Plant Defense Regulated by EDS1 in response to herbivory attack

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Abstract

Spodoptera exigua, is a beet armyworm that causes extensive damage to farm crops. They are also resistant to most insecticides, so a molecular approach is necessary to combat their damage. Plant response to stresses are carried out locally and systemically by jasmonates and salicyclic acid. *Eds 1-2* and are genes along these pathways that regulate the production of jasmonic acid and salicylic acid. It has already been established by previous research that mutations in *eds 1-2* increase salicylic acid accumulation. To determine the extent, and effect of mutations on genes along these pathways, experiments were performed on three different *Arabidopsis thaliana* genomes (Col – wild type, and mutations on *eds 1-2,).* The cannibalism assay was conducted to see the extent of cannibalism between the insects when allowed to feed on these plants, and the plant performance assay to determine the growth of plants if not for the damage caused by insects. The insect assay results showed there is a greater variance between actual growth and expected growth in *Col,* as compared to and *eds 1-2*. That is, the insect did not cause much damage to the *eds 1-2* plants which suggests that it may have not liked to eat the *eds 1-2* due to increased accumulation of salicylic acid as the others. The cannabilism assay results suggest a possible acclimatization of insects to the defense response, or a reduced defense response to a second herbivory attack in a short period of time. Further research involving the measurement of the products produced by these defense pathways at different timepoints will help answer the questions if the amount of salicylic acid produced a second time is less, and if so what is the minimum time before the plant is able to produce the original amount of salicylic acid. This can also help determine if the insects get acclimatized to the salicylic acid or not.

Background and significance

Herbivory is a growing concern for farmers. Arthropod pest damage to crops causes an upward of 15% crop loss. Most methods of combatting these pests include pesticides or chemicals that do not selectively harm the pests, but the plants as well. *Spodoptera exigua*, the beet armyworm is a prevalent pest in southern parts of USA (Bessin, 2004). It preys on alfalfa, beans, beets, cole, corn, cotton, lettuce, onion, peppers, peas, potatoes, and tomatoes (Bessin, 2004). The significance of this project lies in the fact that the beet armyworm is resistant to most insecticides, so a molecular approach is necessary to combat it (Bessin, 200).

To an extent, plants have ways to deter or stop herbivores. Therefore, there is a growing need of establishing and exploiting internal plant defense mechanisms so as to promote sustainability. Once the nuances of the genes, and cross-regulation of pathways is completely understood, herbivory can be stopped by an internal, chemical route. Many genes have been identified in these essential plant defense mechanisms, including *eds1*, but the true extent of its impact on the entire pathways, and how the insects that have been subject to it cope later has not been fully explored.

Plant response to biotic stress are carried out locally and systemically by jasmonates (Turner et. al. 2002). Jasmonate production is activated by the jasmonic acid pathway (Turner et. al. 2002). Another important molecule in plant defense is Salicylic acid (Ng et. al 2011). EDS1 (*Enhanced Disease Susceptibility)* gene is not directly involved in the biosynthesis of salicylic acid, but mutations in EDS1 leads to compromised accumulation of salicylic acid and is therefore important in its regulation (Ng. et. al. 2011). *Eds 1-2* has been shown to suppress *acd6-1,* a gain of function mutation that leads to severe cell death and dwarfism (Ng et. al, 2011).

This project will be able to determine the importance of this gene, and whether investing in this gene in particular is advantageous to the farmer.

Research objectives

Plants are subjected to various attacks of predation, especially by herbivory. Plants utilize defense pathways to protect themselves, and one such pathway is the antagonistic regulations between the salicylic acid (SA) and jasmonic acid pathways. The enhanced disease susceptibility gene (*eds1-2)* has been established as a positive SA pathway regulator by previous research.

We will employ the wild type Col, and its mutants eds1-2 and for both types of feeding. This project serves to explore the extent of resistance by *Arabidopsis thaliana* mutants *eds1-2* and in response to *Spodoptera exigua* feeding. The specific objectives for this project are as follows:

Objective 1: Insect Assay

The full plants will be subjected to herbivory to determine the extent of damage the insects are able to cause on different genotypes. Before and after pictures of leaf tissues will be analyzed to measure the plant and insect performances for tow different genotypes Col, *eds1-2*.

Objective 2: Cannibalism

A group of six S. exigua larvae were placed inside a deep petri dishes to feed on fresh leaf tissues or partly eaten leaves, to compare the cannibalism levels between the cannibolic rates between these two types of feeding among two genotypes, to understand the effects of activated defense pathways in plants on the cannibalism of insects.

1- Insect Assay

The insect assay was conducted to see whether insects have a preference between the plants. To determine the extent of plant damage that can be caused, two insects of the second instar of *Spodoptera exigua* were placed on each experimental plant and a tall cages placed around the plants. The control plants will not have any insects, and a tall cage will be placed around the plants. They were allowed to feed for seven days. A picture was taken using the WINDAS plant area analyzer software on the first and the last day of the experiment period. This data was then plugged into an excel sheet that calculated the average growth percentage of the controls for each genotype, and used this value to calculate the expected growth for every experimental plant. The data is shown for the average and each replicate below.

1- Plant performance:

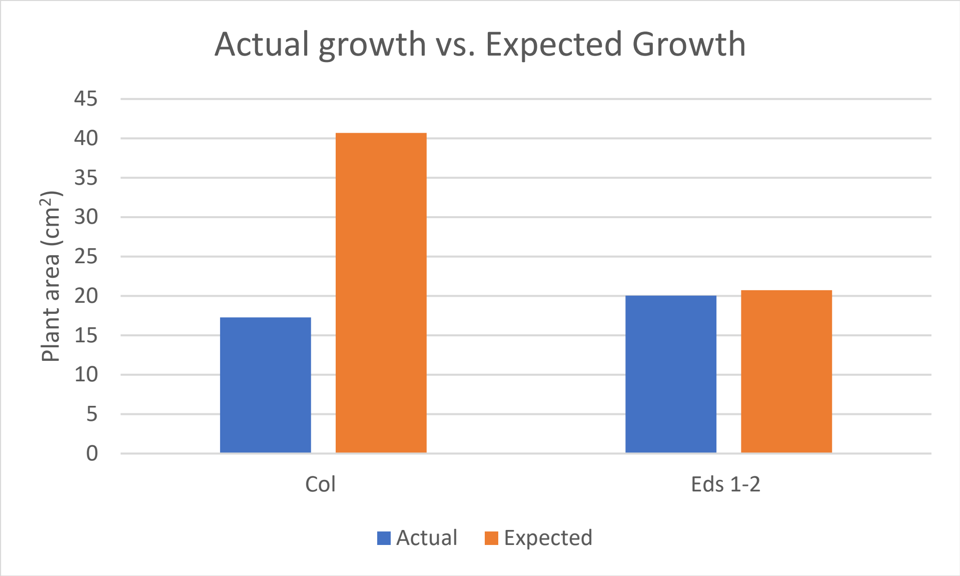

Figure 1: The average growth of 20 pots per genotype. *Eds 1-2* shows little variance between expected and actual growth as compared to *Col.* This shows that *Spodoptera exigua* did not cause much damage to the growth of *eds 1-2.*

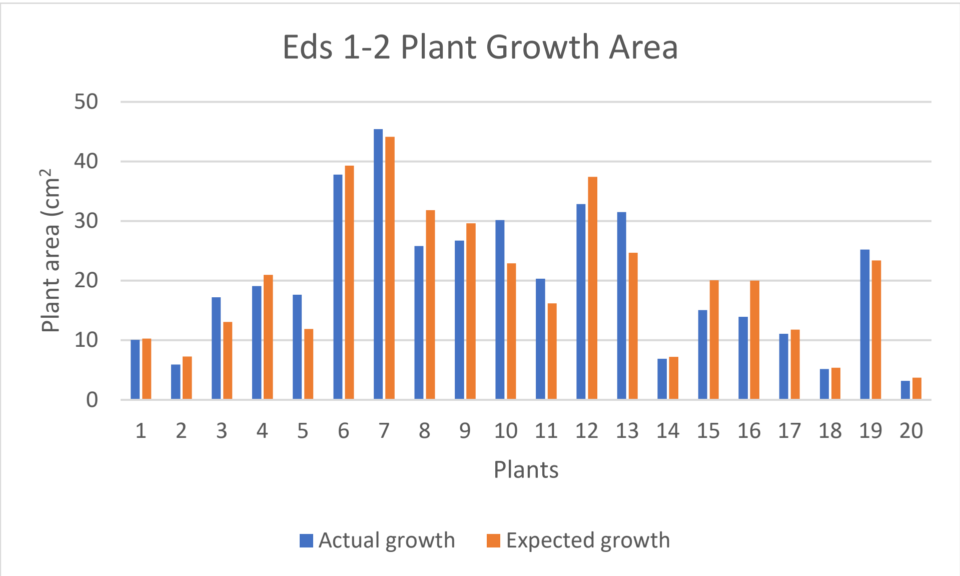

Figure 2: The above graph shows the actual growth vs. expected growth for each replicate of the *eds 1-2* plants.

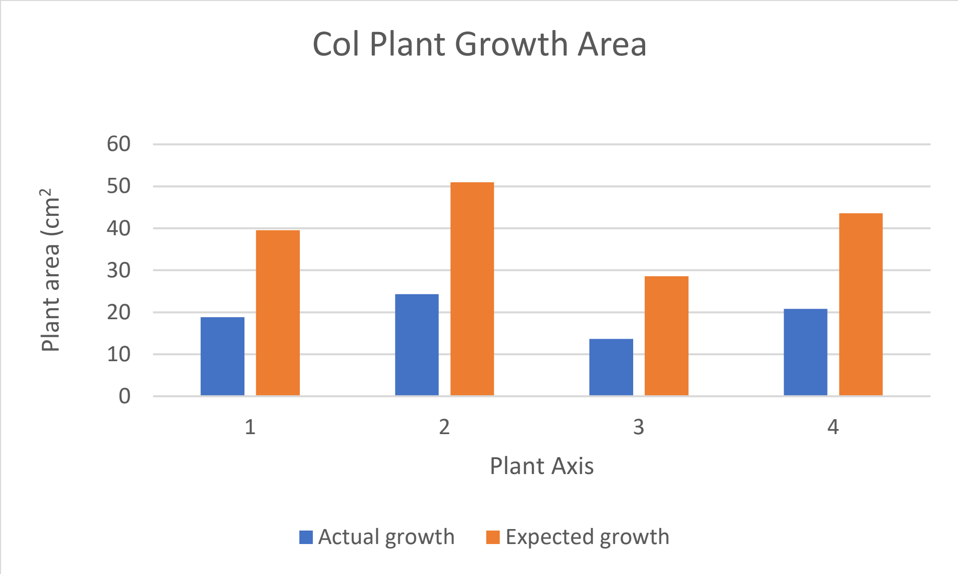

Figure 3: shows the actual growth vs. expected growth for each replicate of the *Col* plants.

As shown by the graphs above, there is a greater variance between actual growth and expected growth in *eds 1-2,* as compared to *Col*. That is, the insect did not cause much damage to the *eds 1-2* plants which suggests that it may have not liked to eat the *eds 1-2* due to an increase in salicylic acid or an increased accumulation of salicylic acid as the others.

<u>2- Cannablism assay</u>

For the cannibalism assay, two insects of the second instar of *Spodoptera exigua* will be placed on each experimental plant and confined in a clear plastic cages covering tightly around each plant pot. The control plants will not have any insects, but the same cages were applied. This insect feeding lasted for three days. On the third day, 300mg of plant tissues from experimental and control plants for each pot will be cut and placed inside petri dishes for experimental ones, along with six fresh insects. Col had three control plants, and 10 experiment plants, while *eds 1-2* had eight controls and 10 experimentals. The number of remaining insects on each plate will be recorded daily for four days.

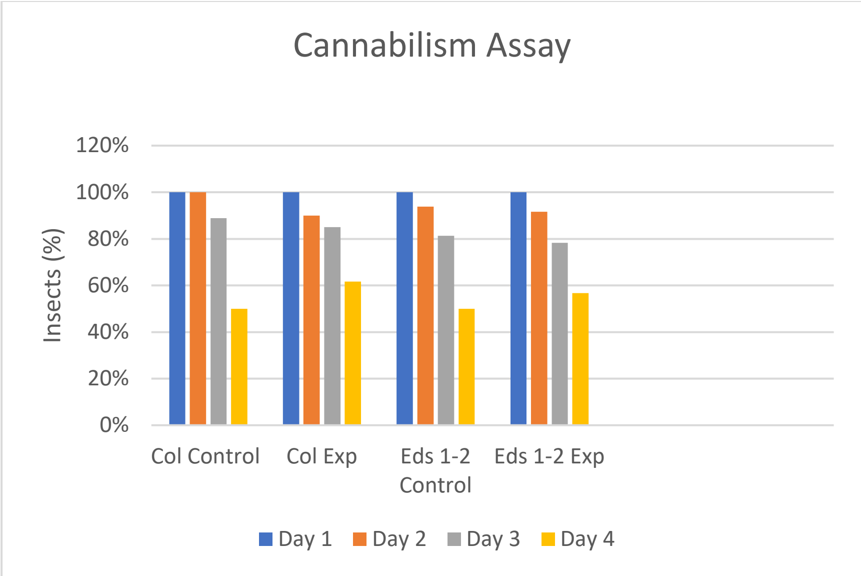

Figure 4: the average numbers of insects remaining on each day for each genotype for control and experimental assays. Interestingly, the insects given controls have reduced more than those given experimental leaves for *Col* and *Eds 1-2* by Day 4.

<u>Discussion</u>

<u>1- Insect Assay:</u>

Figure 1 shows a greater difference between the actual and expected growth in *Col* than in *eds 1-2.* Even with herbivory, the *eds 1-2* plants have managed to grow with little hindrance from the insects. This suggests a fast accumulation of salicylic acid to deter the insects from eating the plant, but does not show the extent of accumulation and responses to further herbivory. This will be seen in the cannabilism assay.

Figure 3 shows a great difference per replicate between the actual and expected growth. Figure 2 shows a smaller difference per replicate that is consistent with previous research (Ng. et. al. 2011) that identified mutations in *eds 1-2* to cause accumulation of salicylic acid. This accumulation may have deterred the insects from eating the plant (Ng. et. al.) But, as the cannabilism assay, the cannabolic rates were higher in the control plants of *eds 1-2* than the experimentals, this suggests a weaker defense response to a second herbivory attack within a short timeframe. Measuring salicylic acid levels at different time points in future research may help answer this question.

<u>2- Cannablism assay:</u>

The insects that were given control leaves for *Col* and *Eds 1-2* had lower cannioalic rates as compared to those given experimental leaves. This suggests that the initial plant defense response (salicylic acid) produced to herbivory is either stronger, or temporary. The control plant tissue (that had not been exposed to herbivory for three days) elicited a strong herbivory response that dettered the insects from eating it. The insects given the experimental leaves (which had been previously exposed to herbivory for three days) had already produced a strong defense response (salicylic acid) and may have not produced a strong enough response for the insects to not eat the plant tissue. Further research involving the measurement of the salicylic acid levels of the plant at different time points after insect infection would help answer these questions.

References

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