

令和 5 年 5 月 8 日現在

機関番号：14603
研究種目：若手研究
研究期間：2019～2020
課題番号：19K16169
研究課題名（和文）Elucidating regulatory mechanism of haustorium number using a model parasitic plant system
研究課題名（英文）Elucidating regulatory mechanism of haustorium number using a model parasitic plant system
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交付決定額（研究期間全体）：（直接経費） 3,200,000円

研究成果の概要（和文）：本研究プロジェクトでは、寄生植物がホルモンを用いて、また環境中の栄養素に反応して、どのように吸器の形成を制御しているのか、そのメカニズムを調べました。本研究では、寄生植物が成長や様々な発生過程を制御するための必須ホルモンである内因性オーキシンを用いて、吸器の数を調節していることを確認し、寄生器官の発生とホルモン制御経路の分子的關係を明らかにしました。また、環境中の栄養素、特に窒素と鉄が、吸器の形成を抑制することも明らかになりました。

研究成果の学術的意義や社会的意義

本研究は、寄生植物が環境シグナルと内因性プログラムを統合して寄生戦略を調整するメカニズムについての知見を提供するものです。これらの成果は、寄生性雑草が農業や生態系に与える被害の予測と制御に貢献するものです。

研究成果の概要（英文）：The research project investigated the mechanisms of how parasitic plants regulate haustorium formation using hormone as well as in response to environmental nutrients. The research reviewed that parasitic plants use endogenous auxin, which is an essential hormone to control growth and various developmental processes to modulate haustorium number, and thus provide an insight into molecular relationship between parasitic organ development and hormone regulatory pathway. It also showed that environmental nutrients, particularly nitrogen and iron, largely suppress haustorium formation.

研究分野：plant research

キーワード：parasitic plant haustorium auxin nutrient hormone development

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1 . 研究開始当初の背景

Haustorium is essential for parasitic plants to obtain water and nutrients from other plants. Haustorium initiation occasionally requires host signal. Controlling haustorium number is thought to benefit the parasites to optimize the growth and increase nutrient competition in complex environments surrounded by biotic and abiotic factors. Therefore, regulation of haustorium number should occur, in principle, in a controlled manner in response to environmental and intrinsic signals. However, the molecular mechanisms underlying haustorium formation and to what extent haustorium number benefits parasitic plants remain almost unknown.

Recent efforts with genomic and transcriptomic analyses in *Phtheirospermum japonicum*, a root parasitic species in the Orobanchaceae, have made prominent progress on the understanding of important parasitic genes required for host interaction (Ishida et al., 2016; Cui et al., 2020; Wakatake et al., 2020). Because the Orobanchaceae family contains many agriculturally important parasitic weeds such as *Striga* and *Orobanche* and covers a full range of parasitic life-style, *P. japonicum* inevitably becomes an excellent model for understanding plant parasitism. Besides, several *P. japonicum* mutants showing defects in haustorium formation or development were timely isolated. Among them, the phenotype of two mutants with defects in root hairs and haustorial hairs (*haustorial hair defective 1 (hhd1)* and 2 (*hhd2*)) were extensively characterized (Cui et al., 2016), revealing the function of haustorial hairs in host tissue attachment which is beneficial for parasitism.

2 . 研究の目的

This research was initially proposed to understand the molecular mechanism of haustorium formation by elucidating the regulatory roles of auxin and nutrients in modulating haustorium number, using *P. japonicum* as a model parasitic plant system. In addition, this research aimed to identify parasitic plant genes that negatively regulate haustorium formation. The results from this project will provide a molecular framework for understanding parasitic organogenesis as well as prediction and control of crop damage posed by parasitic weeds.

3 . 研究の方法

(1) To elucidate the mechanistic role of auxin in haustorium number control, phenotypic characterization of a *P. japonicum* mutant defective in a gene encoding auxin transporter of ATP-binding cassette (ABC) gene family (*Pjhhd2*: *haustorial hair defective 2*; **Fig.1**) was extensively carried out by conducting marker expression analysis and auxin measurement with LC-MS/MS.

(2) To assess how and what nutrients affect haustorium formation, the haustorium induction assay with single nutrient element supplement as well as depletion experiment was conducted in a hydroponic system and agar system.

(3) Mutant screening was carried out in peonidin solution which was confirmed to induce haustorium in HIF-hypersensitive *Pjhhd2* by not in wild type.

This is aimed to isolate HIF-hypersensitive mutants and identify parasitic plant genes repressing haustorium formation or limiting sensitivity against HIFs.

4 . 研究成果

(1) Application of auxin and various auxin analogs with different kinds of HIFs increased haustorium number compared with HIFs treatment alone, without significantly affecting the root architecture, indicating that auxin hormone is a positive regulator for haustorium formation. *Pjhhd2* was initially

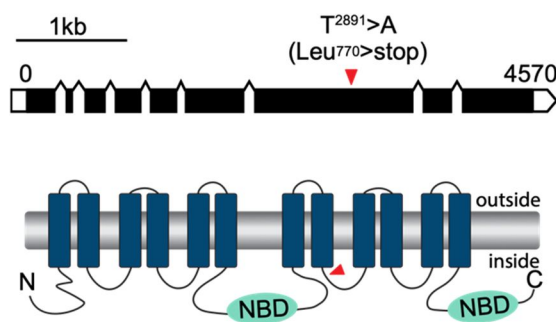


Figure 1. Gene structure and topology of PjABC4 homologous to Arabidopsis membrane localized auxin transporter of ABC family. *Pjhhd2* contains a mutation (red arrowheads) in the middle region of the gene causing stop codon. The mutation was identified by whole genome sequencing of more than 40 mutants from BC1F2 progeny. NBD: nucleotide binding domain.

identified as a mutant defective in both root hairs and haustorial hairs. Later, whole-genome sequencing identified the causal gene encoding auxin efflux carrier ABCB4. Complementation of *Pjhhd2* with a genomic region of *PjABCB4* fully restored root hair formation, indicating that *PjABCB4* is responsible for hair growth phenotype. In great support with exogenous auxin application increasing haustorium number, *Pjhhd2* accumulated more auxin conjugates at root tip compared to the wild type and produced more haustorium against multiple HIFs compared to the wild type. In addition, the auxin response marker showed increased auxin response at root tip of *Pjhhd2* compared to the wild type. Taken together, these results indicate the role of *PjHHD2/ABCB4* in restricting haustorium formation by removing auxin from the cell. Furthermore, it suggests that *PjHHD2/ABCB4* controls auxin homeostasis in parasitic plant roots and modulate haustorium frequency. How cellular auxin level affects cellular potency to HIFs and how auxin signaling cascades interact with haustorial initiation programs remain to be answered. To address this question, comparative transcriptome analysis between wild type and *Pjhhd2* is undergoing. Since ROS was reported to be enhanced by auxin in *Arabidopsis* and is indispensable for haustorium induction in *Striga hermonthica*, a hypothesis was made where ROS level may be altered in *Pjhhd2*. Several genes involved in H₂O₂ production were indeed upregulated in *Pjhhd2*, revealed by qRT-PCR. This new finding suggests that auxin may regulate haustorium induction through ROS. The role of ROS for haustorium formation needs further investigation.

However, an unexpected phenotype occurred in *Pjhhd2*: seedlings of BC2F3 *Pjhhd2* progeny derived from BC2F2 homozygous parents showed segregation in more-haustorial phenotype. Since *Pjhhd2* was initially derived from EMS mutagenesis which generates random single-nucleotide mutations in the genome, the phenotype segregation in *Pjhhd2* homozygous background are highly likely caused by an additional mutation, by either positively or negatively affecting *Pjhhd2* mutation. It is also possible that this unknown mutation rather than the mutation in *PjHHD2* is responsible for more-haustorial phenotype. To address this issue, each of the BC2F3 lines was independently subjected to whole-genome sequencing for identification of additional mutation. Analysis of SNPs data in correlation with the haustorium phenotype successfully identified an additional mutation loci in the gene (named as *PjMF*) encoding a membrane transporter belonging to major facilitator transporter family, causing stop codon at the N terminus. While this project was initially proposed to analyze the role of auxin in controlling haustorium number using *Pjhhd2*, these unexpected results have led us to carefully re-investigate the more haustorium phenotype. With that, *Pjabcb4*, *Pjmf* and *Pjabcb4/mf* were isolated by backcrossing original *Pjhhd2* with WT again and phenotypic analyses showed that *Pjabcb4/mf* rather than single mutants showed more prehaustorium phenotype. More experiments need to be carried out to address whether and how *PjABCB4* and *PjMF* redundantly suppress prehaustorium formation in *P. japonicum*.

(2) Investigation of nutrient effects on haustorium formation was investigated in *P. japonicum*. DMBQ treatment in 1/2MS medium greatly suppressed haustorium formation. To further understand the responsible suppressor element, nutrient depletion and supplementation experiments were carried out. In a hydroponic system, nitrogen and manganese significantly suppressed haustorium formation, while in the agar system nitrogen and iron (Fe) play a major role in haustorium formation inhibition. Effects of nutrients on haustorium formation and host infection were also examined in *Striga* by collaboration with Prof. Charles Melnyk (SLU, Sweden) and identified prominent nutrient components. Together, we identified macronutrient and micronutrient elements suppressing haustorium formation in parasitic plants. These results build an important foundation for the molecular study of parasitic plants' response to the environmental signal, which is a largely unexplored area.

(3) To isolate HIF-hypersensitive mutants, forward genetic screening was carried out by treating the seedlings with 10 μ M peonidin, an anthocyanidin that does not trigger haustorium formation in the wild type but *Pjhhd2* (**Table 1**). 47974 EMS-mutagenized

	M2 (1st screen)		M3 (2nd screen)
	Total	Identified	Confirmed
Lines	2972	38	1
Individuals	47974	81	1

P. japonicum mutant populations from 2972 lines of M2 generation were subjected to screening. 81 individuals from 38 lines were isolated in the first screening, showing haustorium formation in peonidin. Among these, only 1 reproducibly showed haustorium phenotype in the second screening in the M3 generation. The mutant does not induce haustorium in water; therefore, it is highly likely a HIF-hypersensitive mutant. Throughout the project we successfully isolated a mutant defective in the restriction of HIF recognition system, providing a novel genetic resource for understanding the genetic regulation of

haustorium formation in response to the host signal. Further analyzes are needed to understand how the negative regulatory genes integrate external cues with intrinsic pathways to fine-tune haustorium initiation in parasitic plants.

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〔図書〕 計0件

〔出願〕 計0件

〔取得〕 計1件

産業財産権の名称 吸器形成阻害剤	発明者 吉田 聡子、和田 将 吾、Songkui Cui	権利者 同左
産業財産権の種類、番号 特許、P190016314	取得年 2019年	国内・外国の別 国内

〔その他〕

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6. 研究組織

氏名 (ローマ字氏名) (研究者番号)	所属研究機関・部局・職 (機関番号)	備考

7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関		
スウェーデン	SLU		