Molecular biological analysis on arsenic transport and accumulation in Pteris vittata

博 士 学 位 論 文 要 約

Doctoral Dissertation Summary

Molecular biological analysis on arsenic transport and accumulation in *Pteris vittata*

（モエジマシダのヒ素輸送と蓄積の分子生物学的解析）

東北大学大学院環境科学研究科

Graduate School of Environmental Studies, Tohoku University

先進社会環境学専攻

専攻 major／
コース course

氏　名
Name

魏書君

指　導　教　員 Supervisor at Tohoku Univ.	井上千弘　教授
研 究 指 導 教 員 Research Advisor at Tohoku Univ.	簡梅芳　　助教
審　査　委　員 （○印は主査） Dissertation Committee Members Name marked with "○" is the Chief Examiner	○　井上千弘　教授 1　上高原理暢　教授　　2　魚住信之　教授 （工学研究科） 3　簡梅芳　助教　　4 5　　6

Summary
論文要約

Molecular biological analysis on arsenic transport and accumulation in *Pteris vittata*
（モエジマシダのヒ素輸送と蓄積の分子生物学的解析）

Wei Shujun

Graduate School of Environmental Studies
Tohoku University

2021/09/02

Arsenic (As) in soils and waters is of major environmental concern due to its ubiquity and carcinogenicity. Phytoremediation is currently availably applied to cleanup soil As contamination by employing plants to assimilate As from contaminated soil via their roots. *Pteris vittata* (Chinese brake fern) is the first known As-hyperaccumulator, which is highly efficient in extracting As from external environment via root and translocating it to the fronds, making it possible to be used for phytoremediation of As-contaminated soils. In addition, *P. vittata* has served as a model plant to study As metabolisms and correlated mechanisms responsible for As resistance and accumulation in plants.

When AsV was supplied to *P. vittata*, As was transported in the xylem sap predominantly as AsIII (93~98%). Reduction of AsV to AsIII in the roots and efficient loading of AsIII to the xylem, making AsIII as the predominant form being accumulated in the fronds. Curiously, As was unevenly distributed among fronds of *P. vittata*, which was predominantly accumulated in young fronds of *P. vittata* with low As exposure (0.2 and 20 μM) for a week, whereas As accumulation shifted to mature fronds under high As exposure (2 mM). These physiological studies suggested that the mechanisms of As accumulation in *P. vittata* include processes of AsV uptake, reduction to AsIII, and transport of AsIII. In the last few years, few proteins involved in As uptake and transport have been functionally characterized in *P. vittata*. In gametophytes of *P. vittata,* AsV, is taken up by the phosphate transporter PvPht1;3 and reduced to AsIII by the arsenate reductase, PvACR2, and perhaps other reductase. Subsequently, AsIII was transported into the vacuoles from the cytoplasm, mediating by AsIII transporter PvACR3 in gametophytes of *P. vittata*. Since ferns have alternative generations which contain haploid gametophyte and diploid sporophyte, the As response including gene expression should

be demonstrated individually. However, even sporophyte generation is much more active and physiologically intact than gametophyte generation, information including expression timing of these genes and how they collaborated on As processing in sporophyte is still unclear. Further, sporophyte of *P. vittata* contains complex physiological structure, accordingly, the process involving As uptake, transport should be conducted by a network of series critical genes, while the tissue–specific transport system has not been comprehensively investigated in *P. vittata.* After As was transported into cell, *P. vittata* attempt to reduce intracellular As content through strategies such as cell wall immobilization and redistribution. Besides, higher antioxidant capacity of *P. vittata* makes it prevents cell damage from ROS relevant oxidative stress. However, the genetic components relating to As detoxification remain unknown. Therefore, the objective of this study is (1) to investigate expression profile of *PvPht1;3*, *PvACR2* and *PvACR3* and how they collaborated on As processing in *P. vittata* root. (2) to illustrate the overall image of As uptake, transport and metabolism in *P. vittata* root. (3) to investigate the mechanisms of As transport, accumulation and metabolism in different *P. vittata* fronds.

Chapter 1 is the general introduction reviews the background information of As contamination, remediation technology including phytoremediation; physiology report about As movement and metabolism as well as molecular component associated with mechanisms of As hyperaccumulation in *P. vittata*.

Chapter 2 demonstrates the As processing in root of *P. vittata* in phenomenal and genetical level. Results of As addition analyses revealed that *P. vittata* had high-sensitivity to AsV, while this high affinity was inhibited when coexisting with P. Analyses of As speciates and movement in the plant suggested that the roots play an essential role of As uptake, reduction, and translocation. Then expression profile of 3 genes (*PvPht1;3*, a

phosphate (P) transporter gene; *PvACR2*, a AsV reductase gene; *PvACR3*, a AsIII transport gene) was focused on to examine their contributions on As processing in root of *P. vittata*. *PvPht1;3* reacted to AsV sensitively which made a AsV depletion in the solution. The AsV reductase gene *PvACR2* was constitutively transcribed in root, which supported a stable AsV reduction to AsIII in root. It might be the reduced AsIII which induced the transcription of an AsIII transporter gene *PvACR3*. The transcription level of *PvACR3* was correlated to As concentration in root. Together the results, the collaboration of these 3 genes made to a sensitive AsV absorption, a stable AsV reduction and a prompt AsIII transportation as a minimum facility which may contribute to *P. vittata* as an As hyperaccumulator.

Chapter 3 suggests quickly uptake and continuing reduction of AsV, efficient AsIII transport and high tolerance as crucial process in root system of *P. vittata*. Comparative transcriptome analyses identified a large number of differentially expressed genes (DEG) in roots under As exposure, and functional annotation suggested that DEGs mainly associated with transmembrane transport, GSH metabolism, redox control, signal transduction, as well as cell wall biosynthesis. In detail, activated transporter activities and increased expression of GST contribute to sensitive AsV uptake and efficient AsIII transport in *P. vittata* root. Oxidoreductase activities, signaling and cell wall biosynthesis related genes were activated to facilitate the regulation and metabolism of As in *P. vittata* root.

Chapter 4 evaluates the behavior of As translocation and distribution in different *P. vittata* fronds as well as investigate the molecular mechanisms which contribute to As transport and accumulation in different *P. vittata* fronds. Phenomenal results demonstrated that the pattern of As distribution is strongly depending on As uptake stage

and frond status. Higher concentration of As was obviously distributed to young fronds and fiddleheads. Comparative transcriptomic analysis revealed that genes related to activated transporters, cell wall modification and antioxidant activity were special triggered in young frond, thus support the priority of As distribution and accumulation.

Chapter 5 summarizes all of results and the environmental implications of this study. Meanwhile, the limitation of this study and future research focus was proposed.

The present study is one of only a few in which RNA-Seq was used to examine the *P. vittata* transcriptome, which revealed the whole picture of its As response, including potential correlating transport system and tissue-specific stress responses. The results broaden our understanding of molecular mechanisms of As transport and accumulation in *P. vittata* and provide valuable genetic components for potential use in phytoremediation.