

MASTER'S THESIS

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

Chen, Xunwen

Date of Award:
2012

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

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

CHEN Xunwen

A thesis submitted in partial fulfillment of the requirements

for the degree of

Master of Philosophy

Principal Supervisor: Prof. WONG Ming Hung

Hong Kong Baptist University

July 2012

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 ($19.5 \pm 7.2\%$). 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.

Table of Contents

Declaration.....	i
Abstract.....	ii
Acknowledgments.....	v
Table of Contents.....	vi
List of Tables	xiv
List of Figures	xvi
Abbreviations and Acronyms	xix
Chapter 1. General Introduction	1
1.1. Background of Research.....	1
1.1.1. Arsenic Contamination and Human Health	1
1.1.2. Technologies to Reduce Arsenic Accumulation in Rice	8
1.2. Literature Review.....	9
1.2.1. Arsenite Accumulation in Lowland Rice.....	9
1.2.2. Arsenate Accumulation in Plants.....	10
1.2.3. The Role of Arbuscular Mycorrhizal Fungi on Arsenic Accumulation in Plants.....	11

1.2.3.1. What are Arbuscular Mycorrhizal Fungi?.....	11
1.2.3.2. Architectures of Arbuscular Mycorrhizal Fungi Colonization in Root.	12
1.2.3.3. Effects of AMF on Nutrient and Heavy Metal Uptake by Their Hosts	12
1.3. Objectives of the Present Research	18
1.4. Contributions and Significance of the Present Research.....	18
1.5. Framework of Research	20
Chapter 2. Colonization Rates of Arbuscular Mycorrhizal Fungi (AMF) on Lowland Rice (<i>Oryza sativa</i> L.): Field and Greenhouse Studies	21
2.1. Introduction	21
2.1.1. Occurrence of AMF Colonization under both Aerobic and Anaerobic Conditions	21
2.1.2. Methods for AMF Identification	22
2.1.3. Trap Culture of AMF	24
2.1.4. Water Holding Capacity (WHC) in Soils Affects Colonization of AMF	24
2.2. Materials and Methods	25
2.2.1. Collection of Samples	25
2.2.2. Chemical Analyses	26

2.2.3. AMF Studies on the Colonization Rates, PCR Products, Extracted Spores and Moisture Test	28
2.2.3.1. Morphological Observation of AMF	28
2.2.3.2. Polymerase Chain Reaction (PCR) for Amplification of rDNA Fragments of AMF in Rice Root	29
2.2.3.3. Trap Culture of AMF	30
2.2.3.4. Spores Extraction	32
2.2.3.5. Moisture Test for the Moisture Tolerance of AMF Cultured from Soil in Huizhou City	33
2.2.4. Statistical Analyses	34
2.3. Results and Discussion	34
2.3.1. Properties of Paddy Soils Collected from Huizhou City	34
2.3.2. AMF Colonization Rates in Lowland Rice from 7 Different Paddy Fields	37
2.3.2.1. Colonization Rates in Lowland Rice	37
2.3.2.2. Microscopic Observation	40
2.3.3. Identification of AMF Using PCR and Sequencing Techniques	42
2.3.3.1. Target rDNA Fragment Amplified	42
2.3.3.2. PCR Products Sequences	44

2.3.4. Cultured Spores	45
2.3.5. Moisture Test.....	45
2.4. Conclusions	51
Chapter 3 Expressions of Transporters of Arsenite in Rice (<i>Oryza sativa</i> L.) Associated with Arbuscular Mycorrhizal Fungi (AMF) Colonization under Different Levels of Arsenite Stress.....	52
3.1. Introduction	52
3.1.1. Arsenite Pathways from Outside Medium into Rice Plant	52
3.1.2. AMF have Considerable Effects on Nutrients Uptake and Gene Expressions in Plant.....	53
3.2. Materials and Methods	54
3.2.1. Plant, AMF Materials and Growth Conditions	54
3.2.2. Experimental Design	55
3.2.2.1. Screening of AMF from four AMF Species.....	55
3.2.2.2. Germination of Rice Seeds and AMF Inoculation	57
3.2.2.3. Arsenite Treatments	57
3.2.2.4. Harvesting of Plant Materials.....	58

3.2.3. Analyses of Transporters of Arsenite, Total As, As Speciation and As Uptake Ratio	58
3.2.3.1. Lsi1 and Lsi2 Analyses	58
3.2.3.2. Total As and As Speciation.....	61
3.2.3.3. Uptake Ratio	62
3.2.4. Statistical Analysis.....	62
3.3. Results and Discussion	62
3.3.1. Biomass of Rice Plant and Colonization Rates of AMF.....	62
3.3.2. Relative Expressions of Lsi1 and Lsi2 under AMF Inoculation	65
3.3.3. Arsenic Content in Plant Tissues and the Expressions of Lsi1 and Lsi2.....	67
3.4. Conclusions.....	77
Chapter 4. Phosphate Transporters Expression in Rice (<i>Oryza sativa</i> L.) Associated with Arbuscular Mycorrhizal Fungi (AMF) Colonization under Different Levels of Arsenate Stress.....	78
4.1. Introduction.....	78
4.1.1. Arsenate and Phosphate Transport Pathway in Plants.....	78
4.1.2. The AMF Phosphate Pathway	79
4.1.3. AMF Affecting Gene Expression of Plants	80

4.1.4. Expressions of Phosphate Transporters in Rice Associated with AMF.....	80
4.2. Materials and Methods	82
4.2.1. Plant, AMF Materials and Growth Conditions	82
4.2.2. Experimental Design	82
4.2.2.1. Germination of Rice Seeds and AMF Inoculation	82
4.2.2.2. Arsenate Treatments.....	83
4.2.2.3. Harvesting of Plant Materials.....	84
4.2.3. Morphological Observation for Colonization Rates of AMF	84
4.2.4. Phosphate Transporters (OsPT2, OsPT6 and OsPT11) Analyses.....	85
4.2.5. Analyses of Total Phosphorus, Total Arsenic and Arsenic Speciation.....	87
4.2.6. Statistical Analyses	89
4.3. Results and Discussion.....	89
4.3.1. Colonization Rates and Effects of AMF on Plant Biomass	89
4.3.2. The Changes of Relative Expressions of OsPT2, OsPT6 and OsPT11 Genes and P Content	92
4.3.3. Arsenic Concentrations and Speciation in Rice Associated with AMF.....	98
4.3.4. Increased P Content in Plant Tissues Offered Higher P/As Molar Ratio in Rice Associated with AMF	102

4.4. Conclusions.....	104
Chapter 5. General Discussion and Conclusions	106
5.1. Introduction.....	106
5.2. AMF Associated with Rice	110
5.3. Arsenic Accumulation in Rice	115
5.3.1. Arsenite Accumulation in Rice	115
5.3.2. Arsenate Accumulation in Rice	118
5.3.3. Organic Arsenic Accumulation in Rice	119
5.4. Effects of AMF on the Accumulation of As and Gene Expressions in Rice....	122
5.5. General Conclusions	124
5.5.1. Confirmation of Arbuscular Mycorrhizal Fungi Colonization in Lowland Rice	124
5.5.2. Inoculation of AMF Increased Plant Biomass, Conditionally Lowered Relative Expressions of Transporters of Arsenite and Arsenic Uptake Ratio.....	125
5.5.3. Inoculation of AMF Increased Plant Biomass, Conditionally Exerted Different Effects on Different Phosphate Transporters and Lowered Arsenic Uptake Ratio	126
5.6. Future Work.....	127

5.6.1. Monitoring Dynamic Changes of As, P Concentrations, AMF Colonization Rates, Gene Expressions	127
5.6.2. Adding Silicon and Phosphate Treatments	127
5.6.3. Arsenic Transport Activities of Genes	128
5.6.4. Identifying Transporters Responsible for Nutrient Transportation in AMF	129
5.6.5. Field Trials	129
References	130
Publications	159
Journal papers.....	159
Presentations.....	159
Scientific Meetings and Workshops Attended	159
Curriculum Vitae.....	161