

1 **Transport of DMAA and MMAA into Rice (*Oryza sativa* L.) Roots**

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18 Abstract:

19 Arsenate (As(V)) transport into plant cells has been well studied. A study on rice (*Oryza sativa*
20 L.) showed that arsenite is transported across the plasma membrane via glycerol transporting
21 channels. Previous studies reported that the dimethylarsinic acid (DMAA) and
22 monomethylarsonic acid (MMAA) uptake in duckweed (*Spirodela polyrhiza* L.) differed from
23 that of As(V), and was unaffected by phosphate (H_2PO_4). This article reports the transport
24 mechanisms of DMAA and MMAA in rice roots. Linear regression analysis showed that the
25 DMAA and MMAA uptake in rice roots increased significantly ($p \leq 0.0002$ and ≤ 0.0001 for
26 DMAA and MMAA, respectively) with the increase of exposure time. Concentration-dependent
27 influx of DMAA and MMAA showed that the uptake data were well described by Michaelis-
28 Menten kinetics. The MMAA influx was higher than that of DMAA. The DMAA and MMAA
29 uptake in rice roots were decreased significantly ($p \leq 0.0001$ and ≤ 0.0077 for DMAA and
30 MMAA, respectively) with the increase of glycerol concentration indicating that DMAA and
31 MMAA were transported into rice roots using the same mechanisms of glycerol. Glycerol is
32 transported into plant cells by aquaporins, and DMAA and MMAA are transported in a dose-
33 dependent manner of glycerol which reveals that DMAA and MMAA are transported into rice
34 roots through glycerol transporting channels. The DMAA and MMAA concentration in the
35 solution did not affect the inhibition of their uptake rate by glycerol.

36

37 **Keywords:** Arsenic, DMAA, MMAA, Rice (*Oryza sativa* L.), Aquaporins, Influx.

38

39 **Introduction:**

40 Although arsenic contamination in groundwater has been reported in many countries,
41 Bangladesh (Acharyya et al., 1999; Alam and Sattar, 2000; Alam et al., 2002), West Bengal,
42 India (Mandal et al., 1996; Chowdhury et al., 1999), China (Guo et al., 2001; Sun, 2004), and
43 Taiwan (Schoof et al., 1998; Guo et al., 2001) are the mostly affected areas. In Bangladesh and
44 West Bengal (India), the arsenic contaminated groundwater has been used not only for drinking
45 purpose but also for crop irrigation, especially for rice cultivation. Presently, 75% of the total
46 cropped area and 83% of the total irrigated area are used for rice cultivation in Bangladesh (Dey
47 et al., 1996), which are mostly dependent on groundwater irrigation. Survey from Bangladesh
48 show that irrigation with arsenic contaminated groundwater is leading to the elevation of arsenic
49 in paddy soils (Alam and Sattar, 2000). Although the background levels of arsenic in soils of
50 Bangladesh ranged between 4 and 8 mg kg⁻¹, up to 83 mg kg⁻¹ soil arsenic has been reported in
51 areas irrigated with contaminated water (Abedin et al., 2002). Irrigation of arsenic contaminated
52 groundwater during dry season rice production has been adding > 1000 metric tons of arsenic to
53 the soil per year in Bangladesh alone (Alam and Sattar, 2000; Meharg and Rahman, 2002). A
54 substantial amount of arsenic is accumulated from soil and irrigation water and is deposited in
55 rice grain, which has the potential to create health disaster for the population in Southeast Asia
56 (Meharg, 2004). Worldwide market surveys show that rice grain contains considerably higher
57 amount of arsenic than that in other food items (Schoof et al., 1999; Roychowdhury et al., 2002;
58 Williams et al., 2007). Therefore, rice could be substantial for the population of arsenic epidemic
59 areas.

60 Studies on the kinetics of arsenic uptake in plant roots have focused almost entirely on
61 arsenate as this is the dominant form of plant available arsenic in aerobic soils (Meharg and

62 [Jardine, 2003](#)). In flooded condition, arsenite becomes the predominant species of arsenic
63 ([Takahashi et al., 2004](#)). There is evidence of arsenic methylation in paddy soil systems by
64 microorganisms ([Takamatsu et al., 1982](#)). A number of studies have been investigated the
65 mechanisms of arsenic uptake by different plant species ([Meharg and Macnair, 1992](#); [Meharg
66 and Macnair, 1994](#); [Rahman et al., 2008a](#); [Rahman et al., 2008c](#)). Plants take up arsenate through
67 the phosphate transporters ([Meharg and Hartley-Whitaker, 2002](#); [Wang et al., 2002](#)). Although
68 the exact mechanisms of arsenite uptake in higher plants has not been identified, physiological
69 studies suggests that arsenite is transported in rice by aquaporins ([Abedin et al., 2002](#); [Meharg
70 and Jardine, 2003](#)). A recent molecular study explained more clearly that arsenite is transported
71 into rice roots by nodulin 26-like intrinsic membrane proteins (NIPs), one of the major
72 subfamilies of aquaporins transporter that facilitates the transport of neutral molecules such as
73 water, glycerol, and urea ([Ma et al., 2008](#)).

74 Uptake of organoarsenic species by plants is lower than that of inorganic species
75 ([Odanaka et al., 1987](#); [Rahman et al., 2007](#)). [Marin et al. \(1992; 1993\)](#) observed high uptake of
76 inorganic arsenic species, dimethylarsinic acid (DMAA) and monomethylarsonic acid (MMAA),
77 in rice plant in hydroponic culture. Whatever the amount was, previous studies confirmed the
78 uptake of organoarsenic species (DMAA and MMAA) in rice and other plant species. Although
79 the uptake mechanisms of inorganic arsenic species such as arsenate and arsenite in rice have
80 been studied ([Abedin et al., 2002](#); [Meharg and Jardine, 2003](#); [Ma et al., 2008](#)), the uptake
81 mechanisms of organoarsenic species are overlooked. [Rahman, et al. \(2008a\)](#) observed that the
82 DMAA and MMAA uptake in duckweed (*Spirodela polyrhiza* L.) was much lower than that of
83 As(V) and As(III), and the uptake was not influenced by phosphate. This might be because the
84 mechanisms of organoarsenic species uptake in plants differed from that of inorganic arsenic

85 species, and the physicochemical adsorption would be one of the possible mechanisms of
86 DMAA and MMAA uptake in aquatic plants. [Robinson, et al. \(2003\)](#) also proposed
87 physicochemical adsorption as an alternative mechanism for DMAA and MMAA uptake in New
88 Zealand watercress (*Lepidium sativum*).

89 Uptake mechanisms of DMAA and MMAA in rice have not studied extensively. [Abedin](#)
90 [et al. \(2002\)](#) studied the uptake kinetics of arsenic species in rice, and mostly focused on
91 inorganic arsenic species, arsenate and arsenite. This study investigates the uptake kinetics of
92 DMAA and MMAA into rice roots to observe how these species are taken up into the plant cells.
93 Since plant aquaporins transport neutral molecules such as water, glycerol, and urea ([Dean et al.,](#)
94 [1999; Ma et al., 2008](#)), and organoarsenic species are not taken up into plants by phosphate
95 uptake pathway; there is a possibility of DMAA and MMAA uptake through the aquaporins
96 water channels. Studies showed that the rice aquaporin Lsi1 mediates uptake of methylated
97 arsenic species ([Li et al., 2009](#)). In the present study, we investigated the competition between
98 glycerol and DMAA and MMAA for uptake into rice roots.

99

100 **Materials and Methods:**

101 **Seed sterilization**

102 Rice seeds of BRRI (Bangladesh Rice Research Institute) dhan 29 were collected from
103 Bangladesh Rice Research Institute, Gazipur. The seeds were surface-sterilized before using
104 them in the experiment. For sterilization, about 100 g seeds were soaked in 200 mL of 1%
105 methyl-1-butylcarbamoyl-2-benzimidazole carbonate solution for 10 min. After that, the seeds
106 were washed by deionized water (using an E-pure system (Barnstead)) and kept in deionized

107 water at 20 °C for 24 h. The seeds were then washed and transferred to deionized water of 45 °C
108 for 2 min, and of 52 °C for 10 min.

109

110 **Plant growth**

111 Sterilized rice seeds were soaked in deionized water for 48 h, and were germinated on
112 moistened filter paper placed within petri dishes. When the germinated seeds produced enough
113 roots and about 2 cm of shoot, the small seedlings were transferred to a 500-mL polystyrene
114 beaker filled with 400 mL of distilled water. The seedlings were placed on the water with a
115 support in such a way that only the roots of the seedlings emerged into the water. The rice
116 seedlings were allowed to grow for 1 wk in the distilled water. Nutrient salts and other
117 osmoregulators were not added to the water so that they could not alter the arsenic transporter
118 regulation in an unknown manner (Meharg and Jardine, 2003). Rice seedlings were grown in a
119 plant growth chamber, and the conditions in the chamber were set as 14:10 h light/dark schedule,
120 100-125 $\mu\text{E m}^{-2} \text{s}^{-1}$ light intensity, 22(\pm 2) °C temperatures.

121

122 **Uptake kinetics**

123 After 1 wk growth, sufficient numbers of roots were produced from the basal node.
124 Replicated rice seedlings were then transferred to aerated water solution (having no nutrient
125 salts) for 30 min at room temperature. They were then incubated in aerated test solutions
126 (distilled water without nutrient salts) for 1 h with different concentrations (ranged between 0.1
127 and 0.7 mM) of DMAA and MMAA for concentration-dependant uptake experiment. Replicated

128 rice seedlings were incubated in test solution (distilled water without nutrient salts) with 0.1 mM
129 arsenic for time-dependant uptake experiment, and the samples were collected at a 10 min
130 interval. In DMAA and MMAA transport assay, 0.3 mM or 10 μ M DMAA or MMAA was
131 added to 10, 50, 100, 500, and 1000 mM glycerol solutions. Replicated rice seedlings were
132 incubated into these solutions for 1 h. The test solution was adjusted to pH 7 using weak
133 solutions of HCl or NaOH. Stock solutions of DMAA and MMAA were prepared from
134 dimethylarsinic acid ($((\text{CH}_3)_2\text{AsO}(\text{OH}))$) and $(\text{CH}_3\text{AsO}(\text{OH})_2)$, respectively. Glycerol was
135 purchased from Kanto Chemical Co., Japan (purity 99.0%).

136

137 **Sample preparation and chemical analysis**

138 After the set time, the roots were quickly rinsed in ice cold distilled water, and then
139 placed in aerated ice cold distilled water for 20 min (Meharg and Jardine, 2003). The roots were
140 washed once again with distilled water and blotted dry with tissue papers. Now the roots of the
141 rice seedlings were excised at the basal node, and the fresh weight of the roots were determined.
142 The roots were then taken into 50-mL polyethylene digestion tubes, and 3 mL of 65% HNO_3
143 were added to the samples and allowed to stand for 12 h. The samples were heated on a heating
144 block at 95 $^\circ\text{C}$ for 90 min. After cooling to room temperature, 2 mL of 30% hydrogen peroxide
145 were added, and heated again at 105 $^\circ\text{C}$ for 30 min. On cooling, the residue was taken was
146 diluted to 10 mL with deionized water, and analyzed for total As. At least one reagent blank and
147 two certified standard reference materials (1573a, tomato leaf from National Institute of
148 Standards and Technology (NIST), Department of Commerce, United States of America) were
149 included in the digestion. Chemical analysis for arsenic was performed by graphite-furnace

150 atomic absorption spectrometer (Z-8100, Hitachi, Japan). Certified standard reference material
151 1573a (tomato leaf from NIST, USA) was used to check the accuracy of analysis. Arsenic
152 concentration in certified standard reference materials was $0.112 \pm 0.004 \mu\text{g g}^{-1}$ dry weight while
153 the measured concentration was $0.114 \pm 0.002 \mu\text{g g}^{-1}$. The concentrations detected in all samples
154 were above the instrumental limits of detection ($\geq 0.01 \mu\text{M}$ in water sample).

155 All chemical reagents used in this experiment were of analytical grade. Glassware and
156 dishes were washed with detergent and 1 N HCl solution, and rinsed with deionized water for
157 eight times before use.

158

159 **Statistical Analysis**

160 Data were analyzed for linear and nonlinear regression using GraphPad Prism (v5)
161 (GraphPad Software, Inc., CA, USA). Kinetic parameters for DMAA and MMAA uptake were
162 calculated from mean arsenic influx ($n = 3$) by linear and nonlinear regression models.

163

164 **Results and discussions:**

165 **Uptake kinetics of DMAA and MMAA**

166 Time- and concentration-dependent uptake kinetics of DMAA and MMAA were
167 determined to assess the pattern and efficiency of organoarsenic species influx in rice roots.
168 Since the uptake kinetic is calculated from the influx (uptake into the plant cells) across the
169 plasma membrane, it is important to measure the adsorption of arsenic on rice root surfaces. The

170 adsorption of As(V) and As(III) on roots of terrestrial and aquatic plants has been reported by
171 several researchers (Otte et al., 1995; Hansel et al., 2002; Blute et al., 2004; Chen et al., 2005;
172 Rahman et al., 2007; Rahman et al., 2008c). A significant amount of As(V) is adsorbed on Fe-
173 oxides (Fe-plaques) on rice root surfaces (Chen et al., 2005; Rahman et al., 2008b) because of
174 high adsorptive affinity of As(V) to Fe-oxides, while DMAA and MMAA adsorption, either on
175 Fe-plaques or on rice roots, is negligible (Rahman et al., 2007).

176 Even though there was a little chance of chemical adsorption of arsenic on Fe-oxides in
177 the Fe-free experimental solution (distilled water without nutrient salts), Meharg and Jardine
178 (2003) reported significant physical adsorption of As(V) and As(III) on rice roots. Meharg and
179 Jardine (2003) evaluated the desorption of arsenic from rice root surface by ice cold distilled
180 water and NaCl (0.1 M) solution. Since there was no significant differences of these two washing
181 methods in arsenic desorption from rice root surface, they proposed ice cold distilled water
182 washing as the appropriate method. Therefore, rice roots were washed with ice cold distilled
183 water in this study to remove arsenic physically adsorbed on rice roots.

184 Time-dependent uptake showed that the influxes of both DMAA and MMAA were linear
185 upon 60 min. of exposure (Fig. 1). The DMAA and MMAA uptakes were well described by a
186 linear function ($r = 0.688$ and 0.756 for DMAA and MMAA, respectively), and their uptakes
187 were increased significantly ($p = 0.0002$ and < 0.0001 for DMAA and MMAA, respectively)
188 with the increase of exposure time (Table 1). Meharg and Jardine (2003) reported that As(III)
189 influx was linear up to 30 min, and further influx did not occurred probably due to the
190 toxicological inhibition as As(III) exhibits phytotoxicity through binding with protein -SH
191 groups. Present result showed that DMAA and MMAA did not show phytotoxicity up to 60 min.
192 Although organoarsenic species are generally considered to be less toxic than inorganic species

193 to a wide range of organisms including aquatic plants, animals and humans (Tamaki and
194 Frankenberger, 1992), long-term arsenic uptake studies showed that the phytoavailability of four
195 arsenic species to *Spartina patens* in hydroponic systems followed the trend: DMAA < MMAA
196 \cong As(V) < As(III), while the order of phytotoxicity was As(V) \cong As(III) < MMAA < DMAA.
197 Studies also suggests that organoarsenicals would be more toxic than inorganic arsenic species
198 (Carbonell-Barrachina et al., 1998). In another study with arsenate, arsenite, and DMAA influx
199 in maize (*Zea mays* L.), Abbas and Meharg (2008) found low toxicity of DMAA compared with
200 arsenate and arsenite, and the relative toxicity of arsenic species on maize was As(V) > As(III) >
201 DMAA. The phytoavailability of arsenic by rice, however, in long-term hydroponic culture was
202 DMAA < As(V) < MMAA < As(III), and the order of phytotoxicity was the same as the order of
203 phytoavailability (Marin et al., 1992). Moreover, short-term uptake of MMAA and DMAA was
204 considerably less than that of As(V) and As(III) in rice (Abedin et al., 2002), which is consistent
205 to the time-dependent DMAA and MMAA uptake in rice roots of present study. Thus, from the
206 above discussions it could be assumed that the uptake and toxicity of arsenic species are related
207 to the plant species as well as to the exposure time depending on their resistance mechanisms.

208 Concentration-dependent influx showed that the DMAA uptake was poorly described by
209 Michaelis-Menten kinetics ($r^2 = 0.688$), but well explained by linear function ($r^2 = 0.837$) (Fig.
210 2; Table 1). On the other hand, MMAA uptake showed a hyperbolic increase with the increase of
211 MMAA in the experimental solution (Fig. 3). The MMAA uptake fitted well to the Michaelis-
212 Menten kinetics ($r^2 = 0.914$) as well explained as linear function ($r^2 = 0.904$) (Table 1). These
213 results are also in consistent with those of Abedin et al. (2002). The MMAA influx was higher
214 than that of DMAA (Figs. 2 and 3). At a substrate concentration of 0.7 mM, the uptake rates of
215 DMAA and MMAA were 1.25 and 1.74 $\mu\text{mol g}^{-1}$ fresh weight h^{-1} , respectively. Kinetic

216 parameters also showed that V_{\max} for DMAA and MMAA were 0.757 and 3.619 $\mu\text{mol g}^{-1}$ fresh
217 weight h^{-1} (Table 1). [Abedin et al. \(2002\)](#) also reported similar results for DMAA and MMAA in
218 rice roots.

219

220 **Inhibition of DMAA and MMAA uptake by glycerol**

221 Water channels or water channel proteins (WCPs) are transmembrane proteins that have a
222 specific three-dimensional structure with a pore that permeates water molecules ([Benga, 2009](#)).
223 The WCPs belong to the superfamily of major intrinsic proteins (MIPs) (over 800 members) that
224 are present in plants, animals, and microorganisms. The WCPs include three subfamilies: i) the
225 water specific aquaporins (AQPs), ii) aquaglyceroporins (permeable to water, glycerol, and/or
226 other small, neutral molecules), and iii) superaquaporins or subcellular AQPs ([Agre, 2004](#);
227 [Benga, 2009](#)). In addition to water, some MIPs seem to be specific to other molecules such as
228 urea, glycerol or even CO_2 ([Maurel et al., 1994](#); [Baiges et al., 2002](#)).

229 The competition between glycerol and arsenite for uptake into rice (*Oryza sativa* L.)
230 ([Meharg and Jardine, 2003](#)) and *Saccharomyces cerevisiae* ([Wysocki et al., 2001](#)) reveal that
231 arsenite is transported across the plasma membrane through WCPs/aquaporins. Previous studies
232 reported that the DMAA and MMAA uptake mechanisms into plant tissues differ from those of
233 arsenate ([Mkandawire and Dudel, 2005](#); [Rahman et al., 2008a](#)). In the present study, we
234 investigated the effect of glycerol on DMAA and MMAA uptake in rice roots to understand the
235 uptake mechanisms of these organoarsenic species. Results showed that glycerol inhibited
236 DMAA and MMAA uptake in rice roots significantly ($p \leq 0.0001$ and 0.0077 for DMAA and

237 MMAA, respectively; [Table 2](#)) in a concentration dependant manner ([Fig. 4](#)), which is consistent
238 to the arsenite uptake in rice roots ([Meharg and Jardine, 2003](#)).

239 Among DMAA and MMAA, the DMAA influx was about two times greater than that of
240 MMAA. DMAA and MMAA influx in rice roots were higher at low glycerol concentrations (10-
241 50 mM) than those at higher concentrations (100-1000 mM) ([Fig. 4](#)). [Meharg and Jardine \(2003\)](#)
242 elucidated the possible explanations for the inhibition of arsenite uptake in rice roots by glycerol
243 which can be applicable for DMAA and MMAA too. The explanations are: i) glycerol closes
244 aquaporin channels, ii) glycerol causes general physiological stress disrupting arsenite
245 transporter, and iii) high levels of glycerol rapidly down-regulate aquaporin channels. Since
246 glycerol has been widely used for aquaporins assay ([Biela et al., 1999](#); [Dean et al., 1999](#)) and
247 since glycerol has low phytotoxicity ([Meharg and Jardine, 2003](#)), the first two explanations do
248 not elucidate adequately. Moreover, the third explanation is also not agreeable because transport
249 activity would be constant for the short exposure time (1 h) ([Meharg and Jardine, 2003](#)).
250 Therefore, inhibition of DMAA and MMAA influx by glycerol indicates that they are
251 transported across the plasma membrane via same transporter such as MIPs/aquaglyceroporins.

252 Inhibition effect of glycerol on arsenic uptake at low (10 μ M) and high (0.3 mM) DMAA
253 and MMAA concentration was investigated. Results show that the rates of DMAA and MMAA
254 influx were almost similar for both concentrations, and the uptake of both arsenic species was
255 linear rather than hyperbolic ([Figs. 4 and 5](#)). Linear regression analysis of mean arsenic influx in
256 rice roots reveals that the mean r^2 values for DMAA and MMAA at 0.3 mM concentration were
257 0.891 and 0.525, respectively. The values were 0.509 and 0.354 for DMAA and MMAA at 10
258 μ M concentration, respectively ([Table 2](#)). Thus, it can be revealed that the aquaglyceroporin
259 channels facilitate DMAA uptake more frequently compared to that of MMAA.

260

261 Conclusions

262 Uptake of organoarsenic species in plants is lower than those of inorganic species.
263 Although several reports have been described the uptake mechanisms of arsenate and arsenite in
264 plants, little is known about uptake mechanisms of organoarsenic species. The results of this
265 study show that the DMAA and MMAA follow the uptake mechanisms of glycerol in rice roots.
266 The glycerol transporter in plasma membrane (aquaglyceroporins) facilitates DMAA and
267 MMAA uptake in rice roots indicating that these arsenic species are transported in rice via
268 MIPs/aquaglyceroporins.

269

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274 References:

275 Abbas, M., Meharg, A., 2008. Arsenate, arsenite and dimethyl arsenic acid (DMA) uptake and
276 tolerance in maize (*Zea mays* L.). *Plant Soil* 304, 277-289.
277 Abedin, M.J., Feldmann, J., Meharg, A.A., 2002. Uptake kinetics of arsenic species in rice
278 plants. *Plant Physiol.* 128, 1120-1128.

- 279 Acharyya, S.K., Chakraborty, P., Lahiri, S., Raymahashay, B.C., Guha, S., Bhowmik, A., 1999.
280 Arsenic poisoning in the Ganges delta. *Nature* 401, 545-545.
- 281 Agre, P., 2004. Aquaporin water channels (Nobel Lecture). *Angew. Chem. Int. Ed.* 43, 4278-
282 4290.
- 283 Alam, M.B., Sattar, M.A., 2000. Assessment of arsenic contamination in soils and waters in
284 some areas of Bangladesh *Water Sci. Technol.* 42, 185-193.
- 285 Alam, M.G.M., Allinson, G., Stagnitti, F., Tanaka, A., Westbrooke, M., 2002. Arsenic
286 contamination in Bangladesh groundwater: A major environmental and social disaster. *Int. J.*
287 *Environ. Health Res.* 12, 235 - 253.
- 288 Baiges, I., Schaffner, A.R., Affenzeller, M.J., Mas, A., 2002. Plant aquaporins. *Physiol. Plant*
289 115, 175-182.
- 290 Benga, G., 2009. Water channel proteins (later called aquaporins) and relatives: Past, present,
291 and future. *IUBMB Life* 61, 112-133.
- 292 Biela, A., Grote, K., Otto, B., Hoth, S., Hedrich, R., Kaldenhoff, R., 1999. The *Nicotiana*
293 *tabacum* plasma membrane aquaporin NtAQP1 is mercury-insensitive and permeable for
294 glycerol. *Plant Sci.* 18, 565-570.
- 295 Blute, N.K., Brabander, D.J., Hemond, H.F., Sutton, S.R., Newville, M.G., Rivers, M.L., 2004.
296 Arsenic sequestration by ferric iron plaque on cattail roots. *Environ. Sci. Technol.* 38, 6074-
297 6077.
- 298 Carbonell-Barrachina, A.A., Aarabi, M.A., DeLaune, R.D., Gambrell, R.P., Patrick, W.H., 1998.
299 The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina*
300 *alterniflora* growth and tissue arsenic concentration. *Plant Soil* 198, 33-43.

- 301 Chen, Z., Zhu, Y.-G., Liu, W.-J., Meharg, A.A., 2005. Direct evidence showing the effect of root
302 surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol.*
303 165, 91-97.
- 304 Chowdhury, T.R., Basu, G.K., Mandal, B.K., Biswas, B.K., Samanta, G., Chowdhury, U.K.,
305 Chanda, C.R., Lodh, D., Roy, S.L., Saha, K.C., Roy, S., Kabir, S., Quamruzzaman, Q.,
306 Chakraborti, D., 1999. Arsenic poisoning in the Ganges delta. *Nature* 401, 545-546.
- 307 Dean, R.M., Rivers, R.L., Zeidel, M.L., Roberts, D.M., 1999. Purification and functional
308 reconstitution of soybean nodulin 26. An aquaporin with water and glycerol transport properties.
309 *Biochemistry* 38, 347-353.
- 310 Dey, M.M., Miah, M.N.I., Mustafi, B.A.A., Hossain, M, I.e., 1996. Rice production constraints
311 in Bangladesh: Implications for further research priorities, in: Evenson, R.E., R.W., H., Hossain,
312 M. (Eds.), *Rice research in Asia: progress and priorities*. Wallingford, UK: CAB International,
313 and International Rice Research Institute, Manila, Philippines, pp. 179-191.
- 314 Guo, X., Fujino, Y., Kaneko, S., Wu, K., Xia, Y., Yoshimura, T., 2001. Arsenic contamination of
315 groundwater and prevalence of arsenical dermatosis in the Hetao plain area, Inner Mongolia,
316 China. *Mol. Cell. Biochem.* 222, 137-140.
- 317 Hansel, C.M., La Force, M.J., Fendorf, S., Sutton, S., 2002. Spatial and temporal association of
318 As and Fe species on aquatic plant roots. *Environ. Sci. Technol.* 36, 1988-1994.
- 319 Li, R.Y., Ago, Y., Liu, W.J., Mitani, N., Feldmann, J., McGrath, S.P., Ma, J.F., Zhao, F.J., 2009.
320 The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. *Plant Physiol.* 150, 2071-
321 2080.

- 322 Ma, J.F., Yamaji, N., Mitani, N., Xu, X.-Y., Su, Y.-H., McGrath, S.P., Zhao, F.-J., 2008.
323 Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings
324 of the National Academy of Sciences 105, 9931-9935.
- 325 Mandal, B.K., Chowdhury, T.R., Samanta, G., Basu, G.K., Chowdhury, P.P., Chanda, C.R.,
326 Lodh, D., Karan, N.K., Dhar, R.K., Tamili, D.K., Das, D., Saha, K.C., Chakraborti, D., 1996.
327 Arsenic in groundwater in seven districts of West Bengal, India - the biggest arsenic calamity in
328 the world. Current Science (Bangalore) 70, 976-985.
- 329 Marin, A., Masscheleyn, P., Patrick, W., 1992. The influence of chemical form and
330 concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil 139, 175-183.
- 331 Marin, A.R., Pezeskhi, S.R., Masscheleyn, P.H., Choi, H.S., 1993. Effect of dimethylarsenic acid
332 (DMAA) on growth, tissue arsenic, and photosynthesis of rice plants. J. Plant Nutr. 16.
- 333 Maurel, C., Reizer, J., Schroeder, J.I., Chrispeels, M.J., Saier, M.H., Jr., 1994. Functional
334 characterization of the *Escherichia coli* glycerol facilitator, GlpF, in *Xenopus* oocytes. J. Biol.
335 Chem. 269, 11869-11872.
- 336 Meharg, A.A., 2004. Arsenic in rice - understanding a new disaster for South-East Asia. Trends
337 Plant Sci. 9, 415-417.
- 338 Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant
339 and nonresistant plant species. New Phytol. 154, 29-43.
- 340 Meharg, A.A., Jardine, L., 2003. Arsenite transport into paddy rice (*Oryza sativa*) roots. New
341 Phytol. 157, 39-44.
- 342 Meharg, A.A., Macnair, M.R., 1992. Suppression of the high affinity phosphate uptake system:
343 A mechanism of arsenate tolerance in *Holcus lanatus* L. J. Exp. Bot. 43, 519-524.

- 344 Meharg, A.A., Macnair, M.R., 1994. Relationship between plant phosphorus status and the
345 kinetics of arsenate influx in clones of *Deschampsia cespitosa* (L.) Beauv. that differ in their
346 tolerance to arsenate. *Plant Soil* 162, 99-106.
- 347 Meharg, A.A., Rahman, M.M., 2002. Arsenic contamination of Bangladesh paddy field soils:
348 Implications for rice contribution to arsenic consumption. *Environ. Sci. Technol.* 37, 229-234.
- 349 Mkandawire, M., Dudel, E.G., 2005. Accumulation of arsenic in *Lemna gibba* L. (duckweed) in
350 tailing waters of two abandoned uranium mining sites in Saxony, Germany. *Sci. Total Environ.*
351 336, 81-89.
- 352 Odanaka, Y., Tsuchiya, N., Matano, O., Goto, S., 1987. Absorption, translocation and
353 metabolism of the arsenical fungicides, iron methanearsonate and ammonium iron
354 methanearsonate, in rice plants. *J. Pestic. Sci.* 12, 199-208.
- 355 Otte, M.L., Kearns, C.C., Doyle, M.O., 1995. Accumulation of arsenic and zinc in the
356 rhizosphere of wetland plants. *Bull. Environ. Contam. Toxicol.* 55, 154-161.
- 357 Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007. Arsenic
358 adsorption on iron plaque and uptake in duckweed (*Spirodela polyrhiza* L.) affected by chemical
359 species, in: Starrett, S.K., Hong, J., Wilcock, R.J., Li, Q., Carson, J.H., Arnold, S. (Eds.),
360 International Conference on Environmental Science and Technology (ICEST). American Science
361 Press, Texas, Houston, USA, pp. 319-325.
- 362 Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Rahman, M.M., 2008a. Arsenic uptake by
363 aquatic macrophyte *Spirodela polyrhiza* L.: Interactions with phosphate and iron. *J. Hazard.*
364 *Mater.* 160, 356-361.

- 365 Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Rahman, M.M., 2008b. Influence of chelating
366 ligands on arsenic uptake by hydroponically grown rice seedlings (*Oryza sativa* L.): A
367 preliminary study. CLEAN - Soil, Air, Water 36, 521-527.
- 368 Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Rahman, M.M., 2008c. Influence of
369 phosphate and iron ions in selective uptake of arsenic species by water fern (*Salvinia natans* L.).
370 Chem. Eng. J. 145, 179-184.
- 371 Robinson, B., Duwig, C., Bolan, N., Kannathasan, M., Saravanan, A., 2003. Uptake of arsenic by
372 New Zealand watercress (*Lepidium sativum*). Sci. Total Environ. 301, 67-73.
- 373 Roychowdhury, T., Uchino, T., Tokunaga, H., Ando, M., 2002. Survey of arsenic in food
374 composites from an arsenic-affected area of West Bengal, India. Food Chem. Toxicol. 40, 1611-
375 1621.
- 376 Schoof, R.A., Yost, L.J., Crecelius, E., Irgolic, K., Goessler, W., Guo, H.R., Greene, H., 1998.
377 Dietary arsenic intake in Taiwanese districts with elevated arsenic in drinking water. Human
378 Ecol. Risk Assess.: Int. J. 4, 117 - 135.
- 379 Schoof, R.A., Yost, L.J., Eickhoff, J., Crecelius, E.A., Cragin, D.W., Meacher, D.M., Menzel,
380 D.B., 1999. A market basket survey of inorganic arsenic in food. Food Chem. Toxicol. 37, 839-
381 846.
- 382 Sun, G., 2004. Arsenic contamination and arsenicosis in China. Toxicol. Appl. Pharmacol. 198,
383 268-271.
- 384 Takahashi, Y., Minamikawa, R., Hattori, K.H., Kurishima, K., Kihou, N., Yuita, K., 2004.
385 Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. Environ.
386 Sci. Technol. 38, 1038-1044.

- 387 Takamatsu, T., Aoki, H., Yoshida, T., 1982. Determination of arsenate, arsenite,
388 monomethylarsonate, and dimethylarsinate in soil polluted with arsenic. *Soil Sci.* 133, 239-246.
- 389 Tamaki, S., Frankenberger, W.T.J., 1992. Environmental biochemistry of arsenic. *Rev. Environ.*
390 *Contam. Toxicol.* 124, 79-110.
- 391 Wang, J., Zhao, F.J., Meharg, A.A., Raab, A., Feldmann, J., McGrath, S.P., 2002. Mechanisms
392 of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate, and
393 arsenic speciation. *Plant Physiol.* 130, 1552-1561.
- 394 Williams, P.N., Raab, A., Feldmann, J., Meharg, A.A., 2007. Market basket survey shows
395 elevated levels of As in south central U.S. processed rice compared to California: Consequences
396 for human dietary exposure. *Environ. Sci. Technol.* 41, 2178-2183.
- 397 Wysocki, R., Chéry, C.C., Wawrzycka, D., Van Hulle, M., Cornelis, R., Thevelein, J.M., Tamás,
398 M.J., 2001. The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in
399 *Saccharomyces cerevisiae*. *Mol. Microbiol.* 40, 1391-1401.

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408 Table 1: Kinetic parameters for time-dependent DMAA and MMAA uptake in rice roots. Kinetic
 409 parameters were calculated from mean As influx ($n = 3$) by Michaelis-Menten function
 410 (nonlinear regression) and linear regression model using the GraphPad Prism (v5)
 411 (GraphPad Software, Inc., CA, USA).

As Species	Nonlinear Regression			Linear Regression			
	V_{\max} ($\mu\text{mol g}^{-1} \text{ f. wt.}$)	K_m (mM)	r^2	a	b	r^2	p
Time-dependent							
DMAA				0.007±0.001	0.119±0.045	0.688	0.0002
MMAA				0.002±0.000	0.014±0.007	0.756	< 0.0001
Con.-dependent							
DMAA	0.757	0.140	0.688	0.644±0.116	0.253±0.048	0.837	0.0014
MMAA	3.619	0.762	0.914	2.399±0.318	0.186±0.133	0.904	0.0003

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420 Table 2: Kinetic parameters for the uptake inhibition of DMAA and MMAA in rice roots by
 421 glycerol. Kinetic parameters were calculated from mean As influx ($n = 3$) by linear
 422 regression model using the GraphPad Prism (v5) (GraphPad Software, Inc., CA, USA).

As Species	<i>a</i>	<i>b</i>	r^2	<i>p</i>
Glycerol + As (0.3 mM)				
DMAA	$- 3.04 \times 10^{-4} \pm 3.36 \times 10^{-5}$	$3.34 \times 10^{-1} \pm 1.54 \times 10^{-2}$	0.891	< 0.0001
MMAA	$- 4.11 \times 10^{-4} \pm 1.23 \times 10^{-4}$	$11.66 \times 10^{-1} \pm 5.68 \times 10^{-2}$	0.525	0.0077
Glycerol + As (10 μ M)				
DMAA	$- 1.73 \times 10^{-5} \pm 5.38 \times 10^{-6}$	$3.15 \times 10^{-2} \pm 2.46 \times 10^{-3}$	0.509	0.0092
MMAA	$- 6.96 \times 10^{-5} \pm 2.97 \times 10^{-5}$	$1.22 \times 10^{-1} \pm 1.36 \times 10^{-2}$	0.354	0.0412

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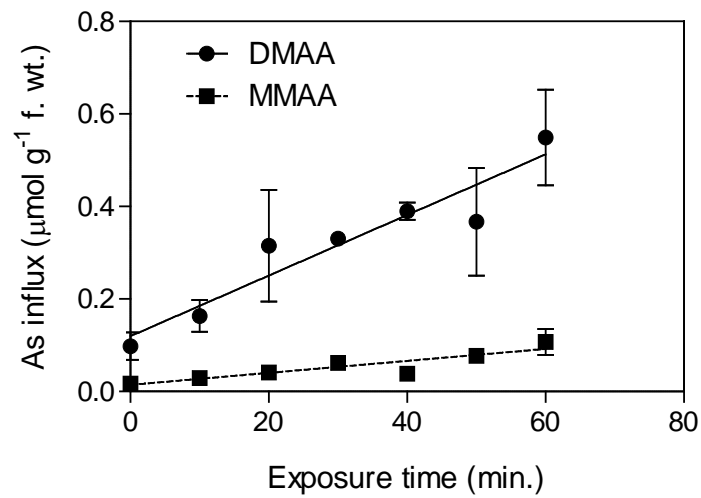
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434 Fig. 1: Time-dependent influx of DMAA and MMAA in rice roots. Arsenic concentration in the
435 solution was 0.30 mM. The graph shows the linear regression lines, and the kinetic
436 parameters are given in Table 1. Each point is the average value of three replicated
437 treatments. Bares represent \pm standard error of the mean (SEM) of the replicates.

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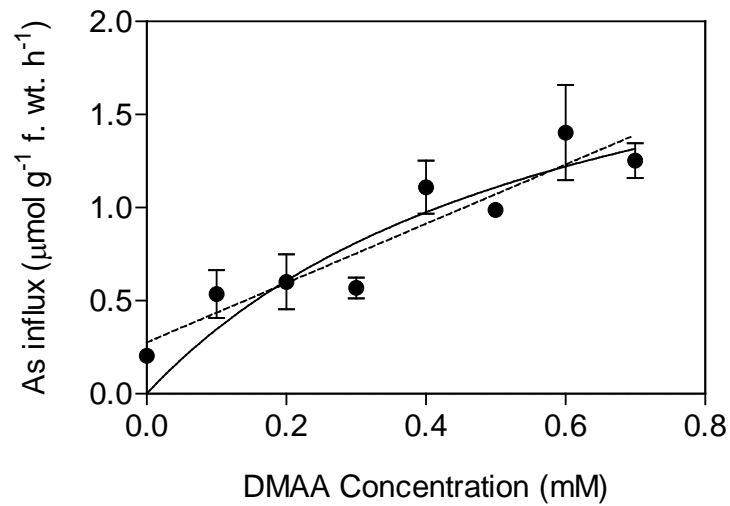
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447 Fig. 2: Concentration-dependent influx of DMAA in rice roots. The graph shows the Michaelis-
448 Menten (nonlinear regression) curve and linear regression line, and the fits are given in
449 Table 1. Each point is the average value of three replicated treatments. Bares represent \pm
450 standard error of the mean (SEM) of the replicates.

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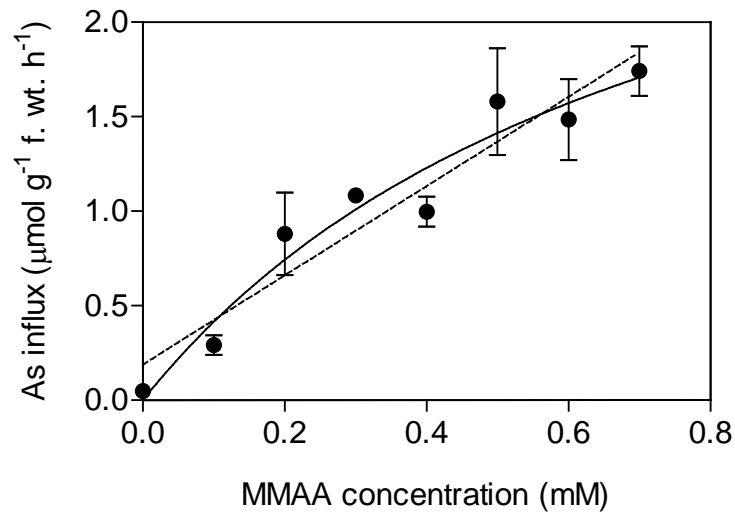
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460 Fig. 3: Concentration-dependent influx of MMAA in rice roots. The graph shows the Michaelis-
461 Menten (nonlinear regression) curve and linear regression line, and the fits are given in
462 Table 1. Each point is the average value of three replicated treatments. Bares represent \pm
463 standard error of the mean (SEM) of the replicates.

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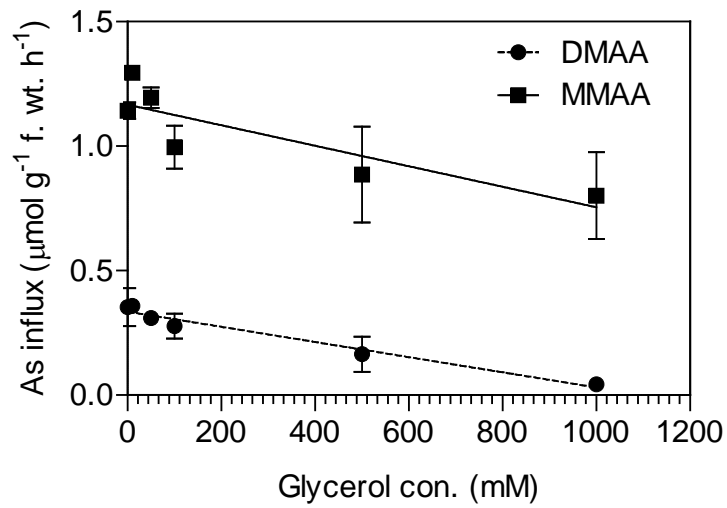
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473 Fig. 4: Inhibition of DMAA and MMAA influx (0.3 mM) in rice roots by different concentration
474 of glycerol. The graph shows the nonlinear regression lines, and the fits are given in
475 Table 2. Each point is the average value of three replicated treatments. Bares represent ±
476 standard error of the mean (SEM) of the replicates.

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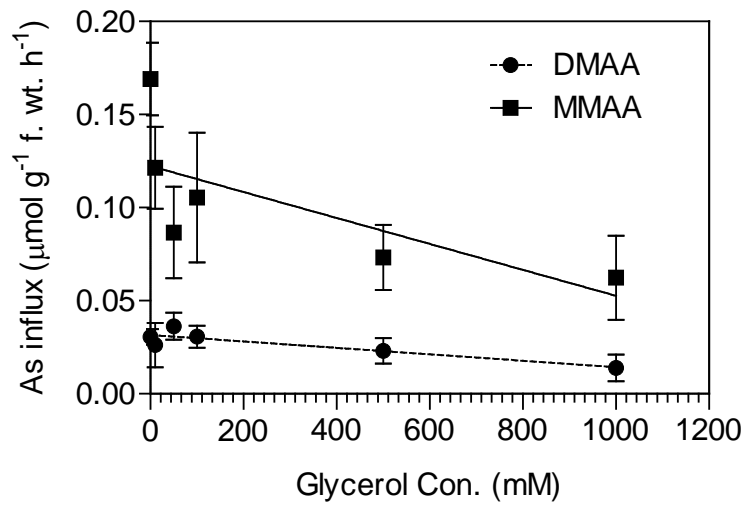
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486 Fig. 5: Inhibition of DMAA and MMAA influx (10 μM) in rice roots by different concentration
487 of glycerol. The graph shows the nonlinear regression lines, and the fits are given in
488 Table 2. Each point is the average value of three replicated treatments. Bares represent ±
489 standard error of the mean (SEM) of the replicates.