# **STRATEGIES FOR REDUCING ARSENIC CONTENT IN RICE: A REVIEW**

# **ÁTTEKINTÉS A RIZS ARZÉNTARTALMÁNAK CSÖKKENTÉSI LEHETŐSÉGEIRŐL**

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# **Abstract**

*Arsenic (As) is one of the most toxic metalloid that can enter the food chain through ingestion of As contaminated water or food, posing a serious threat to human health. Among cereals, rice could contain the highest amount of As because of the special growing conditions. Therefore, the importance of the reduction of As concentration in rice is essential. Many studies have been conducted to understand the mechanism of arsenic uptake, accumulation and translocation. The interactions between As and plants are influenced by soil type and other factors such as pH, mineral contents and redox status of the soil, As speciation, and microbial activity. Different nutrients including phosphates, iron, silicon and sulfur effectively regulate the uptake and accumulation of As in different parts of plants. Genetic variation has also effect on As accumulation of rice grain. Water management practices can help to decrease As content of rice plants due to changing the redox status of the soil. Phosphate and silicon transporters can be used by As to enter the rice root cells, therefore detoxification mechanisms of As in rice greatly depend on the activity of these transporters. In this review, we covered the main factors that affect the uptake, accumulation, and translocation of As in different plant organs in rice. We investigated the different soil factors and plant cell transporters needed to understand the mechanisms. This study may be useful for further research to develop strategies that inhibit As entry and transport in plant cells and contribute to safe food production.*

**Keywords**: *arsenic, varietal variation, water management, mineral nutrients application*

# **Összefoglaló**

*Az arzén (As) az egyik legmérgezőbb félfém, amely a szennyezett vizekkel és élelmiszerekkel bekerülhet az emberi szervezetbe is, és ott súlyos egészségügyi problémákat okozhat. A gabonafélék között a rizs az egyik, amely a legmagasabb koncentrációban tartalmazhat arzént. Ezért az arzéntartalom csökkentése alapvetően fontos a termelés során. Az arzén felvételének, felhalmozásának és a növényen belüli transzportjának a mechanizmusait számos tudományos publikációban vizsgálták. Az arzén felvételét számos tényező befolyásolja, mint a talajtípus, a pH, az ásványi anyagok mennyisége, a talajok redox tulajdonsága, az arzén kémiai formája és a talajok mikrobiológiai aktivitása. A különböző tápanyagok, mint a foszfor, a vas, a szilícium és a kén hatékonyan szabályozzák az arzén felvételét és felhalmozódását a különböző növényi részekben. Emellett a fajták közötti genetikai különbségek is befolyásolják az arzén beépülését a szemekbe. Az agrotechnikai lépések között a megfelelő vízgazdálkodási gyakorlatok*  *segíthetnek az arzénfelvétel csökkentésében a talaj redox tulajdonságainak szabályozása által. A foszfor és szilícium transzporterek segítségével juthat az arzén a rizs gyökérsejtekbe, ezért az arzén detoxifikáció folyamata nagyban függ ezektől a transzporterektől. Ebben az áttekintésben sorra vesszük az arzén felvételében, felhalmozásában és a rizsnövényen belüli transzportjában szerepet játszó főbb tényezőket. Megvizsgáljuk a különböző talajtulajdonságok és a sejttranszporterek szerepét is. Jelen tanulmányunk alapja lehet az arzénfelvétel csökkentését célzó új kutatási programok kidolgozásának és ezáltal hozzájárulhat a biztonságosabb élelmiszerellátáshoz.*

**Kulcsszavak:** *arzén, fajtakülönbségek, vízgazdálkodás, ásványi tápanyag alkalmazás*

#### **Introduction**

Potentially toxic elements (PTEs) negatively affect the environment as well as plants, animals and humans. Most of them are toxic causing abiotic stress; reduce plant growth and affecting food quality by entering the food chain, and ultimately threatening human health (F. J. ZHAO et al., 2009). Among the PTEs, arsenic (As) is considered as one of the most hazardous metalloid causing serious health diseases including cancer (CHANDRAKAR et al., 2016). The source of these elements can be natural or anthropogenic, like industrial emissions, application of fertilizers and pesticides, sewage irrigation, mining etc (ALAM, 2005). As-contaminated groundwater occurs in many regions of the world, the most severe problems reported from Bangladesh, West Bengal, China and Taiwan (WHO, 2001) (ABEDIN & MEHARG, 2002). Also reported groundwater contaminated with arsenic from Chile, Argentina, Mexico United States, India Italy and Hungary (PIGNA et al., 2009). The main forms of As in groundwater are inorganic, like arsenate  $(As(V))$  and arsenite  $(As(III))$  with neglected amounts of methylated As-species including monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (BAKHAT et al., 2017).

Paddy rice (Oryza sativa L.) is the main agricultural crop grown in arsenic-affected areas of Asian countries. Therefore, As accumulation in rice grown in those soils is a concern (ABEDIN et al., 2002b). There are different international standards or regulatory limits for As in food and drinking water. Recommended maximum level (MLs) for inorganic arsenic (iAs) in white rice is 0.20 mg  $kg^{-1}$ , in parboiled rice and husked rice 0.25 mg  $kg^{-1}$ , in rice waffles, rice wafers, rice crackers, and rice cakes  $0.30$  mg kg<sup>-1</sup> and in rice destined for the production of food for infants and young children 0.10 mg  $kg^{-1}$  (FAO, 2016). In aerobic conditions, the As(V) form is dominated which is analogous to the phosphate  $(PO<sub>4</sub><sup>3</sup>)$ . One of the mechanisms to uptake As by rice roots using high affinity  $PO<sub>4</sub><sup>3</sup>$  transporters (ABEDIN et al., 2002a). In rice paddy soils the As bioavailability is high because of the flooded conditions. Anaerobic circumstances contribute to the reduction of  $As(V)$  to  $As(III)$ , which is more soluble therefore can be easily uptaken by rice roots via the silicic acid transporters (X. CHEN et al., 2012; MA et al., 2008). The As(III) is a silicic acid analogous element, therefore its uptake happens through Si transporter aquaporin channels of the plant root cells. Methylated As species (MMA and DMA) can also be taken up similarly (R.-Y. LI et al., 2009b; MA et al., 2008).

Once As get into plants, they may affect many important biological processes inhibiting plant growth and reducing crop yields. As-toxicity causes oxidative stress and inhibits the ATP formation, which leads to lower grain yield (PANAULLAH et al., 2009). Phytotoxic symptoms were also observed by KHAN et al. such as stunted growth, brown spots, and scorching on leaves of plants in soils containing  $>60$  mg kg<sup>-1</sup> total As (KHAN et al., 2010). Other studies showed that excess As-exposure in the soil could negatively affect seed germination, plant shoot, and root growth. It can cause wilting and necrosis of leaf blades, reduces leaf area and photosynthesis (ABEDIN et al., 2002a).

ABEDIN et al. (2002a) also found that the As accumulation in rice tissues increased significantly with increasing soil As concentration. The highest amount of As concentrated in the root followed by straw, husk and grain. Similarly, in KÁDÁR and LEHOCZKY (2008) study, the higher As contaminated soil resulted in higher As accumulation in plant organs. For all the investigated plants, the concentration of As in the roots was higher than that of the above ground parts. In FODOR and SZEGEDI (2015) plot experiment As content was not detected in the grain of wheet, maize and sunflower, even the application of  $270 \text{ kg } \text{ha}^{-1}$  As treatment, however in the straws 5.2, 1.5 and 3.3 mg  $kg^{-1}$  were measured, respectively.

As accumulation and speciation in rice depend on both environment and genotype (NORTON et al., 2009a). The As level and As speciation of the grain are influenced by the redox value, pH, phosphate concentration of the soil, the formation of iron plaque in the rhizosphere, microbial activity and the rice variety. The oxidative rhizosphere in paddy soil modifies As forms associated with rice root surface (LIU et al., 2005).

Williams et al. found large varietal variation in As uptake and transport (WILLIAMS et al., 2005). The study of GENG et al. showed cultivar differences in the growth due to the As exposure (GENG et al., 2006). Based on these findings, the As content of rice grain can be reduced by choosing As-tolerant varieties. In addition, there are some agrotechnical methods for reducing As content in rice, including water management strategies and the application of different minerals e.g. phosphorus (P), silicon (Si), sulfur (S) and iron (Fe). These practices can change the As solubility in soil solution (FAROOQ et al., 2016).

### **Strategies for producing low As containing rice**

#### **Genetical variation in As accumulation**

Recent field studies have shown substantial genetic variation in rice grain As concentration and speciation. NORTON et al. (2009b) examined As concentration in the grains of 76 rice cultivars grown in two fields. The two sites had different levels of As in the soils and in the irrigation water. A strong correlation ( $r = 0.802$ ) was found in grain As concentrations of the 76 cultivars between the two locations, despite the different environments, suggesting stable genetic variations in As accumulation (NORTON et al., 2009b). However, in another study by NORTON et al. (2009a), among six sites, the environment made the most significant contribution to the variation in grain As concentration (61%), followed by genotype (6%) and genotype x environment interaction (19%) (NORTON et al. 2009a). In the studies of NORTON et al. (2009a, b) there was also a significant genotype effect in the percentage of iAs and DMA in grain, but the environmental influence was higher. Similarly, AHMED et al. (2011) investigated 38 Bangladeshi cultivars grown in ten different agroecological zones of Bangladesh. They reported large environmental effect (69–80%) of the variation in grain As concentration, whereas genotype and genotype x environment interactions accounted for only 9–10% and 10–21% of the observed variability (AHMED et al., 2011). Pillai et al. (2010) investigated 25 diverse rice cultivars at one field site over three growing seasons. They reported that the concentrations of total grain As and As species (As(III) and DMA) varied widely among the varieties. The effect of genotype on the As concentration and speciation of the rice grain accounted for about 70% of the variation, and the interaction between year and genotype accounted for about 20% (PILLAI et al., 2010). Comparing the studies of PILLAI et al. (2010), AHMED et al. (2011) and NORTON et al. (2009a), it can be concluded that genetic stability is greater across seasons than in different sites. A study by WILLIAMS et al. (2005) showed that the main As species of the rice grain were As(III) and DMA(V). The proportion of DMA in the grains significantly depended on the variety (P=0.026) (WILLIAMS et al., 2005).

Our previous study examined the As content of shoots of four rice varieties in different irrigation systems (flooded and aerobic). No significant differences were recognized among the varieties in aerobic conditions. In one case, however, we found a significant difference between two tested varieties ('M 488' and 'Nembo') in flooded conditions on soil treated with Humin Power G soil conditioner (SZALÓKI et al., 2022).

#### **Water management**

As discussed above, the anaerobic conditions in paddy soils support the reductive mobilization of As, so the As uptake is can be enhanced greatly by rice roots, leading to excessive As accumulation in rice grain and straw.In contrast, in aerobic treatments, the bioavailability of As reduces dramatically (R. Y. LI et al., 2009a). In a pot experiment, LI et al. (2009a) measured the concentration of total and iAs of rice grains in different water management systems. The continuously flooded rice showed the highest concentration of As in grain compared with the other treatments, followed by flooded-aerobic, aerobic-flooded and continuously aerobic conditions, where the iAs reductions were 25%, 46% and 87%, respectively (R. Y. LI et al., 2009a). In Arao et al. pot study, the results of water management treatments prove that the flooded condition increases the As concentration in the soil solution. The aerobic treatment for 3 weeks before and after heading was the most effective in decreasing the As content in grain, although the concentration of Cd in the grain increased considerably in anaerobic circumstances (ARAO et al., 2009). According to SPANU et al. (2020), intermittent irrigation methods effectively reduce the total amount of toxic elements including As in rice grain. Sprinkler irrigation was more effective method in reducing toxic elements in rice grain (90%) than the saturation irrigation (30%) compared to the continuous flooding. Moreover, the As concentration of the rice grain was decreased by 98.4% with sprinkler irrigation instead of flooded condition (SPANU et al., 2020). NORTON et al. (2019) also found strong correlation between the irrigation practices and As concentration of rice plants. For all tested cultivars, alternate wetting and drying (AWD) significantly reduced the As content of grain and shoot by more than 15 and 27 %, respectively, compared to the plants grown under continuous flooding (NORTON et al., 2019).

The water-saving cultivation methods were described by BOUMAN et al. (2007), such as aerobic rice, alternate wetting and drying (AWD) and raised bed cultivation (BOUMAN et al., 2007). These cultivation methods can also prevent excessive arsenic accumulation in rice. In addition, there is less input of As-contaminated irrigation water into the soil, so the rice absorbs less As (MEHARG & ZHAO, 2012).

A field experiment by TAKAHASHI et al. (2004) verified that As, Fe, and Mn concentrations of soil solution increased under flooded conditions. They also examined the oxidation state of As in soil and water using in-situ spectroscopic method. It was found that the As(III) concentration of soil enhanced from 30% (non-flooded conditions) to 70% under flooded conditions. The higher proportion of  $As(III)$  in the aqueous phase compared to  $As(V)$  suggests that  $As(V)$  is reduced to  $As(III)$ , which explains the increased  $As(III)$  concentration in groundwater (TAKAHASHI et al., 2004).

In our previous greenhouse pot experiment, the As concentration of the above-ground biomass was significantly higher in flooded than in aerobic conditions, as Figure 1. shows (SZALÓKI et al., 2022).



**Figure 1. Arsenic concentration of shoot biomass of four temperate japonica rice varieties in anaerobic (FC, FH) and aerobic (AC, AH) conditions of a greenhouse pot experiment. FC – flooded control, FH – flooded + humic acid, AC – aerobic control, AH – aerobic + humic acid (Szarvas, 2020). Different letters mean significant differences among the examined varieties at level 0.05 (SZALÓKI et al., 2022)**

#### **Role of phosphate**

The application of phosphate fertilizers plays a major role in the control of As uptake by rice in As-contaminated soils (FAROOQ et al., 2016; GENG et al., 2005).

As(V) is chemically analogous of phosphate (Pi) and the major arsenic form of aerobic soils, therefore enters plant root tissues through Pi-transporters (*OsPht1;8*) (WU et al., 2011). KAMIYA et al. (2013) also demonstrated the role of *OsPT1* transporter in As(V) uptake of rice from the soil (KAMIYA et al., 2013). Although As(III) is the main species of the soil solution of flooded paddy fields (TAKAHASHI et al., 2004; XU et al., 2008; LI et al., 2009), As(V) is also considered to be present (STROUD et al., 2011).

In a research by ABEDIN et al. (2002a) rice plants were irrigated with As-contaminated water and two phosphate doses were applied to observe the effect of  $PO<sub>4</sub><sup>3</sup>$  on As uptake and toxicity. The As content of rice parts (root, straw, husk and grain) as well as the agronomic parameters of the plants were not significantly affected by the  $PO<sub>4</sub><sup>3</sup>$  application in flooded (anaerobic) conditions (ABEDIN et al., 2002a).

Several studies showed that the As concentration of the soil solution increases with the application of higher doses of phosphorus fertilizer. The additional phosphate reduces the adsorption capacities of As(V) because of the competition between the two elements (LEE et al., 2016; LIU et al., 2004; ZENG et al., 2012). However, in different soils, the influence of P application on As(V) adsorption may vary (SIGNES-PASTOR et al., 2007). In that study, As solubility increased upon As(V) reduction and Fe was mobilized too. Under highly oxidative conditions, As and Fe solubilities were low and mainly associated with adsorption or coprecipitation onto amorphous Fe oxides. Under aerobic and moderately anaerobic conditions, almost all of the total As was As(V). In contrast, in anaerobic soil solution, the As(V) content was low.

Beside the redox potential, pH also played an important role in controlling the effects of the addition of P-fertilizers on As chemistry (SIGNES-PASTOR et al., 2007).

PIGNA et al. (2009) reported lower As toxicity in wheat according to Pi applications (PIGNA et al., 2009). In LU et al. (2010) study the higher P/As molar ratio in rice shoot resulted in lower As concentration of rice grain (LU et al., 2010). Additionally, the mobility of As can be reduced by co-application of Ca and Pi, as Ca-P-As complex is formed (NEUPANE & DONAHOE, 2013).

#### **Role of silicon**

Silicon (Si) is the second most abundant element in soil. Although Si is not considered an essential element for higher plants, it has a beneficial effect on overcoming stresses (W. CHEN et al., 2011).

It has been reported that Si alleviates the stress caused by salinity, drought, high temperature, and exposure to heavy metals by amplifying the antioxidant enzyme activity, helping to bind heavy metal sequestration in the vacuoles and co-precipitating or chelating with heavy metalloids (W. CHEN et al., 2011; LIMMER et al., 2018; Ma, 2004).

Since As(III) is chemically analogous to Si, it enters rice roots via silicon transporters. These transporters were identified as Nodulin26-like Intrinsic Proteins (NIPs) subfamily of plant aquaporins. OsNIP2;1 (Lsi1) in rice plants have been localized to the plasma membrane, and its main function is silicic acid transport in rice roots (MA et al., 2006). It has been studied that in addition the As(III), methylated As species, i.e., monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA) were also taken up by rice roots via silicon transporter Lsi1 (LI et al. 2009b). While Lsi1 is responsible for As(III) influx to the rice root cells, another transporter, Lsi2 mediates As(III) efflux toward the xylem. Moreover, As(III) transport to the xylem and accumulation in the shoots was inhibited in wild-type rice by addition of Si fertilizer to the nutrient solution (MA et al., 2008). In a greenhouse experiment of LI et al. (2009a) the applied Si fertilizer decreased the total As concentration of rice straw, husk and grain in flooded conditions by 78, 50 and 16 %, respectively. Si treatment also affected As speciation decreasing the content of iAs forms, but increasing the DMA in rice grain. This efficiency of silicic acid/arsenite assimilation and mobilization of As(III) under reduced conditions differentiates rice from crops grown under aerobic soil conditions for high grain As loading (ZHAO et al., 2010).

#### **Role of Sulfur**

Sulphur (S) can influence the regulation and mobility of As within plants due to its complexation with As, resulting in the formation of thio-rich compounds such as glutathione (GSH) and phytochelatins (PCs) (ZHAO et al., 2010). These nonprotein thiol complexes play a crucial role in the detoxification of As (TRIPATHI et al., 2007). SONG et al. (2010) investigated the subcellular localization of thiol compounds in the roots of rice plants and after As exposure, the complexes were localized to the vacuoles (SONG et al., 2014). In a research by DUAN et al. (2011) PCs concentration of rice shoots correlated negatively with the As accumulation of rice grains (DUAN et al., 2011). In DIXIT et al. (2016) study enhanced S doses resulted in enhanced As accumulation in root, but reduced the concentration of As in shoots. It means the translocation factor (root/shoot) of As was decreased by increasing S supplementation indicating less As translocation from root to shoot in 5mM than 0.5 mM S treatment. In addition, higher S level can relieve the As-caused oxidative stress by decreasing H<sub>2</sub>O<sub>2</sub> accumulation in both root and shoot. The activities of arsenate reductase (AS) and ascorbate oxidase (AAO) enzymes were enhanced by the addition of more S. Superoxide dismutase (SOD) activity increased in roots with elevated S levels, but decreased in shoots at the same level of As treatment, as did APX activity. Relative expression of As transporter Lsi1 was upregulated and Lsi2 was downregulated with increasing As content and the S treatments

decreased both of Lsi1 and Lsi2 gene expression compared to the 0 S application (DIXIT et al., 2016). Another effect is the application of S increased concentrations of extractable Fe/Mn in iron plaque formation without As addition. In As treatments the addition of  $SO_4^2$  reduced the concentration of As in dithionite-citrate-bicarbonate (DCB) extracts (HU et al., 2007).

### **Conclusion**

Many experiments were investigated to find answers to As behaviour in rice plants in both aerobic and anaerobic circumstances. In this minor review, the relevant results of researches were gathered about As bioavailability, uptake, mobility and toxicity. The possible strategies for reducing As concentration in rice grains have also been reviewed. BAKHAT et al. summarizes the mechanisms of As uptake, translocation and detoxification and shows the effect of different nutrients on As accumulation and mobilization in rice plants (BAKHAT et al., 2017).

Rice is a more effective plant to accumulate As in its tissues because it is mostly cultivated in flooded conditions where the As becomes available for the plants. Furthermore, the formation of As in anaerobic conditions is arsenite  $(As(III))$  which is more toxic than arsenate  $(As(V))$ , the main form of As in aerobic conditions. However, some possibilities exist to reduce the bioavailability of As, including genetic variations, water management practices or applications of different nutrient fertilizers.

One of the effective irrigation methods to relieve the As toxicity is alternative wetting instead of continuously flooded irrigation. Aerobic rice cultivation can also be a solution if the irrigation system is suitable to cover the high water demand for rice. These practices change the redox status of the soil.

Genetic variation also influences on As concentration of rice. Some studies found significant effect of genotype on As content of rice grain, however the impact of the environment was higher.

The As proportion of rice roots is higher than in above-ground part of the plants, but the appreciable amount of As can be transported to other tissues. Different transporters are used by As to enter the plants, such as Pi- and Si transporters (As(V) and As(III), respectively).

The application of  $PO_4^3$  fertilizers can control the As(V) uptake through competition between the two elements for binding sites. However, the high As concentration in soil with an increasing  $PO_4^3$  addition can enhance the As toxicity. Therefore, in As-contaminated areas it is suggested to apply less amount of soil fertilizer  $PO<sub>4</sub><sup>3</sup>$  with a supplemented foliar fertilizer to avoid As toxicity and environmental problems caused by P.

The main As species in paddy fields is As(III), which uses Si transporters to enter rice roots, as do methylated organic species. Although Si application can increase the As uptake by its desorption from soil particles, the concentration of As in rice shoots and grains is reduced because of the inhibition of As translocation.

Under anaerobic conditions  $SO_4^2$  forms insoluble complexes with As. These non-protein thiol compounds, like glutathione and phytochelatins play an important role in Asdetoxification via sequestration of the complexes to vacuoles and inhibition of As translocation from root to shoot. In addition, S treatment increases iron plaque formation on rice root surface, and iron plaque formation alleviates arsenic toxicity in rice plants.

In summary, the investigations must continue to find sustainable methods that can be effectively applied in Hungarian and other temperate rice-cultivating countries.

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