Transport of DMAA and MMAA into rice (Oryza sativa L.) roots

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

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Abstract:

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

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

Introduction:

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

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

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

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

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

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

Materials and Methods:

Seed sterilization

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

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

Plant growth

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

Uptake kinetics

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

rice seedlings were incubated in test solution (distilled water without nutrient salts) with 0.1 mM arsenic for time-dependant uptake experiment, and the samples were collected at a 10 min interval. In DMAA and MMAA transport assay, 0.3 mM or 10 µM DMAA or MMAA was added to 10, 50, 100, 500, and 1000 mM glycerol solutions. Replicated rice seedlings were incubated into these solutions for 1 h. The test solution was adjusted to pH 7 using weak solutions of HCl or NaOH. Stock solutions of DMAA and MMAA were prepared from dimethylarsinic acid ($(CH_3)_2AsO(OH)$) and ($CH_3AsO(OH)_2$), respectively. Glycerol was purchased from Kanto Chemical Co., Japan (purity 99.0%).

Sample preparation and chemical analysis

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

atomic absorption spectrometer (Z-8100, Hitachi, Japan). Certified standard reference material 1573a (tomato leaf from NIST, USA) was used to check the accuracy of analysis. Arsenic concentration in certified standard reference materials was 0.112±0.004 µg g^{-1} dry weight while the measured concentration was 0.114±0.002 µg g^{-1}. The concentrations detected in all samples were above the instrumental limits of detection (≥ 0.01 µM in water sample).

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

Statistical Analysis

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

Results and discussions:

Uptake kinetics of DMAA and MMAA

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

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

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

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

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

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

parameters also showed that V_{max} for DMAA and MMAA were 0.757 and 3.619 µmol g^{-1} fresh weight h^{-1} (Table 1). Abedin et al. (2002) also reported similar results for DMAA and MMAA in rice roots.

Inhibition of DMAA and MMAA uptake by glycerol

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

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

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

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

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

Conclusions

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

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

As Species	Nonlinear Regression			Linear Regression			
	V_{max} (μmol g^{-1} 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

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

As Species	*a*	*b*	r^2	*p*
Glycerol + As (0.3 mM)				
DMAA	$-3.04\times10^{-4} \pm 3.36\times10^{-5}$	$3.34\times10^{-1} \pm 1.54\times10^{-2}$	0.891	< 0.0001
MMAA	$-4.11\times10^{-4} \pm 1.23\times10^{-4}$	$11.66\times10^{-1} \pm 5.68\times10^{-2}$	0.525	0.0077
Glycerol + As (10 µM)				
DMAA	$-1.73\times10^{-5} \pm 5.38\times10^{-6}$	$3.15\times10^{-2} \pm 2.46\times10^{-3}$	0.509	0.0092
MMAA	$-6.96\times10^{-5} \pm 2.97\times10^{-5}$	$1.22\times10^{-1} \pm 1.36\times10^{-2}$	0.354	0.0412

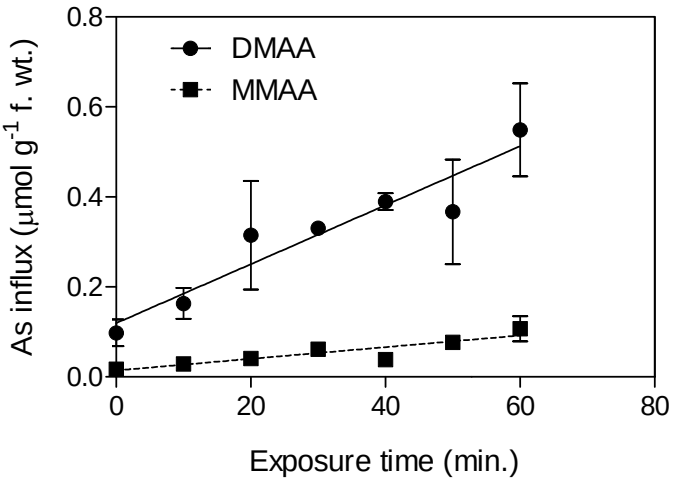


Fig. 1: Time-dependent influx of DMAA and MMAA in rice roots. Arsenic concentration in the solution was 0.30 mM. The graph shows the linear regression lines, and the kinetic parameters are given in Table 1. Each point is the average value of three replicated treatments. Bares represent ± standard error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

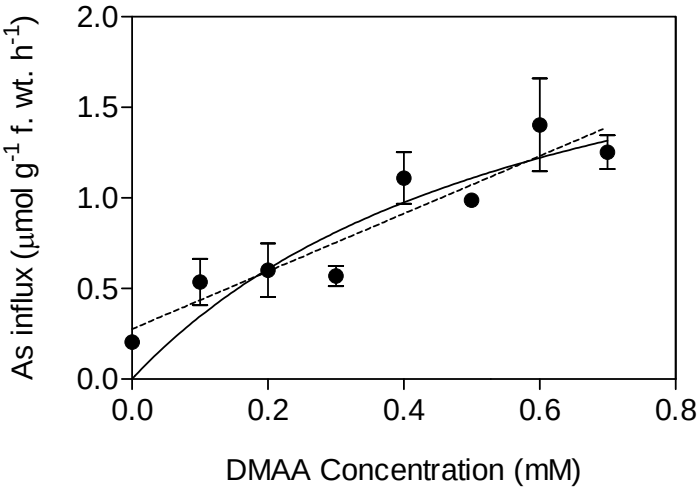
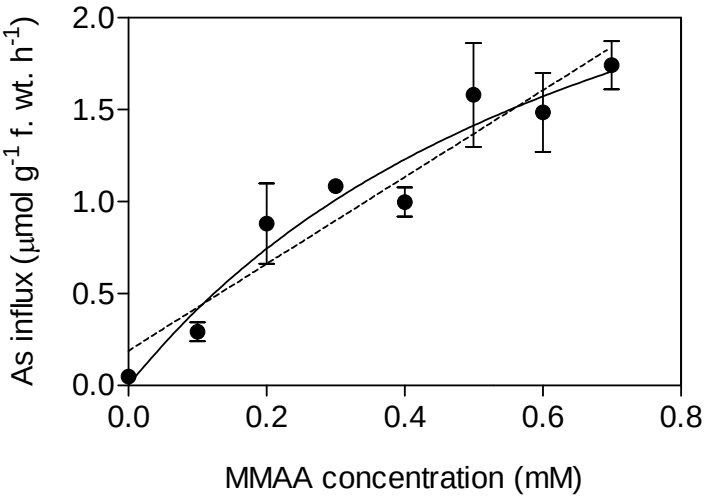


Fig. 2: Concentration-dependent influx of DMAA in rice roots. The graph shows the Michaelis-Menten (nonlinear regression) curve and linear regression line, and the fits are given in Table 1. Each point is the average value of three replicated treatments. Bares represent ± standard error of the mean (SEM)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



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

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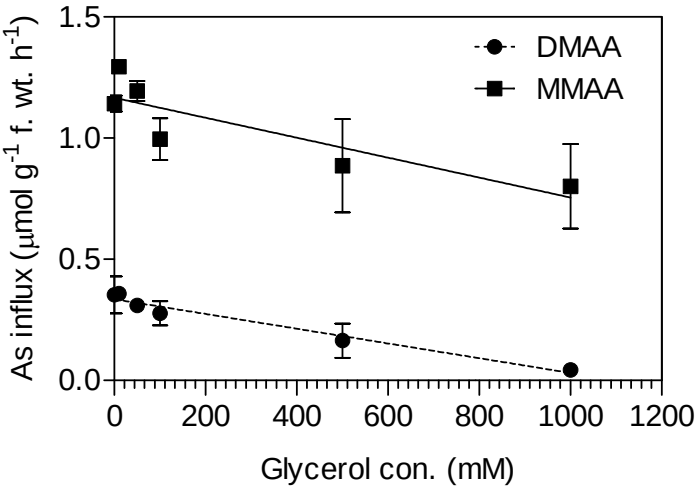

Fig. 4: Inhibition of DMAA and MMAA influx (0.3 mM) in rice roots by different concentration of glycerol. The graph shows the nonlinear regression lines, and the fits are given in Table 2. Each point is the average value of three replicated treatments. Bares represent ± standard error of the mean (SEM)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

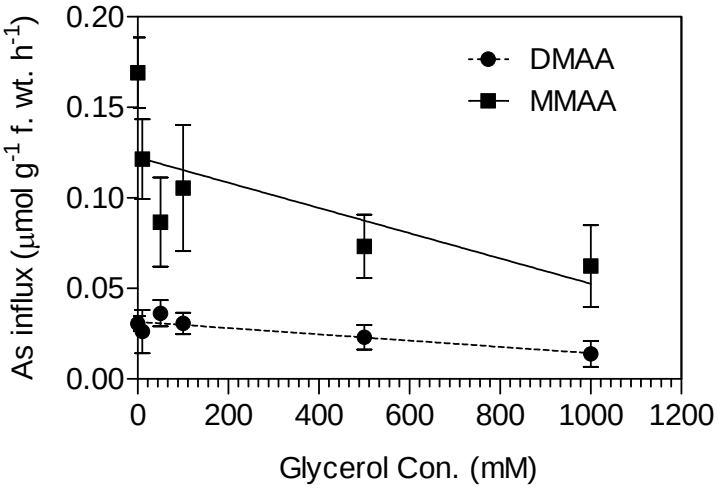

Fig. 5: Inhibition of DMAA and MMAA influx (10 µM) in rice roots by different concentration of glycerol. The graph shows the nonlinear regression lines, and the fits are given in Table 2. Each point is the average value of three replicated treatments. Bares represent ± standard error of the mean (SEM)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