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論　文　名	Molecular mechanisms involved in the early parasitic processes of the stem parasitic plant *Cuscuta campestris* （茎寄生植物アメリカネナシカズラ寄生初期過程の分子機構）
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論文要旨

The stem parasitic plant, *Cuscuta campestris*, establishes a connection of vascular tissues to its host plants, allowing it to absorb water and nutrients. The parasitic process begins when *C. campestris* recognizes the host plant by making contact with its stem surface, which leads to tight coiling around the host stem. This attachment provides mechanical stimuli that trigger the development of a specialized organ called a haustorium in *C. campestris.* The haustorium then starts penetrating into the host stem, a process that involves the degradation of the host's cell walls and forcible separation of host cells. In these early processes of parasitism, the mechanisms by which *C. campestris* perceives mechanical stimuli and how host plants respond to the invasion by *C. campestris* have not been clarified. This study aims to investigate the molecular mechanisms underlying these early parasitic processes in both the parasitic and the host plants.

The first chapter presents a study on identifying a mechanosensitive ion channel (MSC) gene that is involved in haustorium development in response to mechanical stimuli. It is widely accepted that MSC proteins are involved in the perception of mechanical stimuli across various organisms. Therefore, we investigated the potential involvement of MSCs are in haustorium development as well. To investigate whether MSC proteins are involved in haustorium development, we treated *C. campestris* plants with an MSC protein inhibitor, GsMTx-4, which blocks stretch-activated cation-selective channels. At 48 hours after

attachment (haa), treatment with GsMTx4 significantly reduced the density of prehaustoria, which is haustoria in the pre-intrusive stage. To identify the specific *MSC* gene involved in prehaustorium development, we analyzed the known functions and expression patterns of *MSC* genes of a model plant, *Arabidopsis thaliana*, and selected *MID1-COMPLEMENTING ACTIVITY 1* (*MCA1*) as a primary candidate. To further assess the activity of *CcMCA1* as a mechanosensitive ion channel, we confirmed functional complementation assay using the yeast *Saccharomyces cerevisiae mid1* mutant. The results indicated that *CcMCA1* effectively rescued the *mid1* mutant from the mating pheromone-induced cell death phenotype to the same extent as yeast *MID1* and *AtMCA2*. To assess the role of *CcMCA1* in prehaustorium development, *CcMCA1* was silenced using an artificial microRNA (amiRNA) produced by host *Nicotiana tabacum* plants that specifically targets *CcMCA1*. In the *CcMCA1*-silenced plants, the number of prehaustorium per millimeter of stem length decreased, and the spacing between neighboring prehaustoria became longer. Further analysis was conducted using an in vitro haustorium induction system, where stems were mechanically stressed by sandwiching between two glass plates. The results showed that *CcMCA1*-silenced plants had fewer prehaustoria compared to the controls at 48 haa. These findings underscore the critical role of *CcMCA1* in prehaustorium induction in response to mechanical stimuli in *C. campestris*. Next, we investigated how *CcMCA1*-silencing affects the expression of genes related to haustorium development. We evaluated the expression levels of the genes at three time points. 0 haa, which was the time the *C. campestris* stem attached to the second host, and 72 haa, when the prehaustoria grew and began to penetrate the second host, and 120 haa when intrusive haustoria had developed in the second host. the expression level of the *C. campestris* homolog of *LATERAL ORGAN BOUNDARIES DOMAIN 25* (*CcLBD25*), which has been suggested to regulate haustorium development through auxin signaling, was significantly reduced in the *CcMCA1*-silenced plants at all three time points. Next, we measured the expression level of *C. campestris ETHYLENE RESPONSE FACTOR 1* (*CcERF1*), *PECTIN METHYL-ESTERASE INHIBITOR* (*CcPMEI*), and *HOMEOBOX 7* (*CcHB7*), which are involved in haustorium penetration in the host cortex and their silencing has been shown to halt this process. The expression levels of *CcERF1* and *CcPMEI* were significantly reduced in *CcMCA1*-silenced at both 0 and 72 haa compared to the control plants. Conversely, *CcHB7* was not downregulated consistently. We confirmed that *CcMCA1* silencing consistently reduced *CcLBD25*, while *CcERF1* and *CcPMEI* decreased only in the pre-intrusive phase of prehaustorium development. Downregulation of *CcLBD25* prompted us to investigate the impact of

CcMCA1-silencing on the expression levels of genes involved in auxin signaling in *C. campestris*. We tested *PIN-LIKES 3* (*CcPILS3*), which encodes an auxin efflux carrier and co-expressed with *CcLBD25*. Results showed a decreased expression level of *CcPILS3* in *CcMCA1*-silenced at 72 haa. *C. campestris* homologs of *IAA14* and *ARF19*, which are transcriptional regulators known to regulate auxin-inducible expression of *LBD16* in *A. thaliana* showed no significant differences at the three time points. These results suggested that the signaling between *CcMCA1* and *CcLBD25* is potentially mediated by factors that differ from the *IAA14-ARF7/19* regulatory module.

The second chapter discusses how a host plant responds to the invasion of a haustorium. Most previous studies on parasitic plants have concentrated on the behavior of the parasitic plants in establishing their parasitic connections. However, the processes occurring in the host plants have not received much attention. To assess the host response, we compared the molecular responses to haustorium invasion with those to wounding, because haustorium invasion likely causes similar damage as wounding does, including degradation of cell walls and disruption of the transport of water and nutrients. The wound repair process in *A. thaliana* involves key processes: cell division, cell re-adhesion, and vascular regeneration. Two key transcription factors associated with wound repair, *ANAC071* and *AtRAL2.6L*, were found to be increased at the parasitic interface. Additionally, other transcription factors that are known to be upregulated following stem cutting, such as *AtWOX13*, *AtTOM6*, and *AtHCA2*, were also increased at the parasitic interface. This upregulation of transcription factors led us to investigate the expression of genes involved in cell division- and vascular development at the parasitic interface. *AtCYCB1;2*, *AtWOX4*, *AtVND7*, and *AtNEN4* were found to be upregulated, suggesting that cell division and vascular differentiation occur in response to parasitic invasion. We confirmed an increase in the number of cell layers in the cambium. However, unlike the observation in wound repair, the parasitic interface did not exhibit asymmetric auxin accumulation in the upper region of the invading haustoria; instead, auxin accumulated in both the upper and lower regions. To determine whether the upregulation of the genes associated with cell division- and vascular development depends on *ANAC071*, auxin signaling, and ethylene signaling, gene expression was investigated in *anac071/096/011* triple mutants, *auxin responsive factor* (*arf*) mutants, and *acc synthase* (*acs*) octuple mutants. The genes involved in cell division- and vascular development were still upregulated in these loss-of-function mutants. This suggests that the host response to the haustorium invasion is controlled by different signaling mechanisms than those involved in

wound repair.

This study clarified the previously unknown mechanisms involved in the parasite-host interaction during the early stages of the parasitic process. When the parasite attaches to the host's stem, it perceives mechanical stimuli through a mechanosensitive ion channel gene, *CcMCA1*, which subsequently triggers the development of prehaustoria. Silencing the *CcMCA1* gene results in the downregulation of *CcLBD25*, leading to a reduction in haustorium development. Additionally, the invasion of haustoria into the host plant induced responses similar to those caused by wounding. However, the regulation of genes related to cell division- and vascular development at the host-parasite interface differs from typical wound responses, as *ANAC071* and hormones like auxin and ethylene were found to be non-essential. Overall, these findings elucidated regulatory mechanisms involved in parasite-host interactions at the parasitic interface.

審査結果の要旨

寄生植物とは、自身とは別の植物個体に寄生する植物の一群を総称するものである。寄生植物は進化的には特定の単一系統に属するものではなく、進化の過程で複数回にわたって様々な植物系統で独立に発生した。寄生植物は、寄生した相手、すなわち宿主植物から、水や養分を吸収して自身が生長と繁殖をするための資源を得ている。この水や養分の吸収を行うために、寄生植物は吸器と呼ばれる寄生のために特化した器官を発達させ、宿主植物との間に接続を形成する。

寄生植物が寄生を成立させる時に、宿主植物との間には様々な相互作用が発生する。これまでの寄生植物研究においては、寄生植物が宿主植物に物理的に接触する以前の段階での、宿主植物へ屈性を示すために必要な相互作用や、吸器が宿主植物に侵入してから後の過程、例えば吸器が宿主植物の組織内を貫通していく時に見られる細胞壁を介した相互作用、および吸器が宿主維管束組織と接続する時に見られる相互作用、などについて研究が実施されてきた。

こうした流れの中で、寄生初期段階においてアプローチされていないいくつかの相互作用過程があった。その一つとして寄生植物が宿主植物に接触したことをどのように感知しているのかという点、そしてもう一つとしては、宿主植物の側では寄生植物に寄生されたことに対してどのように反応しているのか、という点が挙げられた。本博士論文では、これら二つの寄生初期過程における未解決問題の解明が試みられた。

まず第一章では、茎寄生植物である *Cuscuta campestris*（和名アメリカネナシカズラ、以

下 Cc）が宿主植物に寄生を開始する際に、宿主植物への接触刺激感知に関与する機械的刺激感受性イオンチャネル（MSC）が同定された。Cc は茎に何かが接触すると、それが生物であるか非生物であるかに関わらず吸器の形成を開始する。接触刺激応答性の吸器の形成は、細胞膜が進展することにより活性化するタイプの MSC の阻害剤である GsMTx-4 によって阻害され、MSC の活性が吸器形成に必要なことが明らかとなった。次に、接触刺激応答性吸器形成に関与する特定の *MSC* 遺伝子を同定するために、候補化した *MSC* 遺伝子を Host-Induced Gene Silencing 法により発現抑制した。候補遺伝子の中で *MID1-COMPLEMENTING ACTIVITY 1*（*CcMCA1*）の発現抑制により、接触刺激に応答し形成される吸器の密度が有意に減少した。*CcMCA1* の発現抑制個体では、吸器形成の主たる転写制御因子 *LOB DOMAIN CONTAINING PROTEIN 25*（*CcLBD25*）、ならびに吸器形成に必要な他の遺伝子、*ETHYLENE RESPOSIVE FACTOR 1*（*CcERF1*）ならびに *PECTIN METHYLESTERASE INHIBITOR*（*CcPMEI*）の発現が抑制されており、吸器形成のためのシグナル伝達系を *CcMCA1* が制御していることが示唆された。以上のことから、CcMCA1 が接触刺激に応答した吸器形成に関与していることが明らかとなった。

第二章においては、Cc が宿主植物に吸器を侵入する際の宿主側の反応が明らかにされた。吸器の侵入は細胞壁の破壊や維管束の結合など、既知の茎傷害治癒と形態的に類似していると考えられたが、寄生されている宿主植物における傷害治癒関連遺伝子群の発現応答は解析されていなかった。そこで、Cc に寄生されたシロイヌナズナの茎において、茎傷害応答の主たる制御因子 *Arabidopsis NAC DOMAIN-CONTAINING PROTEIN 71*（*ANAC071*）を含む五つの傷害応答性転写因子遺伝子の発現を解析すると、いずれも寄生によって発現が増加していた。傷害治癒の特徴の一つである細胞分裂の再活性化が寄生部位においても同様に起こっていることが、*CYCLIN B1;2*（*AtCYCB1;2*）の上昇ならびに維管束形成層細胞層数の増加により明らかにされた。しかしながら、細胞分裂再活性化につながるオーキシン蓄積の空間的パターンは、傷害部位では切断箇所上部に局在するのに対し、寄生部位では侵入した吸器の上下に局在しており、オーキシン蓄積機構が異なることが示唆された。さらに、維管束の結合に関連する遺伝子群は、寄生部位では *ANAC071*、オーキシンシグナル伝達系、エチレン合成等に依存せずに発現が上昇することが示された。これらのことから、吸器侵入は形態的には傷害と類似するが、宿主植物の反応の様式は傷害に対する反応様式とは多くの点で異なっていることが明らかになった。

本研究では寄生植物が宿主植物に寄生する初期過程に関して、接触刺激応答性に関与する遺伝子を同定し、また宿主植物側の寄生に対する反応に寄生独自の過程がある事を明らかにした。特に前者の成果は、植物の側生器官形成において機械的刺激受容に関与する遺伝子を明らかにした最初の事例である。これらの成果は、細胞分子生物学や植物生理学的な観点から高く評価できる。よって本論文の審査ならびに最終試験の結果と併せて博士（応用生命科学）の学位を授与することを適当と認める。