(所定書式)

2022年 3 月 1 日

学 位 論 文 の 内 容 の 要 約

氏　　名	王 永浩
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	Analysis of Susceptibility-determining Receptors of *Bombyx mori* for *Bacillus thuringiensis* Cry toxins

【論文の内容の要約】

Field-evolved resistance of insect pests to *Bacillus thuringiensis* (Bt) toxins (Cry toxins) is a threat to the efficacy of Bt-based bio-insecticides and transgenic crops. Recent reports have suggested that ATP-binding cassette transporters and cadherin-like receptor play important roles in conferring susceptibility to Cry toxins. However, the receptors involved in Bt susceptibility in each insect remain unclear.

In the Chapter 1, to determine the receptors that are involved in the susceptibility of *Bombyx mori* to Cry1 toxins (1Ab, 1Ac and 1Fa), I conducted diet overlay bioassay using *B. mori* strains disrupted with one or two receptor (s) among ATP-binding cassette transporter family C2 (BmABCC2), ATP-binding cassette transporter family C3 (BmABCC3), and cadherin-like receptor (BtR175) generated by transcription activator-like effector nuclease (TALEN)-mediated gene editing. The single-knockout strains for *BmABCC2* showed resistance to Cry1Ab and Cry1Ac, whereas only strains with double knockout of BmABCC2 and BmABCC3 exhibited high resistance to Cry1Fa. Progeny populations generated from the crossing of heterozygotes for BtR175 knockout allele included 25% theoretical homozygotes for the BtR175 knockout allele and they showed resistance to Cry1Ab and Cry1Ac. Then, through a cell swelling assay using Sf9 cells ectopically expressing the receptor, I analyzed the mechanisms underlying the different contributions of BmABCC2, BmABCC3, and BtR175 to larval susceptibility. The receptor activity of BmABCC2 for Cry1Ab and Cry1Ac was far higher than that of BmABCC3, and BtR175 synergistically enhanced the receptor activity of BmABCC2. This result well explained the important involvement of BmABCC2 and BtR175 in the larval susceptibility to Cry1A toxins. By contrast, the receptor activities of BmABCC2 and BmABCC3 for Cry1Fa were observed at a similar level and synergistic effect of BtR175 was small. This finding explains the equal importance of BmABCC2 and BmABCC3 and very small contribution of BtR175 on larval susceptibility to Cry1Fa. Thus, I demonstrated the different importance of BmABCC2, BmABCC3, and BtR175 to various Cry1 toxins as susceptibility-determining factors in *B. mori* larvae and the underlying basis for the observed differences. Furthermore, a weak correlation was indicated between the binding affinity and receptor activities of BmABCC2 and BmABCC3 to Cry1 toxins.

In the Chapter 2, I investigated whether BmABCC1 and BmABCC4 could be functional receptor in *B. mori* to serval Cry toxins, Cry1Aa, Cry1Da, Cry8Ca and Cry9Aa. Firstly, I did a cell swelling assay using BmABCC1 and BmABCC4 expressing Sf9 cells to explore whether they do have a receptor function to serval Cry toxins. The BmABCC1 showed receptor activity to Cry9Aa, and BmABCC4 showed receptor activity to Cry1Aa, Cry1Da, Cry8Ca. However, BmABCC1 knockout *B. mori* larvae did not show resistance to Cry9Aa and BmABCC4 knockout *B. mori* larvae did not show resistance to Cry1Aa, Cry1Da, Cry8Ca. These results suggest that BmABCC1 and BmABCC4 can function as low performance receptors to Cry1Aa, Cry1Da, Cry8Ca and Cry9Aa toxins because BmABCC1 and BmABCC4 did not contribute to the determination of susceptibility of *B. mori* larvae to those Cry toxins and a low binding affinity of BmABCC1 with Cry9Aa, and BmABCC4 with Cry1Aa, Cry1Da and Cry8Ca was also observed in SPR analysis.