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学位論文の内容の要約

氏　　名	李　校一
学位の種類	博士（農学）
学府又は研究科・専攻	大学院生物システム応用科学府　食料エネルギーシステム科学専攻
指導を受けた大学	東京農工大学
学位論文題目	Identification of BmABCA2 as a Functional Receptor in the *Bombyx mori* Midgut for Cry2A Toxins カイコガ中腸ABCトランスポーターA2分子のCry2A毒素受容体としての役割と機能に関する研究

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1. Introduction

Cry toxins are insecticidal proteins produced by *Bacillus thuringiensis* (Bt). They are used commercially to control insect pests since they are very active in specific insects and are harmless to the environment and human health. Cry toxins are used widely in pest control. As a gene source, Bt toxin genes have been used efficiently to make transgenic crops (Bt crops) that resist pests. Cry 2A toxins can effectively control lepidopteran and Dipteran. However, the Cry2A toxin that presents intense evolutionary pressure was expressed in transgenic crops. It was toxicity and cause of death for target pests but represented a strong selectivity for resistance, which presents a significant threat to crop benefits. Therefore, to study the functional receptors for Cry2A toxin is essential for delay insect resistance. The gene encoding ATP-binding cassette subfamily A member 2 (ABCA2) was identified in a previous analysis of Cry2A toxin resistance genes. However, we do not have direct evidence for the role of ABCA2 in the mode of action of Cry2A toxins and do not know the reasons why Cry2A toxin resistance does not cross to other Cry toxins. Therefore, the author performed two experiments. First, the ABCA2 gene in *Bombyx mori* (Bm) was edited by a research collaborator Dr. Watanabe using transcription activator-like effector-nucleases (TALENs) and the susceptibility-determining ability of the ABCA2 gene was confirmed by the author with a diet overlay bioassay. Second, the author conducted a cell susceptibility test referred to as swelling assay using BmABCA2 heterologous expressing HEK293T cells. Those demonstrated that BmABCA2 is a functional receptor in the midgut epithelial cells and susceptibility determining factor in *B. mori* for Cry2A toxins.

2. Research Methodologies

2.1 Diet overlay assay

In traditional insecticidal systems, only one Cry toxin has been used. To delay the selection and evolution of resistance in exposed insect populations, current commercial insecticidal systems are constructed based on the combination of two or more Cry toxins since they are bind to different receptors in target pests. However, the generation of resistant insects and cross-resistance to other

Cry toxins are still problematic. Therefore, it is necessary to confirm the susceptibility determinant factor of Cry2A toxins in *B. mori* and confirm whether it is narrowly tuned by one susceptibility determinant factor. In the study, the larvae of *B. mori* were genome-edited by TALEN at *BmABCA2, BmABCC2, BmABCC3*, and *BmABCC4* locus and several mutant strains were created regarding each locus as model systems to clarify susceptibility determining the mechanism of Cry2Aa and Cry2Ab toxins. Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, Cry1Da, Cry1Fa, and Cry9Aa have been assayed the susceptibility against mutant strains of BmABCA2, BmABCC2, BmABCC3, or BmABCC4 as well.

2.2 Cell swelling assay with heterologous expression using HEK293T Cell

To identify how the BmABCs involved in the mode of action of Cry toxin, a heterologous expression system was used to display the receptor function. Since the function of *Spodoptera exigua* ABCC3 (SeABCC3) as a receptor for Cry1Aa and Cry8Ca were successfully shown using human embryonic kidney 293T cell (HEK293T). In the study, BmABCA2, BmABCC2, and BmABCC3 were heterologously expressed in HEK293T cells. After that, the function of BmABCA2 in Cry2Ab intoxication was demonstrated by Cell swelling assay. Cry1Aa, Cry1Ac, or Cry9Aa were also assayed with the heterologous expressed HEK293T cells to confirmed the ability of specific intoxication.

3. Results

The results mutant BmABCA2 strains in diet overlay assay showed highly resistant to Cry2A toxins compared with wild-type, but not to Cry1A, Cry1Ca, Cry1Da, Cry1Fa, and Cry9Aa toxins. It indicates that BmABCA2 plays an essential role in determining the susceptibility of *B. mori* to Cry2A toxins, but not play a susceptible determining for the other toxins in *B. mori*. On the other hands, the strains with mutant strains of BmABCC2, BmABCC3, and BmABCC4 were administrated with Cry2Aa and Cry2Ab as well, no resistance was observed, which suggested that BmABCA2 alone plays an important role in the mechanism of action of Cry2A toxin. Then, using HEK293T cells expressing BmABCA2 to demonstrate whether the BmABCA2 specific function to Cry2A toxin. BmABCA2-expressing HEK293T cells were susceptible to Cry2Ab, but not to Cry1A or Cry9A toxins. Moreover, BmABCC2 and BmABCC3-expressing HEK293T cells were showed susceptible to Cry1A toxins, but not to Cry2Ab. Therefore, the results suggest that the specificity of BmABCA2 as a Cry toxin receptor is narrowly tuned to Cry2A toxins.

4. Discussion

The results of mutant BmABCA2 strains did not show highly resistant to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, Cry1Da, Cry1Fa, and Cry9Aa. They presented the same level resistance as the wild-type strain. It suggested that Cry2A toxins do not share the same susceptible determining factor (BmABCA2) with these toxins, which means the BmABCA2 deficiency−dependent Cry2A resistance does not confer cross−resistance to Cry1A, Cry1Ca, Cry1Da, Cry1Fa, or Cry9Aa toxins. It would provide necessary evidence of new co-expressing strategies in Bt crops. Because so far, the main combinations of co-expressing Cry toxins are Cry1A and Cry2 A toxins. Therefore, the results can support to explore new strategies for co-expression of Cry toxins and ultimately aims to control insect resistance.