Review Article

MITIGATION OF ARSENIC CONTAMINATION THROUGH BIOTECHNOLOGICAL APPROACHESIN RICE

Abstract

Arsenic poisoning negatively impacts plants, soil, water, and human health, posing a serious threat to sustainable agriculture. Pesticides, fertilizers, and industrial processes that contain arsenic are widely used, which leads to soil contamination and reduces soil fertility and productivity. The drinking of groundwater contaminated with arsenic affects around 300 million people globally. Prolonged exposure to arsenic has been linked to a number of health concerns, including as cancer, developmental abnormalities, and skin sores. Concerns have been raised over the possible health effects of arsenic, which is mostly exposed to consumers through the consumption of contaminated food. Rice is one of the most impacted crops from arsenic-polluted irrigation water and soil, making it a common vehicle for dietary intake of arsenic. In present review, the challenges related to arsenic toxicity and its possible solutions in rice have been discussed.

Keywords: Arsenic, Brassinosteroid (BR), Salicylic acid (SA), QTL, CRISPR-Cas9

Introduction

Arsenic toxicity poses a significant threat to sustainable agriculture by adversely affecting soil, water, plants, and human health (Prasad et al., 2020). The widespread use of arsenic-containing pesticides, fertilizers, and industrial processes contributes to the contamination of soil, compromising its fertility and overall productivity. Moreover, arsenic can leach into groundwater, further contaminating water sources used for irrigation and exacerbating the risk of crop uptake. Crops such as rice, leafy vegetables, and root crops are particularly susceptible to arsenic accumulation, presenting a direct threat to food safety. The consumption of arsenic-contaminated crops can lead to various health issues, including skin lesions, cancer, and neurological problems, underscoring the interconnectedness of environmental and human well-being. Additionally, the economic sustainability of agriculture is at stake due to potential trade restrictions on arsenic-contaminated agricultural exports.

Arsenic (As), a metalloid, ranked first in a list of 20 major hazardous substances by the Agency for Toxic Substances and Disease Registry and United States Environmental Protection Agency (Roy and Saha., 2002). Due to its high toxicity, it is also known as ‘king of poison’ (Shaji et al., 2021).Recent study showed that nearly 108 countries are affected by arsenic contamination in groundwater (with concentration beyond maximum permissible limit of 10 ppb recommended by the World Health Organization (WHO) (Shaji et al., 2021). The highest among these are from Asia (32) and Europe (31), followed by regions like Africa (20), North America (11), South America (9) and Australia (4). More than 300 million people worldwide are affected by the consumption of arsenic contaminated groundwater (Kumar et al., 2021). The South and Southeast Asian Belt is considered as the most arsenic polluted areas including India, Bangladesh, Nepal, Vietnam and China (McArthur, 2019; Shaji et al., 2021). In India, 20 states including West Bengal, Jharkhand, Bihar, Uttar Pradesh, Assam, and 4 Union Territories have so far been affected by arsenic contamination in groundwater (Shaji et al., 2021). Bihar has shown an upsurge in cases affected by arsenic poisoning (Kumar et al., 2021).

The adverse human health effects resulting from chronic As consumption include increased risk of cancers, hyperkeratosis, birth defects, cardiovascular disease, neurotoxicity, and diabetes (Phan et al., 2010; Flanagan et al., 2012; Kumar et al., 2021). Skin lesions are a common sign of arsenic poisoning and indicate prolonged exposure. Even more concerning, the International Agency for Research on Cancer has categorized arsenic as a Group 1 human carcinogen, connecting it to malignancies of the skin, lungs, bladder, and other organs. Prolonged exposure to arsenic has also been linked to developmental defects, cardiovascular disorders, and neurological impairments. The negative health effects are more severe in areas where arsenic poisoning is common, which emphasizes how urgently this public health issue has to be addressed.

Use of contaminated groundwater for crop irrigation may result in the accumulation of As in agricultural soils, eventually resulting in decreased crop yields and impaired human health (Brammer and Ravenscroft, 2009; Kumar et al., 2021). The threat of As to humans is usually exacerbated in countries that have high population densities, use groundwater as their primary drinking water source, and rely heavily on large quantities of irrigation for agriculture, such as those within South and Southeast Asia. However, contamination of agricultural soils from arsenic in groundwater is truly a global problem, with geographically dispersed countries (e.g., Mexico, Chile, Argentina, Greece, and the USA) experiencing varying degrees of soil contamination (Smedley and Kinniburgh, 2002; Sahoo and Kim, 2013; Shaji et al., 2021).

Rice (*Oryza sativa* L.) arsenic contamination is a pressing concern in global food safety, as rice is known to accumulate higher levels of arsenic compared to other staple crops (Upadhyay et al., 2020; He et al., 2021). The rice plant effectively absorbs arsenic from the soil, especially inorganic arsenic, which results in higher amounts in the grain. Eating rice exposes one to arsenic over time, which has been related to cancer, developmental disorders, and skin sores, among other health problems. The natural ability of the plant to take up arsenic, in combination with many agronomic, geochemical, and hydrological factors, leads to As accumulation in rice (Zhao et al., 2010). The management of the rice plant frequently consists of controlled flooding in order to eliminate competition from weeds, reduce herbicide needs, and increase yields (IRRI 2015). To create flooded conditions, large quantities of irrigation water may be used in both dry and wet seasons, depending on the ability of a rice paddy to receive adequate natural saturation. Flooding changes natural soil hydrodynamics, results in high loadings of arsenic in soil, if contaminated groundwater is utilized for irrigation.

South and Southeast Asia are the areas of greatest concern for As accumulation in rice-paddy soils following irrigation with contaminated groundwater. Throughout the densely populated river basins that drain the Himalayas, rice is a staple crop, and natural arsenic contamination of groundwater has been used extensively for irrigation of dry-season rice for several decades (Fendorf et al., 2010; Chakraborty et al., 2015). The Bengal Delta of Bangladesh and India, one of the most severely impacted and extensively studied areas, As concentrations in irrigation water applied to rice-paddy fields frequently exceed 50 μg/L (Williams et al., 2006; Khan et al., 2010; Chakraborty et al., 2015), reaching as high as 1800 μg/L (Alam et al., 2003). As a consequence, soil arsenic concentrations in polluted fields can reach up to 95 mg/kg, and rice grains may have As concentrations of up to 1835 μg/kg (Khan et al., 2010). Rice has the ability to accumulate arsenic, amassing concentrations ten times higher than other cereals such as wheat (Karagas et al., 2019). High concentrations of iAs have also been found in rice-based products, including those consumed by infants and young children (Davis et al., 2017). Rice constitutes approximately 66 % of the caloric intake in the region and up to 50 % of the daily arsenic consumption for some people (Liao et al., 2010). Rice is a major contributor to arsenic intake in humans (Williams et al. 2005; Meharg et al. 2009; Gilbert-Diamond et al. 2011), because rice is mainly cultivated in anaerobic paddy soil, where arsenite [As(III)] is more available (Takahashi et al.2004).

The identification and introgression of QTLs related to arsenic are among the preferred strategies for arsenic mitigation in crop plants. The genome wide association (GWA) mapping for arsenic in shoots and grains of rice grown under continually flooded (CF) and alternate wetting and drying (AWD) has identified a large number of 74 individual QTLs for arsenic, with six QTLs showing stability across years and/or water treatments (Norton et al., 2019). Recently, a machine-learning Bayesian network approach along with high-resolution GWAS were performed to identify QTL for grain-arsenic and straight head disorder (StHD resistance (Pinson et al., 2021). For each As-associated trait, several QTL (ranging from 9 to 33) were identified (Pinson et al., 2021). Using a doubled haploid population created by anther culture of F1 plants from a hybrid between a Japonica cultivar CJ06 and an Indica cultivar TN1, the quantitative trait loci (QTLs) linked to arsenic accumulation in rice were mapped (Zhang et al., 2008). The map revealed four QTLs for the concentrations of arsenic. At the seedling stage, a single QTL for As concentrations in roots was found on chromosome 3, and a single QTL for As concentrations in shoots was mapped on chromosome 2 with a 24.4% phenotypic variation. Two QTLs with 26.3 and 35.2% phenotypic variance, respectively, were discovered on chromosomes 6 and 8 at maturity for As concentrations in grains (Zhang et al., 2008).

Phytohormones play a key role in mediating cellular responses to various stress stimuli (Vos et al., 2015; Ku et al., 2018). The protective role of salicylic acid (SA) in plants against abiotic and biotic stress are well documented (Prasad et al., 2009; Noriega et al., 2012; Kumari et al., 2022; Sahni et al., 2021; Sahni and Prasad 2022). Recent studies showed the modulation of genes related to heavy metal stress in plants (Noriega et al., 2012; Singh et al., 2015). The exogenous application of SA was found effective in mitigating arsenate (AsV) toxicity in rice by lowering translocation to the shoots (Singh et al., 2015). The amount of As in the shoot was positively connected with the expression of *OsLsi2*, an efflux transporter that moves As from the root to the shoot as arsenite (AsIII) (Singh et al., 2015).

Brassinosteroid (BR), a plant steroidal hormone, is one of the recognized phytohormones that is important for plant growth and development. It also modulates stress responses and can shield plants from heavy metal poisoning (Sahni et al., 2016; Xu et al., 2018). According to Xu et al. (2018), there is preliminary evidence that treating rice plants cultivated in hydroponic culture with BRs prevents arsenic accumulation. Recently, we have unequivocally demonstrated that the application of Brassinosteroid, BR mediated suppression of *OsLsi1* and *OsLsi2* had a beneficialeffect in reducing the arsenic accumulation in straw and grain of rice plant (Chaudhary et al., 2023). Further, BRs lowered (by up to 50%) the uptake of arsenic in plants grown in naturally arsenic-contaminated soil (Chaudhary et al., 2023). Overall, BR therapy had a net positive effect on rice plant arsenic absorption, highlighting BR's higher-level modulation of this feature in plants' hierarchical response system. A solid foundation for creating BR-based arsenic mitigation techniques in rice is provided by gene expression analysis and the decreased absorption of arsenic in rice seeds and straw in EBR-treated rice plants compared to mock-treated rice plants.

In recent years, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) has become the method of choice for genome editing in crop plants including rice (Zafar et al., 2020; Kumari et al., 2022). The well-developed rice transformation protocol facilitates faster adoption of gene editing technology in rice (Prasad et al., 2008, 2009; 2016). The emerging reports in targeting genes associated with arsenic uptake, translocation and detoxification of arsenic in rice has pave the way of developing low arsenic mitigation in rice using gene editing (Chen et al., 2017; Fiaz et al., 2019; Chaudhary et al., 2023). The role of *OsLsi1* and *OsLsi2* in arsenic translocation in rice plants are well described (Chen et al., 2017; Singh et al., 2015; Chaudhary et al., 2023). The editing of *OsLsi1* and *OsLsi2* genes in rice might be helpful in reducing the arsenic accumulation in straw and grain of rice plant without yield penalties.

Conclusion

Addressing arsenic toxicity in agriculture requires a comprehensive approach, including the development of arsenic-resistant crop varieties and adopting new biotechnological tools. The management practices and use of phytohormones have the potential to significantly lower the arsenic uptake in rice. Further, the plant breeding approaches amalgamated with genes will pave the way for developing arsenic-free rice.

References

Alam MGM., Snow ET., Tanaka A. (2003) Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. Sci Total Environ., 308(1-3):83-96.

Brammer H., Ravenscroft P. (2009) Arsenic in groundwater: a threat to sustainable agriculture in South and South-east Asia. Environ Int., *35*(3): 647-654.

Chakraborty M., Mukherjee A., Ahmed KM. (2015) A review of groundwater arsenic in the Bengal Basin, Bangladesh and India: from source to sink. Curr Pollut Rep., 1(4):220-247.

Chaudhary, B., Prasad, B.D., Sahni, S. *et al.* Brassinosteroid Mediated Modulation in the Gene Expression Analysis of Genes Associated with Arsenic Uptake and Transport in Rice (*Oryza sativa* L.) for Effective Mitigation of Arsenic. *J Plant Growth Regul* (2023). https://doi.org/10.1007/s00344-023-11073-1

Chen Y., Sun SK., Tang Z., Liu G., Moore KL., Maathuis FJM. et al. (2017) The nodulin 26-like intrinsic membrane protein OsNIP3;2 is involved in arsenite uptake by lateral roots in rice. J Exp Bot., 12:e0173681.

Davis MA., Signes-Pastor AJ., Argos M., Slaughter F., Pendergrast C., Punshon T., Gossai A., Ahsan H, Karagas MR (2017) Assessment of human dietary exposure to arsenic through rice. Sci Total Environ., 586:1237-1244.

Fendorf S., Michael HA., van Geen A. (2010) Spatial and temporal variations of groundwater arsenic in South and Southeast Asia. Science., 328(5982):1123-7.

Fiaz, S.; Ahmar, S.; Saeed, S.; Riaz, A.; Mora-Poblete, F.; Jung, K.-H. Evolution and Application of Genome Editing Techniques for Achieving Food and Nutritional Security. *Int. J. Mol. Sci.* **2021**, *22*, 5585. https://doi.org/10.3390/ijms22115585

Flanagan SV., Johnston RB., Zheng Y. (2012) Arsenic in tube well water in Bangladesh: health and economic impacts and implications for arsenic mitigation. Bull World Health Organ., 90:839-846.

Gilbert-Diamond D., Cottingham KL., Gruber JF., Punshon T., Sayarath V., Gandolfi AJ., Baker ER., Jackson BP., Folt CL., Karagas MR. (2011) Rice consumption contributes to arsenic exposure in US women. Proc Natl Acad Sci USA., 108(51):20656-60.

He Y., Zhang X., Shi Y., Xu X., Li L., Wu J-L. (2021) Correction to: PREMATURE SENESCENCE LEAF 50 Promotes Heat Stress Tolerance in Rice (*Oryza sativa* L.). Rice 14:63.

Karagas MR., Punshon T., Davis M., Bulka CM., Slaughter F., Karalis D., Argos M., Ahsan H. (2019) Rice Intake and Emerging Concerns on Arsenic in Rice: a Review of the Human Evidence and Methodologic Challenges. Curr Environ Health Rep., 6(4):361-372.

Khan MA., Islam MR., Panaullah GM., Duxbury JM., Jahiruddin M., Loeppert RH. (2010).Accumulation of arsenic in soil and rice under wetland condition in Bangladesh. *Plant and Soil*, *333*(1), 263-274.

Ku YS, Sintaha M, Cheung MY, Lam HM. Plant Hormone SignalingCrosstalks between Biotic and Abiotic Stress Responses. Int J Mol Sci. 2018 Oct 17;19(10):3206.

Kumar A., Ali M., Kumar R., Kumar M., Sagar P., Pandey RK., Akhouri V., Kumar V., Anand G., Niraj PK., Rani R., Kumar S., Kumar D., Bishwapriya A, Ghosh AK. (2021) Arsenic exposure in Indo Gangetic plains of Bihar causing increased cancer risk. Sci Rep., 11(1):2376.

Kumari, D., Prasad, B.D., Dwivedi, P. *et al.* CRISPR/Cas9 mediated genome editing tools and their possible role in disease resistance mechanism. *Mol Biol Rep* **49**, 11587–11600 (2022).

Liao CM., Lin TL., Hsieh NH., Chen WY. (2010) Assessing the arsenic-contaminated rice (*Oryza sativa*) associated children skin lesions. J Hazard Mater., 176(1-3):239-51.

McArthur JM. (2019) Arsenic in groundwater. In GroundwaterDevelopmentand *Management*. pp. 279-308.

Meharg AA, Williams PN, Adomako E. et al. (2009) Geographical variation in total and inorganic arsenic content of polished (white) rice. Environ Sci Technol. 2009 Mar 1;43(5):1612-7.

Noriega et al., 2012;

Noriega G, Caggiano E, Lecube ML et al. (2012) The role of salicylic acid in the prevention of oxidative stress elicited by cadmium in soybean plants. Biometals (2012) 25:1155–1165.

Norton GJ., Deacon CM., Xiong L., Huang S., Meharg AA., Price AH. (2010) Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. Plant Soil., 329:139–153.

Phan K., Sthiannopkao S., Kim KW., Wong MH., Sao V., Hashim JH., Mohamed Yasin MS., Aljunid SM. (2010) Health risk assessment of inorganic arsenic intake of Cambodia residents through groundwater drinking pathway. Water Res., 44(19):5777-5788.

Pinson SRM, Heuschele DJ, Edwards JD, et al. (2021) Relationships Among Arsenic-Related Traits, Including Rice Grain Arsenic Concentration and Straighthead Resistance, as Revealed by Genome-Wide Association. Frontiers in Genetics. 12. https://doi.org/10.3389/fgene.2021.787767

Prasad BD, Creissen G, Lamb C, Chattoo BB (2009) Overexpression of Rice (Oryza sativa L.) OsCDR1 Leads to Constitutive Activation of Defense Responses in Rice and Arabidopsis. Mol. Plant-Microbe Interact. 22 (12), 1635-1644.

Prasad BD, Jha S, Chattoo BB (2008) Transgenic indica rice expressing Mirabilis jalapa antimicrobial protein (Mj-AMP2) shows enhanced resistance to the rice blast fungus *Magnaporthe oryzae.* Plant sci. 175 (3), 364-371.

Prasad BD, Kumar P, Sahni S, et al. (2016) An improved protocol for agrobacterium-mediated genetic transformation and regeneration of indica rice (oryza sativa l. Var. Rajendra kasturi). J Cell Tissue Res 16(2):5597–5606.

Prasad BD., Mandal J., Kumar S., Sohane R K. (2020) Proceedings-cum-Abstract book National Webinar On Arsenic Mitigation: A Nexus Approach. pp. 1-131.

Roy P., Saha A. (2002) Metabolism and toxicity of arsenic: A human carcinogen. Curr. Sci., 38-45.

Sahni S and Prasad BD (2022) Salicylic acid induced resistance against mungbean yellow mosaic virus (MYMV) and enhanced seed yield in resistant and susceptible urdbean [Vigna mungo (L.) Heper] genotypes. Legume Res. 45 (1), 97-103.

Sahni S, Kumar S, BD Prasad BD (2021) Integration of salicylic acid, vermicompost and bioagent for effective management of chickpea wilt disease. Journal of Environmental Biology 42 (5), 1274-1280.

Sahni S., Prasad B., Liu Q. et al. (2016) Overexpression of the brassinosteroid biosynthetic gene *DWF4* in *Brassica napus* simultaneously increases seed yield and stress tolerance. Sci Rep 6:28298.

Sahoo PK., Kim K. (2013) A review of the arsenic concentration in paddy rice from the perspective of geoscience. Geosci J., 17:107–122.

Shajia E, Santosh M, Saratha KV, Prakasha P, Deepchand V, Divya BV (2021) Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. Geosci. Front. 12(3): 101079.

Singh AP., Dixit G, Mishra S, et al. (2015) Salicylic acid modulates arsenic toxicity by reducing its root to shoot translocation in rice (*Oryza sativa* L.). Front. Plant Sci. 6.https://doi.org/10.3389/fpls.2015.00340

Smedley PL.,.Kinniburgh DG. (2002) A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem., 17(5): 517-568.

Takahashi Y., Minamikawa R., Hattori KH., Kurishima K., Kihou N., Yuita K. (2004) Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. Environ Sci Technol., 38(4):1038-1044.

Upadhyay MK., Majumdar A., Kumar JS., Srivastava S. (2020) Arsenic in Rice Agro-Ecosystem: Solutions for Safe and Sustainable Rice Production. Front Sustain Food Syst., 4:53.

Vlot et al., 2009;

Vos IA, Moritz L, Pieterse CM, Van Wees SC (2015) Impact of hormonal crosstalk on plant resistance and fitness under multi-attacker conditions. Front Plant Sci 6:639.

Williams et al. 2005;

Williams PN., Islam MR., Adomako EE., Raab A., Hossain SA., Zhu YG., Feldmann J., Meharg AA. (2006) Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environ. Sci. Technol., 40(16):4903-4908.

Williams PN., Price AH., Raab A., Hossain SA., Feldmann J., Meharg AA. (2005) Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environ Sci Technol., 39(15):5531-5540.

Xu B., Yu JY., Xie T., Li YL., Liu MJ., Guo KX., Li LH., Yu Y., Zheng CY., Chen YH., Wang G. (2018) Brassinosteroids and iron plaque affect arsenic and cadmium uptake by rice seedlings grown in hydroponic solution. Biol Plant., 62:362–368.

Zafar SA., Zaidi SSEA., GabaY., Singla-Pareek SL., Dhankher OP., LiX., Mansoor S., Pareek A. (2020) Engineering abiotic stress tolerance via CRISPR/Cas-mediated genome editing. J Exp Bot., 71(2):470-479.

Zhang, J., Zhu, Y. G., Zeng, D. L., Cheng, W. D., Qian, Q., Duan, G. L. (2008). Mapping quantitative trait loci associated with arsenic accumulation in rice (*Oryza sativa*). *New Phytol.* 177, 350–356. doi: 10.1111/j.1469-8137.2007.02267.x

Zhao FJ, McGrath SP, Meharg AA. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annu Rev Plant Biol. 2010;61:535-59.