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Research Focus

Arsenic in rice – understanding a new disaster for South-East Asia

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A study by Yongguan Zhu and co-workers has added greatly to our understanding of arsenic dynamics in the rhizosphere of paddy rice. Their finding that arsenic is sequestered in iron plaque on root surfaces in plants, regulated by phosphorus status, and that there is considerable varietal variation in arsenic sequestration and subsequently plant uptake, offers a hope for breeding rice for the new arsenic disaster in South-East Asia – the contamination of paddy soils with arsenic.

The sinking of tubewells into Holocene aquifers (formed over the past 10 000 years) in South East Asia to provide disease-free drinking water to some of the most densely populated areas of the globe, such as the Bengal and Red River delta, has resulted in the worst chemical disaster in human history [1]. Aquifers that provide drinking water to tens of millions of people are arsenic contaminated, resulting in widespread arsenic-related disease. But tubewells are not just used for drinking water; they are also widely used for rice cultivation, particularly during the dry season. This has led to an arsenic build-up in paddy soil, and has resulted in a ten-fold elevation in arsenic levels in rice grain [2]. There are also extensive areas of arsenic pollution from metal mining in Thailand and China, resulting in widespread contamination of paddy field soils and elevation in grain arsenic [3]. For populations living on subsistence rice diets, this arsenic contamination of rice grain contributes greatly to dietary arsenic exposure. When drinking water levels of arsenic are at the World Health Organization's 10 mg/l limit, 0.05 mg/kg arsenic in rice contributes ~60% of dietary arsenic exposure. Rice with arsenic levels of 1.8 mg/kg has been recorded in the arsenic-affected tubewell areas of Bangladesh [2]. Even at dangerous levels of arsenic in drinking water of 1 mg/l, rice arsenic levels of 1.8 mg/kg contribute ~30% to dietary arsenic intake. Arsenic contamination of rice is a newly uncovered disaster on a massive scale. It is crucial that the physiology and genetics of rice uptake of arsenic is understood to counteract this widespread contamination of the food chain.

Iron plaque binding

Liu *et al.* [4] have made an important contribution to our understanding of arsenic interaction with paddy rice (*Oryza sativa*). It has been known for some time that paddy rice, like other plants adapted to grow on anaerobic flooded soils, oxygenate their rhizosphere resulting in the formation of an iron oxyhydroxide plaque [5]. Observations on emergent macrophyte roots in flooded sediments found a considerable bioconcentration of arsenic in root surface iron plaque [6]. In their experiments with paddy rice, Lui *et al.* [4] proved that the same arsenic concentration occurs in rice root iron plaque, and additionally, that the plaque formation was governed by plant phosphorus status. Under low phosphorus conditions, plaque formation is greatly increased. Importantly, given the considerable food chain concerns about arsenic in rice, there are significant varietal differences in plaque formation and, therefore, arsenic sequestration in the plaque. Varieties that sequester more arsenic in plaque translocate less arsenic to aboveground tissues.

Complexity of the rhizosphere

The rhizosphere chemistry of arsenic is complex [4] (Figure 1). Under paddy field conditions, inorganic arsenic introduced into the soil is inter converted between the reduced inorganic species arsenite and the oxidized species arsenate. Soil microbes can methylate inorganic arsenic to give monomethylarsonic (MMA) acid and dimethylarsinic acid (DMA) [7]. Arsenite dominates under paddy conditions, but arsenate, MMA and DMA are also present in significant quantities [8]. However, it is arsenate that has a high affinity for iron plaque, reacting with Fe (III) to give the highly insoluble iron arsenate. Arsenate behaves as a phosphate analogue [7], and like phosphate is relatively immobile in soil. Arsenate enters into plant root tissues via phosphate co-transporters. Furthermore, iron plaque also locks up phosphate and rice roots excrete phytosiderophores to complex Fe (III) to liberate iron-associated phosphate. Rice can also exhibit iron deficiency as a result of plaque formation, so phytosiderophores also have a role in plant iron acquisition. Arsenite is more mobile. The first study on arsenite transport into roots was for rice [8], which showed high rates of active transport of arsenite into the roots. This study also showed that MMA and DMA influx was at a much lower level than either arsenite or arsenate. Subsequently, arsenite has been shown to be transported into rice roots via aquaporins [9]. At neutral pH, arsenite is uncharged and behaves as a water analogue with respect to plasma membrane transport. This had previously been observed in yeast [10].

The dynamics of arsenic in the rhizosphere are controlled by plant phosphate status, regulating iron

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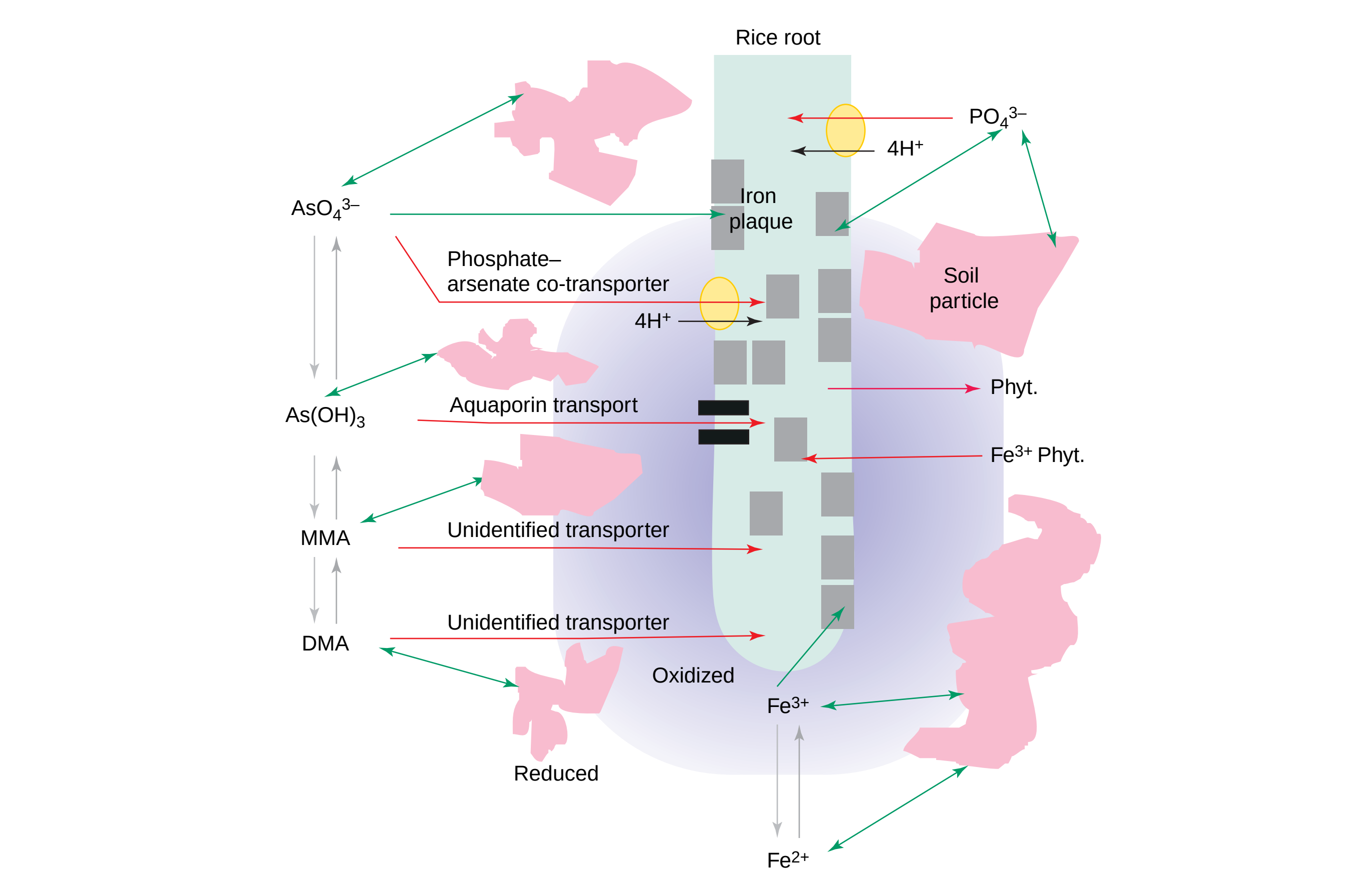

Figure 1. Dynamics of arsenic species in the rhizosphere of rice. Green arrows show adsorption to soil particulates. Red arrows show active plant transport processes. The blue circle illustrates the zone of oxygenation around the growing rice root. Abbreviations: AsO_4^{3-}, arsenite; $As(OH)_3$, arsenate; DMA, dimethylarsinic acid; Fe^{2+}, iron (II); Fe^{3+}, iron (III); H^+, proton; MMA acid, monomethylarsonic acid; Phyt., phytosiderophores; PO_4^{3-}, phosphate.

plaque formation and also feedback regulation of arsenate uptake via phosphate transporters; by soil redox potential, regulating inter-conversion between arsenate and arsenite; and by microbial oxidation or reduction and methylation of arsenic, producing MMA and DMA, which are poorly transported across the plasma-membrane of root epidermal cells. Soil mineralogy will also play an important role, regulating the soil solution concentration of arsenic species because of surface binding and precipitation of poorly soluble arsenic salts.

Breeding rice for low grain arsenic

Creating a model of arsenic dynamics in the rhizosphere enables strategies to be devised for selecting or breeding rice varieties suitable for growing on arsenic-contaminated soils. Liu *et al.* [4] have shown that there were considerable varietal differences in shoot transport of arsenic, with the varieties that produce more plaque able to sequester more arsenic in this plaque, thus reducing arsenic uptake into plant tissues. Another important recent finding is that there are varietal differences in arsenate resistance and that this resistance is conferred by a single gene [11]. Dose–response curves for arsenate toxicity to the rice varieties Azucena and Bala differ greatly, with root elongation in Azucena being inhibited by 90% at 1 mg/l arsenate compared with 50% inhibition in Bala. When F6 inbred lines of crosses between Azucena and Bala were tested for arsenic resistance, there was a clear segregation into tolerant and non-tolerant phenotypes. Quantitative trait locus (QTL) analysis showed that this segregation was under the control of a single gene and that this gene mapped to a site on chromosome six where a phosphate–arsenate co-transporter resides. Arsenate tolerance has been widely investigated in wild grasses where arsenate tolerance is regulated by a single gene; that gene is polymorphic in populations of *Holcus lanatus* [7] from arsenic uncontaminated soils, and in a range of other grass species (A.A. Meharg, unpublished). It appears that rice also exhibits this polymorphism for arsenate tolerance [11]. It has been argued [7] that the polymorphism is because of phosphorus nutrition, not arsenic contamination. Arsenic tolerance is conferred through differences in phosphorus uptake and use between arsenate tolerant and non-tolerant phenotypes. In wild grasses, tolerance is achieved by suppression of arsenate uptake through the constitutive suppression of high-affinity phosphate–arsenate plasma-membrane co-transporters. The tolerance gene identified in rice is also a phosphate–arsenate transporter. It appears that rice and wild grasses exhibit considerable genetic variation in arsenate uptake because tolerant plants take up less arsenic than non-tolerant plants at sub-lethal concentrations [7]. If this proves to be true for rice, another strategy exists, along with selection of rice lines that produce high levels of iron plaque, to breed rice that translocates low amounts of arsenic to shoots. Other characters, such as low arsenite transport and low transfer of arsenic to grain could also be selected for.

Arsenic contamination of paddy soils over substantial areas of South-East Asia now exists. The soils will stay contaminated, as it is not cost effective to remediate paddy fields. The only option is to breed rice with low levels of arsenic in grain. Variation in arsenic tolerance and iron plaque formation are the starting points for breeding rice for arsenic affected soils.

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