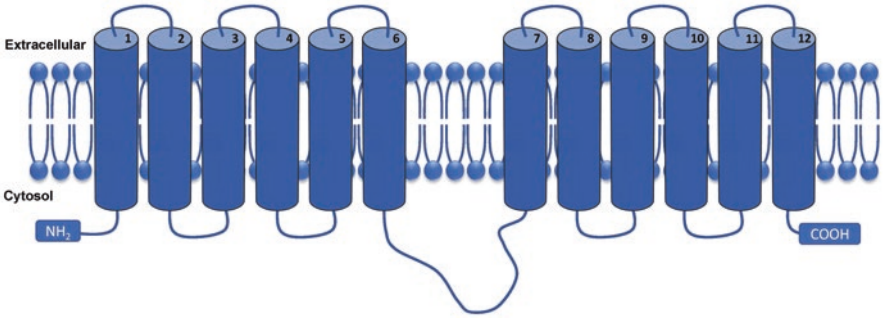

Fig. 1.3 The topology of a plant phosphate transporter with 12 membrane-spanning domains (MSDs), each composed of 17–25 amino acid residues separated by six extracellular and five intracellular hydrophilic loops and centrally located hydrophilic loop