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Review

Arsenic Accumulation in Rice and Probable Mitigation Approaches: A Review

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Abstract: According to recent reports, millions of people across the globe are suffering from arsenic (As) toxicity. Arsenic is present in different oxidative states in the environment and enters in the food chain through soil and water. In the agricultural field, irrigation with arsenic contaminated water, that is, having a higher level of arsenic contamination on the top soil, which may affects the quality of crop production. The major crop like rice (*Oryza sativa* L.) requires a considerable amount of water to complete its lifecycle. Rice plants potentially accumulate arsenic, particularly inorganic arsenic (iAs) from the field, in different body parts including grains. Different transporters have been reported in assisting the accumulation of arsenic in plant cells; for example, arsenate (As^{V}) is absorbed with the help of phosphate transporters, and arsenite (As^{III}) through nodulin 26-like intrinsic protein (NIP) by the silicon transport pathway and plasma membrane intrinsic protein aquaporins. Researchers and practitioners are trying their level best to mitigate the problem of As contamination in rice. However, the solution strategies vary considerably with various factors, such as cultural practices, soil, water, and environmental/economic conditions, etc. The contemporary work on rice to explain arsenic uptake, transport, and metabolism processes at rhizosphere, may help to formulate better plans. Common agronomical practices like rain water harvesting for crop irrigation, use of natural components that help in arsenic methylation, and biotechnological approaches may explore how to reduce arsenic uptake by food crops. This review will encompass the research advances and practical agronomic strategies on arsenic contamination in rice crop.

Keywords: arsenic; rice; uptake; transporters; crop

1. Introduction

Arsenic (As) is a toxic metalloid that is ubiquitous in the environment. It has raised serious concern from both environmental and human health perspectives. Elevated level of As in soil accrued from both geogenic and anthropogenic activities, which include, metal mining and smelting, use of arsenic-containing pesticides, herbicides, wood preservatives, food additives etc., and irrigation with arsenic-contaminated water. United States (US) Environmental Protection Agency (EPA) classified arsenic as a potent human carcinogen and a leading cause of serious health problems, including cancers of the skin, lung, bladder, liver, and kidney, as well as adverse effects on cardiovascular, neurological, hematological, renal, and respiratory systems [1–3]. Arsenic contaminated groundwater used for drinking purpose is likely the major pathway of human exposure [4]. However, food crops, specifically rice, serve as a major source of arsenic being the dietary staple of half of the world's population [5–7]. In Asian countries like Bangladesh, India, China, Korea, Taiwan, and Thailand, arsenic intake from rice diet is significantly higher, as rice plants have a special ability to take up arsenic from the soil and water used for irrigation [8–13]. The transfer of arsenic from soil to plant systems is a serious issue that leads to considerable human exposure [14].

Arsenic exists in the environment in several inorganic and organic forms. In paddy fields, arsenite (As^{III}) is the dominant arsenic species, comprising 63% of total arsenic in soil, followed by arsenate (As^{V}) at 36%, and methylated arsenic species [15]. All of these arsenic species enter to the plant cell through specific transporter proteins. Being a phosphate analog, arsenate interferes with phosphate metabolism in plants, while the binding of arsenite to sulfhydryl groups of proteins affects their structures and/or catalytic functions [12]. Phytotoxic effect of arsenic in plants is evidenced by physiological changes like reduced root extension, chlorosis in leaves, shrinking, and necrosis in aerial plant parts, etc. [16]. Following root to shoot translocation, arsenic can severely impede plants' growth by arresting biomass accumulation, reducing reproductive capacity through impaired fertility, yield, and fruit production [17]. Toxicity symptoms of rice plants grown in soils (containing >60 mg kg^{-1} total arsenic) include stunted growth, brown spots, and scorching on leaves [10]. A study of Duxbury and Panaullah [18] showed that an increase in arsenic concentration in soil from 12 to 60 mg kg^{-1} in conventional paddy fields of Bangladesh resulted in decreased rice yields from 7.5 to 2.5 t ha^{-1}. At a higher concentration, arsenic interferes with various metabolic processes, adversely affects the plant metabolism, and consequences in death. Plant transpiration intensity was found to be reduced after arsenic exposure [19]. Being a redox active metalloid, both the inorganic species of arsenic are known to induce the production of reactive oxygen species (ROS) beyond control level and create oxidative stress in plants [20]. To subsist with arsenic induced oxidative stress, plants have evolved ROS-scavenging enzymatic and non-enzymatic antioxidants [21]. Rice is the most severely affected staple food crop to arsenic contamination as compared with other crops like wheat, maize, and barley due to its cultivation in flooded conditions as compared to non-flooded for wheat. The predominance of arsenite over arsenate is the result of reducing conditions in soils due to water submergence that affect the growing plants [10]. Roots are the major part to get exposure and accumulation of arsenic that may affect its elongation and proliferation. Usually, due to translocation in plants, the accumulation of arsenic decreases from root to above ground parts. As for example, in the Guandu wetland of Taiwan, arsenic concentration in plant parts of *Kandelia obovata* was decreased from the roots (19.74 mg kg^{-1}) to the stems (1.76 mg kg^{-1}), leaves (1.71 mg kg^{-1}), and seedlings (0.48 mg kg^{-1}) [22,23]. A similar observation was also found in the Ratna variety of *Oryza sativa* (Aman rice), where, bioaccumulation of arsenic was found in decreasing order: root > basal stem > median stem > apical stem > leaves > grains [24,25]. As reported in various species, about 40% of the total translocated arsenic is in the form of As^{V} [26,27]. In plants like rice, rhizosphere aeration through downward supply of oxygen from leaves to the roots and bacterial community also help in oxidation at the roots level [28,29].

Rice cultivation requires a huge amount of water, as most of the cultivable varieties are sensitive to water shortage. Flooded soil is also having characteristics like, reduced build-up of pathogens, nematodes and weeds, and an increased amount of nutrient availability [30]. Under flooding or anaerobic conditions in paddy soils, reductive mobilization of arsenic greatly enhances the bioavailability of arsenic leading to excessive accumulation of this metalloid in rice grain and plant [31]. In greenhouse experiments where soil is maintained under aerobic conditions, a significant decrease in arsenic concentration was found in rice grain and straw by 10–20, and 7–63 fold, respectively, when compared with anaerobic rice [32,33]. The pot study by Arao et al. [34], further supports the notion that aerobic treatment can effectively reduce the grain arsenic. New cultivation methods, such as aerobic rice, alternate wetting and drying, and raised bed cultivation may prove to be highly effective in reducing accumulation of arsenic in rice, not only because these water-saving methods are likely to maintain soil under more oxic conditions and hence less arsenic mobilization, and less input of arsenic into the paddy field from irrigation of arsenic contaminated groundwater where elevated arsenic in groundwater intensifies grain arsenic levels [31].

As rice is consumed all over the world, arsenic in rice has become a global concern [11,35]. Extensive research in plant physiology has uncovered the response of plants in arsenic-stress. Alteration of plant growth conditions through water management practices and supplementation of other mineral nutrient such as phosphorus (P), silicon (Si), iron (Fe), and sulfur (S) in soil, to reduce the solubility and availability to plants by changing the exchangeable fraction of arsenic [10,36]. In this review article, an attempt has been made to summarize the data related to arsenic bioavailability to rice from soil, its uptake, accumulation, and oxidative stress in rice and possible cost effective agronomic strategies to reduce arsenic contamination in rice.

2. Arsenic Uptake, Translocation and Accumulation in Rice Plant

2.1. Uptake and Transport of Inorganic Arsenic Species

The uptake of inorganic species of arsenic by rice roots occurs by two mechanisms. The transport of As^V from soil solution to aerial parts of the plants occurs through high affinity phosphate transporter (PT) [37,38]. The phosphate transporter gene family of rice includes 13 OsPT genes encoding the transporters range from OsPT1to OsPT13 [39]. Among them, the roles of OsPT1 and OSPT8 in arsenic transport have been investigated in details by Kamiya et al. [40] and Wang et al. [41], respectively. OsPT1 mediates arsenate transport from root to shoot [40]. Whereas, OsPT8 is a key transporter protein for arsenate uptake into rice roots, and a profound toxic effect on root elongation was exerted after arsenate uptake mediated by OsPT8 [41]. In addition, overexpression of OsPT8 resulted in an enhanced arsenic accumulation in plants [41]. The second route by which As^{III} is taken-up by root cell is aquaporin channels [32,42]. In root cells of rice, As^{III} enters through Lsi1, a nodulin 26 like intrinsic protein (OsNIP2;1), a major influx transporter for silicic acid [43,44], while another protein Lsi 2, a silicon efflux transporter mediate As^{III} efflux to the xylem in rice plant [45]. In the rice cultivars Oochikara, T-65, and Koshihikari As^{III} is transported in the form of arsenous acid $As(OH)_3$ through Lsi1 and Lsi2 transporters [42,46]. In the rice mutant for Lsi2, a significantly decreased rate of As^{III} transport to xylem and accumulation in shoots and grain were found [42]. After As^{III} is taken up by the root cells, some of it is instantly released into the rhizosphere by the bidirectional function of Lsi1protein channel [47]. Inside plant tissues, As^V is reduced to As^{III}; As^{III} is sequestered into root vacuoles or is translocated to the shoots and it is disseminated to various organs [46,47]. Rice is unable to methylate inorganic arsenic species, thus methylated species of arsenic most likely come from the rhizosphere via microbial methylation [48].

2.2. Uptake and Transport of Organic Arsenic Species

Arsenite is the predominant species in the submerged soil and microbial transformation of inorganic species to organic form produces considerable quantities of methylated arsenic species dimethylarsinic acid (DMA) and smaller amounts of monomethylarsonic acid (MMA) in the paddy soil [11]. This transformation to organic form is beneficial because methylated arsenic species are less toxic than the pentavalent arsenic species. The uptake mechanisms of methylated species are less extensively studied than inorganic arsenic species. MMA and DMA are taken up through the nodulin 26-like intrinsic protein. Inorganic arsenic species (As^{III} and As^V) are more efficiently taken up by roots than methylated arsenic species (DMA and MMA), but the translocation rate in plant shoot of inorganic arsenic species is much lower than methylated arsenic species [49]. The reduced complex formation of methylated arsenic-species with the ligands (glutathione/phytochelatin) may be the reason for the better translocation of methylated-arsenic species [49]. As^{III} was found to be the most abundant species in the rice grain, followed by DMA with low concentrations of As^V, MMA, and other two unidentified arsenic species, as suggested by analysis of 121 samples of 12 rice types [50]. On the other hand, in rice straw, As^V is a predominant species followed by As^{III} and DMA [15].

2.3. Accumulation of Arsenic in Rice Grain

Rice is one of the most efficient transporters of Si among all crop plants and inadvertently passes arsenite through silicic acid transporter [51]. This factor contributes to higher concentrations of arsenic in rice grain, greater than the recommended safe limit [12,52,53].

However, Yamaji and Ma [54] observed that transportation of arsenic in rice through Lsi1 and Lsi2 are restricted in the root, and xylem loading of arsenic mainly contributes to the accumulation in vegetative parts but not in the grains. More than 90% of As^{III} uploading into the grain is contributed from phloem transport [55].

Reduction of arsenate may be the first step of arsenic detoxification in rice plants. Researchers have identified different arsenate reductases in rice, like, OsHAC1;1, OsHAC1;2, [56], and OsHAC4 [57], which regulate the conversion of arsenate to arsenite. These genes are expressed mainly in the roots, and catalyse the reaction in the outer cell layer of root, thereby, facilitating arsenite efflux from the root to soil. OsHAC1;1 is abundant in epidermis, root hair, and pericycle, while OsHAC1;2 being predominant in epidermis, outer cortex layer, and endodermis [56]. OsHAC4 is localized mainly in root elongation and mutation zone in epidermis and exodermis [57]. Overexpression of OsHAC1;1 and OsHAC1;2 significantly increased arsenite efflux into external medium and decreased arsenic accumulation in rice [56]. On the other hand, mutation of OsHAC1;1, OsHAC1;2, and OsHAC4 led to decrease arsenate reduction in root, lessen arsenite efflux, and increase arsenic accumulation in root and grain [56,57].

Besides arsenate reduction, phytochelatin-arsenite complexation and subsequent sequestration into the vacuoles constitute another important pathway of arsenic detoxification in plants [58]. Being the precursor compound of phytochelatin (PC) synthesis, glutathione (GSH) plays a crucial role in arsenic tolerance. GSH is synthesized by ATP dependent reaction from Gly and ɣ-glutamylcysteine (ɣ-EC). The intermediate ɣ-ECs are synthesized within plastid and are exported from plastid to cytosol by CRT like transporter protein (CLT) in plants [59]. Similar transporter OsCLT1 in rice plays a role in GSH homeostasis by mediating transport of ɣ-EC and GSH form plastid to cytoplasm. Under arsenic treatment OsCLT1 mutant rice plants exhibit lower concentration of PC content when compared to wild type plants, resulting in lower arsenic accumulation in roots but higher arsenic accumulation in shoots [60].

The presence of a tonoplast transporter in phloem companion cells (OsABCC1) increase the arsenic sequestration in vacuoles, which help in reduced arsenic translocation into rice grains [58]. However, methylated arsenic species, especially DMA is mobilized at a higher rate than inorganic species [55,61] and its redemption in aleurone, endosperm, and embryo may reduce the seed setting rate and induce spikelet sterility and a reduced yield [62]. Arsenic accumulation in rice grain varies according to the genotype of the plant [51]. The genotypes TD71 and Yinjingruanzhan contain less inorganic arsenic in their grains than genotypes IAPAR9 and Nanyangzhan [63]. More than 1700 rice varieties are investigated around the world for their differences in arsenic accumulation, and about 20 fold variations was found among various strains of rice [64]. Arsenic concentration in rice grains vary to 6 and 7 folds in different countries, while this concentration varies up to 40-folds in rice varieties within same country. The findings help in hypothesizing that both the genotypes and environmental factors play very important roles to control arsenic accumulation in rice grains [65]. The major factors that influence the arsenic accumulation in rice grain include the type of the rice cultivar, plant physiology, the place where the plant was cultivated, and the method of processing of rice [10]. It was found that concentration of arsenic in brown rice was higher than white rice [10].

3. Factors Influencing Arsenic Mobilization and Intake in Rice Plant

In soil, metal-metal interaction and their dynamic equilibrium between various chemical forms are governed by different factors, like metal-soil particle affinity, and the physical, chemical, and biological properties of soil [66,67]. The microclimate present in root rhizosphere, i.e., the association of microbes with root and root exudates, also contributes to concentration of metal ions from soil [68]. The factors controlling the mobilization and uptake of arsenic are discussed below:

3.1. Arsenic Speciation

Both inorganic and organic forms (species) of arsenic are present in the soil. The most common inorganic species are arsenate (As^{V}) and arsenite (As^{III}), while the most common organic species are monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The toxicity of arsenic species is in the following order $As^{III} > As^{V} >$ MMA > DMA [69]. Usually, arsenite predominate in reducing environment (anaerobic soil), such as submerged paddy soil. However, arsenite can be transformed into organic forms by methylation promoted by microbial actions in paddy soil [70]. Marin et al. [71] found that the bioavailability of arsenic to rice plants follows the order $As^{III} >$ MMA $> As^{V} >$ DMA; all of the species are taken up by rice roots, but the rate of organic species uptake is much lower than that of inorganic arsenic [15].

3.2. Effect of Redox Condition and Soil Texture

Arsenic speciation and mobility in soil that cause the accumulation of the metal in paddy is predominantly controlled by redox chemistry [72]. While, in aerobic soil (oxidized conditions) arsenic prevails as arsenate (As^{V}) and is adsorbed on Fe-oxyhydroxide phases restricting the arsenic availability to plants [73]. However, in reducing environment like submerged paddy field, arsenite (As^{III}) is predominant, and more available for plant uptake due to the dissolution of Fe-oxides [74] as well as due to the reduction of As^{V} to As^{III} through microbial processes [70]. Some microbial community residents of rhizosphere in rice plants can increase the bioavailability of arsenic by solubilizing ferric ion in the rhizosphere by exuding siderophores to the root–plaque interface [75,76], and as a result accumulated arsenic concentration can reach up to 160 mg kg^{-1} in the root of rice plants [77].

Soil texture is another important factor affecting arsenic solubility in soil and the bioavailability to rice plant [78]. Silt and clayey soil have finer texture, much more surface area than sandy soils and in addition, have a higher arsenic scavenging potential because of the presence of Fe oxides [79]. Therefore, plants grow in clayey soils show less toxic effects of arsenic, in contrary; phytotoxicity of arsenic is five times more in sandy and loamy soils [79].

3.3. Effect of Soil pH

Arsenic speciation and leaching depends on soil pH, and therefore, the solubility and bioavailability of arsenic is directly affected by soil pH [79,80]. Arsenic uptake and accumulation by rice plants are influenced at both lower and higher pH. It may be due to the fact that at very low pH ($pH < 5$) arsenic-binding species, such as Fe-oxyhydroxide compounds, becoming more soluble [81] and enhancing the arsenic uptake by plants. A negative relationship between arsenic concentration in rice and soil pH was also supported by Bhattacharya et al. [82]. On the other hand, a positive relationship between arsenic accumulation and soil pH is also supported by many authors [83,84]. Higher soil pH (usually pH 8.5) increases the negative surface charges, such as hydroxyl ions, facilitating desorption of arsenic from Fe-oxides leading to the mobilization of arsenic in the root vicinity, which, in turn, enhances arsenic accumulation in the plant [84].

3.4. Effect of Organic Matter

The mobility of arsenic is regulated by the soil organic matter (OM), and its chemical nature and complexes (soluble or insoluble) [85]. Pikaray et al. [86] reported that arsenic solubility become reduced in soils having high amount of OM, which in turn affect its availability to plants, as organic matter has a greater affinity for arsenic sorption due to the formation of an organo-arsenic complex. Similar findings of reduced arsenic content in the grains were reported from other studies, where, rice plants were grown in soil having higher OM content [87,88].

However, on the contrary, a positive correlation between soil OM and arsenic accumulation in rice grain is also reported by different researchers. An increase in the organic matter in soil can enhance the mobility of arsenic from solid phase through increasing the microbial activity and decreasing the soil redox potential [89], a condition favourable for the reductive dissolution of Fe-oxyhydroxides linked to OM [90].

3.5. Genotype Variation in Rice

It is evident that arsenic accumulation in rice grains differs with the rice varieties [51]. The highest accumulation was found in the BR11 variety (1.77 mg kg^{-1}) than in the others [91]. Among the various rice varieties, IR 50, White Minikit, and Red Minikit were efficient accumulators of arsenic (0.24–0.31 mg kg^{-1}) as compared with Nayanmani, Jaya, Ratna, Ganga-kaveri, and Lal Sanna (0.14–0.20 mg kg^{-1}); maximum accumulation was found in White Minikit (0.31 mg kg^{-1}), and the minimum was in Jaya (0.14 mg kg^{-1}) [92]. The largest arsenic concentrations in root and vegetative parts were found in cultivar 'TN1' and 'ZYQ8', while cultivar 'JX-17' had the lowest arsenic concentration. Arsenic concentration in shoot or root of 'JX-17' was about 50% of that in cultivar 'ZYQ8' [93].

This variation may be influenced by environmental conditions, genetic differences, and the presence of a different level of arsenic in the irrigation water and soils [93,94]. Norton et al. [94] conducted a field-based experiment in Bangladesh with 76 rice cultivars and in multiple environments at two field sites each in Bangladesh, India, and China [95]; 4–5 fold variations were observed in the grain arsenic concentration among cultivars. The difference in arsenic accumulation in grains may be due to differences in root anatomy, which controls root aeration, porosity [38], Fe-plaque formation on the root surfaces [96], Phytochelatins (PCs) [97], rhizosphere interactions, and differences in the arsenic tolerance gene [98].

4. Arsenic Induced Oxidative Stress and Response in Rice Plant

A number of reports exist on oxidative stress and defence mechanisms in plants under arsenic stress [99–102]. Arsenic generates reactive oxygen species (ROS) during the reduction of As^{V} to As^{III}, which is followed by methylation, a redox driven reaction that may give rise to ROS [103]. Methylated form of arsenic such as monomethylarsonic acid (MMA), dimethylarsinicacid (DMA), and tetramethyalarsoniumion and trimethylarsonium oxide (TMAO), reacts with molecular oxygen and gives rise to ROS within the cellular environment [104]. Dimethylarsinic acid (DMA) causes iron-dependent oxidative stress, which is based on iron released from ferritin and leads to the damage of DNA [105]. Production of ROS in plants after exposure to inorganic arsenic species is well documented, which includes superoxide ($O_2^{\bullet-}$), the hydroxyl radical (•OH), and H_2O_2 [106,107]. ROS can damage proteins, amino acids, purine nucleotides, and nucleic acids, and cause the peroxidation of membrane lipids [108]. Arsenic toxicity at the cellular level impels electrolyte leakage due to membrane damages and is often accompanied by oxidative stress due to increased production of malondialdehyde, a by-product of lipid peroxidation [109]. Arsenic induced lipid peroxidation in several arsenic hyperaccumulator plants [109,110] indicates that ROS production is common and that the magnitude of the redox imbalance in the cell may be an important determinant of ROS-induced toxicity [109].

In plants, oxidative stress is combated by increasing the production of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, or peroxidase [111], and different compounds, including ascorbate, the γ-Glu-Cys-Gly tripeptide glutathione (GSH), GSH oligomer (γ-Glu-Cys]n-Gly) phytochelatin (PC) throughout the plant, but particularly in the roots [112–115] and accumulation of anthocyanin in leaves [116]. Several intrinsic mechanisms have evolved in plants for counteracting arsenic toxicity in plants, including phytochelatin (PC) dependent detoxification [117]. Arsenic tolerance in rice is achieved by the synthesis of a higher level of PCs due to increased PC-synthase activity along with coordinated thiol metabolism [20]. Furthermore, complexation of

PC-arsenite in rice leaves reduces the translocation of arsenic from leaves to grains [97]. In addition, metallothioneins (MTs) also have a potential role in arsenic detoxification in rice, as reported by several authors [118]. Iron (Fe) is an essential element in plants required for various metabolic activities like, photosynthesis, respiration, DNA synthesis, and co-factors for several enzymes. Additionally, Fe helps to reduce arsenic accumulation in rice plant. In soil, it is mainly present in insoluble oxidized (Fe^{III}) form and in a flooded rice field Fe^{III} is converted to ferrous (Fe^{II}) form, quickly released from the soil and sequester arsenic [74]. Apart from preventive role in arsenic accumulation and speciation, Fe also has an ameliorative function in modulating oxidative stress responses during arsenic toxicity, as reported by Nath et al. [119]. Their study reported that As^{V} inhibits growth in the treated plants, while Fe supplementation resulted in an improved growth response and low As^{V} accumulation in the exposed plant. Further, increased levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) generated due to As^{V} exposure for 24 and 48 h duration, were significantly reduced in Fe-supplemented plants in comparison to As^{V} alone.

5. Concentration of Arsenic Species in Rice Grain

The toxicity of arsenic depends not only on total concentrations, but also its chemical forms because there is a large difference of toxicity among inorganic and organic arsenic species. Accurate arsenic speciation is therefore essential to evaluate the impact of arsenic toxicity in rice on human health. Rice from different parts of the globe varies greatly in arsenic concentration and speciation. The first elaborative and comparative study of market rice was made by Williams et al. [120]. Quantification and qualification of low level of arsenic required to survey arsenic speciation in rice grain became possible due to the development of inductively-coupled plasma mass spectrometry (ICP-MS) as an ultra-sensitive arsenic detector, combined with High Performance Liquid Chromatography (HPLC) [120]. HPLC-ICPMS, an internationally validated method, requires an intermediate heating step with diluted nitric acid to extract arsenic species.

The report of Williams et al. [120] revealed that rice produced in the US and European Union (EU) having a high percentage of DMA when compared to Bangladeshi and Indian rice. Chinese rice was dominated by inorganic arsenic as opposed to DMA [11]. According to the report of Meharg et al. [11], rice of Ghana had the lowest median arsenic concentration (20 ng g^{-1}), followed by India (50 ng g^{-1}), while, the USA, Italy, and Thailand had the highest arsenic concentration, with China and Bangladesh being intermediate. An extensive survey of US rice along with a smaller numbers of samples for Spain, Italy, India, Thailand, Pakistan, and Venezuelan produced rice was published by Zavala and Duxbury [121] revealed similar results with the report of Meharg et al. [11]. Another finding of Zavala and Duxbury, [121] related to color of the rice showed that arsenic concentration in brown rice is 0.196 ± 0.111 mg kg^{-1}, in white rice is 0.127 ± 0.087 mg kg^{-1}, and 0.07 ± 0.05 mg kg^{-1} for other colors. The highest amount of arsenic is in brown rice because its outer layers have a higher content of the metalloid [122].

6. Risk of Arsenic from Rice Diet to Human Health

Rice is the primary route of arsenic exposure in many countries being the staple food and has adverse health outcomes [14,123,124]. A substantial relationship of rice intake with both urinary arsenic and prevalent skin lesions has been revealed from a study on 18,470 persons of Bangladesh [125]. A similar report from USA showed a positive correlation between rice consumption and the concentration of urinary arsenic [35]. In a study, Banerjee and co-authors [5] showed that daily consumption of 500 g cooked rice containing arsenic above 200 mg kg^{-1} can trigger genotoxicity in humans.

Rahman et al. [124] reported that certain varieties of protein-rich rice promote arsenic bio-accessibility, as thiol groups are strongly bound to As^{III}. Consumption of vegetable rich diets, on the other hand, reduces the chance of developing arsenic induced skin lesions due to differences in the rate of arsenic metabolism [126]. However, gut microbial population also plays an important role in the arsenic speciation and absorption into the blood [63]. Arsenic undergoes a series of biotransformation in the gastrointestinal tract, including oxidation, reduction, methylation, and thiolation [127]. The acidic pH in the stomach increases arsenic bio-accessibility in comparison to the intestine [127]. During gastrointestinal digestion, As^V is released more easily than DMA^V from the rice matrix. Inorganic arsenic species are more likely to bind to the thiol containing amino acid in the endosperm cells of rice seed [127]. Different studies support the view that real exposure to arsenic through foods depends on the method of food processing, temperature, duration, and medium of cooking. Well-cooked rice with profuse water reduces the arsenic concentration in rice [124].

The processes of bioavailability, root uptake, rhizosphere, transport, accumulation, and grain unloading of arsenic are imperative aspects of research to alleviate arsenic in rice [42,128,129].

7. Agronomic Strategies for Mitigating Arsenic Accumulation in Rice

Several agronomic methods may be adopted as strategies to decrease the effects of arsenic accumulation in rice, which include, aeration of soil by water management and preventing the reduction of arsenic; creating the condition that favors the formation and precipitation of insoluble arsenic in soil; and, decreasing arsenic uptake and translocation in rice plants by augmenting mineral nutrients in soil that competes with arsenic uptake. There are effective remedies present that may help to decrease the risk associated with arsenic in plants [10].

1. Fertilization of soil with minerals
2. Water management and irrigation practices
3. Bioremediation strategy

7.1. Fertilization of Soil with Minerals

Supplementation of soil with specific mineral nutrients like Fe, S, P, and Si can significantly decrease the arsenic accumulation in edible plant parts by minimizing its uptake and translocation in food crops [10].

7.1.1. Role of Fe

Iron (Fe) is an essential plant mineral nutrient and plays an important role in decreasing the absorption of arsenic in rice [96,119]. The exogenous application of Fe leads to the deposition of Fe-oxide or formation of Fe-plaque around roots of rice plants and decreases arsenic uptake, increase co-precipitation of Fe and arsenic and decreased availability of soluble As^V to rice plant due to an adsorption of As^V on Fe surface [10].

Rice cultivation in anaerobic conditions promotes the Fe-plaque formation around the rice roots, which consists of ferrihydrate (63%), goethite (32%), and siderite (5%) [96]. Fe-plaque plays an important role in reducing arsenic uptake in rice because it has high affinity towards As^V and is able to sequester the arsenic, which ultimately leads to decrease the translocation of arsenic from roots to shoots [96]; concentration of Fe oxides also increases in the rhizosphere that consequently decrease arsenic uptake in rice plants [130,131].

Application of metallic Fe and Fe-oxide in rice field has found to significantly reduce arsenic concentration in rice grains by 51 and 47%, respectively [132]. The application of steel slag (rich in Fe and silicate) is a common in rice production systems in Southeast Asia. Arsenic uptake by rice plants mainly depends on the arsenic bioavailability rather than the total arsenic concentrations in soil [133] and binding of arsenic with Fe-(hydr)oxides in the soil reduce the arsenic mobility in soil solution [134] (Figure 1). Fe oxide can act as a sink for arsenic, therefore, increasing Fe oxide in soil leading to decrease in uptake and accumulation of arsenic in rice [135]. Fe supplementation helps to reduce arsenic induced oxidative stress in rice plants, as reported from the study of Nath et al. [119].

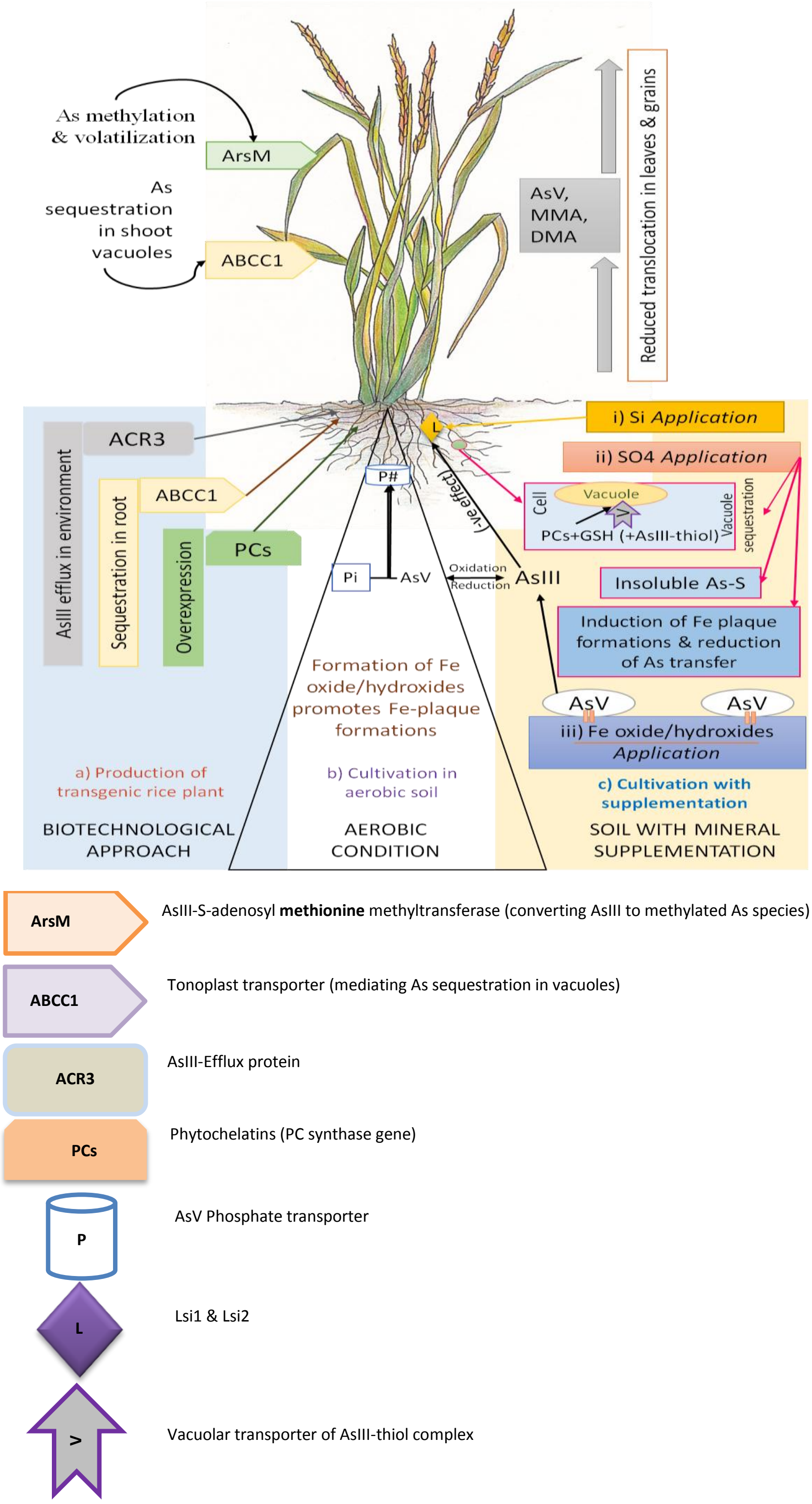

Figure 1. Schematic diagram representing the agronomic strategies and biotechnological approaches for mitigating arsenic accumulation in rice. (**a**) Production of transgenic plants to yield low arsenic-rice is the biotechnological approach, where overexpression of one or more gene(s) in plants, like, Overexpression phytochelatin synthase (PCS) gene (higher phytochelatin production in roots leads to increase arsenic-thiol complexation and decreased arsenic translocation to shoot), ABCC1 transporter protein (in roots and shoot leads to vacuolar sequestration of arsenic within roots and nodes), ACR3 gene in root cell (enhances AsIII efflux), ArsM gene (leads to arsenic methylation and volatilization) are

the basis of the technology. Cultivation practices in aerobic (**b**) soil is another options, where, Fe plaque formation around root surface and increased affinity of arsenic for soil minerals reduce arsenic mobility and bioavailability; further, oxidative condition inhibits reduction of AsV to AsIII (having higher solubility, plant availability, and toxicity). Soil supplementation with minerals (**c**) can reduce the arsenic contaminations—silicon (Si) application subdues expression of Lsi1 and Lsi2 transporters and competes with arsenic for the same transporter (L) during uptake and thus impacted negatively on arsenic uptake; SO_4 application has couple of benefits: induction to formation of Fe plaques, enhancement of the synthesis of thiol ligands (GSH/PC), and increased arsenic-thiol complexation and production of insoluble arsenic-S complex due to strong affinity to arsenic under reducing environment. Fe oxide/hydroxide application in flooded paddy fields leads to reduce arsenic bioavailability to plants due to adsorption of AsV on Fe-oxide surface. Figure modified from [2,10,135–139]. MMA: Monomethylarsenic acid; DMA: dimethylarsenic acid. Legends: ArSM:AsIII-S-adenosyl methyl transferase (converting AsIII to methylated As species); ABCC1: Tonoplast transporter (mediating As sequestration in vacuoles); ACR3: AsIII-Efflux protein; PCs: Phytochelatins (PC synthase gene); P: AsV Phosphate transporter; L: Lsi 1 & LSi2; V: Vacuolar transporter of AsIII-thiol coplex; MMA: Monomethylarsonic acid; DMA: dimethylarsinic acid).

7.1.2. Role of Phosphorus

Phosphate (P) is an important parameter in the paddy field for arsenic solubility in soil and its uptake by plants as it competes with arsenate (As^V) at the same sorption sites in soils or Fe-plaque via ligand exchange mechanisms [140]. Numerous studies supported that the application of phosphate in soil decreases arsenic content in Fe-plaques leading to an upsurge in arsenic solubility and bioavailability in the soil and rhizosphere [67,141–143]. However, at critical concentration, phosphate has an inhibitory effect, at which it competes with arsenate for the same transporter during uptake by the plasma membrane [15,144] and increasing the phosphate concentration in the solution leads to decreases in arsenate uptake by the plant [87,145].

Lee et al. [146] suggested three important factors controlling the effect of P on arsenic mobility in soil and its uptake in rice: (1) the competition between arsenic and P for adsorption sites on soil particles, (2) the antagonistic effect between inorganic phosphate (Pi) and arsenic during uptake in rice roots, and (3) the role of Pi in translocation of arsenic from root to shoot. Arsenic toxicity in plants depends on the As/P ratio in the soil rather than the absolute arsenic concentration. A survey of rice fields in China reported that by altering the status of P in shoots, arsenic accumulation could be decreased in rice grains [147]. Additionally, in arsenic enriched soils, the application of calcium in addition to P forms Ca-P-As complex and it causes a reduction in arsenic mobility [148].

7.1.3. Role of Silica

Silicon (Si) is a beneficial element for tropical grasses such as rice [149]. Among different soluble Si forms present in soil, plants can use only mono silicic acid, [150]. Si solubility in soil depends on soil pH, the most important determinants in soil solution [151]. Si supplementation improves crop yield by enhancing both the number of spikelets per panicle and, most particularly, the percentage of filled spikelets [152]. Detmann et al. [153] has revealed that Si nutrition results in alteration of primary metabolism and stimulating amino acid remobilization in rice.

Uptake of the arsenite occurs through nodulin-26 like intrinsic proteins (NIPs), the same transporter for silicon (Si) uptake and translocation [44]. As silicon competes with arsenite during uptake [26], the presence of high silica available in the soil reduces the arsenite uptake by rice [154]. The interaction of Si with arsenic has received attention in recent years [42,47,155–157] as it has a role in mitigating arsenic toxicity (Figure 1). A significant negative correlation was observed between Si concentration and uptake of inorganic arsenic species, and also for arsenic concentration in rice seedlings [136,158] because Si supply subdues the expression of the Si transporters Lsi1 and Lsi 2, the other mediators of arsenite uptake [2,42,143]. The application of Si in soil decreased the total arsenic accumulation in rice straw and grain by 78 and 16%, respectively [32,43]; strongly decreases the arsenic concentration in leaves and restricts the negative impacts on the photosynthetic apparatus [159]. In a study made by Marmiroli et al. [155], it was observed that the growth of shoot was adversely affected by the As^{III} and As^{V} exposure in the absence of Si supplementation. Tripathi et al. [136] demonstrated that Si mediated decreased uptake of As^{III} and improve the antioxidant defence response in plants. In Southeast Asia, the application of Fe and silicate containing materials such as furnace slag and calcium silicate slag is a very common practice [10]. However, the application of silica gel (10 g kg^{-1} of soil) was much more effective to decrease the arsenic concentration in flag leaf, straw, husk, and grains of rice as suggested by some authors [132,160]. Approximately 33% reduction of As^{III} concentration in polished rice was found as a consequence of ~50% reduced vascular transportation of arsenic after silicon application under flooded condition of paddy field [128,160].

7.1.4. Role of Sulfur

Sulfur is an essential element required for plant growth [161] and also plays a crucial role in reducing arsenic accumulation and translocation in plants [137,162,163] (Figure 1). According to Hu et al. [137] application of sulfur significantly reduces arsenic accumulation in rice, by three probable mechanisms (1) sulfur induces the formation of Fe plaques on the root surface and in the rhizosphere, which reduces the arsenic concentration in soil; (2) SO_4 may enhance the desorption of arsenate (As^{V}) from Fe- plaques; and, (3) at the cell membrane transport site SO_4 can inhibit arsenate transport into cells similar to the extent that phosphate competes with arsenate for transport and metabolism. Sulfur metabolism plays a central role in the arsenic detoxification process and is critical for plants survival in arsenic contaminated soil [109]. Arsenic exposure in plants induces the synthesis of low molecular weight sulphur rich ligands like glutathione and phytochelatin (GSH and PC, respectively) that requires adequate supplies of the GSH-building blocks Glu, Cys, and Gly. Detoxification of arsenic is achieved by conversion of As^{V} to As^{III}, which binds with the sulfhydryl groups of GSH and PC and subsequently transported to vacuoles [164]. As-thiol complexation plays an important role in arsenic mobility, decreasing As translocation from root to shoot or by efflux of arsenic from root to the growing medium [67,163]. Rice grain arsenic accumulation was negatively correlated to the concentration of PCs, as reported by Duan et al. [97]. Higher concentration of sulfur (5 mM) treatment resulted in an increased accumulation of arsenic in roots due to enhanced thiolic ligand synthesis (glutathione and phytochelatins) and consequent enhanced arsenic complexation in roots, thus restricting arsenic translocation from roots to shoots [138]. The genes involved in SO_4 uptake, transport, and metabolism were found to up-regulated in rice when it is exposed to As^{V} [165]. The application of sulfate (SO_4) in paddy soils has another advantage as SO_4 has a strong affinity towards arsenic under reducing conditions, leading to its precipitation as insoluble arsenic-sulfide [81].

7.2. Water Management and Irrigation Practices

Water management in paddy field is one of the best approaches in controlling arsenic bioavailability in the soil-plant system [166]. A water-saving regime has been reported to be an immediate and sustainable solution to decrease arsenic contents in rice [34]. As discussed in earlier sections, under flooding conditions, arsenic mobility is largely increased by the reductive dissolution of Fe-(oxyhydr)oxides [74]. Water saving efforts changes the redox status of soil and promotes oxidation condition that consequently impedes the reduction of As^{V} to As^{III}, the most toxic arsenic species, which has prominently higher solubility, plant availability, and toxicity [74]. In aerated soil or oxidized condition, affinity of arsenic enhances for soil minerals and also the oxidation of Fe consequences to Fe plaques formation around root surface [96,167] (Figure 1). The overall effect is to decrease arsenic mobility, and accordingly, less arsenic is available for the plants [33,74]. The report of Talukdar et al. [168] support the observations that, under aerobic water management practices, rice takes up less arsenic (0.23–0.26 ppm) than under anaerobic practices (0.60–0.67 ppm). To reduce the arsenic in rice grains, sprinkler irrigation practice is also having positive impacts [169,170]. Differential irrigation practices also influence the Fe-plaque formation and arsenic uptake by rice [166]. Under the flooding conditions, Fe-reducing bacteria are abundant that reduce Fe-oxyhydroxides and increase the arsenic solubility in soil [171]; it also promotes the transformation of As^{V} to As^{III} and methylated arsenic species. The experiment conducted by Somennahally et al. [166] in both continuous and intermittent flooding showed that total arsenic concentrations in the rhizospheric soil and grains were significantly decreased in intermittent flooding conditions than continuous flooding, which is supported by other studies [167,172].

7.3. Bioremediation Strategy

7.3.1. Role of Soil Microorganism

Soil microorganisms control the concentration of mineral in soil through various mechanisms including mineralization, immobilization that directly affects the fate and transport of arsenic in the environment [173]. Soil microorganisms detoxify arsenic species through sorption at their extracellular surface which have uronic acids, proteins and amino sugars with a hydrogen bonding potentials [10]. Adsorptions of various inorganic and organic species of arsenic have been reported by various soil bacteria: *Bacillus* sp. [10] *Rhodococcus* sp., *Halobacterium* sp. [174]. Another possible pathway of arsenic detoxification in soil microorganism is the formation of amorphous Fe hydroxides on the cell surface via the formation of inner-sphere complexes [175].

Arbuscular mycorrhizal fungi (AMF) play a protective role in arsenic translocation that suppresses mRNA expression of OsLsi1 and OsLsi2, the mediators of As^{III} transport [176]. Thus, AMF helps in biomass and grain yield without accelerating the accumulation of arsenic in grain under As stress [176,177], which might be an interesting approach to develop a cost-effective mitigation strategy [29].

7.3.2. Restriction of Arsenic at Underground Level

Arsenic mitigation in rice can be improved by inducing the increased synthesis of chelators such as glutathione (GSH) and phytochelatins (PC) in rice plants. In plants, the overexpression of phytochelatin synthase (PCS) gene showed promising result [178,179]. Arsenic build up in rice grain can be reduced by increased level of arsenite-thiol complexation, thus phytostabilize the metalloid in underground biomass or inedible parts [180]. Very recently, heterologous expression of PCS gene from *Ceratophyllum demersum* (CdPCS1) in rice enhanced arsenic accumulation in the roots and decreased arsenic accumulation in aerial part including the rice grain [181] (Figure 1).

7.3.3. Increase AsIII Efflux Rate

Transgenic rice plants expressing the *S. cerevisiae* ACR3 gene encoding arsenite efflux protein increased As^{III} efflux and also lowered arsenic accumulation in rice grain (Figure 1). Transgenic rice plants exhibited 30% lower arsenic concentration in root and shoot in comparison to wild type plants having similar arsenic translocation factor [53,182].

7.3.4. Volatilization of Arsenic

Volatilization of arsenic can be done by conversion of inorganic arsenic to methylated organic species like MMA and DMA and finally to the gaseous trimethylarsine (TMA) through the production of transgenic plant harboring the bacterial gene As^{III}-S-adenosyl methioninemethyltransferase ArsM [139,183] (Figure 1). A report from Meng et al. [184] suggested that transgenic rice plant expressing ArsM produced 10 fold higher volatile arsenical maintaining low arsenic level in rice seed along with organic arsenic species MMA^{V} and DMA^{V} in the root and shoot of transgenic rice.

8. Concluding Remarks

Wide ranges of issues are prevailing related to arsenic content in rice and factors controlling arsenic bioavailability, uptake, accumulation, and toxicity. All the factors, including the oxidative stress in rice, and possible cost effective agronomic strategies and biotechnological approaches to decrease uptake, translocation, and accumulation of arsenic in rice have been reviewed. Arsenic enters into the plants through phosphate transporter (arsenate) and aquaporin or silicic acid transporter (arsenite and methylated species of arsenic). Methylated species of arsenic are less toxic and among the inorganic arsenic species, As^{III} is more toxic than As^{V}. Oxidative stress generated due to production of ROS after arsenic exposure in plants is counteracted by production and complexation with thiol rich compounds like glutathione and phytochelatins (PCs). Though thiol-complexation leads to sequestration of arsenic in the vacuoles within root cell, still appreciable amount of arsenic can be transported to rice shoots and grains depending on genotype and soil conditions. Besides, arsenic-contaminated irrigation water greatly influences the increase of arsenic level in soil and its subsequent accumulation in rice grains. Arsenic accumulation in rice is largely dependent on its bioavailability, even in the arsenic rich soil, and is influenced by a variety of factors like soil types, physicochemical parameters, presence of other elements, and mineral composition such as iron, phosphorus, sulfur, and silicon in soil, soil-rhizosphere-plant system; rhizospheric microorganisms and their activities; organic matters and related microbial populations, water regime, nutritional status, biochar etc. Modification in agricultural practices and bioremediation methods may be a viable strategy to mitigate arsenic accumulation in rice. Some practices like rain water harvesting for crop irrigation, bioremediation by microbes resistant to arsenic, use of natural arsenic chelators, genetically modified rice plants, and the aerobic farming of paddy crops are effective to mitigate arsenic contamination. Changes in the cultivation practices like sprinkler irrigation method reported to decrease arsenic accumulation in rice plant. Although researchers are working on different genes in the rice plant that are responsible for arsenic uptake, transport and/or detoxification to produce more viable crop for consumption, however, application at the varied field conditions and subsequent quality production of rice is a serious topic. Gene-editing technologies are recent developments that help a researcher in the context of characterizing gene function and improved crop production. Based on the CRISPR-Cas (Cluster Regularly Interspaced Short Palindromic Repeats -associated nuclease) of bacteria, precise gene-editing is being tried with RNA-guided CRISPR/Cas9 system, apart from other technologies like Transcription Activator—Like Effector Nucleases (TALENs) and Zinc-Finger Nucleases (ZFNs) to get precise requirement [139]. The advent of molecular biology technologies has opened up the several opportunities to make rice grain safer by reducing arsenic accumulation. The approach from agriculture practices to potential rice plant development and subsequent field implementations are required. Detailed multidimensional integrated investigations are required to provide a sustainable way to remediate the arsenic polluted soil and their judicious use to reduce the extent of bioaccumulation in economically important crop to meet to human food demand.

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