Application of molecular methods to identify genes involved in CpGV-resistance of the codling moth, *Cydia pomonella*

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Abstract

The codling moth, Cydia pomonella*, occurs worldwide in apple growing regions and is regarded as one of the most severe insect pests in apple orchards. The larvae can also damage quinces, cherries, plums, apricots, walnuts, and pears. The* C. pomonella *granulovirus (CpGV) is one of the most powerful methods for reducing* C. pomonella *populations, especially in organic farming. Since 2003 less sensitive codling moth populations against CpGV-M (Mexican strain; found 1963 in an apple orchard in Mexico) emerged in Germany and in other European countries during the following years. The respective populations showed a sensitivity that was reduced up to 1000-fold. Single cross experiments showed that the putative CpGV-M resistance gene is located on the Z-chromosome (sex chromosome) of codling moths (Asser-Kaiser et al.2007).*

For identifying genes potentially involved in the development of CpGV resistance, a gene expression profiling approach was chosen. CpGV-M resistant codling moth larvae (4th instar) were reared on virus-contaminated and virus-free diet via "droplet feeding". Two different CpGV strains were involved in this assay: CpGV-M, where resistance is known to exist in the field, and CpGV-I12, a new isolate, with apparent resistance breaking capacities. A virus-free diet was used as a control. Complementary DNA-amplified fragment length polymorphism (cDNA-AFLP) analysis followed by sequence analysis of the respective genes was applied to identify and to compare the expression levels of different genes putatively involved in the CpGV resistance process and in virus-host-interactions.

Keywords: codling moth, resistance, CpGV, cDNA-AFLPs

Introduction

The codling moth (*Cydia pomonella* L) is the most important insect pest in nearly all apple growing areas worldwide. Without any control damage by larvae can reach up to 95%. Cydia pomonella granulovirus (CpGV) is one of the most powerful bioinsectides to control *Cydia pomonella*, in particular in organic farming programs. In Germany CpGV is registered since 1992. Since 2004 it is known that some *C. pomonella* populations in Germany are resistant to the Mexican isolate of this granulovirus (CpGV-M) (Fritsch et al., 2005). The resistant populations showed an up to 1000-fold reduced sensitivity against CpGV-M. Single cross experiments revealed that the putative resistance gene(s) is located on the Z-chromosome (sex chromosome) of *C. pomonella*. (Asser-Kaiser et al., 2007). In the meantime resistant populations are also present in Italy, Austria, France, Switzerland and the Netherlands.

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To obtain information which genes are putatively involved in the CpGV resistance mechanism a study on analysis of differences in gene expression was designed: CpGV-M resistant 4th instar codling moth larvae were reared on virus contaminated (CpGV-M, the Mexican isolate, where the resistance is known in field populations; CpGV-I12, a new isolate found in Iran, with resistance breaking capacities) and virus free (water control) artificial diets. Complementary DNA-amplified fragment length polymorphism (cDNA-AFLP) analysis was performed to find differences in the banding pattern between the three samples.

Material and Methods

Resistant codling moth larvae were reared on artificial diet. Larvae in the 4th instar were starved for 3 to 14 hours. Afterwards they were fed with a piece of artificial diet containing either a drop (=1 µl) of CpGV-M or CpGV-I12 (1000 OBs/1 ml, respectively) or water as a control. The larvae were given 180 min to feed the whole piece of diet, only larvae who had fed the complete piece were included in the experiment. After 10, 15, 20 and 24 h larvae were killed in liquid nitrogen (Fig. 1).

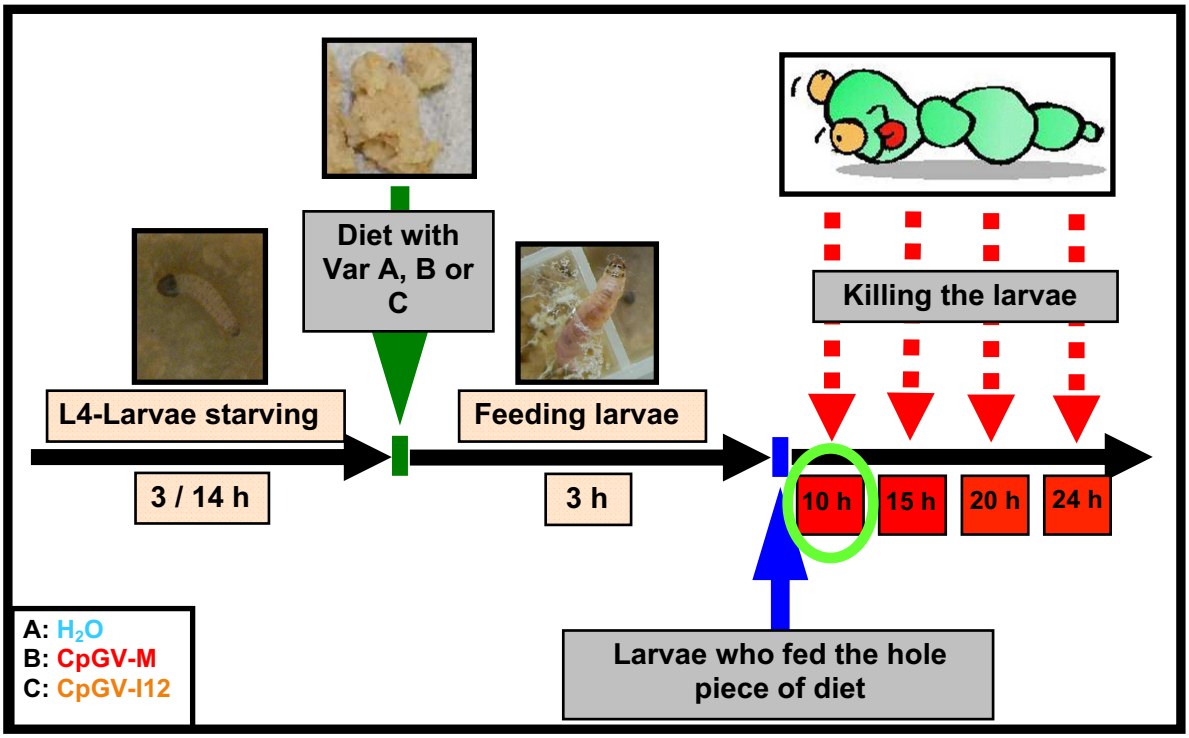

Figure 1: Experimental scheme of *C. pomonella* droplet feeding

RNA was extracted from the midgut of larvae from the 10 h treatment and cDNA was synthesized. cDNAs from larvae fed on either of the three diets were subsequently used for AFLP reactions. 65 different AFLP primer combinations were tested. Bands that were present in one of the three variants were cut out and sequenced.

Results and Discussion

A number of bands were differentially expressed in virus-fed on non-virus fed *C. pomonella* larvae (see Fig. 2 as an example). Twelve bands were cut out from cDNA-AFLP gels and were sequenced. A Blast search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with these twelve sequences revealed homologies for seven of them (table 1).

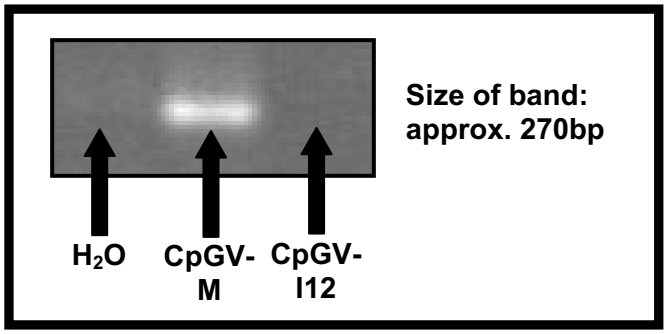

Figure 2: Part of a cDNA-AFLP gel comparing gene expression patterns in *C. pomonella* larvae after feeding on three different artificial diets (arrow). In larvae fed on diet containing H_2O and CpGV-I12 no AFLP-band is visible, while larvae fed on CpGV-M show a specific band.

Table 1: Identified cDNA-AFLP bands with the size of the band, the primer combination used and the obtained homologies in the database "GenBank".

cDNA-AFLP band identified in…	Primer combination	Homology	Band size
Water	Mse AC + Eco AG	Serine Protease	200bp
CpGV-M	Mse CA + Eco G	Phoshorine Aminotransferase 1	200bp
CpGV-I12	Mse ACT + Eco G	Ubiquitin Thioesterase	200bp
CpGV-M	Mse AA + Eco G	Homocysteine-S-Methyltransferase	270bp
CpGV-I12	Mse CT + Eco T	Insect Intestinal Mucin IIM86	300bp
Water	Mse GC + Eco T	RRP12-like Protein	220bp
CpGV-M	Mse CT + Eco T	Ankyrin Repeat Containing Protein	320bp
CpGV-M	Mse CC + Eco T		270bp
CpGV-M	Mse AC + Eco AA		180bp
CpGV-I12	Mse AG + Eco C		200bp
CpGV-I12	Mse CC + Eco C		150bp
CpGV-I12	Mse AC + Eco AC		130bp

The putative genes identified via cDNA-AFLPs are likely to be involved in immune reactions or in digestion processes. Among them, a homologue to an intestinal mucin is an interesting candidate, since it is a major protein constituent of the peritrophic membrane of the insect gut and protects insects from microbial infections. Baculoviruses, however, have evolved a strategy to overcome this barrier, in particular with the help of the protein enhacin, which has a mucin-degrading activity (Wang & Granados, 1997). Further studies will now be conducted to elucidate the role of mucin in CpGV resistance.

In summary, the genes identified in the present study are a first step in understanding the mechanisms involved in both the evolution of CpGV resistance as well as in virus-host-interactions between codling moth and granulovirus.

References

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