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RNA Isolation of Aphids

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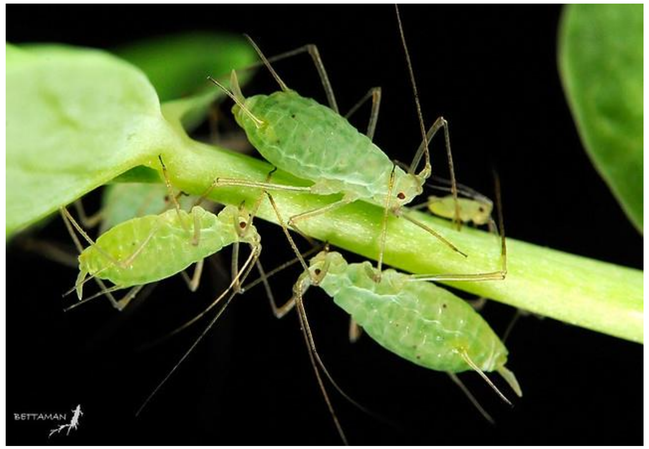
Introduction

Pea aphids (*Acyrthesiphon pisum*) are the model organisms of aphid species. These aphids pose enormous economic stress when it comes to agriculture, and current management methods involving pesticides have major downsides. RNA Interference provides an alternative management method for this specific species. “The introduction of double-stranded RNA (dsRNA) complementary to each of the target genes of the Unfolded Protein Response (UPR) in the pea aphid, coupled with a carrier, has shown significant changes in expression of gene products and subsequently has shown decreased survivorship and reduced fecundity in the organism.”



Methods

- Countertops were sterilized with RNase Away Reagent
- Transfer 10 adult pea aphids into a 1.5mL micro centrifuge tube and homogenize in 1.0mL of TRIzol Reagent. Spin in refrigerated centrifuge at 12,000 x g at 4˚C for 10 minutes.
- The sample was decanted into clean 1.5mL micro centrifuge tube and 200µL of chloroform were added and vortexed.
- The sample was spun again in a refrigerated centrifuge at 12,000 x g at 4˚C for 10 minutes.
- The upper aqueous layer was removed by pipette and transferred into a clean micro centrifuge tube containing 500µL of cold isopropanol and incubated on ice for 5 minutes to facilitate RNA precipitation.
- The sample was spun in a refrigerated centrifuge at 15,000 x g at 4˚C for 10 minutes.
- The liquid was decanted, and the pellet was washed with 100µL of cold absolute ethanol. The ethanol was decanted, and the pellet was incubated at room temperature until the residual ethanol evaporated.
- 50µL of Rnase-free water were added to the pellet, and the sample was incubated at 37˚C for one minute to facilitate RNA solvation.
- We then measured the quantity using a Nano drop One spectrophotometer

Discussion & Conclusion

Several trials of the listed methods were conducted by the collection of data from the Nano drop One spectrophotometer resulting in whole RNA isolation concentrations varying between 721.4-12,784.9 ng/µL. The purpose of these measurements were to reach a reasonable RNA purity by measuring the A260/A280 ratios. An ideal ratio varied between 1.8 and 1.9 as high sample quality is essential to continue toward the next steps of this research.

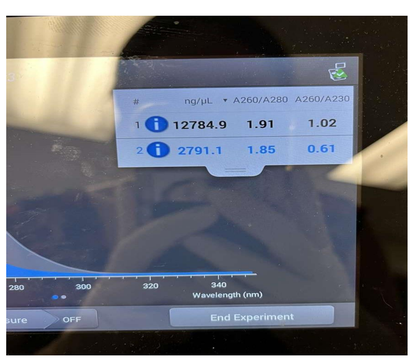
An excessive amount of absorbance at 280 indicated the high presence of phenol that hold genes involved with the UPR.

Results of RNA isolation could often be influenced by pH from outside sources such as breathing too close to the sample or working with contaminated lab tools. As stated, the importance of sample quality is detrimental for students to master in their first semester of research before learning to prepare samples for cDNA and dsRNA synthesis. Which lead to the final product used to test the survivability of aphids in response to the RNA interference.

Previously resulting in a 30% decrease of survival of 100 ng groups and 50% decrease of offspring from those 100 ng groups.

Research Relevance

Aphids are well known for attacking cereal crops and are accountable for spreading nearly 40% of all plant viruses. Overall, pests account for 20-50% of crop loss. The success of this study may have huge impacts on the agriculture and medical fields by sharing how RNA interference may be used to reverse the survivability of several diseases and pests across the nation in a way that does not harm outside entities.



Purpose and Goals

Master the method of RNA isolation in order to prepare the anti-gene dsRNA to be fed to controlled groups of aphids and uncover their survival and fecundity under the result of consumption. Allowing for pest prevention utilizing a method that does not harm other species or the environment.

Increasing crop production and ensuring consumer safety.

Similar methods of engineering dsRNA to decrease the survival and fecundity on human cancer cells have been reported to be under progress, but have so far only proven effective for crop protection.

This technique may prove more effective with combined RNA interference by attacking several different genes that may cause misfolded proteins, difficulty of gene replacement, and even cellular apoptosis. This involves genes such as the following: PDIA6, XBP1, VCP, DNAJC3, PFD2, TRAF2, and ATF6.

Acknowledgments

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