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Insect resistance to dietary protease inhibitors

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Introduction

Protease inhibitors (PIs) are plant defensive compounds that are considered as candidates for future genetic modification of crop plants. They **target the digestive proteolytic enzymes in the gut of insects**. However, insect resistance to these antinutritional PIs is frequently observed. The general aim of this research was to identify PI induced compensatory responses in the gut of the African migratory locust, *Locusta migratoria*, an infamous pest insect, capable of forming huge swarms. Using **microarray analysis** we studied transcriptional changes after oral uptake of plant protease inhibitors by the locust.

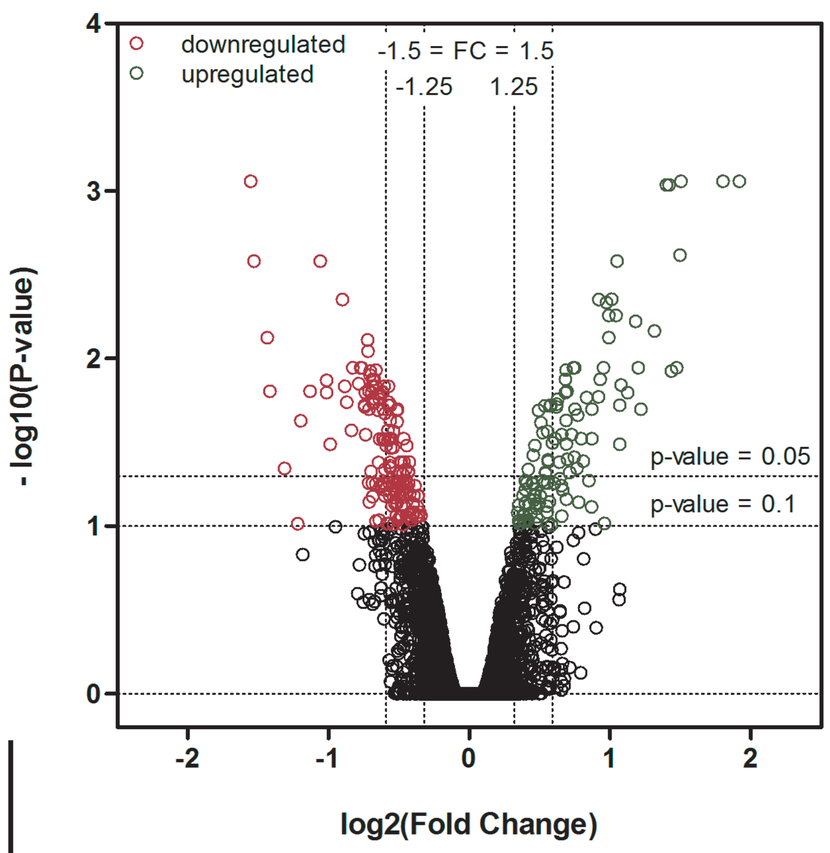
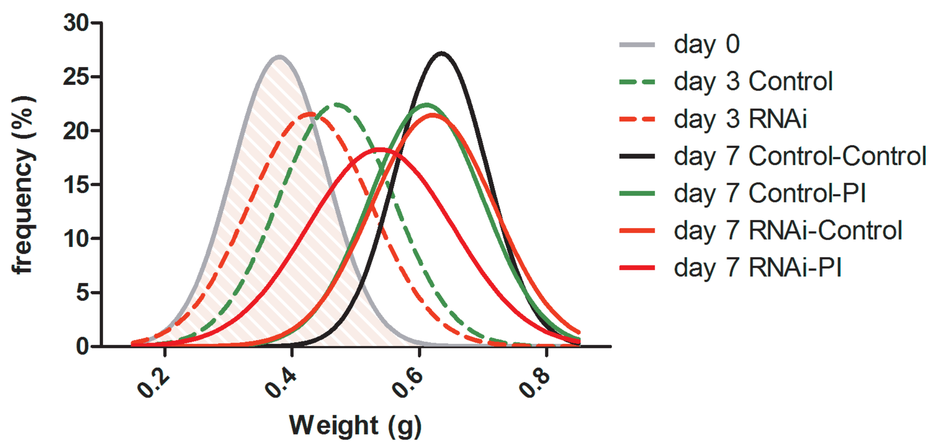


Fig. 1. Volcano plot of retrieved microarray data. Plotting the negative log10 of the adjusted p-value against the log2 of the fold change.

Fig. 2. Frequency distribution of the weight of locust populations after knocking down hexamerin-like transcripts in combination with ingestion of PI.



Results

Table 1. Functional annotation of the upregulated (top) and downregulated (below) transcripts. Annotations were performed using InterPro scan and sorted based on putative function.

Putative function	InterPro	# Seq	Gene description	Fold Change
JH-binding	IPR013788	17	Hemocyanin/hexamerin	1.4 - 2.8
	IPR010562	1	Haemolymph juvenile hormone binding	1.5
Protein digestion	IPR001254	9	Serine protease family S1	1.4 - 1.8
	IPR003146	5	Carboxypeptidase	1.3 - 1.6
Carbohydrate metabolism	IPR001360	1	β-glucosidase	1.6
	IPR000933	1	α-L-fucosidases	1.7
	IPR001139	1	Glucosylceramidase	1.5
Lipid metabolism	IPR025483	1	Lipase	1.6
Detox/Stress response	IPR001128	3	Cytochrome P450	1.3 - 2.0
	IPR002018	1	Carboxylesterase, type B	1.6
Peritrophic membrane	IPR002557	2	Chitin binding peritrophin A	2.0 - 2.1
Other		7		1.3-1.6

Putative function	InterPro	# Seq	Gene description
Structural activity	IPR004000	10	Actin
	IPR001781	3	Zinc finger, LIM-type
	IPR013098	2	Immunoglobulin I-set domain
	IPR002928	1	Myosin
	IPR001715	1	Calponin homology domain
Defense	IPR001304	2	C-type lectin
	IPR008597	1	Destabilase
Carbohydrate metabolism	IPR001360	3	β-glucosidase
	IPR001312	2	Hexokinase
	IPR001701	1	Cellulase
Lipid metabolism	IPR000566	1	Lipocalin/cytosolic fatty-acid binding protein domain
	IPR002172	1	Low-density lipoprotein (LDL) receptor class A
Other metabolism	IPR002198	1	Short-chain dehydrogenase
	IPR002085	1	Long-chain alcohol dehydrogenase
	IPR023186	1	Inosine/uridine-nucleoside hydrolase
	IPR001148	1	Alpha carbonic anhydrase
Protein digestion	IPR000994	1	Peptidase M24, aminopeptidase
	IPR008257	1	Peptidase M19, dipeptidase
Detox/Stress response	IPR002018	2	Carboxylesterase, type B
	IPR010255	1	Haem peroxidase
	IPR002403	1	Cytochrome P450, E-class, group IV
	IPR008978	1	Alpha crystallin/Heat shock HSP20-like chaperone
Other		15	

By using a two-color microarray hybridization setup we identified 114 and 150 transcripts out of a total of 35869 that were respectively up- or down-regulated **(Fig. 1, Table 1)**. A large group of upregulated transcripts encoded hexamerin-like proteins. Knockdown of these transcripts in combination with PI-ingestion resulted in a stunted growth **(Fig. 2)**, possibly due to an inability to regulate the normal response **(Fig. 3)**.

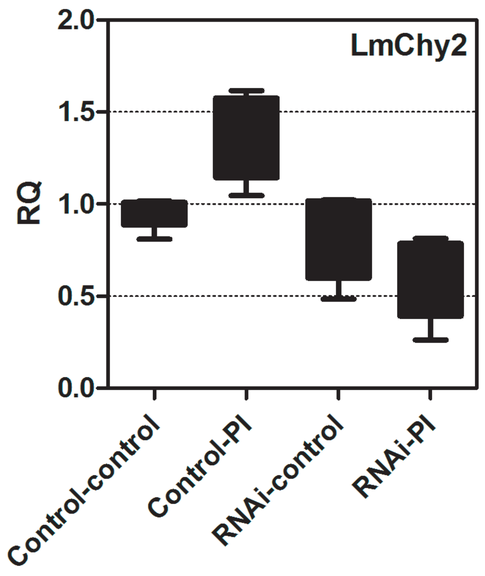
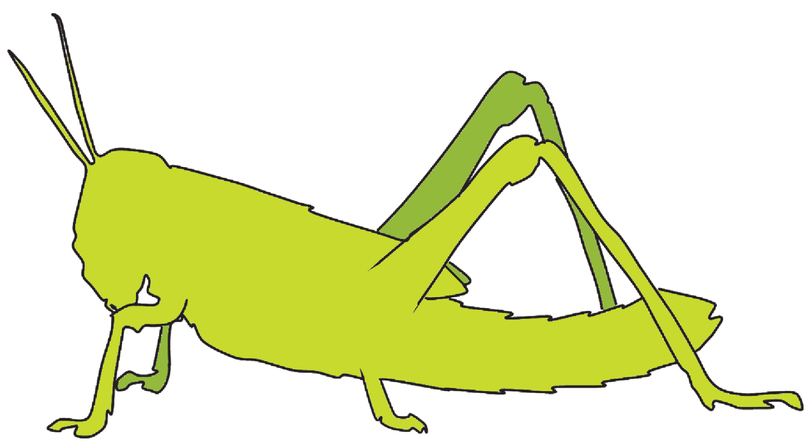


Fig. 3. Relative transcript quantity of LmChy2, an important digestive chymotrypsin in the gut of the locust, after combined hexamerin knockdown and PI-ingestion.



Conclusions

- **Microarray data suggest that during adaptation to ingested PI fewer resources can be invested in defense, stress responses and the maintenance of structural integrity**
- **Knocking down key proteins in this adaptive process could result in impaired response mechanisms leading to improved antimetabolic effects of PI**

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