論文の内容の要旨

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氏名　　ジュティポーン タッサグンパニト
指導教員名　　浅見　忠男

論文題目 Study on Signal Molecules Regulating Strigolactones and Brassinosteroids Function
（ストリゴラクトン・ブラシノステロイドの機能を制御するシグナル分子に関する研究）

Chapter 1. Introduction

Plant hormones are the small molecules which influence physiological processes consisting mainly of growth, differentiation and development in plants. Plant growth regulators are chemicals which mimic natural plant hormones and widely used for increasing crop yield and for studying how plants response to plant hormones. In this study, I focused on two kinds of plant hormone, brassinosteroids (BRs) and strigolactones (SLs).

BRs are a group of steroidal plant hormone. BRs have various effects on a wide spectrum of cellular responses including cell division, cell elongation and so on. SLs were first identified as seed germination stimulants of root-parasitic weeds. SLs are also known as plant hormones involved in the regulation of various phenomena such as the suppression of shoot branching. Identification and characterization of *Arabidopsis* biosynthetic and signaling mutants of BRs and SLs are the basic way for studying BRs and SLs functions. The phenotypes of these mutants as well as the phenotypes induced by their agonists or biosynthesis inhibitors are both important for elucidating signal factors regulating functions of BRs and SLs.

Not only each plant hormone functions but also the crosstalk among hormones is essential for understanding plant responses to plant hormones. Previous data suggested that there are some crosstalks between SLs and BRs in the photomorphogenesis or light-adapted development. As reported in the previous study, STH7, a member of B-box zinc-finger proteins, exists downstream of BR signal and could be one of the candidates that mediate the crosstalk. It was also reported that *STH7* gene expression is up-regulated by SL. Here, to elucidate the crosstalk between BR and SL, I investigated the two possibilities. Firstly, BR mimics exert BR like activity. Secondly, STH7 could be a factor mediating the SL-BR crosstalk.

Chapter 2. Characterization of Synthetic Ecdysteroid Analogues as Functional Mimics of Brassinosteroids

The concentrations of BRs are very low in many plants, and consequently, the yield of naturally occurring BRs from plants is typically poor. Several plant species produce ecdysteroids, which are known as insect molting steroid hormones. In this study, I evaluated the biological activities of three hydroxysteroidal compounds, 20-hydroxyecdysone (ECD), 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), and 7,8-dihydro-5α,8α-20-hydroxyecdysone (α-DHECD), and compared their activities with that of brassinolide (BL), the most potent BR. ECD was obtained at amount of quantity from stem bark of *Vitex glabrata*, a common plant in Thailand. ECD was readily converted to DHECD and α-DHECD, by the catalytic hydrogenation and the subsequent base-catalyzed epimerization.

The potency of these three hydroxysteroidal compounds as functional mimics of BRs in rice and *Arabidopsis* was investigated. In rice, DHECD and α-DHECD enhanced the degree of lamina inclination, as do BRs. In *Arabidopsis*, DHECD and α-DHECD increased hypocotyl length in the wild-type, and also partially overcame the hypocotyl shortening caused by brassinazole (Brz), a specific BR biosynthesis inhibitor. DHECD and α-DHECD partially reduced dwarfism in the BR-biosynthesis-deficient mutant, *det2*. Treatment with DHECD or α-DHECD decreased the expression of the BR down-regulated genes, and increased the expression of BR up-regulated genes, such as *TCH4* and *SAUR-AC1*. Moreover, DHECD and α-DHECD induced the accumulations of dephosphorylated BIL1/BZR1 that enhance BR signaling as a master transcription factor. In contrast, ECD did not show BR mimic activity.

Chapter 3. Strigolactone and Karrikin Promote Light-adapted Development of *Arabidopsis thaliana* in a STH7 Function Dependent Manner

SLs and karrikins (KARs), which are a class of seed germination stimulant containing a methyl-butenolide moiety, were used in this study. Both SLs and KARs have been reported to inhibit hypocotyl elongation which can be thought as one of the light-adapted developments. The previous data of my group showed that SL and KAR can enhance *STH7* expression level. STH7 was reported as a positive regulator of photomorphogenesis. From this reason, STH7 could be a candidate protein that functions in linking SL and KAR to induce light-adapted development.

GR24, a synthetic SL analogue and KAR_1, a KAR, inhibited the hypocotyl elongation under weak light condition. To investigate roles of SLs and KARs in photomorphogenesis of *Arabidopsis* seedlings, *STH7*-overexpressing (*STH7ox*) and functionally defective *STH7* (*STH7-SRDX*) mutants were prepared. *STH7-SRDX* mutants were less sensitive to hypocotyl inhibition induced by both GR24 and KAR_1 treatment under weak light condition. Moreover, *Arabidopsis* SL signal mutant (*max2-1),* biosynthesis mutant (*max3-1*), receptor mutant (*d14-1*) and KAR

receptor mutant (*kai2-1*) were used to investigate the effect of SL and KAR on light-adapted development. The hypocotyl of *max2-1* was insensitive to both GR24 and KAR_1 implying that MAX2 is important for both SL and KAR signal transduction. The effect of racemic mixture of GR24 (*rac*-GR24), pure enantiomer of GR24 that has the same configuration as 5DS ($GR24^{5DS}$), and pure enantiomer of GR24 that has the same configuration as *ent*-5DS ($GR24^{ent\text{-}5DS}$) was compared on those mutants. $GR24^{5DS}$ significantly inhibited *kai2-1* hypocotyl elongation but not *d14-1*. While, $GR24^{ent\text{-}5DS}$ showed a significant reduction of *d14-1* hypocotyls but not of *kai2-1*. These results support that $GR24^{5DS}$ and $GR24^{ent\text{-}5DS}$ is received separately by D14 and KAI2, respectively. Moreover, KAR_1 reduced *d14-1* hypocotyls but could not decrease *kai2-1* hypocotyls indicating that KAR is perceived by KAI2.

The anthocyanin content was increased in *STH7ox* when de-etiolated under light conditions and GR24-treated plants enhanced the anthocyanin production. GR24 and KAR_1 treatment significantly increased the expression level of photosynthesis-related genes, suggesting that SL and KAR induce light-adapted development in the STH7-dependent manner.

Chapter 4. Coordination of STH7 and the Brassinosteroid Regulated Molecules Mediates the Crosstalk between Strigolactone and Brassinosteroid in Light-adapted Development

SLs promote the light-adapted development in *Arabidopsis* hypocotyls elongation, but BRs function oppositely to stimulate hypocotyl elongation. To investigate the possibility of crosstalk between these two hormones, effects of SL and BR on the hypocotyl elongation under weak light condition were investigated. The result showed that BL repressed the effect of GR24 on the inhibition of hypocotyl elongation in wild-type *Arabidopsis*. The quantitative real-time PCR (qRT-PCR) analysis showed that the expression level of *STH7* was synergistically up-regulated by the co-application of GR24 and Brz. To understand functions of STH7, *STH7ox* and *STH7-SRDX* mutants were used. *STH7-SRDX* mutant was insensitive to GR24 and weakly but significantly responded to Brz in the inhibition of hypocotyls. Moreover, Brz enhanced the effect of GR24 on the inhibition of hypocotyl elongation in both wild-type and *STH7ox*. To investigate roles of STH7 in SL and BR crosstalk suggested above, genes both STH7-regulated and BR-regulated were analyzed by using the previously published microarray data. In overlapping genes in the *STH7*-upregulated and the BR-repressed genes, half of them, including *STH7*, *ELIP2* and *CHI*, were the genes related to the light-adapted development. The qRT-PCR showed that the co-application of GR24 and Brz highly up-regulated the transcript level of *ELIP2*, *CHI* and their homologs.

Next, the double mutant analysis between *STH7ox* or *STH7-SRDX* and the BR gain-of-function *bil1-1D* or *bes1-D*, were performed to investigate the SL and BR crosstalk. The *bil1-1D*×*STH7ox* double mutant exhibited a small rosette, a high contents of chlorophyll and

anthocyanin like *STH7ox*. Co-treatment of GR24 and Brz in *bil1-1D×STH7ox* double mutants induced shorter hypocotyls than individual application of either GR24 or Brz did. In contrast both *bes1-D×STH7ox* and *bes1-D×STH7-SRDX* double mutants were insensitive to Brz. Either GR24 or Brz treatment down-regulated the expression of *SAUR-AC1* and *TCH4* in wild-type and *bil1-1D×STH7ox*. This result supports that SL suppresses the transcription of cell elongation-related genes as same as Brz. Moreover, the increase of *STH7* expression by co-treatment of GR24 and Brz was clearly shown in *bil1-1D* but not in *bes1-D* mutant. The results implied that BIL1 might mainly repress *STH7* transcription. Furthermore, the electrophoretic mobility shift assay showed that BIL1 interacted with E-box (CACATG) in *STH7* promoter.

Conclusion

The photomorphogenesis is mediated by various plant hormones including SL and BR. Among three hydroxysteroid compounds, DHECD and α-DHECD had BR-like function. DHECD and α-DHECD activated BR signal transduction pathway by accumulating dephosphorylated form of BIL1/BZR1, which activates BR signaling. Therefore, DHECD and α-DHECD could be used as BR mimic compound to suppress the light-adapted growth.

In contrast with BR, SL and KAR stimulate the light-adapted development. GR24 and KAR_1 up-regulated the expression of *STH7*. The hypocotyl growth in *STH7-SRDX* mutant was insensitive to GR24 and KAR_1. Moreover, treatment of both GR24 and KAR_1 up-regulated the photosynthesis-related genes in the STH7-dependent manner indicating that STH7 is essential for the light-adapted process.

The application of Brz enhanced effects of SL on the inhibition of hypocotyl elongation and on the up-regulation of *STH7*. The *bil1-1D×STH7ox* double mutant displayed the shorten hypocotyl similar to *STH7ox*, whereas *bil1-1D×STH7-SRDX* was insensitive to both SL and Brz. Lastly, the binding of BIL1/BZR1 protein to E-box element on the *STH7* promoter was confirmed. Above results suggested that STH7 works downstream of BIL1/BZR1. I think SL and BR play an antagonistic role in light-adapted development of *Arabidopsis* likely in the STH7-dependence.

Publications

Thussagunpanit J, Nagai Y, Nagae M, Mashiguchi K, Mitsuda N, Ohme-Takagi M, Nakano T, Nakamura H, Asami T. Involvement of STH7 in light-adapted development in *Arabidopsis thaliana* promoted by both strigolactone and karrikin. Biosci Biotechnol Biochem. 2017;81:292–301.

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