論文要約（４（２）関係）

# 博士論文要約（Summary）

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連合農学研究科 Tropical Bioresource and Plant Resource Production
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| タイトル | Molecular Characterization of Cysteine synthase in *Mimosa pudica* and *Leucaena leucocephala* |

**キーワード（Cysteine)(Mimosine) (*Mimosa pudica*) (*Leucaena leucocephala*)(*α*-aminoacrylate)**

## 「序論及び目的」

### Introduction

Environmental inorganic primary sulfur assimilation held in the plant cytoplasm, chloroplast, and mitochondria as cysteine (Bonner et al. 2005). Cysteine is the reduced sulfur donor for the components of the cell that plays the significant role in plant biology as a sensor for protein structure and folding, amino acid metabolism, enzyme regulation, many secondary metabolites production etc. (Bonner et al. 2005).

Cysteine biosynthesis is elevated during the sulfur (Saito et al. 1994) and heavy metal stress (Dominguez-Solis et al. 2001). Thus it protects plants against oxidative stress. Cysteine contains thiol (sulfide) that participates actively in the redox reaction as it contains strong nucleophilic properties (Hell and Wirtz 2011). Cysteine and its related molecules demonstrate predominant roles in plant photosynthesis, cope with harsh environmental conditions, plant immunity, autophagy, and the development of root structure (Romero et al. 2014).

*L. leucocephala* foliage contains mimosine (β-[N-(3-hydroxy-4-pyridone)-L-2-aminopropanoic acid]). Mimosine (N-heterocyclic nonprotein amino acid) is considered as toxic for the prokaryotes (Soedarjo et al. 1994) as well as eukaryotes (Lalande 1990). Mimosine usually chelates the metallic ions (bivalent). Thus it cannot act as a co-factor for various metallic ion-

dependent enzymatic reactions (Dai et al. 1994; Hashiguchi and Takahashi 1977; Negi et al. 2014). It can also form stable complex with PLP. Hence, it inactivates the activity of the PLP dependent enzymes (Crounse et al. 1962). By the inactivation of different enzyme activities mimosine causes various health hazard for the cattle after ingestion of its leaves such as enlarged thyroid glands, infertility, birth defects, loss of hairs etc. (Crounse et al. 1962; Dewreede and Wayman 1970; Hamilton et al. 1968; Joshi 1968; Negi et al. 2014).

Mimosine demonstrates potential defense mechanism against invading viruses by chelating the iron of ribonucleotide reductase (Dai et al. 1994) that is essential for DNA synthesis for viruses. Mimosine degrading enzymes from the seedling extract of *L. leucocephala* [mimosinase, (Smith and Fowden 1966)] and *M. pudica* (Suda 1960) catabolize the mimosine into 3.4 DHP, pyruvic acid, and ammonia (Negi et al. 2014). Hence, this ammonia may serve as the reservoir of carbon and nitrogen. Plants containing mimosine degrading enzymes may utilize this ammonia (mimosine degradation product) for their existence under the nutrient-limiting soil environment (Negi et al. 2014).

Cytosol is the prime place of cysteine synthesis in the cell (Haas et al. 2008; Heeg et al. 2008; López-Martín et al. 2008a; Watanabe et al. 2008a; Watanabe et al. 2008b) and Cy-OASTL is the major player for cysteine synthesis (Romero et al. 2014). Cytosol has 300 μM or more cysteine concentration (Romero et al. 2014). Hence, another part of the cell has less than 10 μM cysteine (Krueger et al. 2009). Cysteine is considering toxic to the cell if it is exceeding its threshold point due to the highly reactive nature of thiols in its structural backbone (Romero et al. 2014). Cysteine homeostasis perceived in the cell cytosol by converting L-cysteine into sulfide, ammonia, and pyruvate through DES 1 (L-Cys desulfhydrase) (Álvarez et al. 2010; López-Martín et al. 2008a). Cytosolic cysteine concentration administers redox signaling regulatory role in all plant biochemical processes, defense responses to biotic and abiotic stresses, and induce stress tolerance (Domínguez-Solís et al. 2001; Dominguez-Solis et al. 2004; Romero et al. 2014). Cysteine is also a good indicator of the antioxidative capacity of the cytosol (Álvarez et al. 2010; López-Martín et al. 2008a; López-

Martín et al. 2008b).

Thus OASTL plays an important role in plant metabolism and reduced sulfur assimilation pathway in plants. It also demonstrates sensory roles for biotic and abiotic stresses, plant autophagy, plant life cycle events, plant immunity as well as defense mechanisms.

**Purpose of the Studies**

1. Molecular characterization of cytosolic OASTL switches cysteine metabolism in *M. pudica* ;
2. Molecular characterization of cytosolic OASTL switch cysteine and mimosine production in *L. leucocephala* ; and
3. Molecular cloning of chloroplastic cysteine synthase in *L. leucocephala*.

**「材料及び方法」**

We followed the below methods for this study-

1. Total RNA extraction

2. RT-PCR

3. Restriction enzyme treatment

4. Ligation

5. Transformation

6. Mass culture

7. IPTG induction

8. Enzyme extraction

9. GSTtag purification of enzyme

10.Cysteine synthase activity measurement

11. Mimosine synthase activity measurement

12. Molecular dynamic simulation study

**「結果」**

It is evident that there are multiple copies of OASTLs in plants. All the plant OASTLs demonstrate distinct features. Cy-OASTL of *M. pudica* is only cysteine specific, but it contains some unique features for the sulfate assimilation regulatory network and plays a sensory role in the cell. Furthermore, site directed mutagenesis considering the structural and molecular point will provide us with new insights regarding Cy-OASTL in the cysteine biosynthesis process and sulfate assimilation network in plants.

The Cy-OASTL showed dual functions to produce cysteine and mimosine with reacting respective substrate but the catalytic efficiency is not the same as guided by the common chemical reaction mechanism. CAS and OASTL display little difference in the interacting residues of the cysteine moiety of PLP-Cys. In case of CAS, methionine is positioned at the end of TSGN loop whereas Threonine (Thr 81) in case of Cy-OASTL of *L. leucocephala*. CAS and Cy-OASTL respective reaction schemes are operated by forming of the common AAA intermediate. This AAA (external aldimine) exhibited conformational differences with the internal (open conformation) and external (closed conformation) aldimines (Benci et al. 1997; Tai and Cook 2006). Cysteine and mimosine production reaction is the consequence of adjustment of the conformational patterns (open and closed) in the enzyme (Burkhard et al. 2000). This conformational changes are guided by the reciprocation between the substrate OAS $\alpha$-carboxyl moiety and the substrate binding loop following the closed conformation of the active site of the enzyme (Burkhard et al. 2000). Thus, it allots firmness to the external aldimine and guides the OAS functional group for bona fide alignment with the enzyme (Burkhard et al. 2000), Cy-OASTL functional group Lys 49. Thus, this study, as well as the molecular dynamics simulation, postulated for the first time a putative mechanism of mimosine biosynthesis pathway in *L. leucocephala*. This mechanism will help future research concerning mimosine-free *L. leucocephala* plant development and mimosine as a signaling molecule to cope with the various stress conditions. Furthermore, site-directed mutagenesis and crystallographic analysis of cysteine synthase will provide us new insight about the cysteine and mimosine biosynthesis processes.

Comparing with two sequences of *L. leucocephala* (AHG97874 and LC306827), we observed that Val 181 (LC306827) corresponded to position Ala (AHG 97874) and Thr 271 (LC306827) to the Ser 272 (AHG97874). Thr 81, Ser 181, and Thr 185 make a significant contribution in the OASTL

reaction mechanism (Yi et al. 2012). Different amino acids in the corresponding positions of AHG 97874 might be one reason for its inability to produce mimosine.

Cy-OASTL showed differences in the active site structure of the substrate binding nature related to the possible reaction chemistry of cysteine and mimosine production pathways that accelerated the respective reactions. Additionally, some additional changes occur inside and outside of the active site structure of PLP-Lys 49 which is necessary for the respective reaction mechanism (Yi et al. 2012). Changes in the size of active site gateway from the open to the closed one of the enzyme structure would impart a steric gate to restrain the nucleophile approach to the AAA intermediate (Yi et al. 2012).

Plants use sulfate from soil, as well as $SO_2$ and $H_2S$ from the atmosphere, to obtain cysteine. Cysteine then acts as a donor molecule of reduced sulfur-containing cellular components, such as secondary metabolites, methionine, GSH, and vitamins. During cysteine biosynthesis, a CSC is formed that is associated with a sulfur starvation condition in the cell compartment. Therefore, Ch-OASTL may perform a sensory role in sulfur-constraining conditions, activate sulfur transporter genes, and accelerate the sulfate regulatory network of plant. We can also infer that Ch-OASTL is necessary for the survival of plants, and its regulatory role makes it a sensor and precursor of various metabolic compound molecules. In the future, investigations on the crystallization, and site-directed mutagenesis of Ch-OASTL will give us new perspectives on the sensory and networking functions of Ch-OASTL in cysteine-harboring plant species.

**「結論及び考察」**

Cysteine is the crucial reduced sulfur donor for the plants. It is important to keep up the cellular sulfur homeostasis. Cysteine is the active site for S-metabolism, different enzyme reactions, electron transfer, and biosynthesis of protein, glutathione, and S-containing compounds. Cysteine and Mimosine biosynthesis pathway mainly depends on intermediate OAS followed by the representative amino acids production with their respective substrates.

*M. pudica* and *L. leucocephala* has mimosine in their different vegetative parts. *L. leucocephala* could be considered as a good fodder if it is free from mimosine. Thus it is necessary to find out the cysteine and mimosine channel in these two plants.

In my study, I isolated the candidate cDNA encoding cysteine synthesis (OASTL) from *M. pudica* and *L. leucocephala*. Out of these cDNA clones, I observed that cytosolic cDNA from *M. pudica* and chloroplastic cDNA from *L. leucocephala* is cysteine specific, whereas the *L. leucocephala* cytosolic cDNA has a dual role for cysteine and mimosine production.

Thus, the present study revealed that Cy-OASTL of *M. pudica* and Ch-OASTL of *L. leucocephala* is responsible for the sulfate assimilation regulatory network and plays a sensory role in the cell. Hence, Cy-OASTL of *L. leucocephala* plays a significant role in coping with the various abiotic stress and will help the future researcher to develop mimosine free *L. leucocephala* plant that could be a good source for cattle feed.