学　位　論　文　要　旨

Characterization of seed germination stimulants for root parasitic plants produced by cucumber and maize

キュウリおよびトウモロコシが生産する根寄生植物種子発芽刺激物質の解析

生物生産学科学専攻　生物制御科学大講座

Pichit Khetkam

Witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *Phelipanche* spp.) are devastating root parasitic weeds which cause significant crop losses around the world. Seed germination of these root parasites can only be induced by host-derived signals called germination stimulants produced by and released from plant roots. Among the germination stimulants characterized so far, strigolactones (SLs) are most important as they are indispensable host recognition signals for symbiotic arbuscular mycorrhizal (AM) fungi in the rhizosphere and function as a novel class of plant hormones regulating shoot and root architecture *in planta*. In this study, isolation and structural determination of germination stimulants produced by cucumber (*Cucumis sativus* L.) and maize (*Zea mays* cv. Pioneer 2817) were conducted. The plants were grown hydroponically with tap water and root exudates collected by using activated charcoal for several weeks. The root exudates were extracted with ethyl acetate and the extracts were subjected to bioassay-guided purification with *Orobanche minor* seeds. The structures of all purified compounds were determined by spectroscopic analyses.

Cucumber plants were found to exude at least 12 germination stimulants including 5 known SLs, 7-oxoorobanchol, 7-oxoorobanchyl acetate, orobanchol, orobanchyl acetate, and 4-deoxyorobanchol. Two novel germination stimulants were purified and their structures were determined as 7α-hydroxyorobanchyl acetate and 7β-hydroxyorobanchyl acetate by 1D and 2D NMR spectroscopy, and ESI– and EI–MS spectrometry. The stereochemistry was determined by NOE measurement and comparing the CD spectra with those of the synthetic standards of four stereoisomers of orobanchol.

7α-Hydroxyorobanchol and 7β-hydroxyorobanchol were detected as an inseparable mixture by LC–MS/MS and GC–MS analyses. In addition to these SLs, two other minor novel germination stimulants were obtained. Based on their spectroscopic data, they were suggested to be carlactone-related compounds but not typical SLs. The germination stimulation activities of 7α-hydroxyorobanchyl acetate and 7β-hydroxyorobanchyl acetate on *O. minor* were comparable to those of orobanchyl acetate and 7-oxoorobanchyl acetate. By contrast, 7β-hydroxyorobanchyl acetate was a highly potent germination stimulant for *Phelipanche ramosa* inducing more than 50% germination at 10 pM.

The maize root exudate was found to contain at least 11 germination stimulants including 4 known SLs, orobanchol, sorgomol, strigol, and 5-deoxystrigol. In addition to these known SLs, novel germination stimulants, putatively called SL1 and SL2, and methoxy-5-deoxystrigol isomers were detected. The distribution of germination stimulation activities on *O. minor* seeds after fractionation by RP-HPLC and LC–MS/MS analysis of the crude extract revealed that both SL1 and SL2 were mixtures of isomers. One of SL2 isomers could be successfully purified by HPLCs. The HR–TOF–MS analysis indicated that the molecular formula of the stimulant is $C_{20}H_{24}O_7$. The structure was tentatively assigned as methyl 9-desmethyl-4-hydroxy-5-oxocarlactone-9-carboxylate by NMR spectroscopy and ESI– and EI–MS spectrometry. It is likely that the other SL2 isomers, SL1, and methoxy-5-deoxystrigol isomers are all carlactone-related derivatives but not typical SLs.

It is intriguing that in addition to normal SLs, both dicot cucumber, a host of *Orobanche* and *Phelipanche* spp. and monocot maize, a host of *Striga* spp. have shown to produce carlactone-related compounds; 5-desmethylcarlactone-5-carboxylates and 9-desmethylcarlactone-9-carboxylates. So far, due to their scarcity, we have not yet examined their biological activities on AM fungi hyphal branching or axillary bud outgrowth. These carlactone-related compounds, similar to SLs, would play important roles in the chemical communications in the rhizospere, and development and growth of plants. Further studies are needed to clarify their structures, biosynthetic pathway, and biological functions.