Studies on the role of cuticular function-related genes in insecticide resistance mechanism in red flour beetles, *Tribolium castaneum.*

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Summary

Since the green revolution, agriculture has aimed to manage agricultural pests in order to improve yield production. Agricultural pests consist of weeds, plant pathogens, and insect or other invertebrate pests. Insects are one of the major agricultural pests that cause significant crop yield losses every year. Chemical insecticides have proven to be the most effective method for integrated pest management (IPM) and controlling insect populations.

The insect body is covered with the exoskeleton, which consists of epidermal cells and cuticular layers. The main components of insect cuticles are chitin microfibrils and cuticular proteins. Chitin is the polymer of *N*-acetylglucosamine; in chitin biosynthesis in epidermal cells, UDP-N-acetylglucosamine is incorporated into polymerized chitin by an enzyme called chitin synthase 1 (CHS1). Cuticular proteins are another component of insect cuticles. A number of cuticular proteins have been identified and characterized in model insects, such as the fruit fly, mosquitoes, and red flour beetles.

In insect molting, biosynthesis of new cuticles proceeds. After ecdysis (i.e., shedding the old exoskeleton), the newly synthesized cuticular layer undergoes pigmentation and sclerotization process, which is called tanning. Enzymes such as tyrosine hydroxylase (TH), dopa decarboxylase (DDC), and laccase 2 (Lac2) have been reported to be involved in the tanning process. Through the tanning process, insect integument becomes more rigid with a darker color.

In general, insect epidermal cuticles have been regarded to be crucial for defense against xenobiotics such as entomopathogenic microbes and chemicals. Previous studies on the red flour beetle *Tribolium castaneum* revealed that some cuticular proteins are essential for the defense against the attack of entomopathogenic fungi *Beauveria bassiana* (Sirasoonthorn et al., 2021). However, the role of cuticles as the defense against insecticides has yet to be examined.

In this study, I examined the role of cuticular function-related genes in terms of the resistance (or defense) against topically applied insecticides, using the red flour beetle *T. castaneum*. After the knockdown of a gene of interest by RNA interference (RNAi), the beetles were topically applied with insecticides, and the short-term neurotoxic effects were evaluated. Their external morphology and the cross-sections of the cuticle of the abdominal sternite were also observed. In this study, neonicotinoid insecticides, which inhibit neurotransmission by acting on nicotinic acetylcholine receptor (nAChR) in synapses, were used as the test chemicals with sublethal concentration. This thesis includes the two studies as described below.

Study 1. Functional analysis of cuticular proteins and Chitin synthase 1 (CHS1) in the insecticide resistance mechanism in *T. castaneum*.

RNAi-mediated knockdown of major adult cuticular proteins (*CPR4*, *CPR18*, and *CPR27*) was performed. By observing cross sections of abdominal sternites, it was confirmed that *CPR* knockdown caused thinner and disorganized cuticles. The knockdown of any of the *CPR* genes, either singly or in combination, resulted in enhanced susceptibility of the beetles to insecticides. These results suggested that these CPRs are indispensable in organizing the insect cuticular structure as the defense against exogenous insecticides.

Mild knockdown of *CHS1* also caused disorganized cuticle. However, it did not significantly affect the beetle's susceptibility to insecticides.

Study 2. Functional analysis of genes in the tanning process in the insecticide resistance mechanism in *T. castaneum*.

RNAi-mediated knockdown of genes in the tanning process (*TH*, *DDC*, and *Lac2*) was performed. Mild knockdown of *TH*, *DDC*, and *Lac2* genes resulted in defects in cuticle coloration as well as the disorganized cuticle of the abdominal sternite. However, it did not significantly affect the beetle's susceptibility to insecticides.

In summary, this study revealed that major adult cuticular proteins (*CPR4*, *CPR18*, and *CPR27*) are indispensable for the defense against exogenous insecticides. These CPRs have been reported to be essential for the defense against attack from entomopathogenic fungi (Sirasoonthorn et al., 2021). It was concluded that these CPRs are essential for the defensive function of the cuticle against both insecticides and microbes. Knockdown of other factors (*CHS1*, *TH, DDC,* and *Lac2*) caused disorganized cuticles but did not have an obvious effect on the susceptibility of the beetles to the insecticides. In future studies, it needs to be examined whether more severe knockdown affects the susceptibility of the beetles to the insecticides. These experiments will help to elucidate the factors that are essential for the defense of the cuticle against exogenous insecticides.