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RNA Interference of the Unfolded Protein Response in Acyrthosiphon pisum

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RNA Interference of the Unfolded Protein Response in *Acyrthosiphon pisum*

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ABSTRACT:

Aphid species are distributed worldwide and are persistent agricultural pests. Previous studies have demonstrated the efficacy of dsRNA as a tool to cause selective death in target organisms through RNA interference (Balthazor, J. R., *et al* 2018). Whole RNA was isolated from adult *A. pisum* and used to synthesize cDNA. Synthesized cDNA was then used as a template to produce dsRNA for use in feeding studies with pea aphids. dsRNAs complementary to target mRNAs were fed to adult aphids and survivorship was assessed. Concentrations of target mRNAs were assessed by quantitative real-time PCR (qPCR)

Introduction

Members of the aphid families (Hemiptera: Aphididae) are major parasitic pest; these insects cause major economic damage to agricultural plants around the world (Sabater-Munoz, B., Legeai, F., Rispe, C. 2006). Investigation into a non-organophosphate means of control of the pea aphid is of major ecological and economic interest. The unfolded protein response (UPR) is an interaction of multiple genes and gene products activated in response to the accumulation of unfolded/misfolded peptides within the lumen of the ER. Dysfunction of the ER and subsequent activation of the UPR can result in cell differentiation, reestablishment of function of the ER, or apoptosis of the cell. The UPR is a multifunctional pathway that works by slowing the rate of protein synthesis, degrading unfolded proteins, and activating signal cascades starting at the ER (Ryoo, H.D., and Steller, H. 2007). While the UPR is dictated by the interaction of many genes and gene products, there are three main signal transducers activated in the condition of ER stress: IRE1, PERK, and ATF4 (Hetz, C. 2012). IRE1 acts as a sensor for ER stress, and causes a signal cascade that ultimately results in the upregulation of UPR gene transcripts. The increase in translation of UPR proteins can lead to the resolution of ER stress. PERK acts in the cell to attenuate protein synthesis while the products of the IRE1 signal cascade work to clear misfolded protein within the lumen of the ER. ATF4 acts in the cell to begin transcription of the CHOP gene; expression of CHOP products results in apoptosis of the stressed cell (Ryoo, H.D., and Steller, H. 2007). Using double-stranded RNA, interference of the translation of the gene products of IRE1, PERK, and ATF4 might knock down the gene products of the UPR resulting in the death of this pest insect.

Results & Discussion:

Whole RNA isolation concentrations ranged from 663.3 – 1096.2 ng/µL. The amount of measured RNA depends on the size of the insects isolated from and the efficacy of the DNAse used to remove any genomic DNA. Only samples with an A260/A280 measurement > 1.90 were used for cDNA synthesis. Synthesized cDNA concentrations ranged from 2322.3 – 3272.1 ng/µL. Only cDNA with an A260/A280 value > 1.80 were used for dsRNA synthesis. Synthesized dsRNA concentration ranged from 15342.2 – 17598.3 ng/µL. The survivorship of aphids fed 100 ng/µL of ATF4 dsRNA was statistically significant from the control group by Log-rank test. (z=8.16, p<0.001). No other significant difference is reported. Knockdown of PERK and IRE1 mRNAs may have occurred but death is not observed due to redundant function. Knockdown induced by ATF4 dsRNA is promising as the time needed to kill 50% of the population is less than 30 hours. mRNA concentration of target genes is currently being assessed by qPCR.

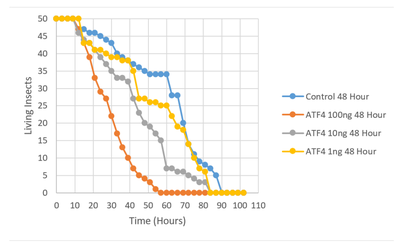

Figure 1. Death curve of aphids fed control diet for 48 hours compared to aphids fed various concentration of ATF4 dsRNA for 48 hours.

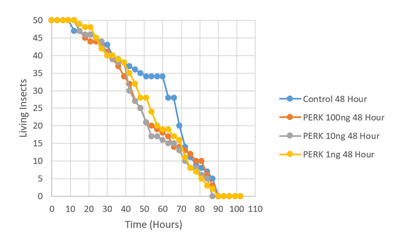

Figure 2. Death curve of aphids fed control diet for 48 hours compared to aphids fed various concentration of PERK dsRNA for 48 hours.

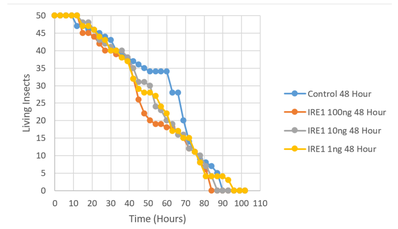

Figure 3. Death curve of aphids fed control diet for 48 hours compared to aphids fed various Concentration of IRE1 dsRNA for 48 hours.

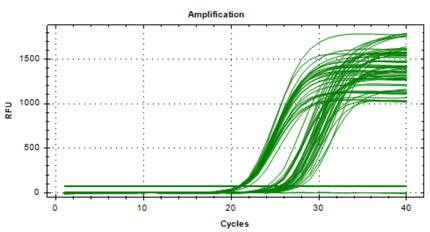

Figure 4. Raw qPCR amplification data of control and dsRNA fed aphids. RPL27 gene used as housekeeping gene.

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Methods

RNA isolation was performed by adding 1000 µL of Trizol reagent (Invitrogen #15596026) to 10 adult *A. pisum* in 50 µL of RNALater in a DNase/RNase free 1.5 mL Eppendorf microcentrifuge tube. A rotating pestle (USA Scientific, 1.5 mL microcentrifuge tube pestle) was used to homogenize the insects with a battery powered rotating tissue homogenizer (Argos Technology, Pestle Motor Mixer) for 1 minute until no tissue remained intact.

The samples were allowed to stand for 3 min at room temperature. After 3 min, 1 µL of gDNA Eliminator from an RNeasy Kit (Qiagen #74104) was added to reduce genomic DNA contamination from the aqueous phase during phase separation. The sample was then subjected to vortexing several times with 200 µL chloroform to ensure even distribution of reagents in the sample. The sample was incubated on ice for 10 minutes, followed by centrifugation at 12000 x g for 10 min at 4°C. Two layers were observed after centrifugation; the top layer was a clear, aqueous layer, with a pink, organic layer on the bottom.

The clear layer was transferred into a new, nuclease free 1.5 mL Eppendorf micro-centrifuge tube, and the organic layer was discarded. Following the sample transfer into the new centrifuge tube, 500 µL of chilled isopropanol was added and was allowed to stand for 10 min at room temperature facilitating the precipitation of RNA. Centrifugation was then performed at 12000 x g for 15 min at 4°C, which formed a pellet of RNA. After removal of the liquid, the RNA pellet was washed twice with 500 µL of chilled ethanol. After washing, the sample was dried at room temperature.

The dried RNA pellet was subsequently dissolved in 50 µL of RNase-Free water (Qiagen #129112). 2 µL was removed for Nanodrop analysis to determine quantity and purity for downstream cDNA synthesis. Synthesis of cDNA was accomplished through the use of a Bio-Rad SingleShot SYBR Green One-Step kit (Bio-Rad #172-5095). 2 µL of the cDNA product was analyzed *via* Nanodrop for quantity and purity.

Custom oligonucleotide primers for dsRNA synthesis were designed using the NCBI Primer-BLAST tool. dsRNA synthesis was attempted with the use of an Invitrogen T7 RNA polymerase kit (Invitrogen #AM1334). The amplicon of dsRNA synthesis was compared to a 1000 KB DNA ladder through gel electrophoresis, and analyzed via Nanodrop for quantity and purity.

50 adult pea aphids were transferred to sterile petri dishes. A layer of parafilm was stretched over the dish and artificial diet was placed on top of it. A second layer of parafilm was stretched over the diet to form a pocket. Aphids were fed on diet containing 100, 10, or 1 ng/µL of anti-gene dsRNA for 48 hours. The diet was removed and the aphids were placed on healthy *Vicia faba* for the remainder of the study. Survivorship was assessed every three hours and dead adult aphids and nymphs were removed. Survivorship curves for each treatment group are shown in Figures 1-3. Knockdown of target mRNAs was assessed by quantitative real-time PCR and shown in Figure 4.

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Acknowledgments

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