Chapter 14
Tolerance Mechanisms of Rice to Arsenic Stress

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14.1 Arsenic Pollution

The outermost layer of the earth comprises of primary igneous olivine rocks, sedimentary sandstone, and metamorphic limestone in which arsenic (As) is also present in high concentrations. In igneous rocks its range is 0.2–10 mg kg^{-1}, while sedimentary rocks contain approximately 0.6 mg kg^{-1} of As (Zhenli et al. 2005). Arsenic (As) has been found to be allied part of various minerals like iron (Fe), oxides/hydroxides of aluminum (Al), manganese (Mn), and sulfides, and it was also reported that sea salt sprigs and volcanic upsurges were among its other sources (Fitz and Wenzel 2002). Soil contains various forms of As complexes of chlorides, oxides, hydroxide, and sulfides chiefly enargite (Cu_3AsA_4), cobaltite (CoAsS), and skutterudite ($CoAsS_4$) (Moreno-Jiménez et al. 2012). According to published reports, the prevalence of As in soils is thought to be caused by natural and anthropogenic sources. According to some reports, high As in soil was attributed to the extensive use of As-containing pesticides during the Green Revolution in the 1970s (Adriano 2001; Ng et al. 2003; Chopra et al. 2007). The associated risk of As human health is mainly owed to the bioavailable species of As (Rodriguez et al. 2003). Total As concentration does not indicate its bioavailability, and even no direct methods could measure the bioavailable As of soils; thus the assessment of risk is cumbersome. Hot acid extraction has been highlighted as the sole method to characterize As in soils and other media.

Quaghebeur and Rengel (2005) reported that As occurs in various chemical forms in environment. Generally, inorganic As is more toxic than organic ones; moreover, As in the trivalent oxidation state is more toxic than those in the pentavalent oxidation state. They differ from each other in physical, chemical properties,

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toxicity, mobility, and bioavailability (Quaghebeur and Rengel 2005). According to Gonzaga et al. (2006), As is not essential for plant growth and is highly phytotoxic in inorganic forms.

As-contaminated drinking water is serious menace for human health in the Southeast Asia and the Bengal Delta (Sharma 2006). Malik et al. (2009) reviewed As contamination and its possible remedies and reported that various aquifers and tube wells contained As above the USEPA's recommended level in Pakistan. Smith et al. (2000, 2002) reported that among 1.4 million global As-contaminated sites, 41% were located in the USA, while the USEPA documented that As concentration was even higher in Australia (>10,000 mg kg^{-1}). Arsenic present at high amounts (10,000–20,000 mg kg^{-1}) in soils may pose serious health risk to human when enters food chain (Davis et al. 2001). It is also reported that high As content was associated with soil and plant samples collected near industrial estates such as Ghari Rahimabad, Pakha Ghulam, Hattar Industrial Estates, Gujranwala Industrial Estate, and Peshawar Industrial Estate of Pakistan (Rehman et al. 2008).

Human health may be seriously affected due to high As exposure and intake. Rathinasabapathi et al. (2006) reported that prolonged As contact may result in various carcinomas of the skin and internal organs, impaired neural dysfunction, and kidney and liver failures. In 1993, the WHO (1993) lowered the guideline value for As in drinking water from 50 μg L^{-1} to 10 μg L^{-1}. On the other hand many developing countries still have 50 μg L^{-1} as MCL (Sharma 2006). As-contaminated groundwater has been reported in various parts of world, such as Vietnam, Massachusetts States, Carolina, Canada, and Bangladesh with 0.305, 30, 2460, 6590, and 0.3990 mg kg^{-1} As (Roychowdhury et al. 2003; Salido et al. 2003; Das et al. 2004; Bonney et al. 2007). Groundwater contamination of As was suggested as the most common consequence of high As concentrations in soil. High dependence of nearly one third of the world's population on groundwater (Erakhrumen 2007) can be a reason of As toxicity in affected regions. As toxicity was considered as the biggest calamity mainly due to the dependence on groundwater as drinking source in Bangladesh (Chakraborti et al. 2009).

14.2 Paddy Pollution Due to Arsenic

Plants require an adequate supply of all nutrients for their normal physiological and biological functions (Gupta et al. 2003). Deficiency of specific nutrient occurs when plant cannot obtain sufficient amount as required, whereas excessive supply of the same, through contaminated soil, results in toxicity in plants. Recommended soil application by the USEPA for As is 41 mg kg^{-1}. The understanding of arsenic (As) biogeochemical cycle in paddy soils is very important which is related with the mobility, solubility, and bioaccessibility of this heavy metal (Lim et al. 2014). The health risks associated with food chain become higher due to the high concentration of soluble and bioavailable As to living organisms (Abdul et al. 2015). Study revealed that the bioavailability of As may depend on the presence of Fe, Al, or

Mn complexes of arsenic and bacterial community of pore water which reduces the As oxides to reductive liquefaction (Yang et al. 2016; Rinklebe et al. 2016). Arsenic release depends on the physicochemical and biological composition of the soil (Wang et al. 2014). In anaerobic paddy soils, sulfur and the sulfur-reducing bacteria can play an important role in the As methylation and biogeochemical cycling of As contamination by decreasing its mobility and bioaccessibility to rice plants (Jia et al. 2015). Biogeochemical cycle of As-contaminated paddy soils show that the nitrate addition reduces the As mobilization and bioavailability due to the actions of anaerobic As (III) oxidizing rhizobacteria (Zhang et al. 2017).

Rhizosphere of paddy soil becomes favorable for the oxidation of AsIII to AsV due to the release of oxygen from roots of rice plants, development of iron plaque, and oxidation activities of rhizobacteria (Jia et al. 2014). It has been analyzed that there are many As-resistant varieties of rice which accumulate 20–30 times less As than others. So As accumulation and uptake in rice grain can be controlled by the selection of As-resistant rice varieties for cultivation (Syu et al. 2015; Zhang et al. 2016). Such specific As-resistant varieties have As-responsive quantitative trait loci which control the uptake, transportation, and accumulation of As in rice grains and prevent food chain relating As toxicity (Zhang et al. 2017; Norton et al. 2014).

To overcome the toxicity of As on metabolic, biochemical, and molecular activities of cells, many plants develop phosphate and hexose carriers, enzymatic and nonenzymatic antioxidants, and synthesis of vacuolar As phytochelatin complexes (Finnegan and Chen 2012; Chen et al. 2017). Many studies revealed that under anaerobic conditions, ferric hydroxide has more affinity for adsorption and desorption of As (III) than As (V) because of possessing variable surface complexes (Ackermann et al. 2010; Postma et al. 2010; Herbel and Fendorf 2006). Anaerobic conditions not only promote dissolution of ferric hydroxide by Fe-reducing bacteria but also produce secondary minerals like magnetite, ferrihydrite, goethite, and zero-valent iron [ZVI] which may enhance sorption capacity of As rather than solubility of As (Tokoro et al. 2009; Wang et al. 2017).

14.3 Dissolution of Arsenic Minerals

Arsenic has various chemical species, but the most commonly studied are as follows: arsenopyrite (FeAsS), arsenian pyrite $Fe(AsS)_2$, orpiment As_2S_3, claudetite As_2O_3, gersdorffite NiAsS, realgar AsS/As_4S_4, and arsenolite (Malik et al. 2009). Dissolution and mobility of arsenic in soils occur through the following steps: (1) reductive dissolution, (2) oxidative dissolution (3), ligand exchange, and (4) ligand-enhanced dissolution.

Reductive dissolution of arsenopyrite discharges As (V) into groundwater, whereas dissolution of claudetite produces As(III) (Foley and Ayuso 2008). Study revealed that acidic conditions and availability O_2 quantity are necessary for the dissolution of arsenopyrite As_2O_3 (Neil et al. 2014). Oxidative dissolution comprises on three main steps: (1) As dissolution and leachability from minerals through oxidation of arsenopyrites (Yunmei et al. 2004), (2) oxidation of arsenian pyrites (Brown and Calas 2012), and (3) carbonation of arsenosulfides (Lim et al. 2009).

Oxidative and reductive dissolution of As from minerals depends on the oxygen availability and pH values of soil and water. At neutral pH some minerals of AS produce secondary minerals like orpiment, realgar, and gersdorffite which on dissolution readily changes into arsenite and thioarsenite (Drahota and Filippi 2009; Wang et al. 2015). Arsenolite is the primary mineral of As which readily dissolved and liberate As directly into water (Haffert et al. 2010). Scorodite is a type of primary mineral that naturally coexists with ferric oxyhydroxide (FO) phase at pH ranges between 2.5 and 3, but at neutral pH it produces arsenate through aqueous dissolution or through weathering of arsenic-containing minerals bedrocks (Langmuir et al. 2006).

Ligand exchange dissolution is the mechanism which is related with the exchange of anion attached on any mineral with another anion like sulfate, phosphate, oxalate, citrate, and malate. For example, exchange As(V) by phosphate from arsenopyrites:

$$\text{Arsenopyrite} + \text{Phosphate} \leftrightarrow \text{Pyrophosphate} + \text{As(V)}$$

Ligand-enhanced dissolution is the type of As dissolution in which the cations of As mineral are replaced by oxalate, malate, and citrate and release As(V) and resulted in the synthesis of complex structures of As salts.

$$\text{Arsenopyrite} + \text{Oxalate} \leftrightarrow \text{Pyro oxalate} + \text{As(V)}$$

The rate of this reaction with organic ligands, such as oxalate, malate, and citrate, varies substantially with mineral phase. The reaction rates decrease in the following trend (Fig. 14.1).

14.4 As Uptake by Rice

Rice (*Oryza sativa* L.) is a most common staple food in Asia and worldwide which uptake and accumulate As in it (Roychowdhury et al. 2002; Khush 2005). As is toxic heavy metal which stands first by the Agency for Toxic Substances and Disease Registry in a list of 20 hazardous substances (Goering et al. 1999). Two most predominantly occurring forms of arsenic (As) in plants are As III and As V, but most of the plants reduce As V to As III which resulted in plants death by disturbing the cellular activities of plants body (Abedin et al. 2002). It has been observed that the uptake of inorganic As species is commonly higher by rice than organic methylated As species, but after uptake methylated As species are efficiently transported to the grains and resulted in spikelet sterility syndrome which lowers down the crop yield (Zhao et al. 2013).

Several research studies have also found high concentrations of arsenic in vegetables and rice in areas where concentrations of arsenic in soil and water are also

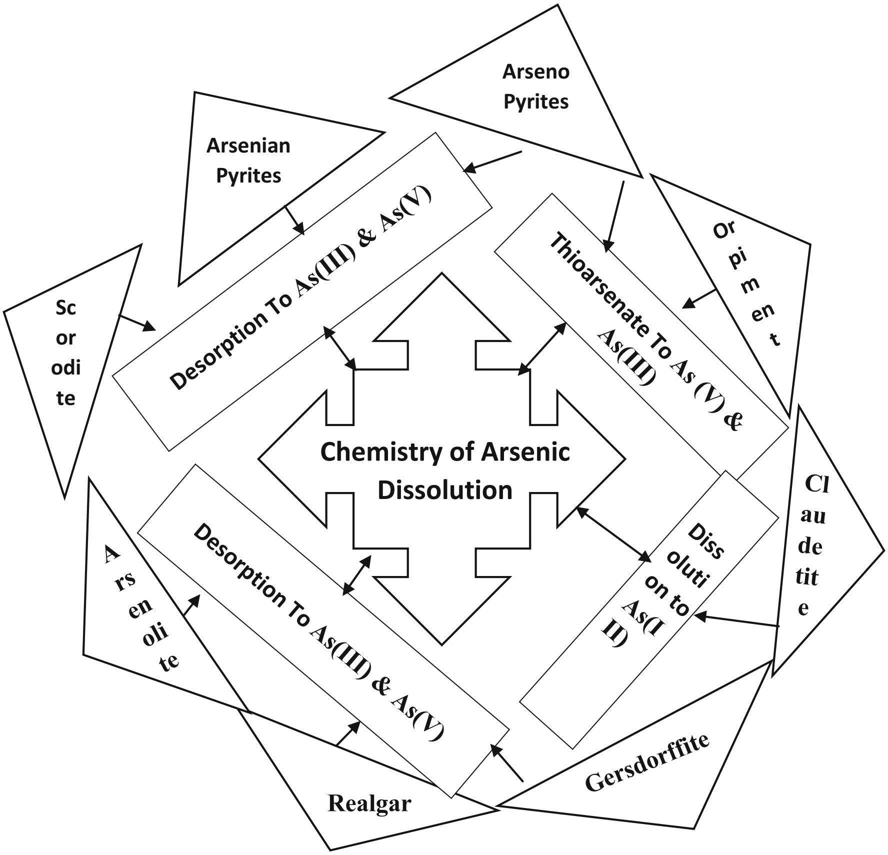

Fig. 14.1 Demonstration of arsenic dissolution at various oxygen and pH conditions from As minerals

high. Higher concentrations of arsenic have been reported in rice plants (boro rice in Bangladesh) in the following orders: rice roots > rice stem > rice leaf > rice grain > rice husk (Chakma et al. 2012; Haq et al. 2012; Rai et al. 2010). Arsenic toxicity disturbs the physiological actions of plants by damaging cellular membranes of plants which ultimately cause leakage of plant electrolytes (Singh et al. 2006).

Chemical species of organic arsenic are translocated by specific aquaporin canals comprising on nodulin 26-like intrinsic (NIP) and by the phosphate transporters, and arsenite and organic As species through the nodulin 26-like intrinsic (NIP) aquaporin channels (Zhao et al. 2010). After entrance in cytoplasm, As species strives with phosphate which produced ADP arsenate by substituting a phosphate group of ATP and disturb the energy flows in cells (Meharg and Hartley-Whitaker 2002).

14.5 Rice Growth and Physiology Under As Stress

Due to irrigation of As-contaminated water to rice paddies, it accumulated in the topsoil in the form of inorganic As(V), arsenite As(III), the organic As(V), dimethyl arsinic acid (DMA), and monomethylarsonic acid (MMA) and becomes available to the next cultivated varieties of rice (Huq et al. 2006, 2008, 2011; Meharg and Rahman 2003; Williams et al. 2005). All forms of As are highly toxic, which affects the yield of rice grains resulting in straight head condition due to improper grains filling in the panicles (Yan et al. 2014). Mechanism of As detoxification in plant cell occurs by the formation of complexes with PCs, which help in the translocation of metal inside vacuole and finally its reduction from a high toxic form, i.e., As(V), to less toxic form As(III) (Rai et al. 2010). It has also been studied that in some plants, PvACR3 accumulates AsIII in the vacuole after sequestration (Indriolo et al. 2010). As toxicity disturbs the metabolism of plants which inhibits not only plants growth but also reduces the biomass, fertility, and yield (Garg and Singla 2011). Recently it has been explored that rice plants uptake AsV which rapidly reduce to AsIII by specific arsenate reductases, namely, HAC1 (High Arsenic Content 1) (Shi et al. 2016). Phytotoxicity due to As contamination of soil and water has shown the following symptoms in rice like stunt growth, reduction in roots elongation, necrosis, and decrease in size of photosynthetic pigments, which hinder the germination of seed which ultimately reduce the fruit and grains yield (Zhao et al. 2009).

14.6 Transcriptomic Study of Rice Under As Stress

Advancement in the field of sequencing technology, genomic exploration, and transcriptomic studies become helpful in understanding the effects of stress conditions in eukaryotes. In this regard the use of RNA-Seq technology is supporting the transcriptional reporting and various genes expression against stress (Wang et al. 2009). Transcriptomics has widely been utilized for the exploration of plant responsive genes under various biotic and abiotic stresses (Zeng et al. 2014; Yamamoto et al. 2015; Shaheen et al. 2017; Chaires et al. 2017). Isayenkov and Maathuis (2008) described that the AtNIP7;1 protein may contribute in the transportation of As in *A. thaliana*. It was highlighted that the As toxicity boosts a number of genes in rice related with metal transportation, metal-binding proteins, and antioxidant responding (Rai et al. 2010).

The entrance of arsenic species into the rice roots is possible by silicon pathway which has been elaborated by the identification of silicon transporter genes OsNIP2;1 or Lsi1 in rice plant (Ma et al. 2008). It has been studied that the silicon transporter Lsi1 in rice (*Oryza sativa*) also uptakes the methylated As species, i.e., monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA) from paddy soil (Li et al. 2009). Previously the transcriptomic study of *Arabidopsis* plant showed the AsIII accumulation and translocation by the expression of heavy metals stress-responsive genes NRAMP (natural resistance-associated macrophage protein) transporter. The expression of the same protein OsNRAMP1 in rice shows that these genes may involve for the translocation and accumulation AsIII in rice. So in rice OsNRAMP1 genes may confine the epidermis and pericycle which cause the uptake of AsIII into root xylem to shoot xylem (Tiwari et al. 2014).

Other arsenate As(V) transporters in plants roots are phosphate transportation (Pi) pathways (Zhao et al. 2009). A research was conducted on comparative analysis of rice plants with phosphate (Pi) transporter OsPT8 with a rice mutant defective phosphate (Pi) transporter OsPHF1 and observed that rice having OsPT8 had higher capacities of Pi and arsenate uptake and translocation than rice with OsPHF1 (Wu et al. 2011). The As-resistant varieties of rice may be developed for the overexpression of OsABCC1 because its overexpression in wild and mutant varieties was differential upon exposures of various As concentrations. In mutant rice OsABCC1 genes were expressed equally even against low concentrations of As due to the biosynthesis of thiol complexes in the epidermis and pericycle of plant, while these genes were not expressed at low As concentration in wild type of rice (Song et al. 2014).

14.7 Remedial Measures

Arsenic remediation options in suffering countries could be possible by taking following check and balances:

1. In As-contaminated sites, the wells and tube wells should be dig deeper not shallow.
2. Rain water should be harvested.
3. Phytoremediation by growing As hyperaccumulating plants species (duckweed) can also improve the conditions of polluted soil and water bodies (Ng et al. 2017).
4. As filters should be available to community at low cost.
5. Safe water supply should be made possible.
6. As tolerant and hyper tolerant varieties of rice should only be referred for contaminated areas.
7. Removal of As from contaminated water by using iron-coated sand is a very useful technique (Chang et al. 2012).
8. Treatment of As-contaminated water with the exposure of gaseous chlorine, permanganate, hydrogen peroxide, Fenton’s reagent, and ultraviolet (UV) radiation is a useful technique for purification (Litter et al. 2010).
9. Awareness programs should also be launched for education on As pollution.
10. Fertilization practices can also be helpful as As mitigation strategy (Barbafieri et al. 2017).

11. Bioavailability of As in the soil can also be helpful for its phytoremediation of paddy soil by the addition of phosphate-containing fertilizers (Lewinska and Karczewska 2013; Niazi et al. 2017).
12. Bioadsorption is also a useful technology for the adsorption of As(III) and As (V) by a biomass or biofilm of living or dead organisms such as algae, bacteria, macrophytes or microphytes, and biopolymers (Dickinson et al. 2009).
13. Adaptation of proper irrigation system can control the As contamination in rice. A research work clearly demonstrated the impact of sprinkler irrigation over flood irrigation, and the results have shown that total concentration of As in rice kernels under sprinkler irrigation was 50 times less than the constant flooding irrigation (Spanu et al. 2012).
14. Biochar addition has also been studied as a best remediation for As release from contaminated sites (Li et al. 2016; Choppala et al. 2016; Yin et al. 2016).

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