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研究課題名（和文）植物・昆虫インターフェイスで繰り広げられる分子間相互作用

研究課題名（英文）Molecular interactions at plant-insect interface

研究代表者
Ｉｖａｎ　Ｇａｌｉｓ（Galis, Ivan）

岡山大学・資源植物科学研究所・教授

研究者番号：９０３６０５０２

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研究成果の概要（和文）：本研究において、イネ-植食性昆虫間の相互作用解析を行う上で、有用な研究基盤を構築した。イネの防御応答において、OsJAR1を介して植物ホルモンであるジャスモン酸イソロイシン（JA-Ile）が蓄積すること、またJAやJA-Ileの蓄積は、主要な生合成経路であるリノレン酸量により制御されることを示した。一方で傷害応答との比較解析より、咀嚼性植食性昆虫の吐き戻し液に含まれるエリシターに特異的に応答して蓄積する、新規なフェノールアミド化合物を同定した。さらに、フェノールアミド化合物やROS蓄積を指標に、クサシロキヨトウの吐き戻し液に含まれる、新規と推定されるエリシターの部分精製を行った。

研究成果の概要（英文）：We established a novel system to study rice-insect interactions. We showed that the accumulation of active defense hormone jasmonoyl-L-isoleucine (JA-Ile) in rice depends on the function of OsJAR1 enzyme. JA and JA-Ile accumulations in rice were controlled by the availability of the main pathway substrate, α-linolenic acid. In comparison to wounding, rice plants specifically responded to elicitors present in chewing herbivore oral secretions. We identified two novel herbivory-related phenolamide phytoalexins. Using ROS and rice phenolamides as molecular markers, we identified and partially purified a new putative insect elicitor from Mythimna loreyi oral secretions.

研究分野：生物学

キーワード：防御応答　昆虫エリシター　植食生昆虫　ジャスモン酸　Oryza sativa　ファイトアレキシン　イネ　傷害

１．研究開始当初の背景 (Introduction)

Plants accumulate many toxic defense metabolites in response to herbivore attack. Compared to mechanical wounding of leaves, herbivore attack elicits quantitatively as well as qualitatively different changes in plant defense metabolite profiles. It is because plants are able to perceive elicitors released from herbivores during feeding. For example, the presence of insect FAC-type elicitors is tightly connected with the activation of an important transcription factor (MYB8) that controls biosynthesis of several defense metabolites against insects in tobacco plants (Kaur et al. 2010). Insect elicitors and their corresponding plant receptors are therefore two most important factors that determine the outcome of plant defense against insects in natural environment. This system is comparable to the function of microbe-associated molecular patterns (MAMPs) that trigger plant defense against pathogens. However, compared to MAMPs, insect elicitors and their function are much less understood. Only few insect elicitors have been characterized so far, and no plant receptors of insect-derived elicitors have been reported to date. In addition, downstream signal transduction of herbivore elicitors and their interaction with canonical jasmonic acid (JA) signaling pathway in plants remain elusive. Therefore, identification of novel elicitors present in rice herbivores is necessary to advance our understanding of innate defense mechanisms against insects in this important crop.

２．研究の目的 (Purpose of research)

We aimed to investigate major factors involved in recognition of herbivore attack that leads to activation of innate defense mechanisms against herbivores in rice plants. We focused mainly on ① novel types of herbivore-associated elicitors that function in rice plants and ② analysis of signal transduction pathways mediating defense signaling in rice. This research was designed to provide a broad theoretical and practical base for development of novel environment-friendly methods of crop protection in the future.

３．研究の方法 (Methods)

We established stable colonies of three Lepidopteran insect species feeding on rice to enable this work (*Mythimna loreyi*, *Parnara guttata*, and *Spodoptera mauritia*). In addition, we adopted brown planthopper (*Nilaparvata lugens*) as sucking specialist insect by obtaining and propagating this insect in the laboratory. To estimate degree and specificity of rice responses to herbivores, we analyzed the amounts of plant hormones in wounded and artificial herbivory-treated samples. In order to dissect herbivory signaling, we obtained and characterized TOS17 mutant of rice deficient in the last step of JA signaling required to form the active hormonal signal JA-Ile. Using untargeted metabolomics (UPLC-ESI-microTOF-MS), we identified large diversity of herbivory-induced metabolites in rice. By targeted chemical analysis using HPLC-MS/MS, we further refined responses of rice to herbivores at metabolic level. Finally, we established a new semi-high throughput monitoring system to trace novel insect elicitor activity in rice cells. Reactive oxygen species (ROS), phenolamide and diterpene class of metabolites were used as major molecular markers to monitor rice response to individual herbivore-derived elicitors.

４．研究成果 (Results)

At first, it was necessary to establish a suitable research system. Because plant-insect interactions are highly specific, we first observed field populations of herbivores and selected three insect species for our further work (*M. loreyi*, *P. guttata* and *S. mauritia*). Next, we included sucking insects represented by brown planthopper (*N. lugens*) into our research scope.

(1) Response of rice plants to insect derived elicitors. In this part, we used *S. mauritia* as our primary experimental model. Oral secretions (OS) were extracted from insect larvae feeding on rice leaves and these extracts were applied in diluted form to rice leaves wounded with a fabric pattern wheel on both sides of the leaf lamina. Compared to wounds treated with water, diluted oral secretions elicited significantly higher amounts of JA and JA-Ile defense signals. Other hormones such as abscisic acid (ABA) and salicylic acid were not affected by insect elicitors (data published in Fukumoto et al. 2013). Nipponbare rice SA levels were notably high compared to those found in other

plants such as tobacco or Arabidopsis.

(2) OS-mediated increase in JA-Ile is associated with OsJAR1 transcript levels. By analyzing transcript levels of *OsJAR1* and *OsJAR2* genes, we found that only *OsJAR1* profile corresponds to that of the JA-Ile accumulation. In addition, *OsJAR1* transcript levels were higher in OS treated leaves, suggesting that OsJAR1 is one of the key signaling components in the insect elicitor-mediated pathway (data published in Fukumoto et al. 2013).

(3) OsJAR1 mutant plant phenotypes. Based on the expression profile of *OsJAR1*, we confirmed expected function of OsJAR1 in the biosynthesis and accumulation of JA-Ile in herbivory stimulated rice leaves. Osjar1 mutant plants contained highly reduced amounts of JA-Ile while free JA levels increased in direct compensation response, and inability to conjugate the hormone (data published in Fukumoto et al., 2013). Because OS was used to treat mutant and control plants, it is safe to conclude that insect elicitor signaling towards the accumulation of active JA signal, JA-Ile, is dependent on OsJAR 1 activity while OsJAR2 is likely to play other roles in rice. In addition to function in herbivore-elicited hormone levels, we found that OsJAR1 and JA-Ile are essential for fertility in rice. Osjar1 mutant plants were male sterile that could be complemented by the application of the synthetic JA-Ile to developing flowers (data published in Fukumoto et al., 2013).

(4) JA biosynthesis in rice. It is important to know how JA and insect elicitor signaling pathways are interconnected. In the previous paragraph, we showed that elicitor signal connects to transcriptional activation of JA conjugating enzyme, OsJAR1 and thus regulate the active hormone levels. However, it was not clear to which extend other regulatory steps in JA biosynthesis are dependent on the presence of wound- and elicitor-derived signals. We used the advantage of previously developed analytical methods to establish basic mechanisms for wound-induced JA biosynthesis in rice (data published in Christeller and Galis, 2014). Using crushed leaves and biochemical approaches, we established that the production of JA in rice is strictly dependent on the release of the initial pathway substrate α-linolenic acid by lipases. Treatment of crushed leaf extracts with lipase inhibitor lipstatin inhibited the accumulation of JA intermediate, 12-oxo-phytodienoic acid (OPDA), which was complemented by the addition of free α-linolenic acid to the reactions. In summary, we showed that rice JA biosynthetic pathway is fully enzymatically primed for JA biosynthesis. We provided direct evidence for this postulate by supplying α-linolenic acid to intact unwounded rice leaves (applied as DMSO solution). α-linolenic acid treated intact leaves rapidly accumulated JA and JA-Ile, while these compounds remained at low levels in DMSO (solvent) control treated leaves. Importantly for the scope of our research, we found that addition of OS to the *in vitro* reaction mixtures increased the amounts of OPDA produced by crushed leaf extracts. It suggests that a component in OS can directly stimulate enzymes in the pathway. A likely target of OS could be the lipase protein, thus modulating the rate of α-linolenic acid release from cell lipids and JA biosynthesis.

(5) Metabolic responses of rice to herbivory. At the time of initiation of this project, almost nothing was known about the low molecular weight compounds and their function against herbivores in rice. As we needed suitable molecular marker(s) to trace insect elicitor activity, apart from already known JA and JA-Ile hormonal signals, we conducted an unbiased metabolomics analysis of rice leaves subjected to herbivory. Using highly sensitive UPLC system coupled to a microTOF-MS detector, we identified a large number of ions associated with potential metabolites that responded specifically to herbivory (data summarized in Alamgir et. al., submitted). Amongst them, phenolamides *p*-coumaroylputrescine (CoP) and feruloylputrescine (FP) were identified as two novel rice metabolites closely associated with herbivory in rice. Using targeted analytical approach and highly selective HPLC-MS/MS method, we show that feeding of at least three chewing herbivores, and a sucking insect *N. lugens*, all induced substantial accumulations of FP in the attacked leaves (Fig. 1). It suggests that FP may function in rice as

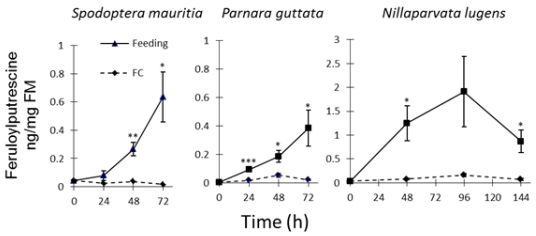

Figure 1 Herbivory elicits accumulation of FP. Rice seedlings were subjected to herbivory by *S. mauritia* and *P. guttata* (larvae), or *N. lugens* (adult insects) for designated time-periods and FP content was determined by HPLC-MS/MS. Full line, feeding; dashed line, control leaf. Data from Alamgir & Galis, et al., submitted.

universal defense metabolite against insects. It also enabled the use of FP and related CoP as metabolic markers to trace novel insect elicitors from complex initial mixtures present in crude insect OS, which was one of the major tasks set in this project.

(6) *In vitro* system to study herbivore elicitor functions in rice. Low sensitivity, high labor and demand for constant supply of plant material are major constrains in finding novel insect elicitors using intact rice plants. With novel systems and tools developed above, we therefore designed a more efficient method to monitor rice insect elicitors (this part of work is currently under preparation for publication, authored by Shinya & Galis, at al.). At first, we adapted rice cell cultures as crucial part of our novel monitoring system. Rice cells can be cultivated in the laboratory and aliquots can be easily treated with crude OS as well as individual purified fractions. In principle, *M. loreyi* OS was used as our starting material for characterization of novel insect-associated elicitor components. This OS contained typical insect elicitors known as fatty acid-amino acid conjugates (FACs), which was confirmed by HPLC-MS analysis. Crude OS at 1:100 to 1:500 dilutions elicited reactive oxygen species (ROS) in cells within minutes of application. After 24 hours, the amount of CoP metabolic marker also significantly increased in the cells (Fig. 2). At the same time, rice diterpenes, momilactone A and momilactone B, also increased in the cells. Next, crude OS was subjected to separation and purification using SPE chromatography, size exclusion methods such as dialysis etc. to remove low molecular substances from the sample. At this point, FACs have been removed but the major elicitor activity remained associated with the high molecular fraction. We then examined if the new putative elicitor and FACs can interact at molecular level.

By combining the newly isolated active elicitor fraction and synthetic FAC, we found that both components show synergistic effect on ROS production in the rice cell system. We are now conducting extensive structural analyses to identify this novel elicitor from *M. loreyi* OS. Similar activity was found also in the *P. guttata* OS, suggesting that we have likely identified a novel class of elicitor present in more than one Lepidopteran species.

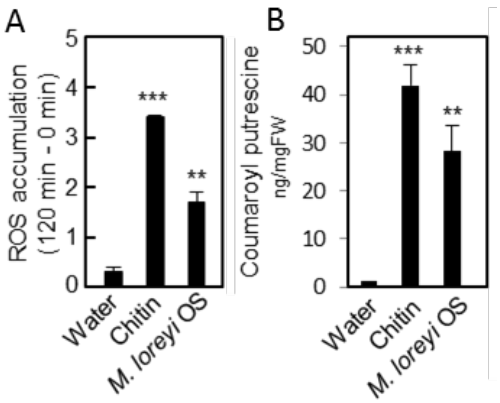

Figure 2 Treatment of rice cells with OS elicits (A) ROS and (B) CoP production. Chitin elicitor was used as positive control in the experiment at 10 nM concentration. Data from Shinya & Galis, et al., in preparation.

(7) Conclusions

We established a suitable system to study rice-insect interactions based on native herbivores of rice in Japan. We defined hormonal and metabolic responses in rice to show specific changes in the amounts of these compounds in plants subjected to herbivory. We contributed to understanding of JA production in rice and point out major control elements in the process. Finally, we established a new monitoring system and successfully used this method to trace putatively novel

elicitor in rice herbivore OS that is different from the already known FAC-associated insect elicitor function. This elicitor was partially purified and it is now being characterized at molecular level.

Additional reference cited in text:

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５．主な発表論文等（Research outputs）

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計3件）

〔Main papers〕(3)

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〔Conference presentations〕(13)

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② Shinya T., Hojo Y., Alamgir Md. K., Desaki Y., Shibuya N., Galis I. Isolation of novel insect elicitors from rice herbivores. 56th Annual Meeting of JSPP 2015, MAR 16-18, 2015, Tokyo.

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⑨ Alamgir K. Md., Galis I., Kim C.-S. Constitutive defense in finger millet against whitebacked planthopper and inducible defenses in rice plants against brown planthopper. 54th Annual Meeting of JSPP, MAR 21-23, 2013, Okayama.

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〔その他〕Others

ホームページ等 Homepage

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６．研究組織

(1)研究代表者

IVAN GALIS（GALIS IVAN）

岡山大学・資源植物科学研究所・教授

研究者番号：90360502

(2)研究分担者

新屋 友規（SHINYA TOMONORI）

岡山大学・資源植物科学研究所・助教

研究者番号：80514207

谷 明生（TANI AKIO）

岡山大学・資源植物科学研究所・准教授

研究者番号：00335621