Weed Genetics Project Update

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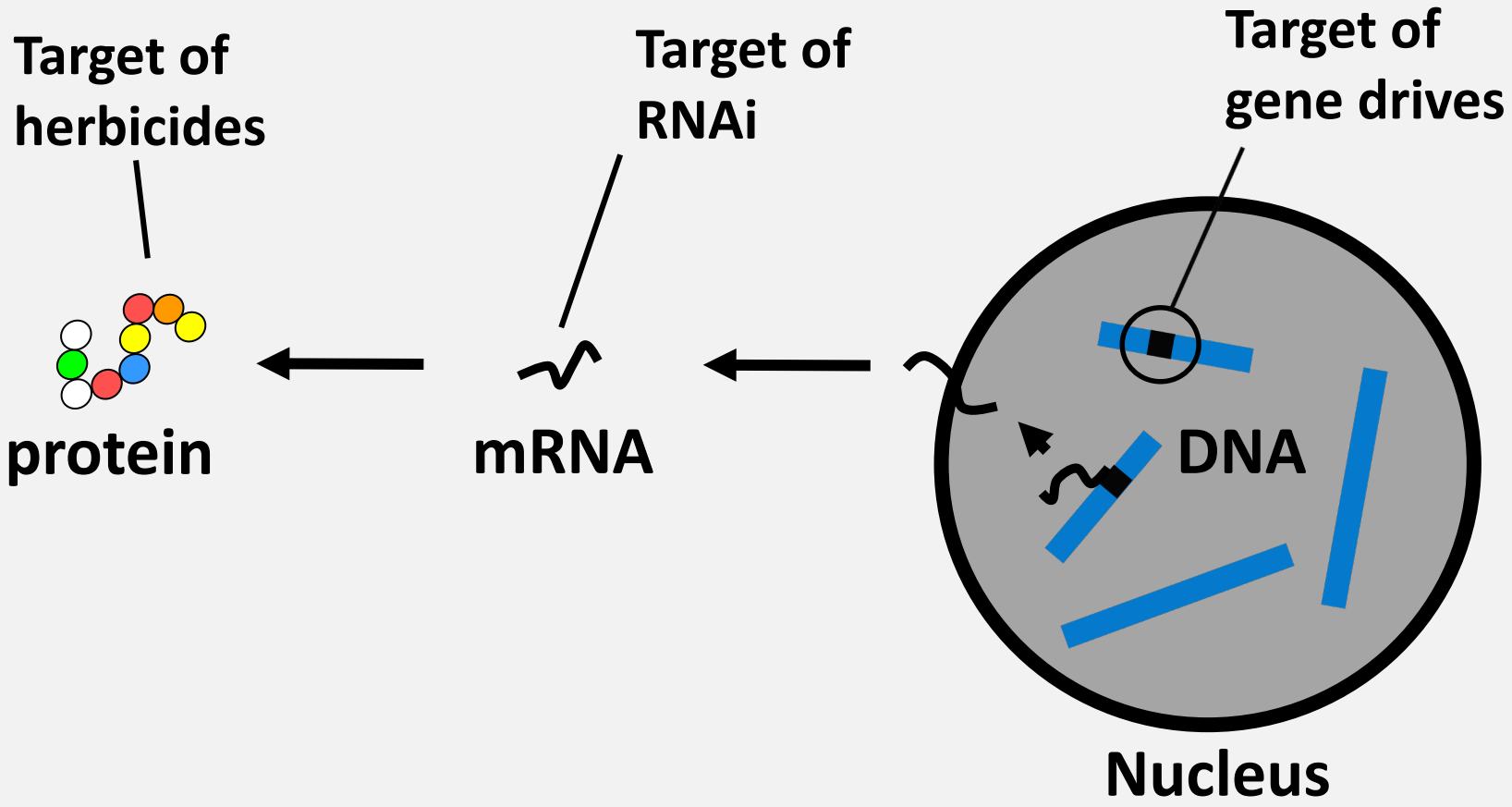
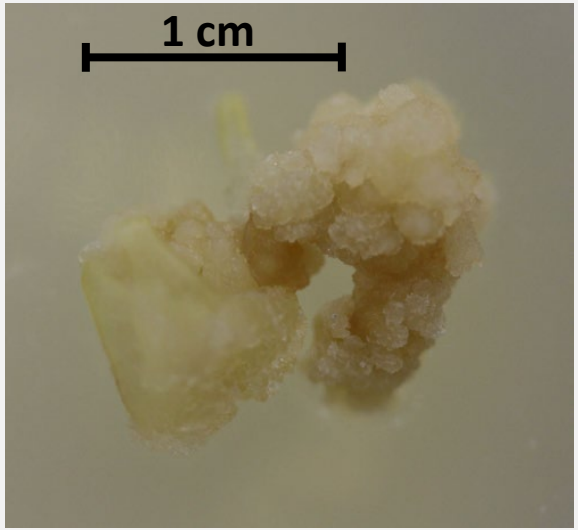
Genetic Biocontrol



Waterhemp Tissue Culture

Germinated seeds

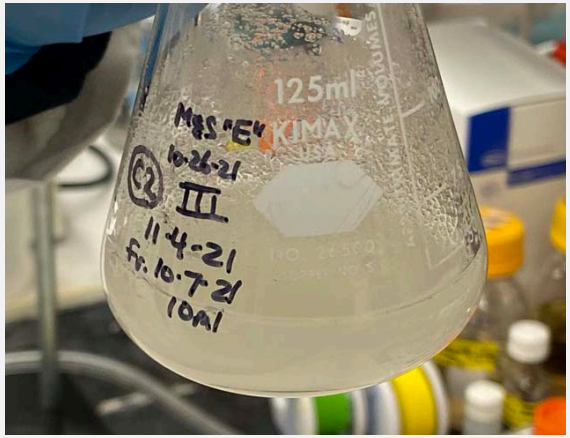
Remove a portion of the stem (hypocotyl)



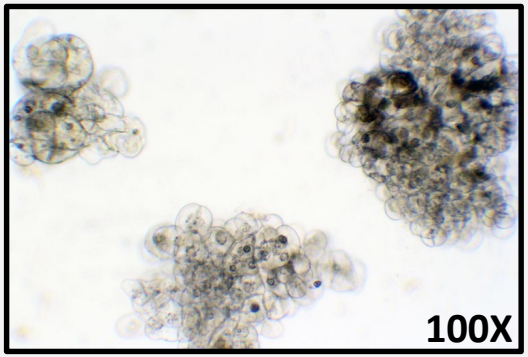
Callus tissue

Waterhemp Cell Suspension Culture

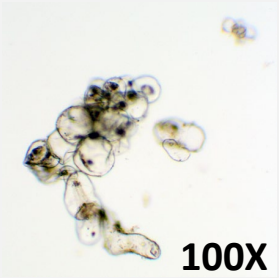
Callus tissue

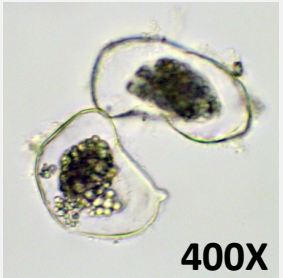


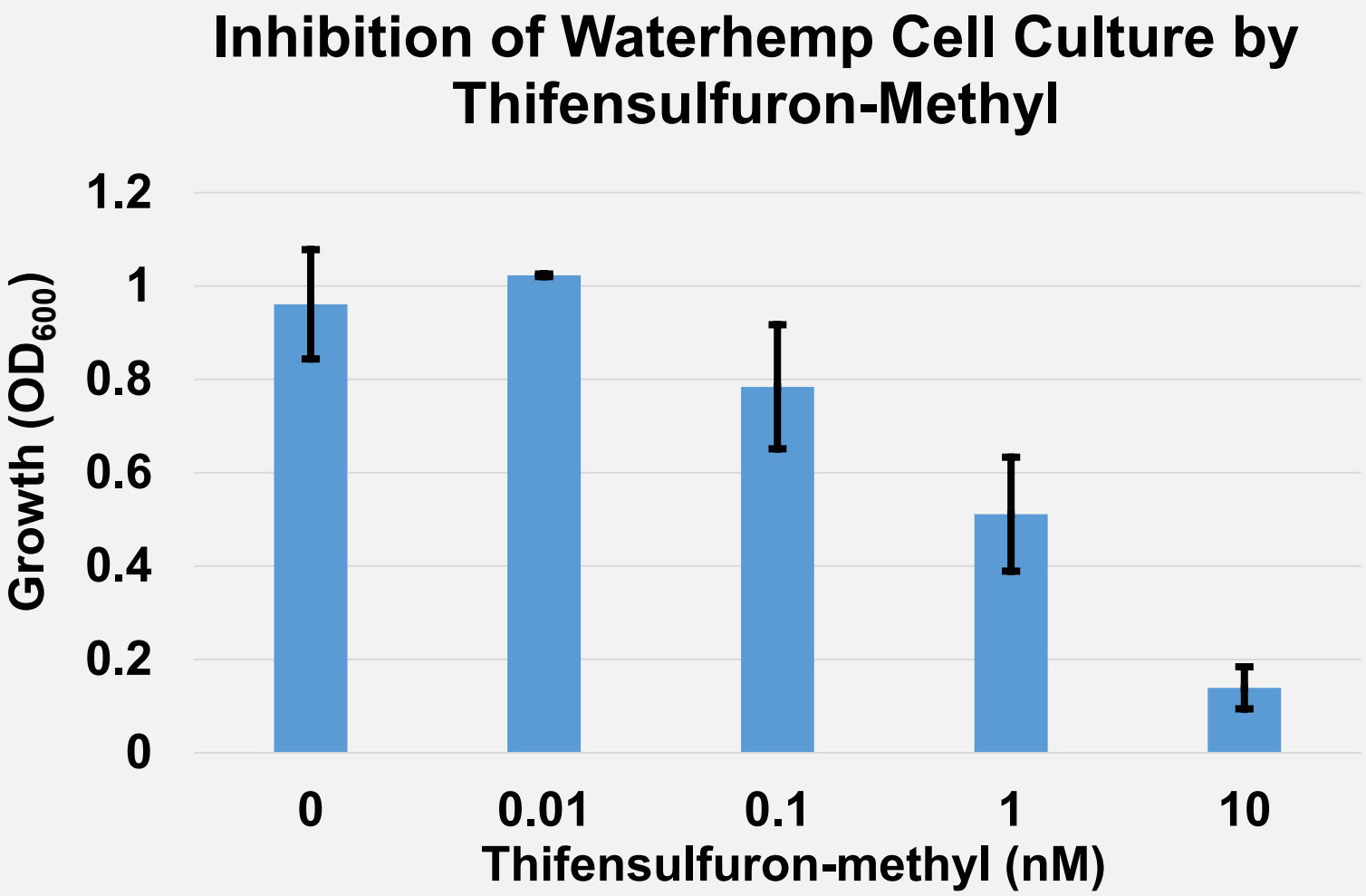
Cell suspension culture



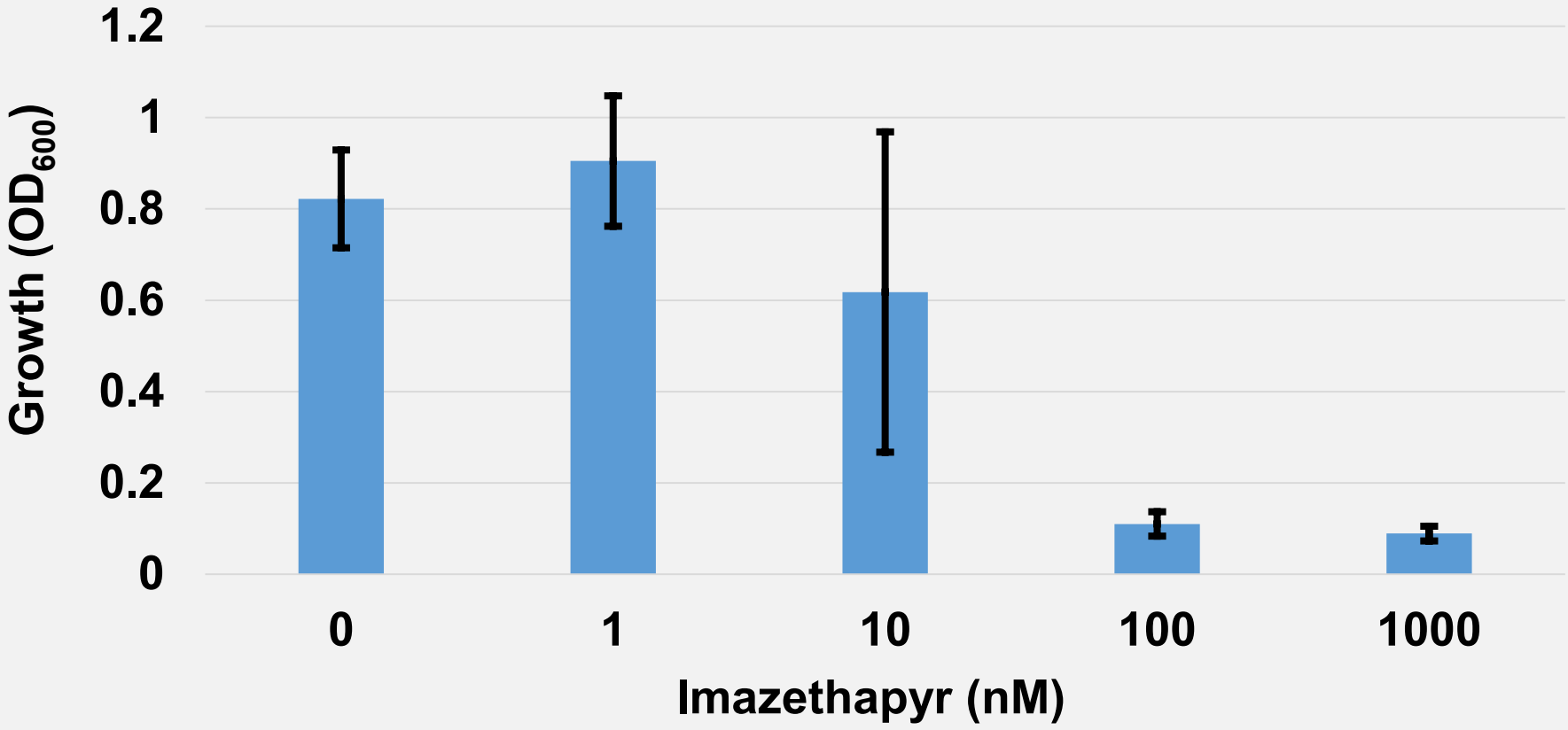
Subculture



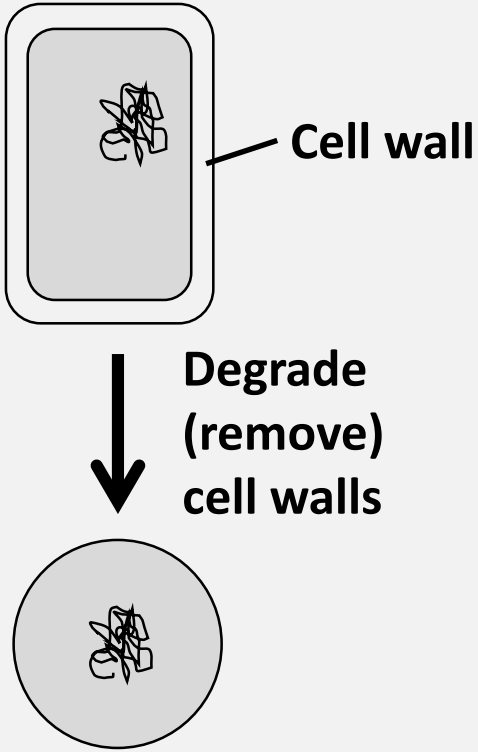



Inhibition of Waterhemp Cell Culture by Thifensulfuron-Methyl
Growth (OD_{600})
1.2
1
0.8
0.6
0.4
0.2
0
0
0.01
0.1
1
10
Thifensulfuron-methyl (nM)

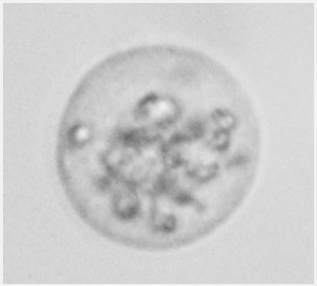
Inhibition of Waterhemp Cell Culture by Imazethapyr



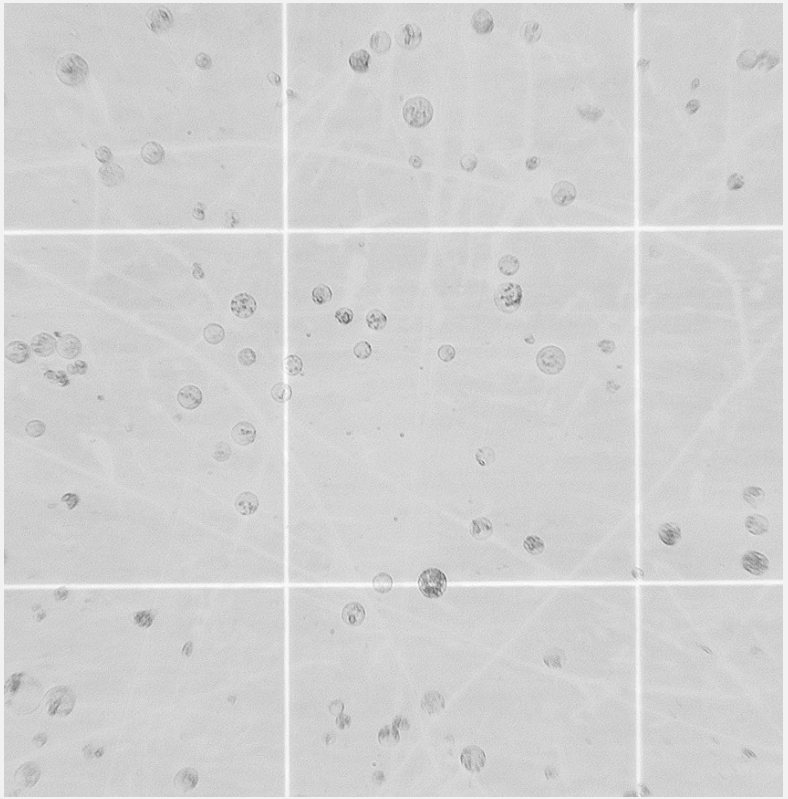
Successful Production of Waterhemp Protoplasts



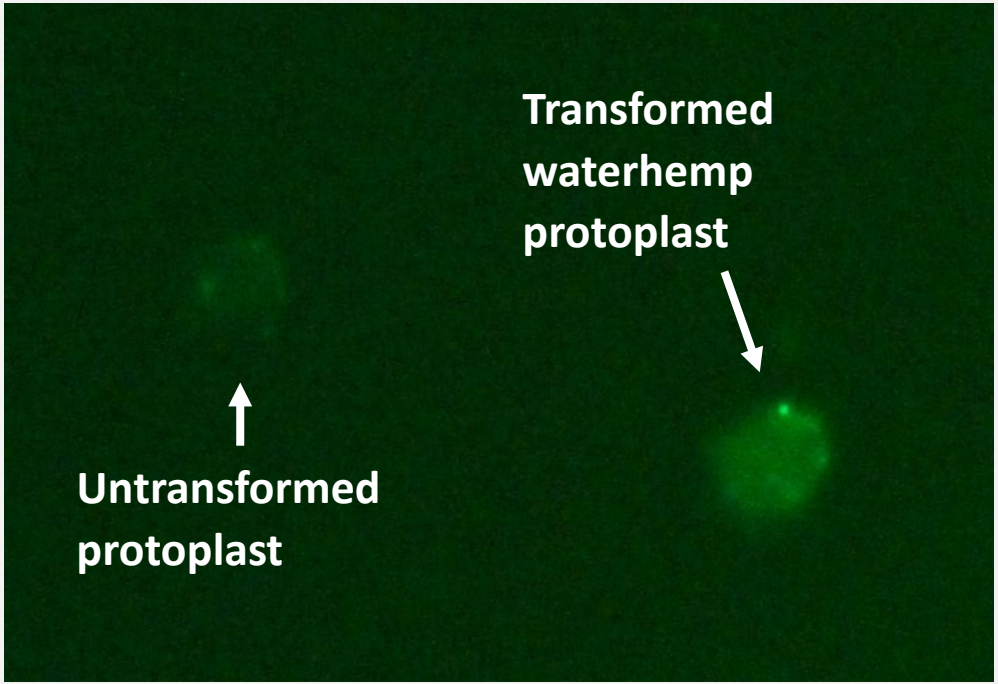
Waterhemp protoplast



Waterhemp protoplasts

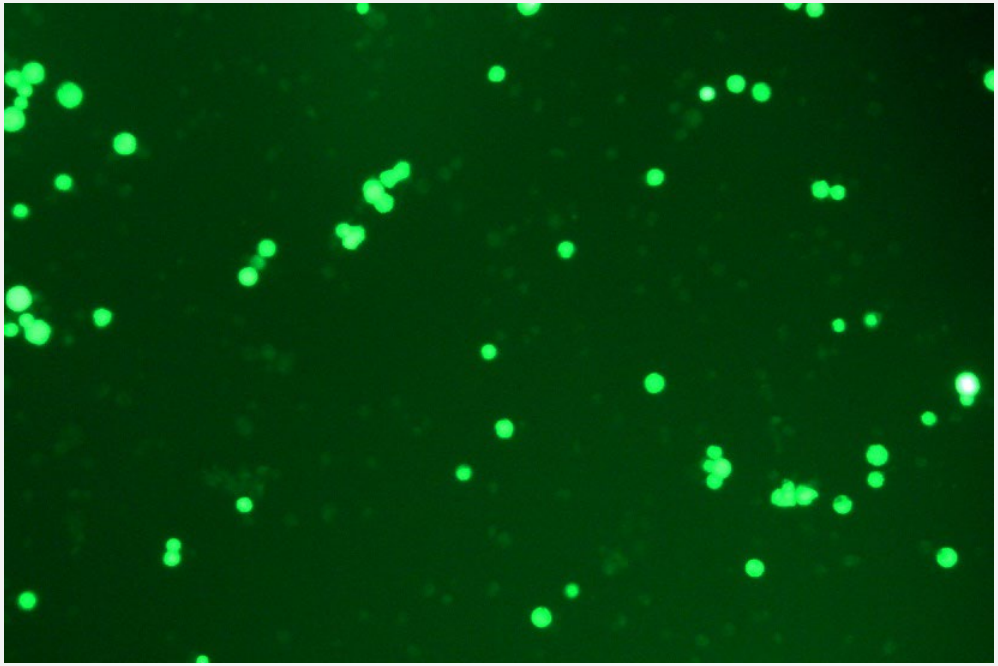


Protoplast Transformation with Protein



Green fluorescence indicates successful transformation of waterhemp protoplasts with protein

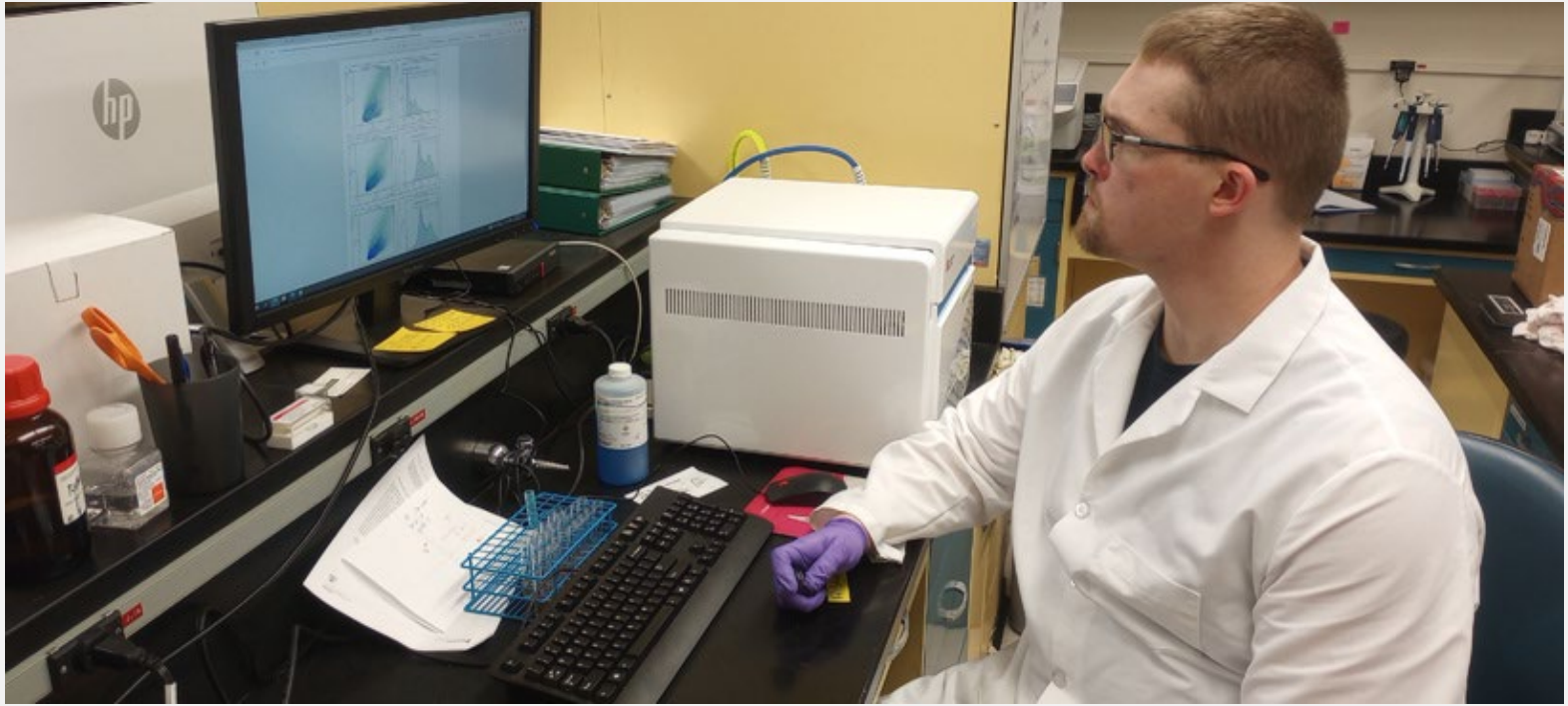
Testing for Oxidative Stress



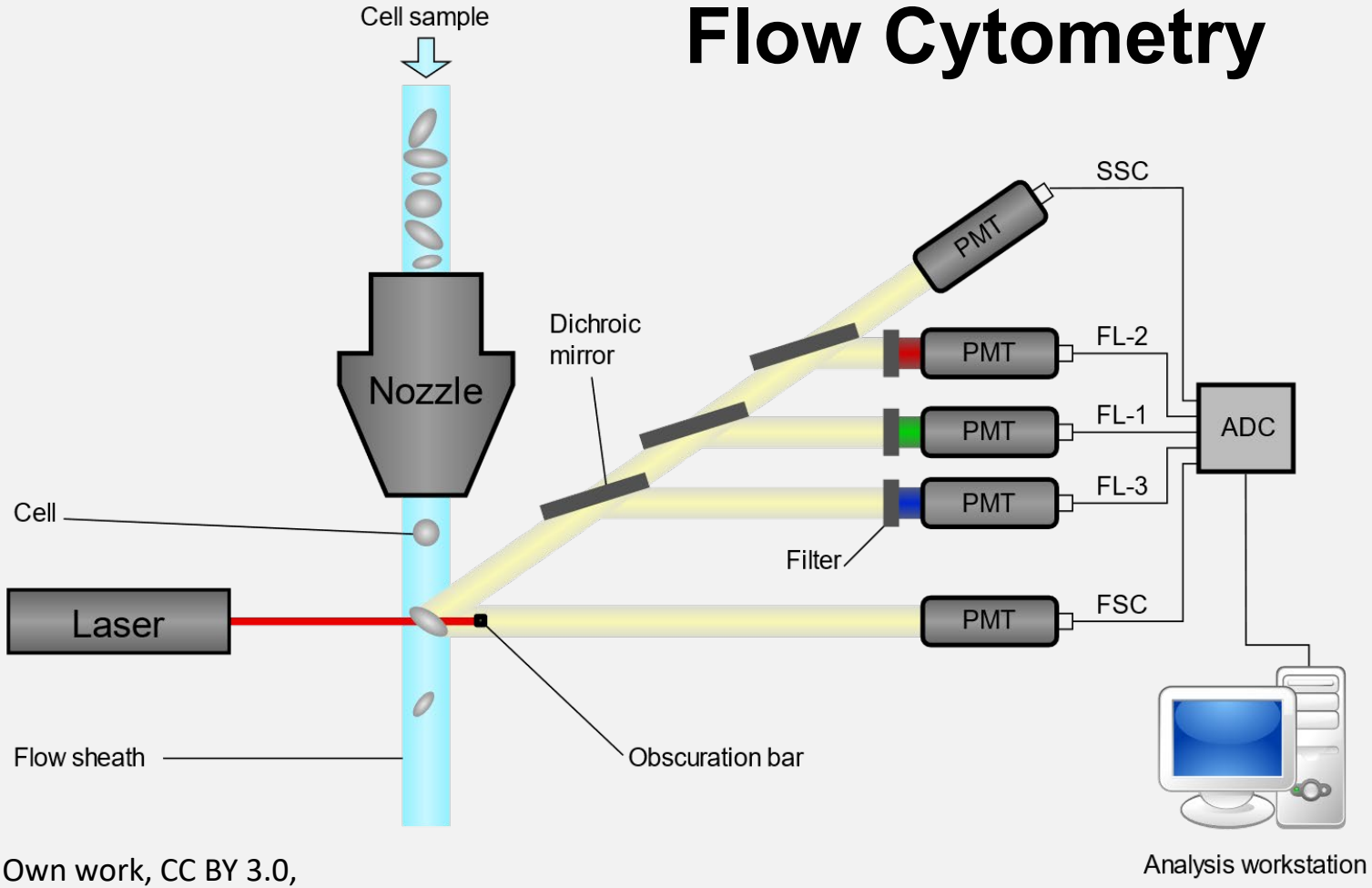
In this case, green fluorescence is bad

Indicates that waterhemp protoplasts are under oxidative stress due to reactive oxygen species (ROS)

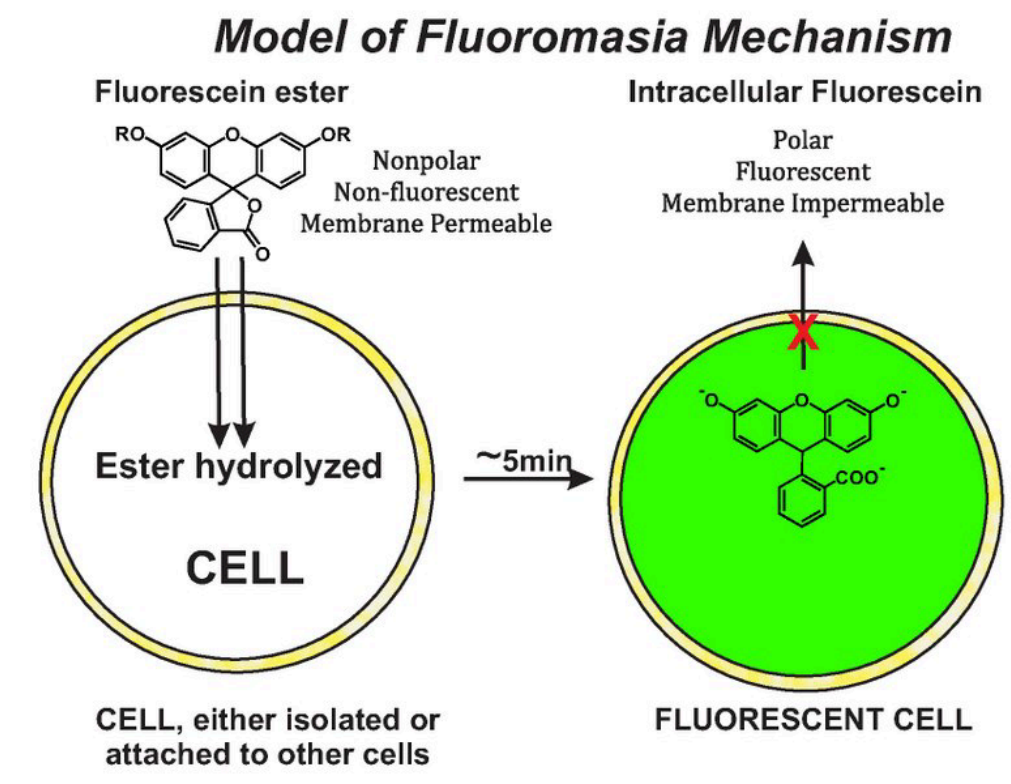
Flow Cytometry



Flow Cytometry



Measuring Oxidative Stress



Fluorescein diacetate (FDA) is used to measure protoplast vitality (see figure)

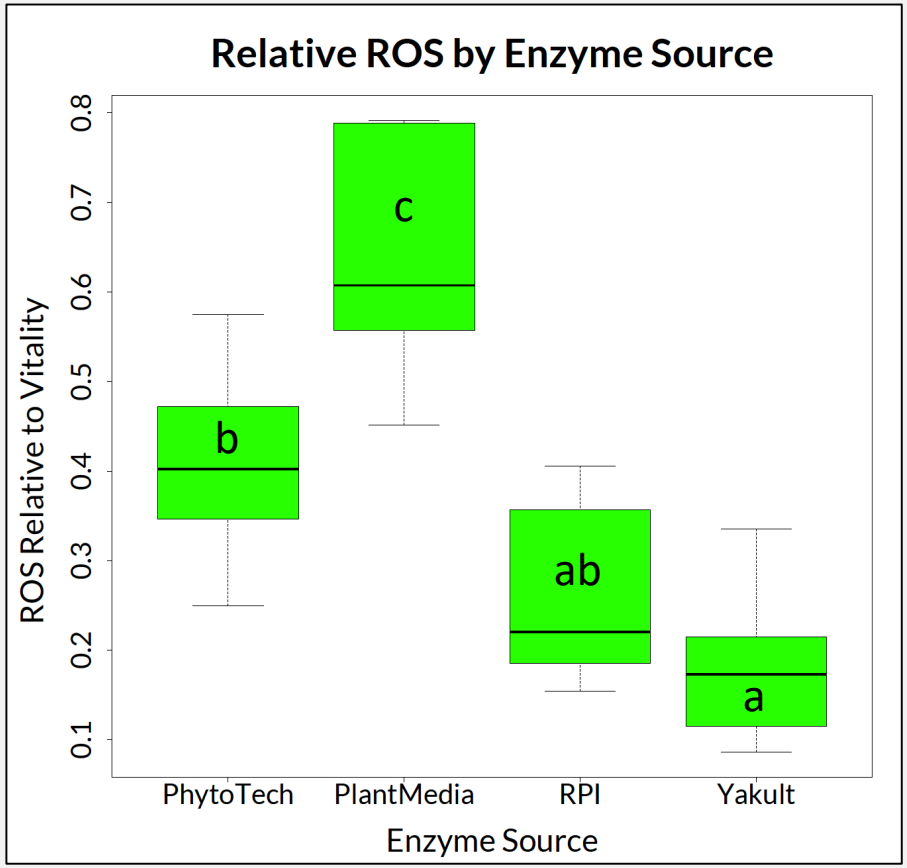
Dichlorodihydrofluorescein diacetate (DCFHDA) fluoresces by the same mechanism, but <u>also</u> needs to be oxidized in the cell

Comparing DCFHDA to FDA fluorescence using flow cytometry measures ROS and oxidative stress

By Autoctono~enwiki - Own work, CC BY-SA 4.0, https://en.wikipedia.org/wiki/File:Schematic_diagram_of_fluorochromasia_molecular_mechanism.pdf

Oxidative Stress by Enzyme Source

- Yakult enzymes found to produce significantly lower ROS than PhytoTech and PlantMedia
- RPI enzymes not significantly different from PhytoTech or Yakult
- Optimization of enzyme source for waterhemp protoplast production should provide healthier protoplasts for transformation and other genetic experiments



Acknowledgments

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