Weeds and Herbicide Resistance

Rise of the mutant weed

What bioinformatic tools can help us locate potential mutations linked to herbicide resistance in weeds? How can we identify weed species based on a DNA sequence?

Goal 1: Locate potential mutations associated with herbicide resistance

Goal 2: Identify the weed species by comparing DNA sequences using a library of known sequences

Background

Weeds are simply unwanted plants. In commodity agriculture, the goal is to keep competition from unwanted plants at a minimum. How does a farmer do that? She might use an herbicide, a specific chemical that is formulated to kill weeds. There are many types of herbicides and each is classified by its mode of action or the biological pathway the herbicide disrupts. For more information on modes of action, see: https://extension.okstate.edu/fact-sheets/herbicide-how-to-understanding-herbicide-mode-of-action.html Unfortunately, using an herbicide with only one mode of action every year, or applying less herbicide than what is recommended, allows weed populations to evolve resistance. Many times, herbicide resistance is caused by a genetic mutation, which increases in frequency with each generation.

Pigweeds are some of the most destructive and resilient weeds in agriculture. During the growing season, soybeans and other crops are highly vulnerable to interference and competition from pigweeds. Yield losses can reach 75% within fields where pigweeds are not controlled with herbicides. Over the last decade, 30+ states have reported herbicide resistance (HR) in two pigweed species, Palmer amaranth and waterhemp, with nearly half of those states also reporting resistance to multiple herbicides. The lack of new herbicides being registered for pigweed control has led to a flurry of research into understanding the mechanisms that drive herbicide resistance.

Herbicides often disrupt important physiological processes within the plant (photosynthesis, amino acid biosynthesis, etc) by attacking specific molecules or target sites in the plant, which end up killing the weed. In some cases, mutations at these target sites have allowed pigweeds to escape the full effects of herbicides. Several mutations within the genome have been linked to herbicide resistance in waterhemp and Palmer amaranth. Once mechanisms for herbicide resistance are found, rapidly identifying and eradicating pigweed populations carrying those traits becomes critical to reduce further spread of mutant pigweeds.

You will use basic bioinformatic tools to search for the presence of a mutation as well as identify the species of pigweed using a DNA library.

Your task

A weed scientist has collected pigweed seeds from a sunflower processing factory and planted them in the greenhouse. Shortly after emergence, she sprayed them with PPO-inhibiting herbicide. After ten days, she noticed that several plants survived and appeared resistant. She sent a leaf sample to the diagnostic lab and received back the DNA sequences for the gene that codes for the protein that the herbicide targets. Your job is to identify the species of pigweed and find mutations that may provide herbicide resistance.

1. Upload DNA sequences (in FASTA format) to a bioinformatics tool - DNA subway.
2. Compare an unknown sequence to a library of known sequences from various pigweeds.
3. Use two bioinformatic tools (MUSCLE and PHYLIP) to search for mutations and identify the species of pigweed.

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Procedure

1. Download to your desktop the two text files at the link: https://grownextgen.org/go/pigweed named [1] unknown pigweed and [2] pigweed library. The files are in FASTA format and can be opened by a text editor, such as notepad or notebook. FASTA format follows this structure:

```
>Sequence Name1
ATCGATCG…….

>Sequence Name2
ATCGATCG.......
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2. Go to https://dnasubway.cyverse.org, then follow the steps below to analyze your sequences.

3. On the DNA subway page, enter as a guest.

4. Click on 'Determine Sequence Relationships' (blue square)

5. You are now on the blue line of the DNA Subway. Complete the following steps:

- Select 'Project Type - DNA'.
- Select 'Enter sequences in FASTA format'.
- Open previously downloaded file 'unknown pigweed' on desktop. Copy and paste the unknown-pigweed (name and sequence) into the textbox.
- Give a title under 'Name Your Project'.
- Click 'Continue'.

STOP 1 Assemble Sequences

1. Click 'Sequence Viewer'.

2. In the window that opens, the DNA sequence will be displayed with the name (Unknown-pigweed) and the nucleotide sequence. A scroll bar will appear below the DNA sequences which will allow you to view the entire sequence.

3. Close the window by clicking on the x in the upper right hand corner.

STOP 2 Add Sequences

1. Click 'Upload Data'.

2. In the window that opens, select 'Enter Sequences in FASTA format' and copy and paste all contents of the previously downloaded file 'pigweed library'.

3. Click on 'Add sequences'. This uploads the known pigweed sequences for analysis.

STOP 3 Analyze Sequences

1. Click 'Select Data'.

2. In the window that opens, click the 'Select all' checkbox. There should be 7 total items selected. Then click 'Save Selections' to the right.

3. On the main page, click 'MUSCLE' (Multiple Sequence Comparison by Log-Expectation) to run the algorithm, then click 'MUSCLE' again to view.

4. In the window that opens, zoom in by clicking the + several times or use a single click to the ATCG icon to see specific nucleotide differences found across the DNA sequence alignment. This allows you to scroll the

entire sequence length and see specific nucleotide differences across the analyzed sequences. If a sequence has a different nucleotide at a position than the consensus (majority of the sequences), the unique nucleotide at that position is displayed inline.

Reflection

a. In the zoomed in or out view, which sequence in the MUSCLE analysis shows the most differences?

Notice that some sequences have gaps indicated by dashes where there are no corresponding base pairs. Be sure to take note of where they are located and how they compare to the unknown species.

b. What is the difference in sequences between Waterhemp PPO-R (herbicide resistant) and Waterhemp PPO-S (herbicide susceptible)?

c. What can you infer about the unknown pigweed based on the Waterhemp samples?

d. Is the unknown pigweed likely susceptible or resistant based off the library?

5. In this same window, click 'Sequence Similarity' to get a matrix of similarity comparisons. The table shows the pairwise comparisons between the different sequences in the alignment. The top diagonal and bottom diagonal give the same pairwise results (mirrored).

a. What sequence has consistently the lowest similarity percentages when compared to the other sequences? What is the lowest percent similarity?

6. Close the page by clicking on the x in the upper right corner.

7. On the main page click 'PHYLIP ML' (Phylogeny Inference Package using Maximum Likelihood methods). After running the analysis, click 'PHYLIP ML' again to view.

The window that opens displays the genetic distance between the DNA sequence in the analysis. The length of the line is determined by how many nucleotide differences there are among these sequences and the grouping is based on relatedness (or sequence similarities). If not pre-selected, have Beet-root be the Outgroup (in the dropdown box).

a. What species is the unknown pigweed? Defend your explanation.

Explanation of results

This activity provides an introduction into one herbicide (PPO-inhibitors; Group 14) and the mutation (ΔG210) that allows Palmer amaranth and waterhemp to be resistant. The mutation, ΔG210, describes the location of the mutation (210th amino acid) and the amino acid at the location (G; Glycine). The delta (Δ), defined as a change, provides the completed description of the mutation: a change in the glycine at the 210th position of

the PPO2 enzyme. The specific change is a complete deletion of the G at the 210th amino acid in the resistant (R), compared to susceptible (S) or wildtype weeds. This deletion causes a change in the shape of the PPO2 enzyme, which reduces the effectiveness of PPO-inhibitor herbicides to interact (inhibit) the enzyme.

Herbicides, classified as PPO-inhibitors, disrupt the normal enzymatic function of the PPO2 molecule for photosynthesis in susceptible plants. Inhibiting PPO2 results in a buildup of harmful by-products that break down cell membranes.

Additional Resources
Learn more about pigweeds and the highly invasive palmer amaranth from OSU Extension.

https://u.osu.edu/osuweeds/super-weeds/palmer-amaranth/

Search the international weed database for herbicide resistance in pigweeds and other agricultural significant weeds.

http://weedscience.org/summary/moa.aspx?MOAID=12