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RNA Interference of X Box Binding Protein 1 in Acyrthosiphon pisum

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RNA Interference of X-Box Binding Protein 1 in *Acyrthosiphon pisum*

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ABSTRACT

Pea aphids, *Acyrthosiphon pisum*, are a significant pest to legumes (*Febaceae*) throughout the world, primarily due to the species serving as a vector to many *Febaceae* viruses. Current management of *A. pisum* includes use of insecticides and the introduction of natural predators, neither of which is ideal because it can have detrimental effects on organisms other than *A. pisum*. The utilization of RNA Interference (RNAi) presents an alternative, pest-specific targeting of *A. pisum*. X-Box Binding Protein 1 (XBP1) is involved in the regulation of the unfolded protein response to promote proper folding in the endoplasmic reticulum (ER). RNAi targeting the XBP1 gene could result in the accumulation of misfolded proteins in the ER lumen, which downstream results in death of the cell and organism. RNA was isolated from *A. pisum* and reverse transcribed to synthesize cDNA. The cDNA was used as a template for XBP1 primers to synthesize XBP1-dsRNA for use in RNAi feeding studies. RNA interference of XBP1 resulted in a fifty percent decrease in fecundity and a thirty percent decrease in survival of *Acyrthosiphon pisum*. RNA interference of XBP1 has shown to be a promising alternative to the management of *A. pisum*.

INTRODUCTION

Acyrthosiphon pisum is an insect that is a significant pest to alfalfa and other legumes. Currently, the main mechanisms used to protect fields against pea aphids are insecticides and early cutting of the field (McCornack, Zukoff, Whitworth, Michaud, & Schwarting, 2017). Making genetically modified plants that can alter protein functions within aphids, leading to death of the aphid, would be the ideal situation to control the aphid population. As soon as aphids begin to feed on the crop, their protein functions would change, and they would be killed. Before this is possible, a gene must be identified that kills aphids efficiently to be worth modifying into crops. *A. pisum* are used as model insects because their genome is entirely sequenced (International Aphid Genomics Consortium, 2010) and they are easy to sustain in laboratories, growing on fava beans, *Vicia faba*. X-Box Binding Protein 1 (XBP1) is involved in the regulation of the unfolded protein response to promote proper folding in the endoplasmic reticulum (ER). RNAi targeting the XBP1 gene could result in the accumulation of misfolded proteins in the ER lumen, which downstream, results in death of the cell via apoptosis

PURPOSE

This study aims to investigate the effects of XBP1-dsRNA on the fecundity and survival of adult *Acyrthosiphon pisum*. Species-specific interference of *A. pisum* XBP1 would allow for the prevention of pea aphid infestations without the use of insecticides and the possibility of harming other species and the environment. Ideally, transgenic legumes could be produced to endogenously express dsRNA to interfere with pea aphid XBP1. I hypothesize that RNA interference of the XBP1 gene by treatment with XBP1-dsRNA will result in a decrease in survival and fecundity of *A. pisum*.

METHODS

1. Whole RNA Isolation

- Add 1 mL Trizol to 10 adult *A. pisum*, homogenize in microcentrifuge tube, centrifuge to fraction cellular components down
- Add supernatant to chloroform, vortex to mix, centrifuge to separate into upper aqueous layer and lower organic layer
- Add aqueous layer to new microcentrifuge tube with isopropanol, vortex briefly, centrifuge to isolate pellet of RNA
- Wash pellet with ethanol, allow to dry
- Dissolve pellet in Ultrapure Water
- Nanodrop sample to determine concentration and purity

2. cDNA Synthesis

- Begin with 1 microgram of whole RNA
- Follow protocol from SingleShot SYBR Green Kit for cDNA synthesis from RNA sample
- Run gel electrophoresis to ensure synthesis of cDNA
- Nanodrop sample to determine concentration and purity

3. dsRNA Synthesis

- Begin with 1 microgram of cDNA
- Follow protocol from MEGAscript Kit to synthesize dsRNA from a starting sample of cDNA with primers designed for *A. pisum*-XBP1
- Run gel electrophoresis to ensure synthesis of cDNA
- Nanodrop sample to determine concentration and purity

4. Preliminary Fecundity Study

- Add 50 adult aphids to petri dish, spread parafilm to contain aphids
- Add XBP1-dsRNA to Akey-Beck diet at specified concentration (100 ng/µL, 10 ng/µL, 1 ng/µL) to top of parafilm
- Spread second layer of parafilm over petri dish with diet between two layers
- Count number of aphids in each petri dish every 12 hours

5. Feeding Studies

- Add 50 adult aphids to petri dish, spread parafilm to contain aphids
- Add XBP1-dsRNA to Akey-Beck diet at specified concentration (100 ng/µL, 10 ng/µL, 1 ng/µL, 0 ng/µL) to top of parafilm
- Spread second layer of parafilm over petri dish with diet between two layers
- Count number of original aphid deaths every 3 hours until 50 deaths
- Perform log-rank test to compare survival

RESULTS

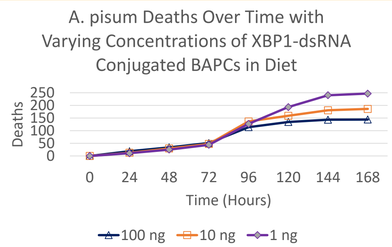
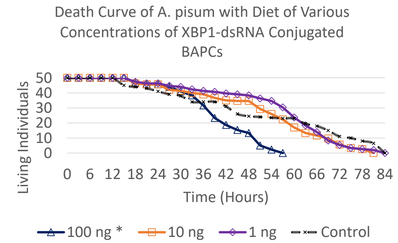


Figure 1 (Left) The initial study was to observe the differences in fecundity of *A. pisum* when treated with varying concentrations of XBP1-dsRNA conjugated to BAPCs. As expected, the lowest concentration of dsRNA resulted in the lowest number of initial deaths and the greatest fecundity of the three treatments. The 100 ng treatment resulted in a fifty percent decrease in fecundity.

Figure 2 (Right) The death curve of the feeding study with various concentrations of XBP1-dsRNA conjugated to BAPCs in the diet until death can be found below. Statistically significant differences were found between the 100 ng* treatment and the control group using a log-rank test. The 100 ng treatment resulted in a thirty percent decrease in survival.



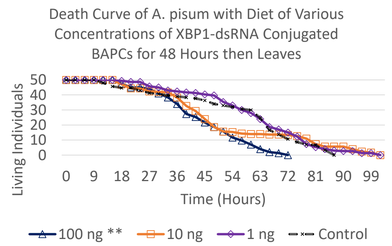


Figure 3 (Left) The death curve of the feeding study with various concentrations of XBP1-dsRNA conjugated to BAPCs for 48 hours followed by a normal diet can be found below. Statistically significant differences were found between the 100 ng** treatment and the control group using a log-rank test. A similar decrease in survival of thirty percent in the 100 ng treatment is observed.

CONCLUSIONS

- 30 percent decrease in survival of 100 ng groups in comparison to control groups.
- 50 percent decrease in number of offspring of 100 ng group in comparison to the 1 ng group in the fecundity study.
- Statistically significant differences were found between the 100 ng treatment groups and the controls.

This study has begun to convey that survival and fecundity of *Acyrthosiphon pisum* can be decreased by means of RNA Interference. Future work will investigate quantified differences in knockdown of XBP1 between treatment groups, expecting that increasing concentrations of dsRNA conjugated BAPCs results in an increased knockdown of XBP1 RNA available for transcription in *A. pisum*.

DISCUSSION

Future work will aim to quantify the differences in protein knockdown levels between the control group and three treatment groups in the feeding studies via qPCR. It is expected that there will be a decreasing level of XBP1 protein expression as the XBP1-dsRNA treatment concentration increases . Work is also underway in testing the effects of XBP1-dsRNA on human cancer cell (HeLa) lines, which we expect to see no change with treatment. Future work will also investigate if treating *Acyrthosiphon pisum* with a combination of dsRNAs rather than solely XBP1-dsRNA will result in a further decrease in survival and fecundity in comparison to treatment with XBP1-dsRNA alone. If a combination of treatments is more effective, we will have shown that RNAi of *Acyrthosiphon pisum* is a novel technique to manage pea aphid population in a pest-specific manner.

REFERENCES

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