2版

様　式　C－１９、F－１９－１、Z－１９　（共通）

科研費 KAKENHI

科学研究費助成事業　　研究成果報告書

令和　６年　５月２３日現在

機関番号：１５３０１

研究種目：基盤研究(B)（一般）

研究期間：2021～2023

課題番号：２１H０２１９６

研究課題名（和文）Integrated analysis of mineral ion dynamics in rice crop exposed to acute herbivory stress

研究課題名（英文）Integrated analysis of mineral ion dynamics in rice crop exposed to acute herbivory stress

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

交付決定額（研究期間全体）：（直接経費）　　13,500,000円

研究成果の概要（和文）：本研究はミネラルを、植物の成長における重要な成分という認識から、植食性昆虫の攻撃を含む生物的ストレスに対しても不可欠な要素として認知するとういうパラダイムシフトを起こすことを目的としている。我々は特にイネの防御機構におけるケイ素（Si）の役割について注目し、ケイ素が咀嚼性植食性昆虫に対する物理的な防御を調節することを示した。加えてケイ素は、様々な一次代謝産物や二次代謝産物の蓄積を含む、イネの防御関連代謝に大きな影響を示した。さらに我々は、ストレスを受けた植物が窒素を利用することを想定し、防御の促進におけるニッケル（Ni）の重要性を提案した。

研究成果の学術的意義や社会的意義
Minerals elements play important role in plant growth and subsequently yield of crops, including rice. However, part of the yield is annually lost due to pest damage. Our research results propose novel ways for increasing the crop resistance by supplementation and optimal use of mineral elements.

研究成果の概要（英文）：The aim in this project was to introduce a paradigm shift in perception of minerals as essential components of plant growth to including them as indispensable players in plant defense against biotic stress, including herbivore attack. We particularly highlight the role of silicon in rice defense by showing that Si modulates mechanical resistance of rice against chewing herbivores. In addition, Si shows profound effects on rice defense metabolism, including levels of various primary and secondary metabolites. We further envisage the utilization of nitrogen in stressed plants and propose the importance of nickel in promotion of defense.

研究分野：Plant-Insect Interactions

キーワード：Mineral　Herbivory　Defense

※科研費による研究は、研究者の自覚と責任において実施するものです。そのため、研究の実施や研究成果の公表等については、国の要請等に基づくものではなく、その研究成果に関する見解や責任は、研究者個人に帰属します。

１．研究開始当初の背景

Macro and micronutrients in soil which are essential for plant growth are obtained by roots, translocated to the shoots and then distributed (or redistributed) in the whole plant according to the demands. Under optimal conditions, plants utilize mineral resources to reach their maximal fitness (seed number), which is equivalent to yield in case of cereal crops. However, stress factors often limit the crops from optimal performance. Herbivory is a major plant stress that occurs rapidly and unexpectedly (reviewed in Wari (Galis) et al., 2022). Much attention has been paid to biological systems for biosynthesis of rice defense metabolites against herbivores but mechanisms for mobilization of mineral resources under biotic stress conditions remained poorly understood. Although it has been formulated in the Mineral Balance Hypothesis by Phelan, Norris and Mason (1996) that optimal nutrient balance results in both, good plant growth and resistance to herbivores, this possibility has never been addressed at molecular level. Therefore, our aim in this project was to introduce a paradigm shift in perception of minerals as essential components for growth and development to including them as indispensable players in the plant defense systems with respect to biotic stresses, such as herbivore and pathogen attacks.

２．研究の目的

The main purpose of this study was to (1) construct a comprehensive spatiotemporal map of mineral use (ionome) in rice challenged by various types of herbivores using ICP-MS, and (2) identify major mechanisms involved in herbivory-triggered mobilization of mineral resources in rice. In the proposal, we have been focusing on utilization of nitrate (ammonia and amino acids); calcium; phosphate; iron; zinc; manganese; and silicon that are closely associated with structural and functional defense systems in plants. Based on research outcomes of this study, we further aimed for design of novel strategies for plant protection against herbivores, which would benefit from (i) optimized mineral supply (e.g., by smarter fertilizer applications) and (ii) optimized mineral use (e.g., by genome editing of mineral transported genes) in herbivory-stressed crops.

３．研究の方法

We used rice as our main research model, which is highly relevant to agricultural needs in Japan. Specifically, cultivar Nipponbare was selected because it the most frequently used rice model plant in Japan, and it is supported by multiple bioinformatics resources. Rice seedling were cultivated in hydroponic culture using Kimura B solution to allow modulation of mineral supply and facilitate collection of intact root samples. We used two types of insects to obtain samples from herbivory stressed rice plants: (1) chewing herbivores, *Mythimna loreyi* (MYL), that were attached in custom clip cages to the rice leaves, similar to (2) sucking-piercing herbivores rice brown planthoppers, *Nilaparvata lugens* (BPH). In addition, we used simulated herbivory as a highly standardized method for induction of stress resembling to herbivore attack, which consisted of introduction of mechanical wounds followed by treatment with the oral secretions derived from MYL larvae. Typically, leaf blades from 6-8 week-old rice plants were used, while categorized in 3 groups as young, mature and old, together with leaf sheaths (basal plant parts) and roots. All material was analyzed for mineral content using Inductively Coupled Plasma-Mass Spectrometer (Agilent 7500 ICP-MS) after mineral extraction by heat disintegration in nitric acid under high pressure in designated START D Microwave Digestion System. Liquidified samples were introduced into ICP-MS by autosampler and multiple mineral elements were simultaneously quantified relative to standard solutions of mineral elements. Gene expression in herbivore stressed rice was examined by RNAseq method (NGS) and conventional RT-PCR. Distribution of abundant minerals, such as silicon in the rice leaves, was examined by Energy Dispersive X-Ray (EDX) spectrometer attached to scanning electron microscope (SEM). Manipulations of minerals in culture media were used to study the concentration effects of minerals on plant defenses. Solid phase extractions were used to purify defense metabolites from plant materials and used for quantification by LC-MS/MS based on our previously established methods. The effect of silicon on emission of volatile compounds from rice was examined after collection of volatiles on column traps and measurement by GC-MS. Effect of minerals (Ni, Si) on primary metabolism of herbivore challenged rice was examined by GC-MS after MSTFA derivatization of plant methanolic extracts.

４．研究成果

The first stage of the project was devoted to large scale time course experiment in the greenhouse to directly compare untreated hydroponic rice plants with those infested with MYL and BPH herbivores. After treatment, young (Y), mature (M) and old (O) leaves, stems (S) and roots (R) (n=4) were collected at 8 different time points (0, 1h, 3h, 6h, 10h, 24h, 48h, 72h) to capture the immediate and late responses to herbivory stress imposed by chewing and sucking insects. In total 440 samples were analyzed as reported below.

4.1. Mineral analyses

Using ICP-MS of nitric acid-hydrolyzed samples, total 34 elements were determined (Li, B, Na, Mg, Al, Si, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Zr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, W, Pb). The content of Si was further re-analyzed by independent colorimetric method as ICP-MS results are not considered quantitative under our ICP-MS instrument specifications. The analysis revealed that majority of mineral elements were stable and did not show very significant changes in response to herbivory stress. However, some elements such as Si, Ni and Cd were affected and therefore used as focal points in our further studies.

4.1.1. Role of Si in plant defense against herbivores as reported in Osibe (Galis) et al. (2024)

4.1.1.1. Si uptake and distribution in herbivore stressed plants

Major changes in mineral content after introduction of herbivory stress were observed in Si content. Plants exposed to MYL showed higher Si contents in locally attacked leaves. In short term Si supply experiments to previously Si-deprived plants in hydroponic culture, we show that (i) herbivore feeding attracts more Si to infested leaf and (ii) supply of Si is essential for rice defense and high mortality of feeding MYL larvae on the rice plants. In particular, we found that rice plants require at least 3 days of continuous supply of Si to mount a strong anti-herbivore defense against MYL feeding. EDX mapping after re-supply of Si showed that Si mainly accumulates in specific cells in the rice leaf epidermis and in spikes on the leaf surface. The silicification of spikes then consistently explained the mode of direct defensive action of Si, already proposed in our previous study with African NERICA rice species that inspired the initiation of the current study (Andama et al, 2020).

4.1.1.2. Study on the role of Si in metabolic defense of rice

It was assumed that Si plays direct defense role by increasing rigidity of plant tissues in Si accumulating plants, such as rice. However, our project revealed multiple additional and beneficial roles of Si in rice that are likely to amplify the importance of Si in the rice field propagation. Firstly, the amounts of defensive metabolites in Si-supplemented rice challenged with MYL and simulated herbivory was significantly higher than in rice without Si supply. Three groups of specialized metabolites were confirmed to increase after Si supplementation in rice laves: isopentylamine, *p*-coumaroylputrescine and feruloylputrescine. Furthermore, metabolomics screening by GC-MS on samples derivatized by MSTFA showed a general trend for Si-supplemented leaves to contain more soluble sugars, including important monosaccharides, glucose and fructose. In contrast, Si-deprived leaves tend to have more amino acids compared to Si-supplied leaves. These metabolic shifts caused by Si were clearly visible in principal component analysis (PCA). The plots showed a separation of replicated samples; (1) based on Si supplementation and (2) based on herbivore treatment (for details see Osibe (Galis) et al., 2024). Furthermore, we found significant changes in the amount of volatile organic compounds (VOCs) released from the rice plants treated with herbivores, in the presence and absence of Si. As VOCs play important role in attraction of natural enemies of herbivores to plants, where they serve as natural plant' s bodyguards, the presence of Si seems even more important for plant defenses in the context of natural environment. As some genes involved in VOC production were upregulated in the presence of Si, it consistently points to Si playing a role of general enhancer of plant metabolism and defense. Si also negatively contributed to herbivore choice of plants for oviposition, adding yet another layer to multiple Si defense functions in rice plants. This can be either explained by surface modifications based on the direct Si incorporation in the leaf epidermis, or changes in volatile profiles described above that are used to navigate insect moths during egg laying behavior. These finding shed a new light into the useful potential of Si and its application in agriculture, especially in plants that can hyper-accumulate this abundant soil element, such as the rice crop.

4.2. Implications from the large scale transcriptomics experiments

In transcriptomics analysis, samples from five parts of rice plants and two herbivore regimes (MYL, BPH) and 8 time points were subjected to RNAseq (NGS) with pools of replicates in each treatment, resulting in total 110 analyzed RNAseq samples. This analysis provided enormous resource for this project, as well as future endeavors in the Group of Plant-Insect Interactions, that will benefit from the identification of hundreds to thousands of herbivore-regulated genes, many of which are novel genes. In the context of this project, we could examine response of every potential mineral transporter and its regulation in rice exposed to two types of herbivory, MYL and BPH. For instance, we identified the elevated expression level of OsSIET4 (Osibe (Galis) et al., 2024) that was recently identified as Si transported responsible for the distribution of Si into epidermal cells. This gene appears to be polarly localized at the distal side of epidermal cells and cells surrounding the bulliform cells (motor cells) of the leaf blade (Mitani et al., 2023). We also identified transcriptional activation of OsLsi6 that unloads Si from xylem elements in the leaves (Osibe (Galis) et al., 2024). These observations complemented the information on preferential accumulation of Si in herbivore attacked leaves as described above and published in Osibe (Galis) et al. (2024). We will continue in data-mining of these transcriptomics data in the future to initiate new hypotheses and unravel novel mechanisms involved in the effectiveness of rice defense against herbivory damage that includes the newly identified potential of Ni in plant' s anti-herbivore defense as described further.

4.3. Identification of Ni as potential new player in plant defense against herbivores

As one original finding in this project, we discovered an increase in uptake of Ni in rice plants subjected to direct herbivory stress. As Ni plays a role as a cofactor of some enzymes in plants, we hypothesize that increased Ni uptake may be motivated by increased metabolic needs and demands for free nitrogen in the stressed plant. In particular, Ni is used as a cofactor in the urease enzyme that is involved in the production of ammonia which acts as an integral part of nitrogen cycle in plants. As nitrogen is structurally needed for the biosynthesis of alkaloids in plants and bioactive amines, such as isopentylamine in rice, we have likely unraveled a new functional bridge between minerals and defense. In addition, as one progress attributed to nitrogen utilization in herbivory stressed rice, we identified an enzyme that is required for the isopentylamine biosynthesis, which happens via decarboxylation of amino acid leucine (Aboshi (Galis) et al, under submission). We are now working on the elucidation of specific mechanisms that will include Ni as an element that potentiates plant defense during herbivore attack, which is one of the major scientific carry-over outcomes in this pioneering research project devoted to making functional connections between minerals and plant defense.

Cited references:

- # Aboshi T, Teraishi M, Galis I, Yoshikawa T, Shinya T. (under submission) Natural variation-based genetic screen in rice identifies the isopentylamine biosynthetic gene that modulates brown planthopper behavior. Plant Biology

- Andama JB, Mujiono K, Hojo Y, Shinya T, Galis I (2020) Nonglandular silicified trichomes are essential for rice defense against chewing herbivores. Plant, Cell and Environment 43 (9): 2019-2032.
- Mitani-Ueno N, Yamaji N, Huang S, Yoshioka Y, Miyaji T, Ma JF (2023) A silicon transporter gene required for healthy growth of rice on land. Nature Communications 14: 6522.
- # Osibe D, Hojo Y, Shinya T, Mitani-Ueno N, Galis I (2024) Comprehensive analysis of silicon impact on defense and metabolic responses in rice exposed to herbivory stress. Frontiers in Plant Science, 15:1399562. doi: 10.3389/fpls.2024.1399562
- Phelan PL, Norris KH, Mason JF (1996) Soil-management history and host preference by *Ostrinia nubilalis*: Evidence for plant mineral balance mediating insect-plant interactions. Environmental Entomology 25: 1329-1336.
- # Wari D, Aboshi T, Shinya T, Galis I (2022) Integrated view of plant metabolic defense with particular focus on chewing herbivores. Journal of Integrative Plant Biology 64: 449-475.

Papers created under main auspices of this project

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　4件／うちオープンアクセス　4件）

１．著者名 Osibe DA, Hojo Y, Shinya T, Mitani-Ueno N, Galis I	４．巻 15
２．論文標題 Comprehensive analysis of silicon impact on defense and metabolic responses in rice exposed to herbivory stress	５．発行年 2024年
３．雑誌名 Frontiers in Plant Science	６．最初と最後の頁 1399562
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fpls.2024.1399562	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Osinde C, Sobhy IS, Wari D, Dinh ST, Hojo Y, Osibe DA, Shinya T, Tugume AK, Nsubuga AN, Galis I	４．巻 18
２．論文標題 Comparative analysis of sorghum (C4) and rice (C3) plant headspace volatiles induced by artificial herbivory	５．発行年 2023年
３．雑誌名 Plant Signaling & Behavior	６．最初と最後の頁 2243064
掲載論文のDOI（デジタルオブジェクト識別子） 10.1080/15592324.2023.2243064	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Osinde C, Sakamoto W, Kajiya-Kanegae H, Sobhy IS; Tugume AK, Nsubuga AM, Galis I	４．巻 18
２．論文標題 Identification of quantitative trait loci associated with sorghum susceptibility to Asian stem borer damage.	５．発行年 2023年
３．雑誌名 Journal of Plant Interactions	６．最初と最後の頁 2153182
掲載論文のDOI（デジタルオブジェクト識別子） 10.1080/17429145.2022.2153182	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Aboshi T, Ittou K, Galis I, Shinya T, Murayama T	４．巻 101
２．論文標題 Glucosylation prevents autotoxicity of stress inducible DOPA in maize seedlings	５．発行年 2023年
３．雑誌名 Plant Growth Regulation	６．最初と最後の頁 159-167
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s10725-023-01009-w	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Wari D, Aboshi T, Shinya T, Galis I	**4．巻** 64
2．論文標題 Integrated view of plant metabolic defense with particular focus on chewing herbivores	**5．発行年** 2022年
3．雑誌名 Journal of Integrative Plant Biology	**6．最初と最後の頁** 449-475
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/jipb.13204	**査読の有無** 有
オープンアクセス オープンアクセスとしている（また、その予定である）	**国際共著** 該当する

〔学会発表〕　計11件（うち招待講演　3件／うち国際学会　3件）

1．発表者名 Galis, I.
2．発表標題 A path for the use of natural products in plant protection against insect herbivores
3．学会等名 3rd International Conference On Tropical Agrifood, Feed & Fuel (ICTAFF) 2023（招待講演）（国際学会）
4．発表年 2023年

1．発表者名 Osibe, D. A., Hojo, Y., Shinya, T., Galis, I.
2．発表標題 Indirect defense in rice: role of silicon in rice volatile production under herbivory stress
3．学会等名 第65 回日本植物生理学会年会, Kobe
4．発表年 2024年

1．発表者名 Galis, I., Kuwabara, T., Hojo, Y., Wari, D. and Shinya, T.
2．発表標題 Systemic defense against herbivores in rice: Not really the case
3．学会等名 3rd ISCE-APACE Joint Meeting, Kuala Lumpur, Malaysia (Online)（国際学会）
4．発表年 2022年

１．発表者名 Osibe, D.A., Hojo, Y., Shinya, T. and Galis, I.
２．発表標題 Dynamics of silicification and the role of silicon in rice defense against chewing and sucking herbivores
３．学会等名 3rd ISCE-APACE Joint Meeting, Kuala Lumpur, Malaysia (Online)（国際学会）
４．発表年 2022年

１．発表者名 Osibe, D.A., Hojo, Y., Shinya, T. and Galis, I.
２．発表標題 Rapid silicon distribution and defense in rice exposed to herbivory stress
３．学会等名 The 64th Annual Meeting of the Japanese Society of Plant Physiologists. Sendai
４．発表年 2023年

１．発表者名 Galis, I.
２．発表標題 Lessons from rice defense against herbivores: What really matters?
３．学会等名 38th IPSR International Symposium and 14th Symposium on Plant Stress Sciences, Okayama, Kurashiki（招待講演）
４．発表年 2023年

１．発表者名 Ono, K., Murayama, T., Aboshi T.
２．発表標題 Dynamics of nitrogen metabolites in rice due to feeding damage
３．学会等名 The 2023 Annual Meeting of the Japanese Society for Bioscience, Biotechnology and Agrochemistry (JSBBA), Hiroshima
４．発表年 2023年

１．発表者名
Mitani-Ueno, N.

２．発表標題
Si in rice: a long journey from uptake to deposition

３．学会等名
38th IPSR International Symposium and 14th Symposium on Plant Stress Sciences, Okayama, Kurashiki（招待講演）

４．発表年
2023年

１．発表者名
Taiga Kuwabara, Yuko Hojo, Tomonori Shinya, Ivan Galis

２．発表標題
Jasmonate-mediated systemic defense in rice leaf

３．学会等名
63rd Annual Meeting of the Japanese Society of Plant Physiologists

４．発表年
2022年

１．発表者名
Namiki Mitani-Ueno, Naoki Yamaji, Yuuma Yoshioka, Takaaki Miyaji, Jian Feng Ma

２．発表標題
SIET4 is required for cell-specific deposition of Si in rice

３．学会等名
63rd Annual Meeting of the Japanese Society of Plant Physiologists

４．発表年
2022年

１．発表者名
Takako Aboshi, Kouhei Ittou, Ivan Galis, Tomonori Shinya, Tetsuya Murayama

２．発表標題
DOPA glycoside accumulation is induced by herbivory in maize

３．学会等名
47th Annual Meeting of the Pesticide Science Society of Japan

４．発表年
2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

－

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
研究分担者	網干　貴子 (Aboshi Takako) (20746705)	山形大学・農学部・准教授 (11501)	
研究分担者	三谷　奈見季 (Mitani Namiki) (40581020)	岡山大学・資源植物科学研究所・准教授 (15301)	

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