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SHORT COMMUNICATION

The relationship between thiamine and two symbioses: Root nodule symbiosis and arbuscular mycorrhiza

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ABSTRACT

Lotus japonicus THIC is expressed in all organs, and the encoded protein catalyzes thiamine biosynthesis. Loss of function produces chlorosis, a typical thiamine-deficiency phenotype, and mortality. To investigate thiamine's role in symbiosis, we focused on *THI1*, a thiamine-biosynthesis gene expressed in roots, nodules, and seeds. The *thi1* mutant had green leaves, but formed small nodules and immature seeds. These phenotypes were rescued by *THI1* complementation and by exogenous thiamine. Thus, *THI1* is required for nodule enlargement and seed maturation. On the other hand, colonization by arbuscular mycorrhiza (AM) fungus *Rhizophagus irregularis* was not affected in the *thi1* mutant or by exogenous thiamine. However, spores of *R. irregularis* stored more thiamine than the source (host plants), despite lacking thiamine biosynthesis genes. Therefore, disturbance of the thiamine supply would affect progeny phenotypes such as spore formation and hyphal growth. Further investigation will be required to elucidate thiamine's effect on AM.

Abbreviation: AM, Arbuscular mycorrhiza; RN, Root nodule

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Legumes establish two typical mutualistic plant–microbe interactions: root nodule (RN) symbiosis with rhizobia and arbuscular mycorrhiza (AM) with AM fungi. Rhizobia and AM fungi, respectively, provide host plants with nitrogen generated by fixation of atmospheric nitrogen and mineral nutrients such as phosphorus.[1,2] Many symbiosis-related genes have been isolated and their functions have been described. Researchers have found both functions common to RN and AM symbioses, as well as RN-specific and AM-specific functions.[1,2] In previous research, we clarified that one of the symbiosis-related genes, the thiamine biosynthesis gene *THI1*, promoted nodule enlargement but did not affect AM.[3]

Thiamine (vitamin B1) is essential for living organisms. Unlike animals, plants can synthesize thiamine through a multiple-step pathway.[4,5] Thiamine is assembled from pyrimidine and thiazole moieties. THI1 and THI2 (a THI1 paralog) catalyze the biosynthesis of the thiazole moiety, and THIC catalyzes the biosynthesis of the pyrimidine moiety.[4,5] Fig. 1 shows the phenotypes of thiamine-deficient mutants of *L. japonicus*. *THIC* is a single copy gene in *L. japonicus* and is expressed in all tissues, including leaves.[3,6] Therefore, the *thiC* mutant displayed chlorosis, which is a typical and lethal thiamine-deficiency phenotype in plants.[3] *THIC* was also expressed in nodules, and the nodule number in the *thiC* mutant was reduced.[3] However, it is not clear whether the nodulation defect in the *thiC* mutant is due to the loss of *THIC* function or to the consequences of chlorosis. In *L. japonicus*, *THI1* is expressed mainly in roots, nodules, and seeds, whereas *THI2* is expressed in shoots.[3,6] Although *THI2*-knockdown plants displayed chlorosis similar to that in the *thiC* mutant, the *thi1* mutant had green leaves and no significant growth defect.[3] By using this mutant, we successfully demonstrated that thiamine affects nodule development in roots by excluding the effects of chlorosis and the growth defect in shoots.[3] Plants treated with exogenous thiamine had enlarged nodules, suggesting that thiamine promotes nodulation.[3] In addition, exogenous thiamine treatment rescued the small nodules and immature seeds of the thi1 mutant.[3] These results indicated that *THI1* is involved in both nodule development in roots and seed maturation in shoots (Fig. 1).

Unlike RN symbiosis, AM colonization was not affected by exogenous thiamine treatment, and AM colonization of the *thi1* mutant root was normal (Fig. 1).[3] However, this result did not rule out the possibility that thiamine contributes to the AM symbiosis. In the *thi1* mutant, the root thiamine content decreased slightly but significantly,[3] and this small decrease might cause no detectable morphological or quantitative changes in AM colonization. In addition, it has been reported that the AM fungus *Rhizophagus irregularis* lacks a thiamine biosynthesis pathway.[7] This suggests that if thiamine is an essential nutrient for this AM fungus, it must be obtained from the host's roots.

To investigate thiamine accumulation in AM fungi, we measured the thiamine content in their spores (Fig. 2A). The

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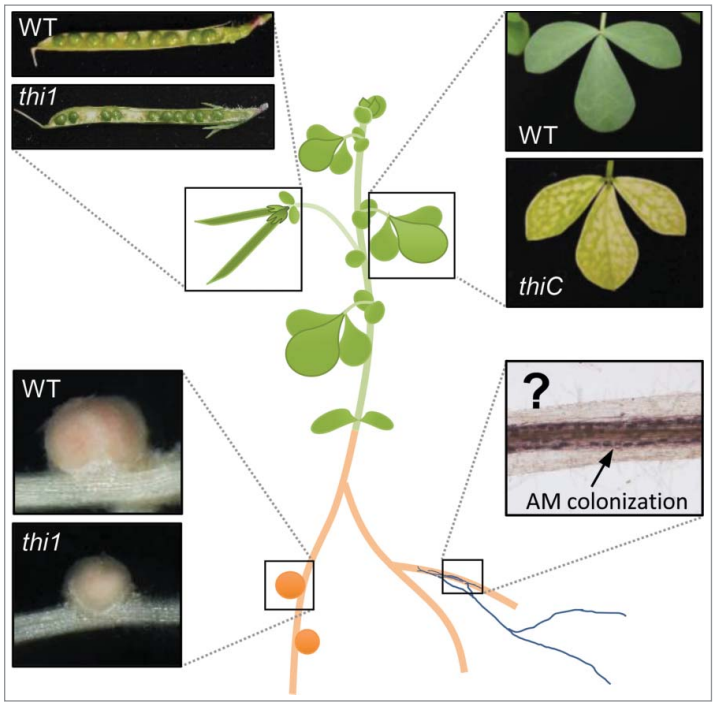

Figure 1. Phenotypes of thiamine-deficient mutants of *Lotus japonicus*. Photographs show the pods, leaves, and nodules of the wild-type (WT) plant and the thiamine-deficient *thi1* and *thiC* mutants. The *thi1* mutant showed abnormal phenotypes in pods and nodules, and the *thiC* mutant showed chlorosis. Although thiamine did not affect AM in our previous study, [3] further investigations will be required to understand its function in AM.

thiamine content in the AM spores was comparable to that in wheat bran, which is a nutrient-rich tissue that contains more thiamine than other plant tissues.[8] For example, the content in the *L. japonicus* roots was smaller than that of AM spores (Fig. 2A). Despite the lack of thiamine biosynthesis genes in *R. irregularis*, the high accumulation of thiamine in its spores suggests that AM fungi positively absorb thiamine from the host plant's roots during the AM symbiosis.

Sequencing the genome of *R. irregularis* also revealed a lack of some genes for fatty acid biosynthesis.[7,9] As in the case of thiamine, it has been reported that large amounts of fatty acids are stored in the spores,[10,11] and we observed this in the form of lipid drops released from the ruptured spores (Fig. 2B). These observations demonstrate that the AM fungus accumulates various nutrients, which they cannot synthesize by themselves, in the spores. These nutrients would be required to sustain the germinating spores until they can reach their next host (i.e., the next source of these nutrients) during their non-symbiotic state, from spore germination through hyphal elongation in the soil and infection of the host root to arbuscule formation. Therefore, the supply of thiamine from host plants during the symbiosis state should be a crucial process for them. Our previous evaluation of AM phenotypes in the *thi1* mutant was limited to infection of the host plant (morphological and

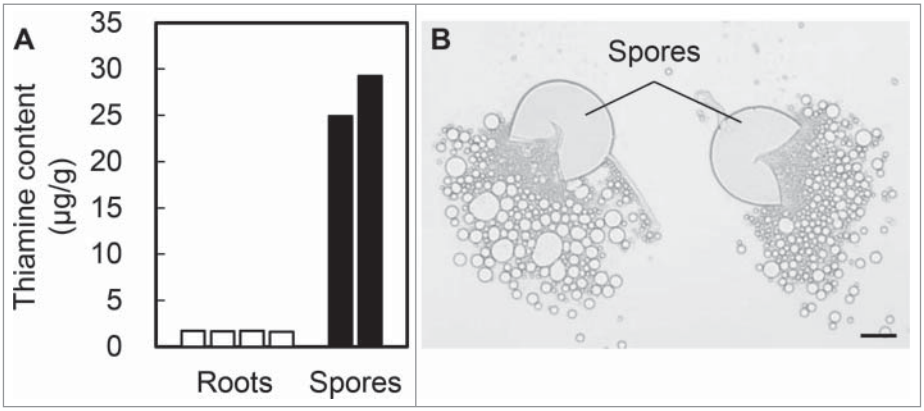

Figure 2. Thiamine content and lipids in the spores of the AM fungus *Rhizophagus irregularis*. (A) Thiamine content of *L. japonicus* roots and AM spores was determined using a VitaFast Vitamin B1 kit (Biopharm) that detects thiamine and its phosphate esters. Three-week-old *L. japonicus* roots and AM spores (DAOM197198; Premier Tech; ca. 40 000 spores) were homogenized and incubated in 71.4 mM citric acid buffer (20 mL; pH 4.5) in the presence of 300 mg Taka-Diastase (Sigma) for 1 h at 37°C, and then for 30 min at 95°C; the solution was then quickly cooled to 30°C and centrifuged at 9200× *g* for 10 min. The supernatants were passed through 0.22-μm mixed cellulose ester filters. Bioassay was performed according to the manufacturer's instructions. Bar plots represent independent biological replicates. (B) Ruptured AM spores releasing lipids. Scale bar, 50 μm.

quantitative analysis of hyphal and arbuscular colonization).[3] However, the later life cycle stages, namely spore formation and growth of spore progeny, may be influenced by a deficiency of key nutrients such as thiamine. Therefore, further investigations will be required to elucidate the effect of thiamine on AM symbiosis and AM fungi.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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