Protocol for measuring corn 2nd and 3rd leaf angles using ImageJ software
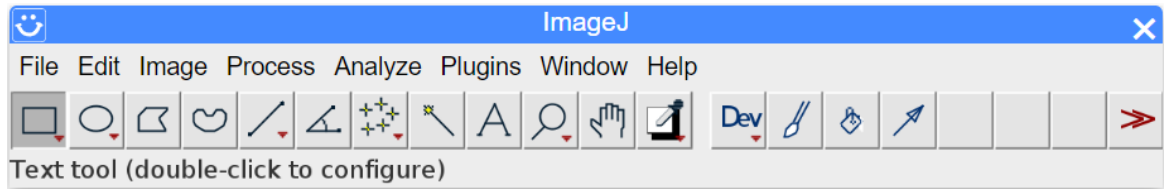
1. Access the ImageJ software by following this link: https://imagej.nih.gov/ij/index.html. Be patient, it takes some time to open the different windows.
2. Click the bullet point labeled "Run ImageJ in Browser!"
3. The following ImageJ window will appear:

   Scroll the cursor over the "File" tab and select open.
4. An "Open File" pop-up will appear, from which the "Select Local File" button should be clicked.
5. Choose an image of one of the maize seedlings to analyze.
6. The image should show the seedling in upright position. If not, select from the menu "Image", "Transform", and then the option that will help you put the seedling in upright position.
7. If the image looks too small, you can resize the image in two different ways. Go to "Image" in the menu and use the "Zoom" option or use the magnifying glass symbol.
8. In the ImageJ window, select the button with the angle symbol.
9. The cursor will become a cross shape when it scrolls over the image. Find the second leaf and position the cursor on the leaf before it starts bending and click. Move the cursor parallel to the leaf towards the ligule region, the region where the leaf meets the stem, and click. This will form a line between the ligule region and the 2nd leaf. Make sure this line is parallel to the base of the 2nd leaf.

10. Continue dragging the cursor upwards so it lines up with the stem in a 180° angle and click to complete the angle.
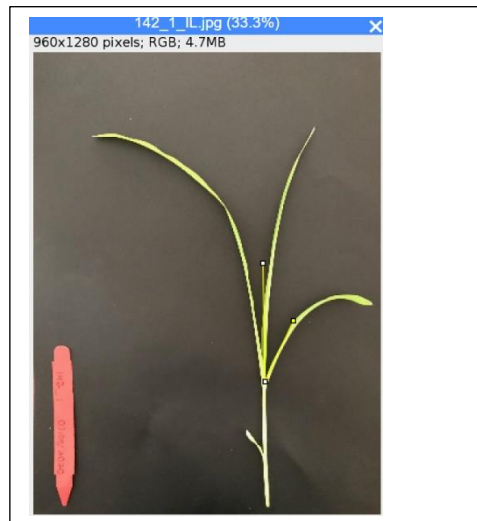
11. To determine the angle, scroll over the "Analyze" tab in the menu, and click "Measure."
12. A results table like the one below will appear, and the value in the "Angle" column is the measurement of the 2nd leaf angle.

13. Record this angle measurement in a data table (by hand or digitally).
14. Repeat this process using the 3rd leaf of the maize seedling.
15. The table will show your second measurement (angle of the 3rd leaf).
16. You can save this table for future reference by clicking on the table menu "File" and "Save As". A window appears showing the table's name. Change the name of the table to the genotype number and replicate seedling number (e.g. 191-2). Keep the .csv extension. Click OK and the table will be downloaded and available to open as an Excel file.