Effects of inducible tolerance to *Bacillus thuringiensis* on the egg transcriptomes and egg parasitism in *Helicoverpa armigera*

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Abstract

In the Australian cotton industry, toxins produced by the soil bacterium *Bacillus thuringiensis* (*Bt* toxins) are utilised to control two lepidopteran pests, *Helicoverpa armigera* (cotton bollworm) and *H. punctigera* (native budworm). *Bt* toxins kill insects by forming pores in the insect midgut, which leads to sepsis. The extensive use of *Bt* toxins, including in the form of transgenic crops, has put strong selection pressure on the pest insects in the field, which can lead to resistance. Understanding the resistance mechanism is essential for planning the resistance management strategy to prolong the effectiveness of the *Bt* toxins.

Previous studies have demonstrated that larvae of cotton bollworm can develop a low-level tolerance to *Bt* toxins after being exposed to a sub-lethal dose. This induced tolerance is associated with increased immune activity in the midgut and haemolymph. In addition, the induced tolerance and the increase in the immune activity can be transferred to the next generation via a maternal effect, and the level of tolerance can increase over generations of exposure. Interestingly, the characteristics of inducible tolerance are also found in a Cry1Ac-resistant strain of *H. armigera* known as the Bx strain (CSIRO, Narrabri, NSW). Even though many studies have reported immune responses against *Bt* toxins, the role of the immune system in facilitating inducible tolerance against *Bt* toxins and its transmission mechanism are still unclear. The primary aim of this study was to investigate the transmission mechanism of inducible *Bt* tolerance.

The effect of the maternal experience on the offspring's immune system (trans-generational immune priming; TGIP) has been demonstrated in several studies. Although there is speculation about the mechanisms of TGIP, such as the insertion of immune substances into eggs and changes in the DNA methylation state of the offspring's genome, the genes and metabolic pathways involved in the transmission mechanisms are still undefined. Given that immune components could be maternally transmitted via eggs,

together with the importance of egg parasitoids to integrated cotton pest management, it is important practically to also understand whether there is any negative effect of *Bt* tolerance/exposure on *H. armigera* eggs with regard to parasitisation.

There are two research questions in this study: 1) what genes are involved in the transmission mechanism of inducible *Bt* tolerance? and 2) what are effects of inducible tolerance on eggs and parasitism?

To address the first question, I investigated the gene expression profiles of eggs. First, two transcriptomic assemblies for eggs of *H. armigera* were generated by combined deep sequencing results from five strains of *H. armigera*: two Cry1Ac-susceptible, Cry1Ac-tolerant (low level *Bt* toxin selection), Cry1Ac-resistant (Bx strain, high level selection, highly resistant), and Cry2Ab-resistant strains. Then, the assemblies were used to compare gene expression profiles of eggs from susceptible and induced tolerant *H. armigera*. Four genes were identified, and confirmed by quantitative RT-PCR, to differentially express between eggs from tolerant and susceptible individuals. These genes are histone cluster 3 H2BB, translationally controlled tumor protein, receptor for activated C kinase, and glyceroldehyde-3-phosphate dehydrogenase. Currently, the roles of these genes in inducible *Bt* tolerance are still unclear. The changes in the expression of these genes could be a part of the mechanism of *Bt* tolerance, or a response to *Bt* exposure. Further investigations on the functions of these genes in inducible *Bt* tolerance are needed.

Since the Cry1Ac-resistant (Bx) strain also has the characteristics of inducible *Bt* tolerance, it is possible that the mechanism of inducible *Bt* tolerance in the Bx strain is the same as the tolerant strain. Interestingly, the four genes mentioned above that were expressed differently between susceptible and Cry1Ac-tolerant eggs (Waite strain) were not expressed differently between eggs of Cry1Ac-susceptible and Cry1Ac-resistant strains. On the other hand, four genes were expressed differentially between eggs of Cry1Ac-susceptible and Cry1Ac-resistant strains. They were pyruvate kinase, olfactory receptor 29,

transmembrane proteins 9, and proteasome 25 kDa. The functions of these genes in eggs and Cry1Ac-resistance are as yet uncharacterized, and need to be further investigated.

The differences in the sets of differentially expressed genes in eggs of Cry1Ac-tolerant and Cry1Ac-resistant strains suggested that the mechanisms of maternally transmitted tolerance/resistance might be different. It is possible that different mechanisms might be necessary to survive the different concentrations of *Bt* toxins that were encountered during the selection process. This might also indicate that there is more than one pathway that leads to the similar immune responses activated in response to *Bt* exposure.

I further investigated whether there was any effect of inducible tolerance on eggs of *H. armigera* and its suitability as a host for egg parasitism by *Trichogramma pretiosum*. Three key measurements were assessed: parasitism success, the number of wasps emerged per host egg, and the proportion of male and female offspring emerged per host egg. The results showed that there was no difference in parasitism success between susceptible and tolerant eggs. However, there was a significant increase in the number of emergent parasitoids, especially male offspring, in eggs laid by tolerant *H. armigera*. Further investigation of the size of host eggs indicated those from Cry1Ac tolerant *H. armigera* were larger than eggs from the susceptible population. The result also showed that the increase in the egg size was correlated with *Bt* exposure. These results confirm that maternally-transmitted *Bt* tolerance affects on the phenotype of the eggs from tolerant *H. armigera*, which consequently affects egg parasitoids. Interestingly, the differences in egg size is correlated with the differences in the egg gene expression profiles, although the link between these two differences remains unclear. However, the differences in egg size and the gene expression profiles did not appear to negatively affect parasitism rates of *T. pretiosum*. In fact, there were more wasps emerged from the larger eggs of tolerant insects compared to eggs of susceptible insects. In conclusion, no negative effect of inducible *Bt*

tolerance on the use of egg parasitoids in cotton pest management systems in terms of the number of wasp progeny produced has been detected.

In conclusion, I identified the genes that were differentially expressed between eggs of susceptible and inducible *Bt* tolerant *H. armigera*. However, the roles of these genes in the transmission mechanism of inducible *Bt* tolerance and in the insect immune system are still unclear, and need further investigation. In addition, inducible *Bt* tolerance or *Bt* exposure has an effect on the egg volume, but this does not have an adverse effect on egg parasitism. Further works should include functional studies on the expression of the genes identified in this study in the larval midgut, and their roles in the transmission mechanisms of inducible *Bt* tolerance.

Thesis Declaration

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Jutamat (Kay) Anantanawat

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Abbreviation list

ALP	Alkaline phosphatase
AMP	Anti-microbial peptide
APN	Aminopeptidase N
Apo III	Apolipophorin III
bp	base pair
Bt	*Bacillus thuringiensis*
C_Contig	Contigs generated from CLC assembler
CAD	Cadherin-like protein
Cry toxins	Crystal toxins
DEG	Differentially expressed gene
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GM	Genetically modified
GO	Gene ontology
GST1	Gluthatione S-transferase I
H2BB	Histone cluster 3 H2B
hr	hour
kDa	kilo Daltons
LC	Lethal concentration
LPS	lipopolysaccharide
MAPK	Mitogen-activated protein kinase
Mb	Megabase pair
min	Minute
min.	Minimum
mM	millimolar
N_Contig	Contigs gneerated from Newbler assembler
NCBI	National centre for biotechnology information
ng	nanogram
nr	non-redundant
nt length	nucleotide length
OLC	Overlap-layout consensus
PAE	Phenoloxidase activating enzyme
PCR	Polymerase chain reaction
PiGV	*Plodia interpunctella* Granulosis Virus
PO	Phenoloxidase
PPO	Prophenoloxidase
PRP	Pattern recognition protein
qPCR	Qantitative Polymerase Chain Reaction (Quantitative PCR)
RACK	Receptors for activated C kinase
RNAi	RNA interference
RPKM	Reads Per Kilobase of exon model per Million Mapped read
RPS15	Ribosomal protein subunit 15
RR	Resistance ratio
RT	Reverse transcription
RT-PCR	Reverse transcript polymerase reaction

s	second
SEM	Standard error of the mean
sptz	spätzle
TCTP	Translationally controlled tumor protein
TGIP	Transgenerational immune priming
ul	microlitre
uM	micromolar

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