論 文 の 内 容 の 要 旨

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氏　　名　井出　曜子
指導教員名　藤原　徹

論文題目　Studies of nutrient deficiency responses in *Arabidopsis thaliana*: Transcriptome and metabolome analysis of molybdenum deficiency and molecular genetic analysis of sulfur deficiency
(シロイヌナズナの栄養欠乏応答の研究：モリブデン欠乏応答のトランスクリプトームおよびメタボローム解析と硫黄欠乏応答の分子遺伝学的解析)

Plants are the producers with photosynthetic ability, and we humans are dependent on them. For example, plants can assimilate inorganic sulfate into cysteine and synthesize methionine, and methionine is an essential amino acid for humans. As land plants are dependent on soils, plants have evolved to respond to nutrient deficiency, and to other biotic and abiotic stresses to survive. It is important to clarify the molecular mechanisms of plant responses to nutrient deficiency, and to utilize the knowledge to improve our life.

In this thesis, I focused on the nutrient deficiency responses in plants using *Arabidopsis thaliana*. This study consists of two parts: The first part is transcriptome and metabolome analysis of molybdenum (Mo) deficiency and the second part is molecular genetic analysis to identify novel factors regulating plant responses to sulfur (S) deficiency. Mo and S are also important for carbon and nitrogen metabolisms. Nitrogen is a major constituent of proteins, whose deficiency is often a limiting factor

for plant growth and productivity.

In the first chapter, I conducted transcriptome and metabolome analysis of plant deficient in Mo. Although Mo is an essential micronutrient in plants, the molecular mechanisms of plant responses to Mo deficiency were not well established. MOT1 is a molybdate transporter belonging to the sulfate transporter family. I described effects of Mo deficiency and defects in MOT1 on plant nitrogen and sulfur metabolisms and transcript accumulations.

In the second and the third chapter, I isolated and analyzed the altered sulfur response (*asr*) mutants. Forward genetics screens are powerful, unbiased approach to identify novel genes responsible for the biological processes of interest. The *asr7-1* showed hypersensitive phenotypes to sulfur deficiency. Genetic analysis identified that ASR7 is CPL1, an RNA polymerase II C-terminal domain phosphatase with two double-stranded RNA-binding domains. Another mutant, *asr2-1*, showed repressed response to sulfur deficiency. Genetic analysis identified that ASR2 is GLU1, a ferredoxin-dependent glutamate synthase important for photorespiration.

The aims of these two approaches were to clarify the molecular mechanisms of plant responses to nutrient deficiency. From the results, I could propose novel factors and pathways involved in the plant responses to Mo or S deficiency. These findings will be helpful to improve plant productivity and quality.

(1) Effects of molybdenum deficiency and defects in molybdate transporter MOT1 on transcript accumulation and nitrogen/sulfur metabolisms in *Arabidopsis thaliana.*

Molybdenum (Mo) is a micronutrient essential for plant growth, as several key enzymes of plant metabolic pathways contain Mo cofactor (Moco) in their catalytic centers. Mo-containing oxidoreductases include nitrate reductase, sulfite oxidase, xanthine dehydrogenase and aldehyde oxidase. These enzymes are involved in nitrate assimilation, sulfite detoxification, purine metabolism or the synthesis of abscisic acid, auxin and glucosinolates in plants. To understand the effects of Mo deficiency and a mutation in a molybdate transporter MOT1 on nitrogen and sulfur metabolisms in *A. thaliana*, transcript and metabolite profiling were conducted using the mutant lacking MOT1 in the presence or absence of Mo. Transcriptome analysis revealed that Mo deficiency had impacts on genes involved in metabolisms, transport, stress responses and signal transductions. Transcript level of a nitrate reductase *NR1* was highly induced

under Mo deficiency in *mot1-1*. In addition, metabolite profiles were analyzed using gas chromatography time-of-flight mass spectrometry, capillary electrophoresis time-of-flight mass spectrometry and ultra high performance liquid chromatography. The levels of amino acids, sugars, organic acids and purine metabolites changed significantly in the Mo-deficient plants. These results are the first investigation of the global effect of Mo nutrition and MOT1 on plant gene expressions and metabolism.

(2) CPL1 regulates sulfur deficiency response and improves sulfur deficiency tolerance in *Arabidopsis thaliana*

Sulfur (S) is a macronutrient essential for plant growth and development. To understand the molecular mechanisms of plant adaptation to sulfur deficiency (–S), EMS-mutagenized M_2 plants of *A. thaliana* were screened for mutants with altered patterns of sulfur-responsive gene expression (altered sulfur response; *asr* mutants). One of the mutants (*asr7-1*) showed reduced –S-inducible reporter gene expression under both normal and –S conditions. In addition, transcript accumulations of other sulfur-responsive genes such as serine acetyltransferase (*SAT1*) or a branched-chain aminotransferase (*BCAT4*) were altered in *asr7-1*. Growth of *asr7-1* under –S conditions was poorer than that of the wild type and –S-responsive amino acid accumulations were altered in the *asr7-1*, indicating the hypersensitivity of *asr7-1* to –S. The *asr7-1* mutation was mapped to a 39.2-kb region in chromosome IV, in which only one mutation in nucleotide sequence was found at an exon–intron junction of the *CPL1* gene. CPL1 is an RNA polymerase II C-terminal domain phosphatase with two double-stranded RNA-binding domains. We obtained four *cpl1* alleles and all of them showed reduced relative growth under –S, indicating that *CPL1* is *ASR7* and is required for maintenance of growth under –S. In addition, gene expressions and amino acid accumulations were altered in the *cpl1* mutants in the same way as *asr7-1*. Taken together, CPL1 is a novel factor involved in the regulation of sulfur metabolism, gene expression, and tolerance to –S stress.

(3) Ferredoxin-dependent Glutamate Synthase GLU1 Regulates Sulfur Assimilation Pathway in *Arabidopsis thaliana*

In order to identify the novel regulatory factors of plant gene expressions in response to sulfur deficiency (−S), we took a forward genetics approach using a transgenic *A. thaliana* line expressing green fluorescent protein (GFP) under the control

of a −S-inducible chimeric promoter. EMS-mutagenized M_2 plants were screened for mutants with altered patterns of sulfur-responsive gene expressions (altered sulfur response; *asr* mutants). One of these mutants (*asr2-1*) showed increased *GFP* expression under normal condition, and reduced *GFP* expression under −S conditions. Other sulfur-responsive genes such as adenosine 5'-phosphosulfate reductase (*APR1*), a sulfate transporter (*SULTR4;2*) and a serine acetyltransferase (*SERAT3;2*) were also repressed in *asr2-1* under −S conditions. *ASR2* was mapped to a 48 kb region in chromosome V, and a mutation was found in a ferredoxin-dependent glutamate synthase (*GLU1*/*GLS1*/*Fd-GOGAT1*) gene. GLU1 is important for reassimilation of photorespiratory ammonium into glutamate in plastid. We obtained other *glu1* alleles (*gls1-30* and *gls1-103*), and they also showed altered response to −S. These results indicate that *ASR2* is *GLU1*, *and* GLU1 is involved in sulfur deficiency responses in addition to photorespiration. Increased sulfate accumulations and altered levels of amino acids in the shoots of *glu1* under −S conditions were consistent with the altered response to −S. These results demonstrated the involvement of GLU1 in the regulation of sulfur metabolism and sulfur-regulated gene expression.

Publication

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