(Form No. 14)

ABSTRACT OF DOCTORAL THESIS

Name: Takaya Tominaga

Title: Molecular mechanisms regulating *Paris*-type arbuscular mycorrhizal symbiosis in *Eustoma grandiflorum*
(トルコギキョウにおける*Paris*型アーバスキュラー菌根共生制御の分子機構に関する研究)

Diverse microbes inhabit the vicinity of land plant roots, the so-called rhizosphere. Rhizospheric microbes affect the host by deterring or potentiating their growth. Arbuscular mycorrhizal (AM) symbiosis is the most ancient and common association between more than 70% of the extant terrestrial plants and Glomeromycotina fungi. Host plants and AM fungi exchange photosynthetic carbohydrates and soil inorganic materials. Whereas AM symbiosis is general, the morphological features found in mycorrhizal roots differ depending on the AM-host species: *Arum*- and *Paris*-type AMs. In *Arum*-type AM, AM fungal hyphae elongate the intercellular space of the root cortex. The AM fungal hyphae penetrate the cortical cells to form tree-like hyphal structures, namely arbuscules, to exchange nutrients efficiently. *Paris*-type AM shows intracellular fungal colonization and hyphal coils. Although each AM morphotype is evenly found in nature, recent studies have mainly focused on *Arum*-type AM hosts because most model plants form the *Arum*-type AM. Here, we investigated how *Paris*-type AM symbiosis is established at the molecular level using the Gentianaceae plant *Eustoma grandiflorum* and the model AM fungus *Rhizophagus irregularis*.

The phytohormone gibberellin (GA) has been known to downregulate the development of *Arum*-type AM symbiosis, which is attributable to the requirement of a GA-degraded DELLA protein for accommodating AM fungi. Indeed, we could confirm that exogenous GA severely inhibited AM fungal infection and arbuscule initiation in *Lotus japonicus* and *Allium schoenoprasum* roots forming *Arum*-type AM. However, GA treatment significantly promoted AM fungal infection and arbuscule formation in *Paris*-type forming *E. grandiflorum* and *Primula malacoides* roots. Thus, GA-mediated regulation of AM symbioses would differ depending on AM morphotypes. Moreover, root exudates collected from GA-treated *E. grandiflorum* roots exhibited significantly high hyphal branching activity, suggesting that unidentified chemical compounds increase the branching of extraradical AM hyphae.

We conducted a comparative transcriptomic analysis to reveal the molecular mechanisms underlying *Paris*-type AM symbiosis. This study used phylogenetically distant AM-host plants, *L. japonicum* (*Arum*-type AM), *E. grandiflorum* (*Paris*-type AM), and *Daucus carota* (Intermediate-type AM). Intermediate-type AM shows intraradical hyphae without hyphal coils.

Regardless of the distinct AM morphotypes among the three host plants, AM fungal colonization considerably enhanced host growth and the expression of conserved and known AM-related genes. These results indicate the responses of the examined host plants to AM fungal colonization. However, GA treatment significantly suppressed the expression of AM-related genes in *L. japonicus* and *D. carota*, whereas that in *E. grandiflorum* was slightly promoted by GA. Interestingly, GA severely reduced a representative signal molecule strigolactone (SL) that triggers AM fungal branching in *L. japonicus* and *E. grandiflorum* roots.

Genetic differences among phylogenetically distant plants might have caused some undesired results in our comparative analysis. Therefore, we also investigated the transcriptional responses of *Solanum lycopersicum* (tomato) forming *Arum*- and *Paris*-type AMs depending on the AM fungal species. We confirmed that tomato roots exhibited *Arum*- and *Paris*-type AMs with *R. irregularis* and another AM fungus, *Gigaspora margarita*, respectively. As found in our former analysis, the biomass of tomato plants and expression levels of some AM-related genes were upregulated during *Arum*- and *Paris*-type AM symbioses. In contrast, the biosynthesis of bioactive GAs and immune-related pathways were transcriptionally activated in *Paris*-type AM roots of tomato plants. Our data suggest that *Arum*- and *Paris*-type AM symbioses would be mutually beneficial; however, bioactive GAs might participate in establishing *Paris*-type AM symbiosis.

In conclusion, our study revealed that the upstream molecular mechanisms regulating AM symbiosis would be insensitive to GA in *Paris*-type AM plants, whereas that in other host plants would be vulnerable to the phytohormone. Furthermore, GA-treated *E. grandiflorum* roots would secrete other chemical compounds than SL to the rhizosphere to stimulate AM fungi. These findings would accelerate our understanding of how diverse the molecular mechanisms underlying AM symbioses are in nature.

* In addition, some of the figures, etc., have been omitted.