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STRATEGIES FOR GROWING LOW GRAIN ARSENIC RICE IN ARSENIC CONTAMINATED SOILS

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Arsenic is a carcinogenic element whose widespread contamination in India and other parts of the world threatens crop cultivation and public health. Rice is one of the major crops affected due to As contamination in field since rice is known to accumulate As most effectively among crop plants. The problem of As in rice grains has been shown to affect few months old children to elderly people. A number of diseases and ailments arise due to As toxicity. Hence, there is a need to grow rice in As contaminated fields sustainably with high yields and with low As in grains. In Indian perspectives, the solutions must be low-cost and feasible for local application considering the poor income status of Indian farmers and small fields. The research efforts have been put towards for the past about one and half decades and various agronomic, biological and transgenic approaches have been tested. The present review article discusses the problem of As in rice and sheds light on prospective solutions to tackle the problem.

Keywords: Arsenic, elemental interaction, microbial inoculation, transgenic rice

Arsenic (As) is present naturally in all soils in several minerals (Smedley & Kinniburgh 2002).Arsenic exists in several inorganic [As(III) and As(V)] and organic [monomethylarsonic acid (MMAV), dimethylarsinic acid (DMAV)] (Planer-Friedrich *et al.* 2017, Awasthi *et al.* 2017). It is recognized as a carcinogenic element by the International Agency for Research on Cancer (IARC). Arsenic exposure leads to several ailments including skin lesions, hyperkeratosis, hyper-/hypo-pigmentation, cardiovascular diseases, diabetes and cancer etc. (Mitra *et al.* 2020). Arsenic finds application in agriculture sector as insecticides, pesticides and herbicides, such as calcium arsenite and dimethylarsenic acid (Bencko & Foong 2017). Arsenic is also used in glass, leather preservatives, poisonous baits, and semiconductors. It also finds application in the treatment of several diseases (Torka *et al.* 2016).

Over the years, As contamination of the environment has continuously increased in several parts of the world owing to natural biogeochemical processes as well as various anthropogenic activities (Ravenscroft et al., 2013). Arsenic contamination is a global menace threatening the health of millions of people (Majumdar and Banik 2019, Ishikawa *et al.* 2019). Arsenic contaminated groundwater is used for drinking and irrigation purposes and therefore it enters into the food chain through crop plants (Tripathi *et al.* 2007; Meharg *et al.* 2009). Arsenic accumulation beyond maximum permissible levels has been reported in a number of crop plants, vegetables, fruits, fishes, mushrooms, sea animals, and a number of food products (Upadhyay *et al.* 2019). Rice is the most affected crop due to As contamination (Upadhyay *et al.* 2019) and As accumulation in rice is expected to increase further in future in the conditions of predicted climate change (Muehe *et al.* 2019).

Rice is widely cultivated throughout the world. In South- and Southeast Asia, rice is commonly cultivated in flooded conditions with rainwater and groundwater. Since the groundwater has become As contaminated, its use has resulted into As build up in soil in past about 30 years (Upadhyay *et al.* 2019). The continuous flooding generates reducing conditions in paddy fields which favourably increase the concentration of As(III) form of As since the speciation of As depends on pH and Eh of soil (Kerl *et al.* 2018, Majumdar *et al.* 2018). Further, the physiological (oxgen release through roots, silica hyperaccumulator plant) and molecular (high expression of silicic acid transporters involved in As(III) uptake and transport) features contribute to high As accumulation in rice (Awasthi *et al.* 2017). Arsenic levels in soil are also influenced by presence of other elements like phosphate, manganese and sulfur and also by organic matter content (Srivastava *et al.* 2016; Zafeiriou *et al.* 2019, Maguffin *et al.* 2020). The factors affecting As concentration and speciation in paddy fields can also be managed to regulate As accumulation in rice plants (Upadhyay *et al.* 2020).

The As accumulating behavior of rice ensues from a number of transporters involved in the uptake, translocation and sequestration of As (Awasthi *et al.* 2017). The aquaglyceroporins mediate As(III) uptake and transport (Ma *et al.,* 2008; Lindsay and Maathuis, 2017) while phosphate transporters perform that for As(V) (Catarecha *et al.* 2007; Wang *et al.* 2016). The vacuolar sequestration of As and hence, tissue specific localization, is mediated by ATP-binding cassette (ABC)-type transporters (Song *et al.* 2010, 2014). However, ABC-type transporters mediate As transport only when it is complexed with phytochelatins (PCs), the oligomers of glutathione (GSH) involved in metal complexation in plants (Grill *et al.* 1987). Thus, enzymes of thiol metabolism and increased synthesis of cysteine, GSH and PCs also play crucial role in As accumulation and localization (Srivastava *et al.* 2010; Shukla e*t al.* 2012). The complexation of As by PCs also helps in toxicity alleviation as As becomes bound and free As ion is not available for reaction with biological molecules (Srivastava *et al.* 2007). One of the important enzymes in As metabolism is arsenate reductase (AR) that mediates the reduction of As(V) to As(III). A few ARs like high As content 1;1/1;2/4 (HAC1;1/1;2/4) have been identified in rice (Shi *et al.* 2016; Xu *et al.* 2017). A number of antioxidants also help in the alleviation of As toxicity through regulation of reactive oxygen species (ROS) and by avoiding oxidative stress (Srivastava *et al.* 2011, Chauhan *et al.* 2020). Various metabolic processes are regulated by molecules (GABA), hormones (jasmonates, abscissic acid (ABA)), microRNAs, kinases and transcription factors (Pathare *et al.* 2013; Srivastava *et al.* 2013, Castrillo *et al.* 2013, Yu *et al.* 2012). The available knowledge about mechanism of As tolerance can be used to develop transgenic rice plants having low grain As (Tripathi *et al.* 2007). The present article focuses on agronomic and molecular strategies that can be implemented to grow safely and to have low As level in rice grains.

Screening of Low grain arsenic accumulating cultivars: After screening of more than 500 rice germplasms, including a collection of popularly cultivated cultivars in India and entries for initial evaluation trails (IETs), were conducted for three years in both seasons (Boro, the summer and Aman, the Rainfed) in collaboration with Rice Research Station (RRS), Chinsurah, West Bengal. On the basis of yield and grain As level, 25 rice germplasms were selected for multi-locational field trials. The trials were conducted at six sites of West Bengal *viz.,* Gaighata, Durgapur, Beldanga, Chinsurah, Purbosthali and Birnagar having different levels of As contamination (10-25mg As/Kg) in soil for three years (2012- 2014). From the multi-locational field trials total nine rice cultivars viz., Nayanmoni, Gotrabhog CN1646-2, CN1643-3, and IET-4786 and popular rice genotypes IR-36, Satya, IR72406-B-R-3-2-2-1 and Pusa Basmati-1 were identified as low grain As accumulating cultivars (Table -1). These low grain As accumulating cultivars has good yield/hectare and will be suitable for cultivation in almost all As affected districts of WB. The benefits of these cultivars to the farmers will be derived from increased income in As contaminated areas due to resistance to As induced yield losses. Some of these variety can also be used by the farmers of other As

Table 1: Grain arsenic accumulation in widely cultivated rice cultivars of West Bengal, India

	Rice Genotypes	District/ Zone in which cultivated	Grain arsenic level ($\mu g\ kg^{-1}$ dw) during field trials at three sites* 2008-2010	Yield/ hectare
A.	**Popular rice genotypes**			
	IR-36	Nadia, Murshidabad, Malda, Bardhaman, Howrah, Hooghly, Bankura	120-911	4.50 – 5.00
	Satya	Different parts of Hooghly	116-336	5.00 – 5.50
	IR72406-B-R-3-2-2-1	Different parts of Hooghly	345-1267	3.00 – 3.50
	Pusa Bas-I	Hooghly, Bardhaman, Nadia, Bankura and some Basmati growing areas of India	129-487	4.00 – 4.50
B.	**NBRI screened low As accumulating rice genotypes**			
	Nayanmoni	Mainly in Nadia district	46-202	3.50 – 4.00
	Gotrabhog (IET 19226)	Nadia, Hooghly and Bardhaman	64-174	3.00 – 3.50
	CN1646-2	Recently developed by RRS and now popularly grown in Hooghly, Bardhaman, Nadia, Bankura, Malda	43-156	5.50 – 6.00
	CN1643-3	Recently developed by RRS and now popularly grown in Hooghly, Bardhaman, Nadia, Bankura.	73-186	4.00 – 4.50
	IET-4786	Almost all rice growing districts of West Bengal like Hooghly, Malda, Murshidabad, Bardhaman, Howrah, 24 Parganas (N&S), Bankura, Midnapore (East & West).	99-241	4.50 – 5.00

contaminated states of India including Indo-Gangetic plain (West Bengal, Chattisgarh, Bihar, Uttar Pradesh, Uttarakhand, Punjab etc.) without any yield penalty as they are well adopted for different agro-climatic zones.

Agronomic strategies for As reduction in rice: Agronomic strategies constitute methods that can be implemented by farmers during rice cultivation so as to reduce As accumulation in rice plants. Arsenic level in soil and its speciation in soil changes according to the cultivation practice and a number of physical, chemical and biological factors of soil (Li *et al.* 2014, Kumarathilaka *et al.* 2018, Muehe *et al.* 2019). Therefore, the appropriate management of rice cultivation practices can reduce As bioavailability and its accumulation by rice plants. A number of agronomic management practices have been tested by the researchers in this direction, such as irrigation management (aerobic irrigation, sprinkler irrigation), elemental supplementation (nitrogen, selenium, silica, etc.), hormonal /chemical supply (salicylic acid, GABA, thiourea etc.), soil inversion, and inoculation of microorganisms (Srivastava *et al.* 2019; Huhmann *et al.* 2019; Seyfferth *et al.* 2019, Li e*t al.* 2019, Awasthi *et al.* 2018, Chauhan et al., 2017; Moreno-Jimenez *et al.,* 2014). The following discussion presents various strategies and positive and negative points associated to each one.

Abiotic arsenic reduction strategies

Elemental supplementation affects As bioavailability and reduces As accumulation in rice: From the rhizosphere in soil to cytoplasm of cell, elements interact and therefore, influence the bioavailability and toxicity of each other in a dynamic manner. The interaction of As with several elements like silica (Si), phosphorus (P), selenium (Se), sulfur (S), and nitrogen (N) has been studied (Tripathi *et al.* 2013, Dixit *et al.* 2015, Chauhan *et al.* 2017, Srivastava *et al.* 2019). Silica has been the most studied as As(III) and Si use the same pathway for uptake and transport in plants and hence it is expected that Si may compete well with As(III) and reduce its accumulation in rice. Further, Si is a beneficial element for rice plants (Tripathi *et al.* 2013). So additional Si supply improves growth of plants and its potential to tolerate As toxicity. Li *et al.* (2009) observed significant reduction in the level of straw and grain total As when rice plants were supplemented with silica gel in a pot experiment. However, an increase in DMA level in grains was noticed. The effect of two different silica containing minerals namely diatomaceous earth and silica gel was tested by Seyfferth and Fendorf (2012). They found that diatomaceous earth did not cause any change or further increased the level of As in grains. However, Si gel addition decreased As in grains. Hence, variable mineralogy and available Si influences As accumulation differently. Tripathi *et al.* (2013) conducted laboratory experiments and reported that Si supplementation reduced As accumulation and improved As tolerance in rice seedlings. Two contrasting rice cultivars i.e. As tolerant (Triguna) and As sensitive (IET-4786) were exposed to two concentrations of AsIII (10 and 25 µM) and silicic acid (0.5 and 1 mM Si). The higher concentration of Si (1 mM) during As stress showed significant reduction of As accumulation in shoot in both the cultivars. Supplementation of Si (1 mM) during As stress ameliorated the As induced oxidative stress in tolerant rice cultivar not only due to lowering of As accumulation but also through improved thiol and antioxidative system of Triguna compared to IET-4786 (Tripathi *et al.* 2013). However, in a field study, Lee *et al.* (2014) observed that Si supply led to increase in both As and Si concentrations in rice plants. Syu *et al.* (2016) reported that application of Si significantly reduced the accumulation of Si in rice shoots due to competition in uptake of As and Si. A study by Khan and Gupta in 2018 reported that priming of rice seed with Si along with As reduced the accumulation of As. Recently, Cui *et al.* (2020) reported that foliar application of nanoparticle of Si provided tolerance against As stress by maintaining the integrity the cell wall and by reducing the oxidative stress and As accumulation. The supplementation of Si rice husk to the soil also helped to reduce As accumulation in rice without hampering the nutrient availability (Seyfferth *et al.* 2016). A recent study by Leksungnoen *et al.* (2019) reported that biochar produced from silica rich rice husk plays an important role in reduction of As accumulation in rice grain (Leksungnoen et al., 2019).

Sulfur (S) is an important element in the context of As detoxification as this is a constituent of GSH and PCs involved in As complexation. It has been noticed that external GSH supply reduces As toxicity and accumulation in rice seedlings (Shri *et al.* 2009). Further, an inhibitor of GSH biosynthesis, buthionine-L-sulfoximine (BSO) was supplied to rice plants. It was found that BSO reduced GSH and PC levels and thus increased As transport to flag leaves and grains (Vezza *et al.* 2019). Dixit *et al.* (2015a) also observed in hydroponics study that supply of high S level reduced As translocation to shoot as this restricted As to roots itself. An opposite result was seen when low S supply was given. The mechanism of S induced As stress tolerance in rice was also evaluated by Dixit et al. (2015b). They showed that S alleviated As toxicity by modulating several protein levels, amino acids and thiol metabolism. Zhang et al. (2016) found that sulfur application decreased As concentration in rice grains by 44%. The response was found to be correlated to changes in expression of a number of genes including transporters. Srivastava *et al.* (2016) conducted a laboratory experiment where they supplied low and zero S level to rice seedlings in presence of As. It was observed that even in condition of zero S supply, plants tend to accumulate PCs to tackle As toxicity. The combined toxicity of As and S depleted condition showed loss of photosynthetic pigments and increased accumulation of anthocyanins. However, even under depleted S supply, increased synthesis and consumption of S-containing thiols was observed upon As exposure. The study inferred that rice plant combated As induced stress and S depleted condition by reduction of As accumulation and by altering subcellular distribution of As (Srivastava *et al.* 2016). Kumar *et al.* (2019) reported that the over-expression of high-affinity sulphate transporters. OsSultr1;1 provides tolerance against different abiotic stresses under limiting sulphur condition. An increased formation of iron plaque on S supplementation is also reported, which act as a barrier for As and leads to reduction in As accumulation in plants (Shakoor *et al.* 2019).

Phosphate is known to compete with As(V) for uptake and transport in plants. Although As exists mostly in the form of As(III) in paddy fields, there is expected conversion of As(III) back to As(V) around rice roots. This is due to the fact that rice roots release oxygen and therefore just close to roots, oxygenic conditions are promoted. The changes in microbial community around roots are also reported (Srivastava *et al.* 2018). Hence, despite the presence of As mostly in the form of As(III), rice rhizospheric As chemistry might be completely different. Considering these facts in mind, phosphate supplementation has also been tested by researchers. However, in most of the studies, phosphate addition caused an increase in As accumulation in rice plants (Hossain *et al.* 2009, Talukder *et al.* 2011, Wu *et al.* 2011). This was considered to be due to replacement of As(V) adsorbed on to soil particles and iron plaque on rice roots and thus making As(V) more bioavailable to plants. Luan *et al.* (2018) reported a major vacuolar phosphate transporter (VPT1) that is involved in vacuolar inorganic phosphate (Pi) homeostasis and thus play an important role in As(V) tolerance in Arabidopsis. This study reported that mutant VPT1 elevated the levels of Pi in the cytosol, thus suppressed the expression of PHT1-type transporters leading to reduced accumulation of As. Another study by Xie *et al.* (2019) reported the role of OsNLA1 in As uptake and tolerance. The increased expression of OsNLA1 is reported during AsV stress and authors revealed that OsNLA1 play an important role in regulation of phosphate transporters and thus provide As tolerance.

Selenium is an important element which acts as an antioxidant and is a constituent of several enzymes (Chauhan *et al.* 2019). Kumar et al. (2014) demonstrated improvement in growth and tolerance of rice seedlings to As stress upon Se supplementation. Chauhan et al. (2017) also reported improved plant growth and nutrient status of rice plants on Se supplementation. A marked reduction in As accumulation as well as increased accumulation of Se in rice plant was observed. The As toxicity amelioration in presence of Se was achieved via increase in enzymatic and non-enzymatic antioxidant, such as phenolic compounds (Gallic acid, protocatechuic acid, caffeic and Rutin) (Chauhan *et al.* 2017) Transcriptome and proteome profiling of rice plant also suggested that supplementation of Se reduced the As induced oxidative stress and toxicity in rice plant by up-regulation of expression of key As transporters and defense responsive genes as well as their respective proteins (Chauhan *et al.* 2020).

Hence, elemental fertilization and optimum management of elemental nutrition of plants can help improve growth of rice plants in As contaminated fields. The strategy can also assist in reducing As level in rice grains and lead to higher yields of better quality rice grains.

Water influences As dynamics in soil and As levels in rice plants: The irrigation of rice fields with groundwater is the major source of As for rice fields. The water is itself an important regulator of As biogeochemistry in soil owing to its influence of soil physicial and chemical properties and microbial activity (Upadhyay *et al.* 2020). Rice is a semi-aquatic plant and sufficient water availability plays a key role in achieving proper rice growth and productivity (Islam *et al.* 2019). Conventionally, the fields are flooded throughout the growth period of rice plants. However, there are other cultivation methods of rice aimed to reduce water use such as aerobic cultivation, alternate wetting and drying and sprinkler irrigation. These approaches were designed to reduce excessive water consumption by rice crops. These also hold worth in the light of increasing water shortage which is expected to worsen further in future. Further, such water conservation approaches might also allow rice cultivation in more aerobic conditions and reduce As accumulation in rice grains.

Duxbury and Panaullah (2007) compared Boro rice grown either in flooded conditions or raised bed cultivation in Faridpur, Bangladesh. They observed decrease in rice yields in both flooded and raised bed conditions. However, raised bed cultivation caused lower decline in rice yields and also reduced grain As level. Talukder *et al.* (2011) demonstrated increase in grain yield (13% increase) and grain As (62% decrease) in raised bed cultivation. Moreno-Jimenez *et al.* (2014) found that sprinkler irrigation application to rice plants reduced grain total As by one-third in only one application as compared to traditional irrigation. When the sprinkler irrigation was used for longer duration, an even greater reduction in As accumulation in rice grains was achieved. However, sprinkler irrigation generates aerobic conditions that increase Cd bioavailability to rice plants. Hence, an increase in Cd concentration in rice grains was observed (Moreno-Jimenez *et al.* 2014). In the experiments of Arao *et al.* (2009) and Li *et al.* (2009) also aerobic rice cultivation resulted in significant increase in Cd concentration while a decline in Se and Fe levels. Spanu *et al.* (2012) reported that sprinkler irrigation is helpful to reduce the As accumulation by 50%. Yang *et al.* (2019) reported alternate wetting and drying of rice during cultivation plays an important role in significant reduction in As accumulation. A study by Islam *et al.* (2019) also reported that water management via alternate wetting and drying is an effective way to reduce As accumulation.

Supplementation of chemical and bio-molecules enhances As tolerance and reduces As accumulation of rice : Srivastava *et al.* (2014) conducted a lab study where they supplemented As exposed rice seedlings with a redox active molecule, thiourea (TU). Thiourea supplementation was found not only ameliorate As stress in rice seedlings but also to reduce As concentrations in shoot parts. The responses could be linked to regulation of As transporters (Lsi1 and Lsi2) in redox dependent manner along with other transporters as well as biochemical modifications.

Singh *et al.* (2015) applied salicylic acid (SA) to rice seedlings and noticed improved growth and stress in rice plants. SA application also decreased As translocation from root to shoot. The positive results were attributable to a number of biochemical and molecular changes. Singh *et al.* (2017) reported that nitric oxide and SA are the signaling molecules and provide protection against As toxicity by modulating signaling response against As. Kumar *et al.* (2017) showed the role of γ-aminobutyric acid (GABA) in As tolerance by modulating expression of As transporters *viz.,* LSi-1 and LSi-2. GABA has been suggested to act as a central regulator of As stress responses of plants in Indian mustard plants (Pathare *et* *al.* 2013). In rice plants also, Pathare *et al.* (2016) suggested involvement of GABA and 14-3-3 protein interaction in As stress responses.

Biotic arsenic reduction strategies

Bacterial inoculants

Microbes constitute a diverse group which includes bacteria, algae, fungi that are associated with improvement of plant growth as well as increase nutrient status. They also play an important role to provide tolerance against As toxicity by As methylation and conversion to non-toxic forms, siderophore procution and by reduction of the bioavailability of As. Dolphen and Thiravetyan (2019) reported that microbes are efficient tool to reduce As accumulation. Inoculation of *B. pumilus* (an endophytic bacterium) with leonardite reduced the accumulation of As in rice by down-regulating of Lsi-1 and Lsi-2. Moens *et al.* (2020) reported that inoculation of a hyperaccumulator rhizobacterial strain, *Ochrobactrum tritici* to the rice during As stress decreased the As induced toxicity in rice and also improved the yield of rice plant. In some studies, the use of more than one bacterium in the form of a consortium was done. A study by Nookongbut *et al.* (2018) reported that microbial consortium of bacteria (*Rhodopseudomonas palustris* C1 and *Rubrivivax benzoatilyticus* C31) not only enhanced the rice growth but also reduced As accumulation in rice by producing siderophores, indole acetic acid and exopolymeric substances. Thus it can be concluded that microbial consortium ameliorates As toxicity and reduces As uptake in rice and also increases the yield.

Fungal inoculants

Arbuscular mycorrhizal fungi impart tolerance against various stresses including heavy metals. Li *et al.* (2016) reported that *Rhizophagus intraradices* significantly reduced As concentrations in rice grains by transforming inorganic As into less toxic organic form in rice. Zhang *et al.* (2016) found that AMF inoculation decreased the proportion of inorganic As in grains of a As tolerant rice variety. Li *et al.* (2011) also demonstrated that *Rhizophagus irregularis* inoculation to lowland rice and *Glomus geosporum.*

inoculation to upland rice resulted in an increase in grain yield and grain P/As ratio. Li *et al.* (2011, 2013) suggested that the response of an inoculum of AMF varies different rice varieties and therefore the best candidate or the best combination of AMF inoculants should be used. Wu *et al.* (2015) reported that inoculation of micorrhizal fungi *Glomus geosporum BGC HUN02C*, *G. versiforme BGC GD01B* and *G. mosseae BGC GD01A* increased the yield of rice plant as well as mitigated As toxicity in rice. Mohd *et al.* (2017) reported that an endophytic fungus *Piriformospora indica* colonized the rice roots during As stress by modulating antioxidant enzyme activities. Thie led to balanced redox status of the cell which and protected photosynthetic machinery of the plants. The supplementation of fungus ameliorated As toxicity by different mechanisms such as by reducing the bio-availability of As to the plant, via bio-transformation of the toxic form of As into insoluble particulate matter and by modulating the antioxidative system of the plant. Several studies also reported that filamentous fungus viz., *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., *Cladosporium* sp., *Rhizopus* sp. and *Westerdykella* sp. are potent candidate to mitigate As toxicity due to their biotransforming potential but among these *Penicillium* sp. and *Aspergillus* sp. are most As tolerant fungal species (Batista *et al.* 2016, Segura *et al.* 2018). Thus the application of fungal sp. in paddy fields might be a good alternative for bioremediation in As-contaminated soils and amelioration of As toxicity in rice as well as for reducing As in rice grains.

Transgenic approaches

For the sustainable solution of the problem and for achieving desired objectives like growth and yield improvement and As level reduction in grains, transgenic variety development has been considered a viable objective. The targeted strategies were either focused to enhance tolerance of rice plants to As stress through changes in the level of antioxidants, GSH and PCs or to reduce As uptake and transport via changes in transporter proteins (reviewed in Srivastava *et al.* 2012). Since, As has some organic species which are volatile, one strategy has been to express bacterial genes in rice plants that can increase As volatilization to the environment and thus reduce As in grains. Meng *et al.* (2011) developed transgenic rice expressing arsM gene (S-adenosylmethyltransferase) of Rhodopseudomonas palustris. They found induced As methylation and eventual volatilization of As (10-fold higher volatile As) in transgenic rice. Duan *et al.* (2012) utilized ACR3 (Arsenic Compounds Resistance 3) gene, responsible for As extrusion from cells to the medium, from *Sachharomyces cerevisiae* for the development of transgenic rice. In the transgenic lines, As concentrations in shoot and root were 30% lower than WT. Importantly, there was a decline in husk and grain As in transgenic lines (about 20-30%) even when grown under continuous flooding. *Oryza sativa* C-type ATP-binding cassette (ABC) transporter (OsABCC1) was chosen to develop transgenic plants and the gene was knocked down. The mutant lines accumulated less As in shoots than wild type. However, mutants showed more As accumulation in flag leaf, husk and brown rice (13-18-fold higher) than WT (Song *et al.*2014). This could be due to the fact that in absence of OsABCC1, lower amounts of As-PC complexes would be sequestered to vacuole. Shri *et al.* (2014) developed transgenic rice lines expressing phytochelatin synthase gene isolated from *Ceratophyllum demersum* for achieving higher As complexation with PCs. In the obtained transgenic lines, significant increase in PCS activity and in the level of PCs was noticed. This led to higher As accumulation in root and shoot owing to greater ability of transgenic rice to complex As. It positively resulted in significant decline in grain and husk As concentration as compared to WT. Hence, altering either the complexation of As or the sequestration of As-PC complexes can be potential strategies for developing low grain As accumulating cultivar in near future. Tiwari *et al.* (2014) has demonstrated involvement of NRAMP1 transporter of rice (OsNRAMP1) in As transport. The transporter was found to be localized in plasma membrane of endodermis and percicyle cells and is involved in xylem loading of As. Expression of OsNRAMP1 in Arabidopsis although provided tolerance to plants but resulted in greater As accumulation n both root and shoot as compared to WT. The transporter there provides an opportunity for low grain As rice development through alteration of its expression. Kumar *et al.* (2013) expressed a rice lambda class of glutathione-S-transferase gene (OsGSTL2) in Arabidopsis and found that it could provide tolerance to heavy metals including As. Glutathione-S-transferases are involved in GSH-mediated detoxification of xenobiotics including metals and hence, this gene can be helpful in the development of rice with improved tolerance to As. The expression of WaarsM gene of Westerdykella aurantiaca in rice was able to convert toxic As into less toxic methylated As and reduced As accumulation in rice by its volatilization and methylation (Verma *et al.* 2018). Shi *et al.* (2016) utilized arsenic reductase genes from rice plants (OsHAC1;1 and OsHAC1;2) and developed knock out and overexpressing lines. The overexpressing rice lines showed greater reduction of As(V) to As(III), higher efflux of As(III) to external medium while reduced As accumulation in shoots. The knock out lines showed an opposite response. Similar results were observed in case of another arsenic reductase gene from rice, OsHAC4 (Xu *et al.* 2017). Thus, increased expression of arsenic reductase gene in roots allows plants to rapidly get rid of As through reduction to As(III) and subsequent efflux. Deng *et al.* (2018) generated transgenic rice plants with the use of two genes. One

Saccharomyces cerevisiae yeast cadmium factor (ScYCF1) and other OsABCC1. Both genes were expressed in the root cortical and internode phloem cells. Further, another gene from Escherichia coli, γ-glutamylcysteine synthetase (γ-ECS) was also expressed. The transgenic rice plants exhibited significantly reduced translocation of As firstly from root-to-shoot and also from internode-to-grain during grain filling stage. An eventual reduction in grains was found to be up to 70% without any negative impact on rice plant growth and development.

Concluding remarks

A number of approaches have been studied at laboratory and field level and mixed responses have been found in terms of As stress mitigation and As reduction in rice. It seems reasonable to cultivate screened and selected low grain As accumulating cultivars for As prone areas of different agro-climatic zones. However, future studies need to be more focused and accurate to provide a novel, feasible and cost-effective solution for growing rice in As contaminated areas. Future research also needs to evaluate the impact of changing climatic conditions and to assess the effects of multiple stressors to come out with sustainable solutions to tackle As problem in rice.

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