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学 位 論 文 要 旨

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題目: 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 narrow space around land plant roots called the rhizosphere. It has been generally known that rhizospheric microbes affect host plants by deterring or potentiating their growth. Regarding symbiotic interaction, arbuscular mycorrhizal (AM) symbiosis is established between more than 70% of the extant terrestrial plants and AM fungi that belong to the Glomeromycotina subphylum. Host plants supply AM fungi with photosynthetic carbohydrates, such as glucose and lipids; in return, AM fungi transfer inorganic phosphate, nitrogen, and water to host plants. Although AM symbiosis is general mutualism established in the plant kingdom, host plants form distinct AM morphotypes depending on AM-host species: *Arum*- and *Paris*-type AMs. In *Arum*-type AM, AM fungi colonize 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. On the other hand, *Paris*-type AM shows intracellular fungal colonization and hyphal coils. *Paris*-type AM is frequently formed in slow-growing understory and woody plants, even in mycoheterotrophic plants exploiting AM fungi for nutrients.

Recent studies have revealed that several phytohormones and their signaling pathways regulate AM symbiosis using *Arum*-type AM model plants, such as *Lotus japonicus* and crop species. For example, host plant roots secrete the phytohormone strigolactones (SLs) biosynthesized from carotene to stimulate AM fungal spore germination and hyphal branching. The attenuation of early AM fungal infection in SL-deficient plants shows that SLs have essential roles in upregulating the so-called pre-symbiotic stage during AM symbiosis. Since AM symbiosis costs host plants up to 20% of photosynthates, host plants can also suppress excessive AM fungal colonization. AM fungal colonization in host roots requires repressors of gibberellin (GA) signaling, DELLA proteins, that are degraded in the presence of GA. In addition, DELLA proteins also upregulate arbuscule formation. Consequently, GA treatment severely inhibits AM fungal colonization and arbuscule initiation in *Arum*-type model host plants. Moreover, in *L. japonicus* and rice, exogenous GA treatment also suppresses the biosynthesis of SLs. Hence, GA has been generally known as a negative regulator of AM symbiosis.

Although host plants that exhibit *Paris*-type AM are generally found in nature, past studies have mainly selected *Arum*-type AM model plants, as mentioned above. The difficulty in growing *Paris*-type AM hosts attributes to the problem. Thus, the understanding of regulatory mechanisms underlying *Paris*-type AM symbiosis remains poor. To this end, we selected the Gentianaceae plant *Eustoma grandiflorum,* which forms *Paris*-type AM and grows under laboratory conditions, to clarify the mechanisms regulating *Paris*-type AM symbiosis at the molecular level.

First, we treated *L. japonicus* and *E. grandiflorum* seedlings with the bioactive GA_3 and inoculated them with the model AM fungus *Rhizophagus irregularis*. As a result, GA treatment

severely suppressed the AM fungal colonization and arbuscule formation in *Arum*-type AM symbiosis in *L. japonicus* and *Allium schoenoprasum* (chive), consistent with previous studies. Conversely, compared with control, AM fungal colonization was drastically increased in GA-treated *E. grandiflorum* roots. Moreover, GA did not affect arbuscule development in the plant. Notably, in the presence of GA, the rhizospheric hyphae of *R. irregularis* highly branched in the vicinity of *E. grandiflorum* roots, indicating that GA treatment primarily upregulated the pre-symbiotic stage of *Paris*-type AM symbiosis. Interestingly, GA enhanced the establishment of *Paris*-type AM symbiosis in another host, *Primula malacoides*, indicating that the responses of AM-host plants to exogenous GA would differ depending on the AM morphotypes.

Next, we performed comparative transcriptomic analyses to clarify how exogenous GA elevated *Paris*-type AM symbiosis in *E. grandiflorum*. In this analysis, *D. carota* was added because the host plant shows intracellular hyphal colonization, as found in *Paris*-type AM. Our transcriptomic analysis revealed that the expression levels of several genes indispensable for AM symbiosis were significantly reduced in *L. japonicus* and *D. carota*, in agreement with the decreased AM fungal colonization in GA-treated these host plants. On the other hand, the AM-related genes were not transcriptionally inhibited by GA in *E. grandiflorum*. Instead, GA increased the expression of AM-related genes along with the enhanced infection of *R. irregularis* in *E. grandiflorum*.

Notably, GA treatment did not change the colonization level of Gigasporacea AM fungus *Gigaspora margarita* establishing *Paris*-type AM symbiosis in *E. grandiflorum* roots. Also, the expression levels of AM-related genes were not affected by GA during *Paris*-type AM symbiosis with *G. margarita*. Therefore, our data suggest that the upstream signaling pathways required for *Paris*-type AM symbiosis would be insensitive to GA, whereas that of *L. japonicus* and *D. carota* would be vulnerable to the phytohormone. Furthermore, it is indicated that GA-treated *E. grandiflorum* produces and secretes some molecules, such as SLs, that possess hyphal branching activity to *R. irregularis* but not to *G. margarita*. Nevertheless, SL biosynthesis was significantly blocked by GA in *E. grandiflorum* roots.

Finally, we identified the *E. grandiflorum*-derived compounds triggering hyphal branching in *R. irregularis* based on the results of our transcriptomic analyses. Our analyses showed that the biosynthetic pathway of Gentianaceae-specific monoterpene glucosides called gentiopicroside (GPS) and swertiamarin (SWM) was transcriptionally upregulated in GA-treated *E. grandiflorum* AM roots. HPLC analysis also supported that GA significantly promoted the accumulation of GPS and SWM in *E. grandiflorum* roots. Therefore, we conducted a bioassay to quantify the hyphal branching activity of GPS and SWM using *R. irregularis*, *G. margarita*, and another *Rhizophagus* fungus *R. clarus,* whose colonization was drastically promoted in GA-treated *E. grandiflorum*. GPS and SWM significantly increased the hyphal branches in *R. irregularis* and *R. clarus* in the 1–100 nM concentration range. However, *G. margarita* did not respond to exogenous GPS and SWM, in agreement with our hypothesis that the *E. grandiflorum*-derived branching factors would be inactive to the fungus. Furthermore, the transcriptional responses of *R. irregularis* to exogenous GPS were analyzed, and we found that GPS activated cytoskeletal functions in the fungus. This result was common in *R. irregularis* treated with a synthetic SL analog, *rac*-GR24.

Expecting the application of GPS as a biostimulant of AM fungi to improve crop cultivation, we treated chive roots with 1–100 nM GPS. Surprisingly, GPS treatment significantly enhanced *R. irregularis* colonization in chive roots in a dose-dependent manner without detrimental effects on chive growth. Furthermore, GPS did not provoke the seed germination of a parasitic plant, *Orobanche minor*, that exploits SLs for germinating.

Our study provided a novel insight that the molecular mechanisms underlying *Paris*-type AM differ from model plants and are insensitive to GA. In addition, our findings suggest that GPS and SWM would upregulate the pre-symbiotic stage of *Paris*-type AM symbiosis in *E. grandiflorum*. The positive effect of GPS on AM fungi also indicates that the metabolite could potentially be a biostimulant for improving the establishment of AM symbiosis in crops.