様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　元 年　 ６ 月 １１ 日現在

機関番号：１４６０３
研究種目：若手研究(B)
研究期間：2017～2018
課題番号：１７Ｋ１５１４２

研究課題名（和文）Molecular mechanism of ethylene on the regulation of haustorium development and function in parasitic plants

研究課題名（英文）Molecular mechanism of ethylene on the regulation of haustorium development and function in parasitic plants

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交付決定額（研究期間全体）：（直接経費）　　3,400,000円

研究成果の概要（和文）：寄生植物は他の高等植物に寄生し、栄養と水を奪う植物である。宿主植物に寄生するために、吸器という特殊な器官を形成する。しかし、吸器がどうやって宿主を認識するのかは未だに未解明である。本研究ではモデル寄生植物コシオガマを用いたエチレン突然変異体の同定および宿主-寄生植物相互作用におけるエチレンシグナル伝達の役割を解析した。寄生植物側のエチレンシグナル伝達は吸器形成過程において、吸器先端細胞の分裂や分化の調節により、宿主感染の有無を決定することを明らかにした。また、宿主植物側のエチレンは寄生に一部寄与することを明らかにした。これはエチレンによって寄生植物の有する宿主感受性をあげていると考えられる。

研究成果の学術的意義や社会的意義
寄生植物は他の植物に寄生し水と栄養を奪う植物である。作物にも寄生できるため、年間１兆円もの農業被害が出ていると推測されている。他の植物に侵入するために吸器と呼ばれる器官を形成するが、その侵入メカニズムについて多くのことがわかっていない。本研究により、寄生に関わる必要な遺伝子の同定やそのシグナル伝達の解明と寄生阻害物質を見つけることができた。これらの成果により農業被害の減少が期待される。

研究成果の概要（英文）：Parasitic plants are one of major agricultural constraints worldwide and use a specialized organ, haustorium, to penetrate host for acquisition of water and nutrients. To date, molecular mechanisms behind how parasitic plants recognize and penetrate host tissue remains unknown. In this study we used model parasitic plant Phtheirospermum japonicum and isolated two mutants defective in ethylene signaling due to mutations in ethylene receptor ETR1 and signaling component EIN2, respectively. This research demonstrats that ethylene signaling in parasitic plant plays an essential role in host infection through regulation of cell division and cell differentiation at haustorial apex. On host side, ethylene also contribute on infection rate and thus confers host susceptibility against parasitic plants.

研究分野：植物生理

キーワード：Parasitic plant　Haustorium　Ethylene signaling

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

Orobanchaceae parasitic members including *Striga* and *Orobanche* spp are among the most devastating agricultural pests by infecting important staple crops such as rice, maize, sorghum, sugarcane, millet and carrot and causing economical loss over 1 billion US dollars per year. Haustorium is the essential organ in all parasitic plants to invade host tissue and acquire nutrient and water from host. Understanding molecular and genetic regulation of haustorium provides an insight into how parasitism is evolved from normal plant-plant interaction and the fundamental knowledge for protecting crops from pandemic parasitic plant infection. To date, however, how parasitic plants recognize and invade host using haustorium remains completely unknown at genetic level.

We initially isolated two mutants from a root parasitic plant *Phtheirospermum japonicum*, a Orobanchaceae member, using forward genetic screening. These mutants were screened with elongated haustorium phenotype compared to the wild type, indicating the abnormal haustorium development in the mutants. Using whole genome sequencing the causal mutations were identified respectively in ETHYLENE RESPONSE1 (ETR1) and ETHYLNEE INSENSITIVE2 (EIN2), both of which are mediating ethylene hormone signaling.

２．研究の目的

This project aims to clarify the role of ethylene in the haustorial development and function in host and parasite interaction using ethylene signaling defective mutants of *P. japonicum* identified from forward genetic screening.

３．研究の方法

To validate the mutation identification results, Pj*ein2* was complemented with genomic region of *P. japonicum* EIN2. Morphological analysis of elongated haustorium phenotype was conducted by histochemical approach. To test whether the elongated haustorium was caused by enhanced cell division or enlarged cell size, the cell size comparison and cell staining with cell division marker EdU were conducted at different stages of haustorium development in the wild type and mutants.

To investigate the role of ethylene signaling in haustorium induction, quantitative analysis on haustorium formation was conducted in the wild type and mutants. In addition, various ethylene chemicals as well as ethylene inhibitors were tested for their effect on haustorium induction. To investigate the role of ethylene in host infection, the infection rate of mutants to *Arabidopsis* and rice was checked. Besides, the effects of various ethylene inhibitors on the infection rate of *P. japonicum* to the host were examined.

The role of host ethylene was investigated using *Arabidopsis* mutants defective in ethylene production, *heptuple* (*acs1-1acs2-1acs4-1acs5-2acs6-1acs7-1acs9-1*) and *octuple* (*acs2-1acs4-1acs5-2acs6-1acs7-1acs9-1amiRacs8acs11*), defective in ethylene signaling (*etr1*, *ein2*) and constitutively activated in ethylene signaling (ctr1).

To understand how ethylene signaling interacts with auxin signaling during haustorium formation, the auxin reporter line DR5:GUS and DR5:3×Venus-NLS was generated and their expression was examined in the wild type and Pj*ein2* mutant.

To investigate spatial-temporal activation of ethylene signaling the ethylene reporters

EBS:3×Venus-NLS and EBS:GUS were generated and their expression was observed during haustorium development. Transcriptomic analysis is under progress to identify key components regulating haustorium elongation and host invasion at downstream of ethylene signaling.

４．研究成果

(1) The role of ethylene signaling in pre-haustorium development is revealed. When haustorium is induced by host signal (haustorium inducing factors) but without actual host attached, the pre-haustorium terminates within 2 days of exposure, whereas those of mutants undergo continuous elongation for additional 2 or 3 days. Histochemical analysis showed that the elongation is caused by extended cell division at haustorium apex. Marker experiment using auxin responsive DR5:3×Venus-NLS showed that auxin response occurs at haustorium tip during haustorium elongation, showing its positive correlation with apex cell division. These indicate that ethylene signaling facilitates termination of pre-haustorium development by regulating cell division and auxin signaling at haustorium apex.

(2) The role of ethylene signaling in host infection is revealed. When haustorium attaches host tissue, ethylene signaling mediated by ETR1 and EIN2 promotes cell differentiation of haustorium apex into intrusive cells which is an essential cell for host tissue invasion, thereby mediating the initial host recognition for the occurrence of later infection process. Notably, nearly all haustoria failed in host invasion in P*jein2* indicating that ethylene signaling is essential for parasitic plant for determining parasitic lifestyle.

(3) The role of host ethylene during plant parasitism is revealed. Host infection assay using *Arabidopsis* ethylene-related mutants showed that ethylene production and signaling in the host partly contribute to parasitic plant infection, conferring host susceptibility against parasitic plant. These results indicate that host ethylene system is targeted by parasitic plant, possibility via ethylene signaling, for efficient infection. Further ethylene treatment experiment reveled that ethylene molecule is not direct signal triggering intrusive cell formation at haustorium apex, indicating that positive effect of host ethylene on invasion process is likely through increasing signaling intensity of ethylene activated in parasitic plant during initial invasion.

(4) Host protection from parasitic plant infection was successfully achieved by pharmaceutical approach. Based the mutant characterization results, the ethylene inhibitors were applied to the medium during host infection events in an effort to inhibit ethylene production or signaling in both host and parasitic plants. As a result, the complete lack of infection could be achieved without significantly affecting the other growth traits of plants. These imply the future implication of ethylene inhibitors on the prevention of parasitic plant infection.

(5) The spatial-temporal activation of ethylene signaling in the haustorium during infection has not been resolved yet. The activity of widely used ethylene responsive EBS promoter was not observed in the haustorium throughout the infection process. When and where the ethylene signaling is activated remains to be solved at cellular level in the future.

５．主な発表論文等

〔**雑誌論文**〕（**計　4　件**）

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3. Songkui Cui, Ken Shirasu, Satoko Yoshida. Ethylene signaling regulates stem-cell like cells in haustorium for host infection in parasitic plant. *Scientific Research on Innovative Areas, Principles of pluripotent stem cells underlying plant vitality,* 2018

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〔**図書**〕（**計　0　件**）

〔**産業財産権**〕

○**出願状況**（**計　1　件**）

名称：吸器形成阻害剤
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種類：特許

番号：2018-010715
出願年：2018 年
国内外の別：国内

○取得状況（計　0　件）

名称：
発明者：
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番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

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