MASTER'S THESIS

Expressions of transporters of arsenite and phosphate in rice (Oryza sativa L.) associated with arbuscular mycorrhizal fungi

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Expressions of Transporters of Arsenite and Phosphate in Rice (*Oryza sativa* L.) Associated with Arbuscular Mycorrhizal Fungi

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Abstract

Arsenic (As) accumulation in rice exerts severe adverse effects on human health. There has been considerable interest in developing mitigation strategies to restrict or decrease As uptake in rice. Arbuscular mycorrhizal fungi (AMF) are able to influence water, nutrients and heavy metal uptake, and also affect the genes expressions of their host plants. The main objectives of this study were to: 1) investigate the colonization rates of AMF associated with lowland rice grown in paddy fields in suburban areas near 7 different cities in Southeastern China, and the moisture tolerance of AMF collected from the fields; 2) investigate arsenite [As (III)] accumulation and the expressions of transporters of arsenite in rice associated with AMF; and 3) study the arsenate [As (V)] accumulation and the expressions of phosphate transporters in rice associated with AMF.

Very few studies have focused on AMF colonization in lowland rice grown under anaerobic field conditions. Being oxygen dependants, AMF were considered to be unable to survive in waterlogged condition. However, it has been proven that AMF are able to colonize semi-wetland and wetland plants. In order to study the colonization rates of AMF in lowland rice grown under waterlogged field condition, lowland rice samples were collected from 7 different sampling sites in Guangdong, Jiangxi, Hubei and Jiangsu Provinces, China. Among all the sampling cities, Huizhou, Heyuan, Shaoguan, Meizhou, Ji'an, Xuzhou and Jingzhou, Huizhou City observed the highest level of colonization rates of AMF in roots of the lowland rice (19.5 ± 7.2%). In general, the colonization rates were significantly correlated with latitudes of the sampling sites (R = -0.68, P = 0.046), but did not correlate with other soil properties

including phosphorus and arsenic. The sequence of amplified rDNA fragments (amplified using polymerase chain reaction technique) showed that the AMF species *Glomus* cf. *clarum* Att894-7 (accession No.: FM865542) colonized rice plant in Pingtan site, Huizhou City. The results of morphological observation showed that AMF existed in the paddy soil and colonized the roots of lowland rice. Based on the moisture tests, it was indicated that most AMF [collected from Shuikou, Pingtan and Liangjing sites, and purchased *Glomus intraradices* (BJ09) *G. mosseae* (GZ01A) and *G. mosseae* (NM01A)] colonized lowland rice under 50-75% water holding capacity (WHC) in soil. Only the AMF collected from 3 sites in Huizhou City (Pingtan, Liangjing and Lianghua) colonized lowland rice under 100% WHC. These AMF may have adapted to the high level of moisture in soil than the purchased AMF which were isolated and cultured from upland plants (*Solanum lycopersicum, Styphnolobium japonicum,* and *Allium tenuissimum*).

As a silicon hyperaccumulator, lowland rice takes up higher levels of As than many other plants due to silicic acid and arsenite sharing the same transporters (Lsi1 and Lsi2). *Glomus intraradices* (AH01) was inoculated to rice under different arsenite concentrations (0, 2 and 8 μM) in order to investigate the interactions between AMF and rice on the accumulation of arsenite. The relative mRNA expressions of Lsi1 and Lsi2 resulted in a down-regulating trend in mycorrhizal plants. Under 2 μM arsenite treatments, Lsi1 and Lsi2 were significantly decreased, by 0.72-fold ($P < 0.05$) and 0.46-fold ($P < 0.01$), respectively, in mycorrhizal plants when compared with non-mycorrhizal plants. This led to the decrease of arsenite uptake per unit of root dry mass. No organic As species were detected in both roots and shoots. The As (III): As (V) ratios indicated that mycorrhizal plants immobilized most of the arsenite proportion in the roots and prevented its translocation from the roots to the shoots.

It is known that arsenate and phosphate (P) share the same transporters in plants, and AMF influence the expressions of P transporters (OsPT1-13) in rice. In order to study the effects of AMF on arsenate accumulation in rice, *Glomus intraradices* (AH01) was inoculated to rice and treated with different levels of arsenate (0, 2 and 8 μM). Results revealed that OsPT11 was increased whereas OsPT2 decreased ($P < 0.05$) in the mycorrhizal plant. The increased expression of OsPT11 was one of the most important factors that led to the significantly higher P concentration ($P < 0.05$) in plant tissues which compensated the down-regulation of OsPT2. The symbiosis of *G. intraradices* with rice slightly decreased ($P > 0.05$) the arsenate concentration in plant tissues but markedly enhanced ($P < 0.05$) plant biomass. The higher P content in mycorrhizal plants led to the higher P/As molar ratio ($P < 0.05$) and lower As uptake ratio ($P < 0.05$) in mycorrhizal plants treated by 2 μM arsenate. Mycorrhizal plants under such an arsenate treatment took up less As by per unit of root dry mass. The inoculation of *G. intraradices* was not able to transform the inorganic As to organic As. Further studies should be conducted focusing on the transport activities of each P transporter using yeast or oocyte expression system to identify which P transporters are responsible for the accumulation of arsenate.

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