



Title	Genetic basis of ionic variations in rice and its implication for the sulfate transporter gene contributing to the sulfur accumulation [an abstract of dissertation and a summary of dissertation review]
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## 学位論文内容の要旨

博士の専攻分野名称：博士（農学）

氏名：Zhang Chengming

### 学位論文題名

Genetic basis of ionic variations in rice and its implication for the sulfate transporter gene contributing to the sulfur accumulation

(イネにおけるイオン変動の遺伝的基盤とそれが示唆した硫黄蓄積に寄与する硫酸トランスポーター遺伝子)

Plant ionome comprises mineral nutrients and trace elements in plants and is a multidimensional dynamic network of elements regulated by genetic and environmental factors. To adapt to a diverse range of geoclimatic environment, there is a high allelic diversity among the natural population of plants. These genotype-to-phenotype links can provide an efficient way to identify the genetic basis of ionic traits. Rice (*Oryza sativa* L.), a staple crop for half the world's population, has a long history of domestication and breeding with widely-spreading genotypes. However, farmers have preferred planting rice varieties adapted to local agroclimatic conditions, with higher yield and better taste, usually not considering the mineral content in practice. Meanwhile, the transportation of elements by the root-to-shoot has been considered a major and common process determining the element accumulation in shoots and grains of rice. Thus, screening elemental properties in shoots and roots is worthwhile for reference to safety biofortification strategies. As a model plant with rich sequence information, an understanding of ionic profiling and associated genetic dissection using natural genetic resources is essential to reveal the genetic basis of rice ionome. In the present study collected rice accessions with known sequence variation from the National Agriculture and Food Research Organization (NARO) GeneBank to investigate the ionic profiles and the associated genetic basis.

### **1. The study of ionic profiling of rice genotypes and identification of varieties with elemental covariation effects**

First, 120 rice varieties collected from NARO GeneBank were cultivated to the seedling stage in hydroponics, and the concentrations of 23 elements and three anions in the shoots and roots of the rice were determined. Although the subspecies effects were limited by the genus *Oryza* pre-framework and its elemental chemical properties, significant differences were found in ionic variations in most elements among the

*aus*, *indica*, and *japonica* subspecies, and these differences are largely defined by geographical and historical distribution. Principal component analysis of the correlations showed that variations in the root-to-shoot ionic transport mechanisms were the leading causes of ionic variations among the subspecies. Furthermore, the correlations were primarily associated with screening varieties for elemental covariation effects. The *japonica* subspecies exhibited the strongest elemental correlations and elemental covariation effects. Therefore, they exhibited greater biofortification advantages than the *indica* and *aus* subspecies, whereas *indica* and *aus* subspecies were likely safer in metal (loid) polluted environments.

## **2. Genome-wide association study on rice ionome and a related sulfate transporter gene analysis**

Based on the genome-wide association study (GWAS) of the ionic data in rice shoot and root, many genomic regions located QTLs related to the concentration of elements were detected. Particularly, a significant single nucleotide polymorphism (SNP) peak for sulfur concentration on chr03, and one 3-bp deletion on chr03:4988355 to chr03:4988357, with changing from ATCAT to AC were determined. This 3-bp deletion is located on the first exon of a high-affinity sulfate transporter gene, *OsSULTR1;1*, which is located on chr03:4988261 to chr03:4988608. The haplotype analysis discovered that the sulfur concentrations in shoot and root of rice haplotype with the 3-bp deletion were significantly higher than that of rice haplotype without the 3-bp deletion. Therefore, amino acid sequencing analysis, which showed that this three-bases deletion exactly constituted one-histidine missing was performed, and it was hypothesized that there was no frameshift mutation leading to gene dysfunction. The following experiment, rice haplotypes with or without the three-base-deletion (TBDs and NBDs, respectively), were contrastively cultivated under normal and low sulfur treatment in hydroponics to evaluate the gene expression to sulfur deficiency. Results indicated that the expression of *OsSULTR1;1* was found only in rice roots and higher under low sulfur treatment. However, the expression of *OsSULTR1;1* in TBDs exhibited significantly lower than that in NBDs, which could not explain the higher sulfur in TBDs, suggesting that the sulfate ion transport activity of this transporter itself, rather than its expression level, is affected by the base deletion.

In conclusion, this study provides a potential insight that combined ionome with genetic basis, can explain the ionic dynamics from a molecular perspective and provides a reference for cultivating safe and nutritious rice crops in practice.