Oxidative Stress in *Brassica rapa* and Humans

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Presentation Overview

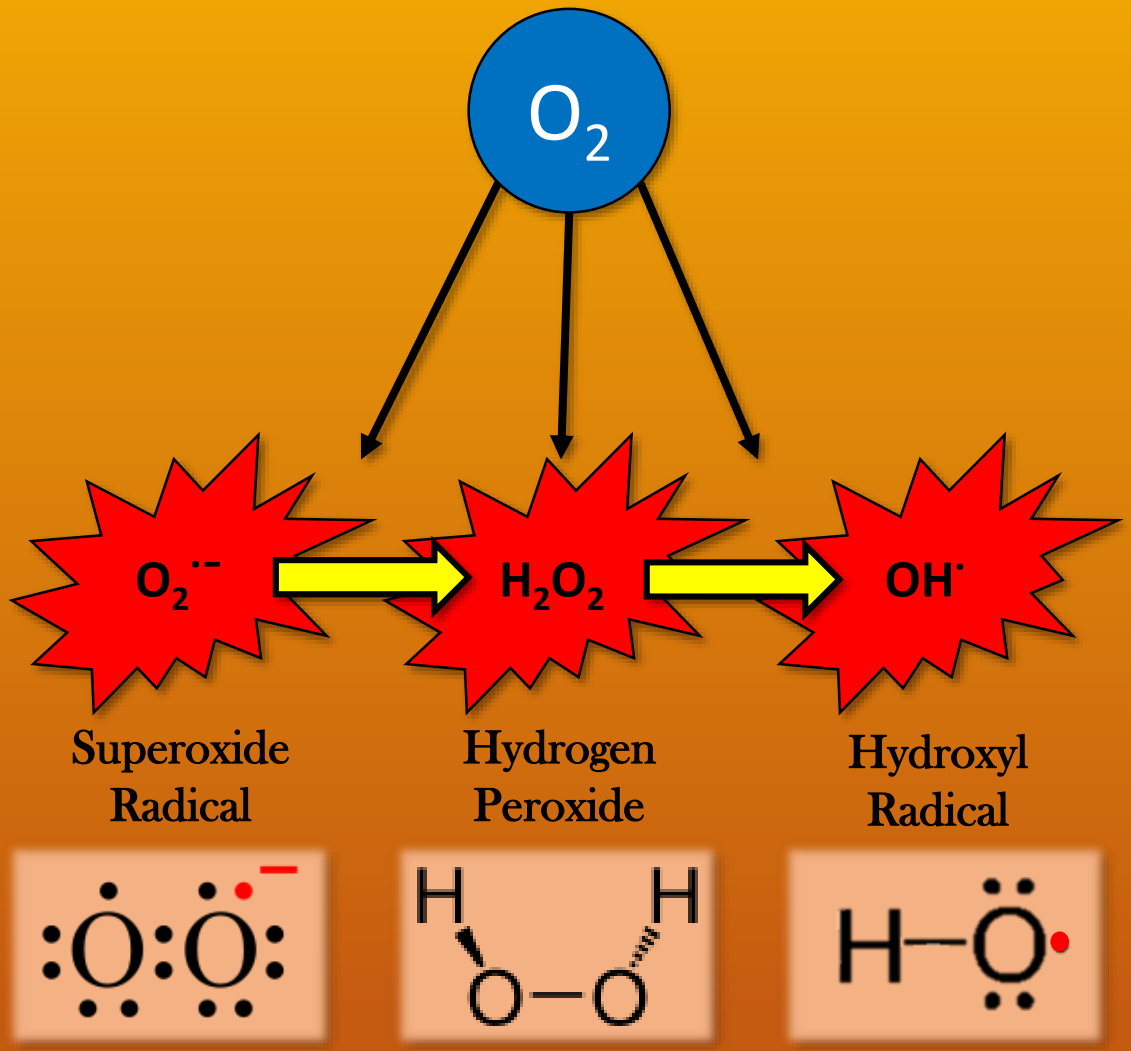
- Reactive Oxygen Species (ROS)
- ROS systemic signaling in Plants
- EPSCoR Project: ROS and Memory Stress in *B. rapa*
- ROS functions in humans

NBT Staining: Detecting ROS in Plants

Reactive Oxygen Species (ROS)

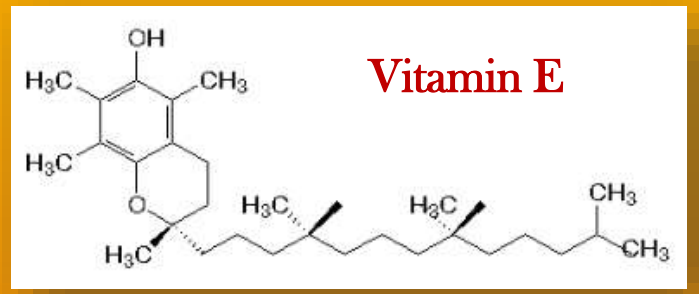
- Natural physiological molecules
- Derived from oxygen
- Both radical and non-radical
- Produced during…
  - Natural physiological processes
  - Stress conditions

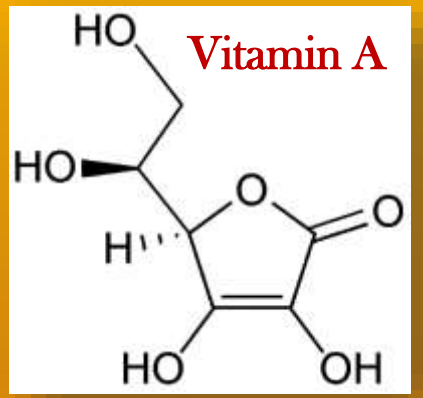
Why are ROS important?

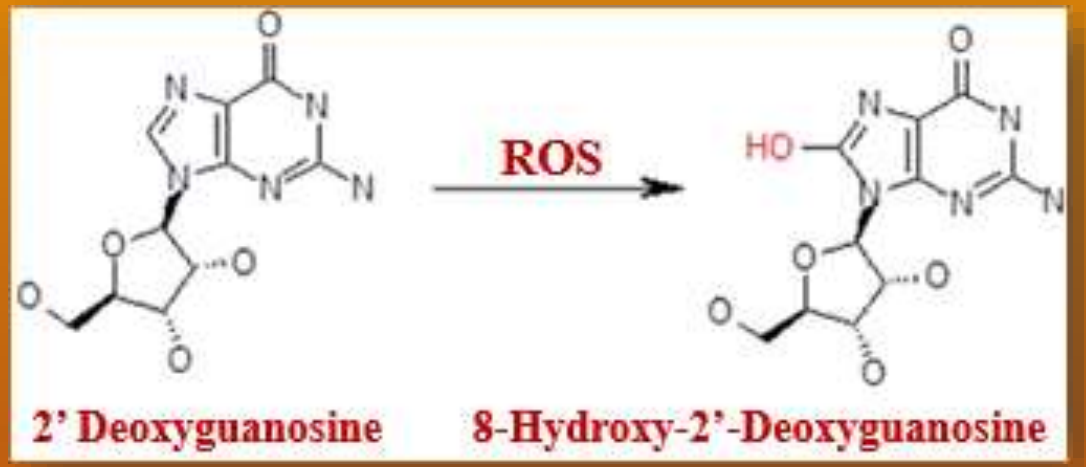


Oxidative Damage

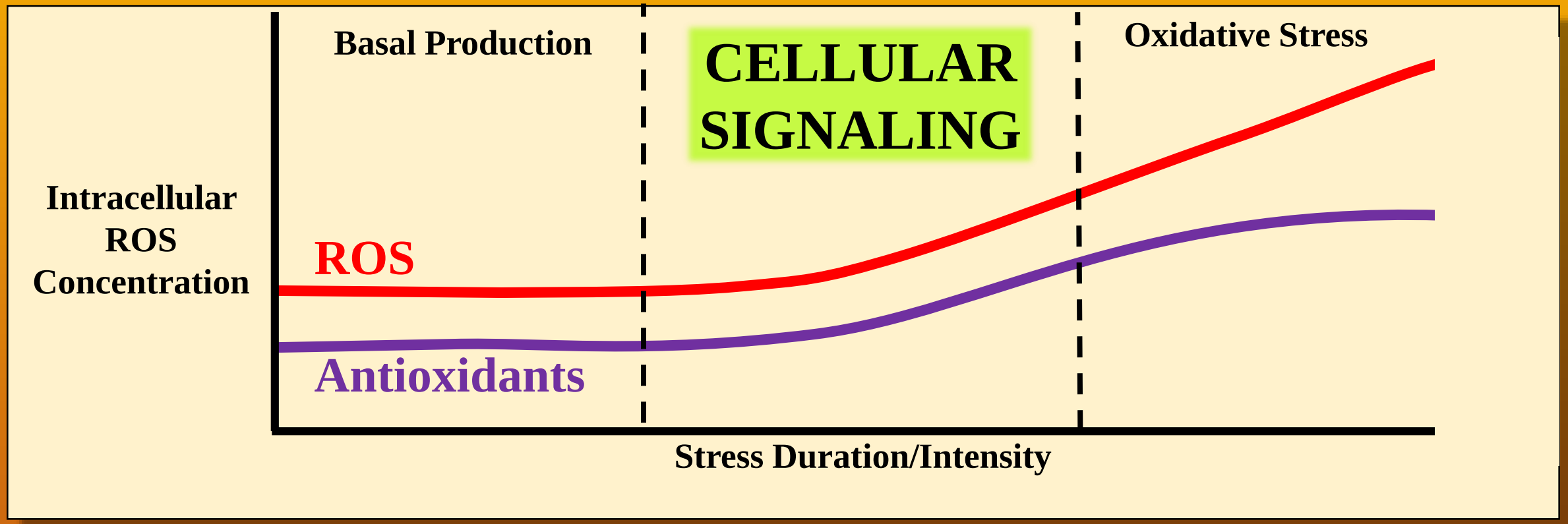
- Basal rate of production
  - Purpose for antioxidants
    - Enzymatic
    - Non-enzymatic (e.g. pigments)
- In high amounts → damage to major macromolecules
  - Proteins
  - DNA
  - Carbohydrates
  - Lipids (e.g. Cell membranes)





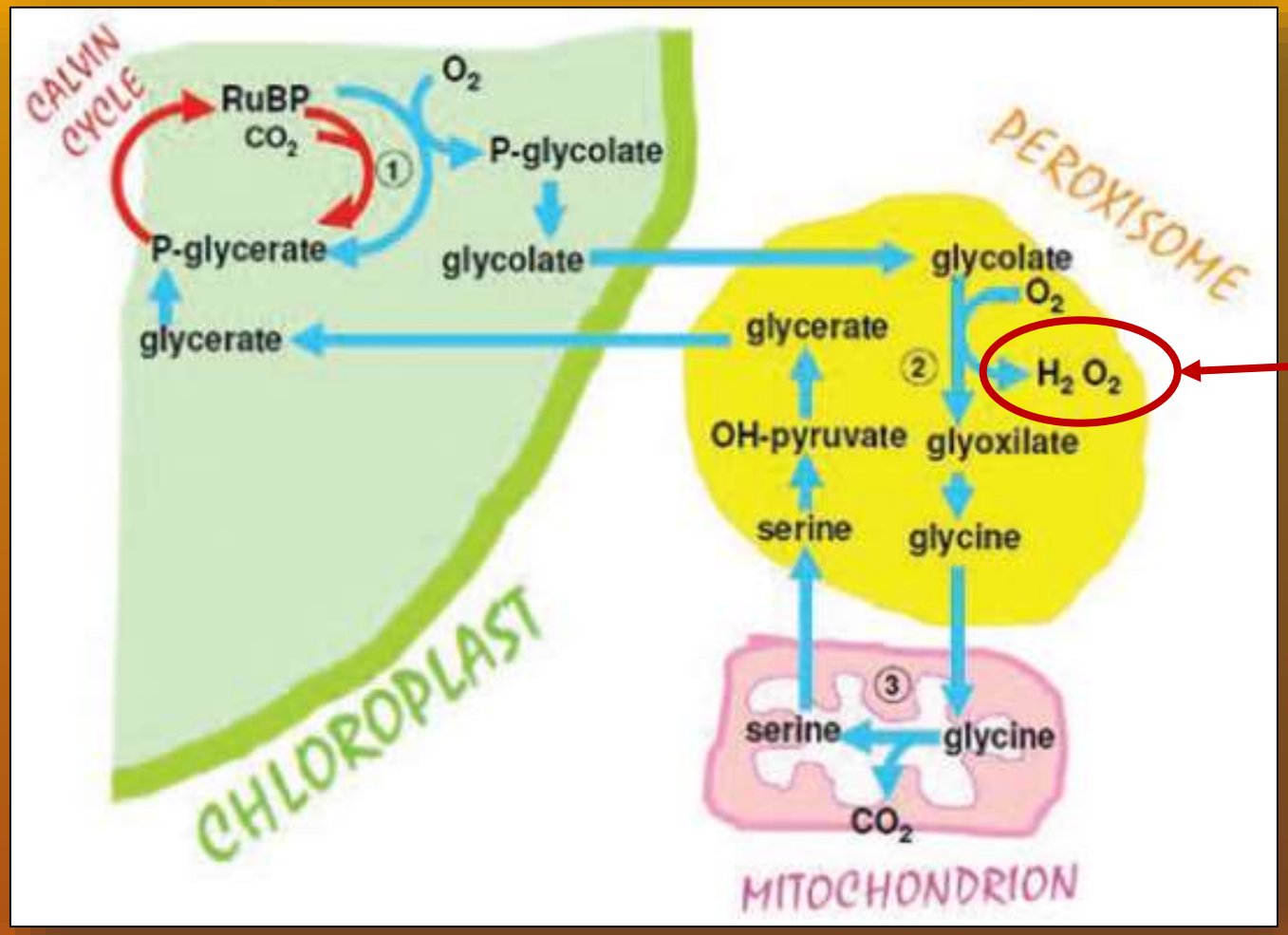


ROS in Cellular Signaling



ROS Signaling in Plants

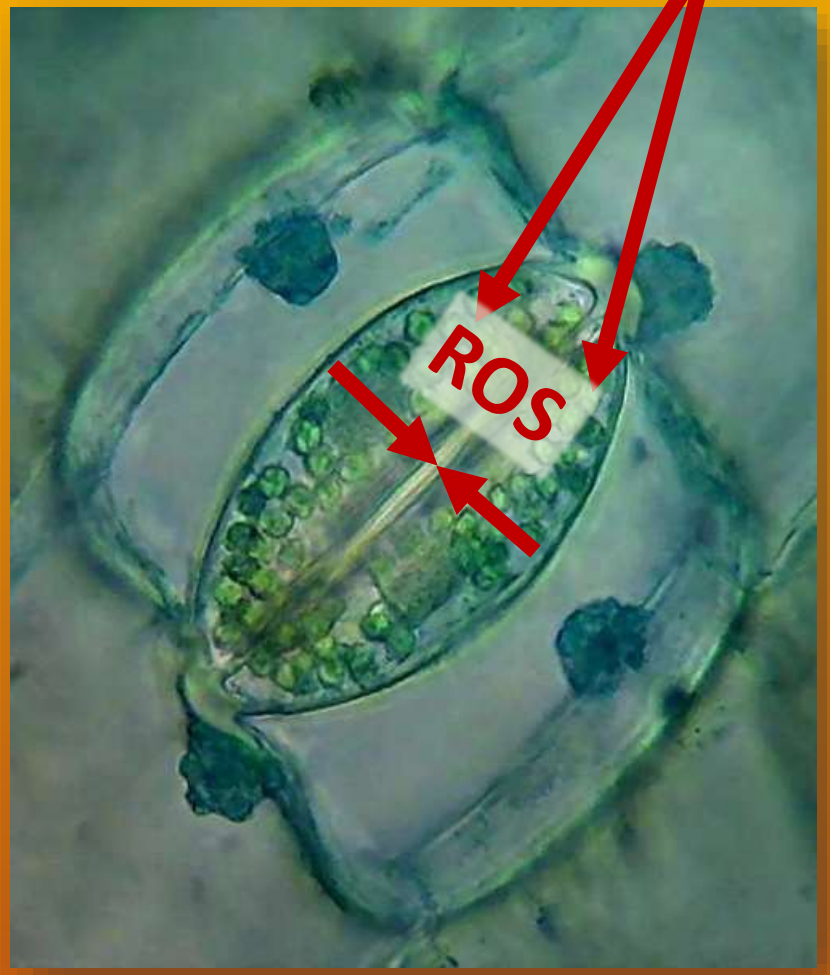
ROS Generation in the Peroxisome



- Sites of cellular ROS production:
  - Chloroplast
    - Electron Transport Chain (ETC) accidentally gives electrons to O_2 instead of CO_2
  - Peroxisome
    - Rubisco accidentally using O_2 instead of CO_2 to create glycolate
  - Mitochondrion
    - ETC accidentally gives an electron to dissolved O_2
  - Various enzymes
    - NADPH Oxidases (NOX)
    - Dual Oxidases (DUOX)

Drought-Induced ROS Signaling

- Some Identified ROS Signaling Functions:
  - Abscisic Acid (ABA) Signaling
    - ABA: drought stress plant hormone ("phytohormone")
    - Required to close stomata of plant leaves
    - H_2O_2 needed in ABA signaling pathway
  - Ethylene Synthesis
    - Ethylene: another stress-associated phytohormone
    - Ethylene also implicated in stomatal closure in some plants
    - H_2O_2 and superoxide assist in inducing its synthesis
  - Inducing *DRT112* Expression:
    - H_2O_2 presence increases expression of a DNA repair protein (**D**NA-damage-**R**epair/**T**oleration)
    - Helping mediate ROS damage to DNA



Stoma (pl. stomata) in *Tradescantia*

Investigating ROS in Stress Memory

Stress Memory:

After an initial exposure to a stress, the ability of an organism to physiologically respond better to a subsequent exposure to the same stress

Research Question:

Could ROS be involved in the development of stress memory in *Brassica rapa?*

EPSCoR Project

Fall 2014 & Spring 2015

- Under Drs. Brent Ewers and Carmela Rosaria Guadagno
- Large-population experiment: Identifying lines of *Brassica rapa* that may efficiently use ROS to develop stress memory
- Four stressors:
  - Drought
  - High Temperature
  - Free-Running Conditions
  - High Light

EPSCoR Project

Fall 2014 & Spring 2015

My role in the project:

- “Tuning” the ROS assay
- Establishing a baseline level of stress measurable and comparable amongst all four stress treatments
  - How **intense** should the stress be applied?
  - How **long** should the stress be applied for?

Experimental Design

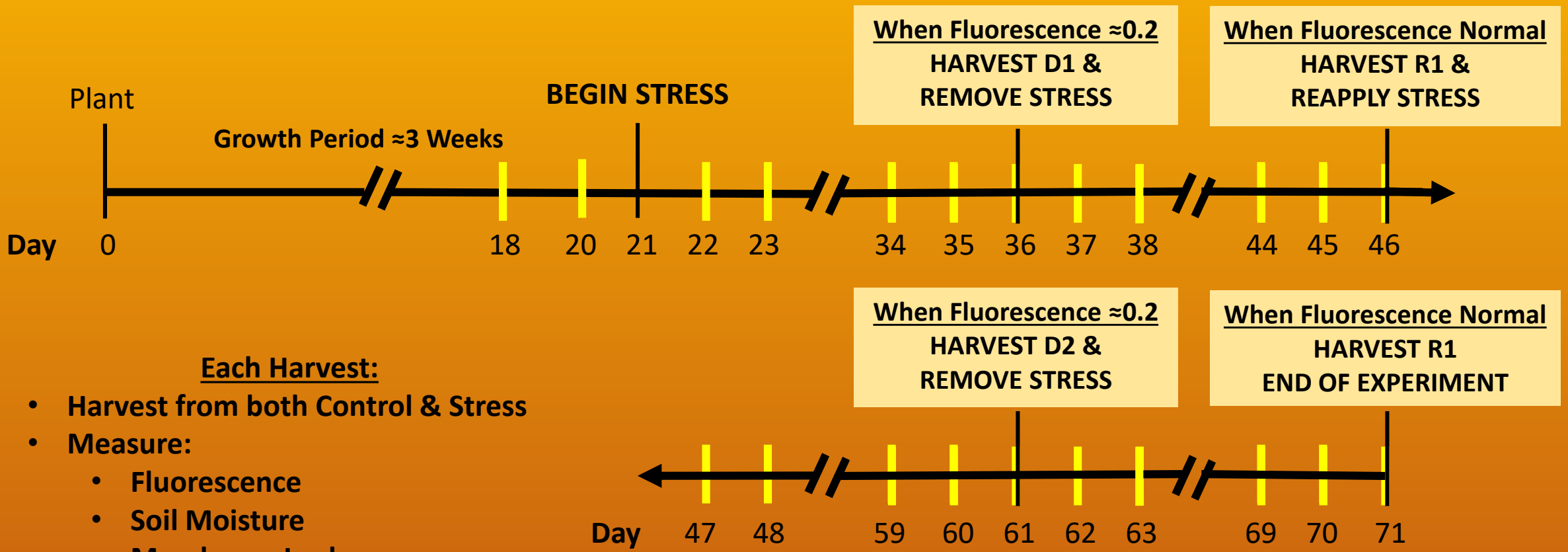
- For each stress (e.g.: DROUGHT):
  - D1 → Drought 1 — 18 Control Plants — 18 Droughted Plants
  - R1 → Recovery 1 — 18 Control Plants — 18 Droughted Plants
  - D2 → Drought 2 — 18 Control Plants — 18 Droughted Plants
  - R2 → Recovery 2 — 18 Control Plants — 18 Droughted Plants

144 Plants

- All plants *B. rapa* (R500 genotype)
- Grown in environmentally identical conditions

Experimental Design

Measure Fluorescence & Soil Moisture



Each Harvest:

- **Harvest from both Control & Stress**
- **Measure:**
  - **Fluorescence**
  - **Soil Moisture**
  - **Membrane Leakage**
  - **Lipid Peroxidation**
  - **Biomass**

Drought Stress

Control

Stress

Day 0

Drought Stress

Control

Stress

Day 5

Drought Stress

Control

Stress

Day 13

BEGIN DROUGHT FOR STRESS

Drought Stress

Control

Stress

Day 33

Drought Stress

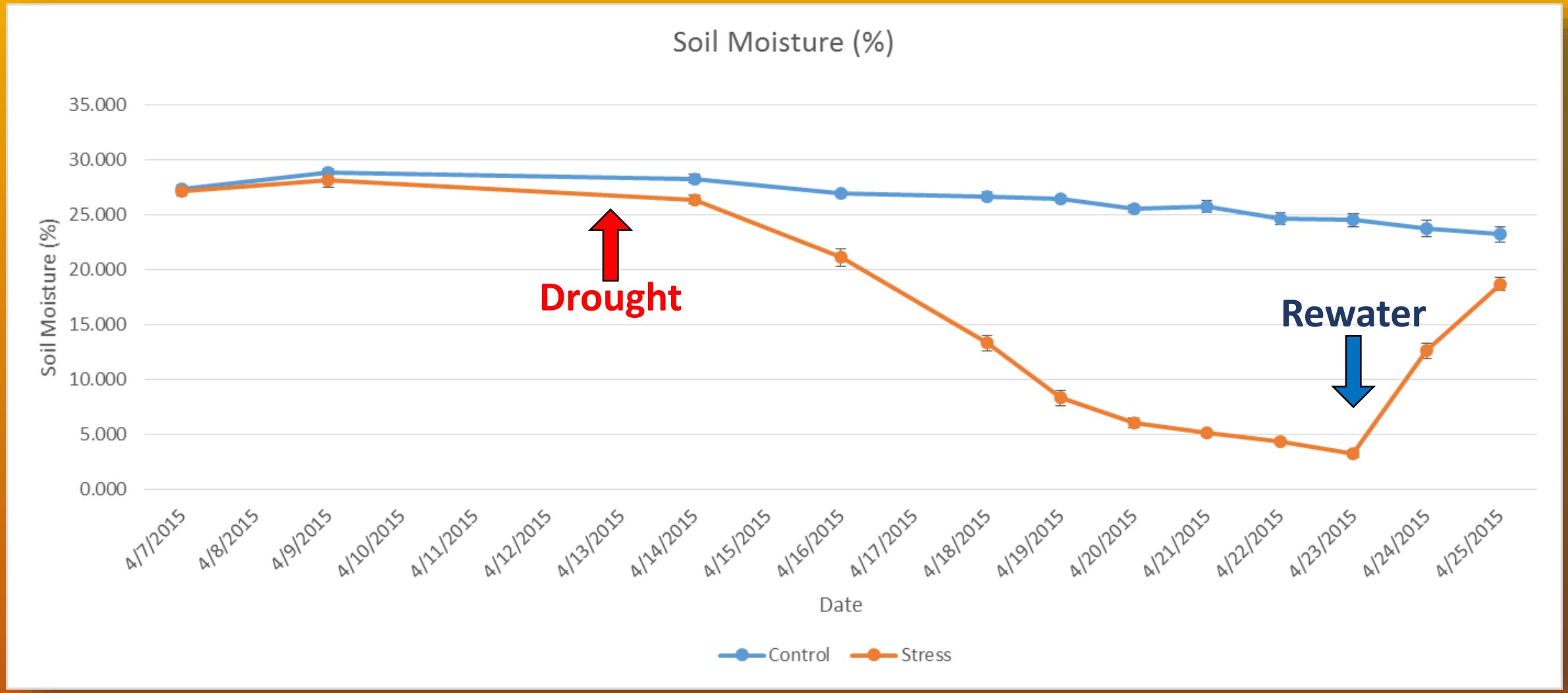
Control

Stress

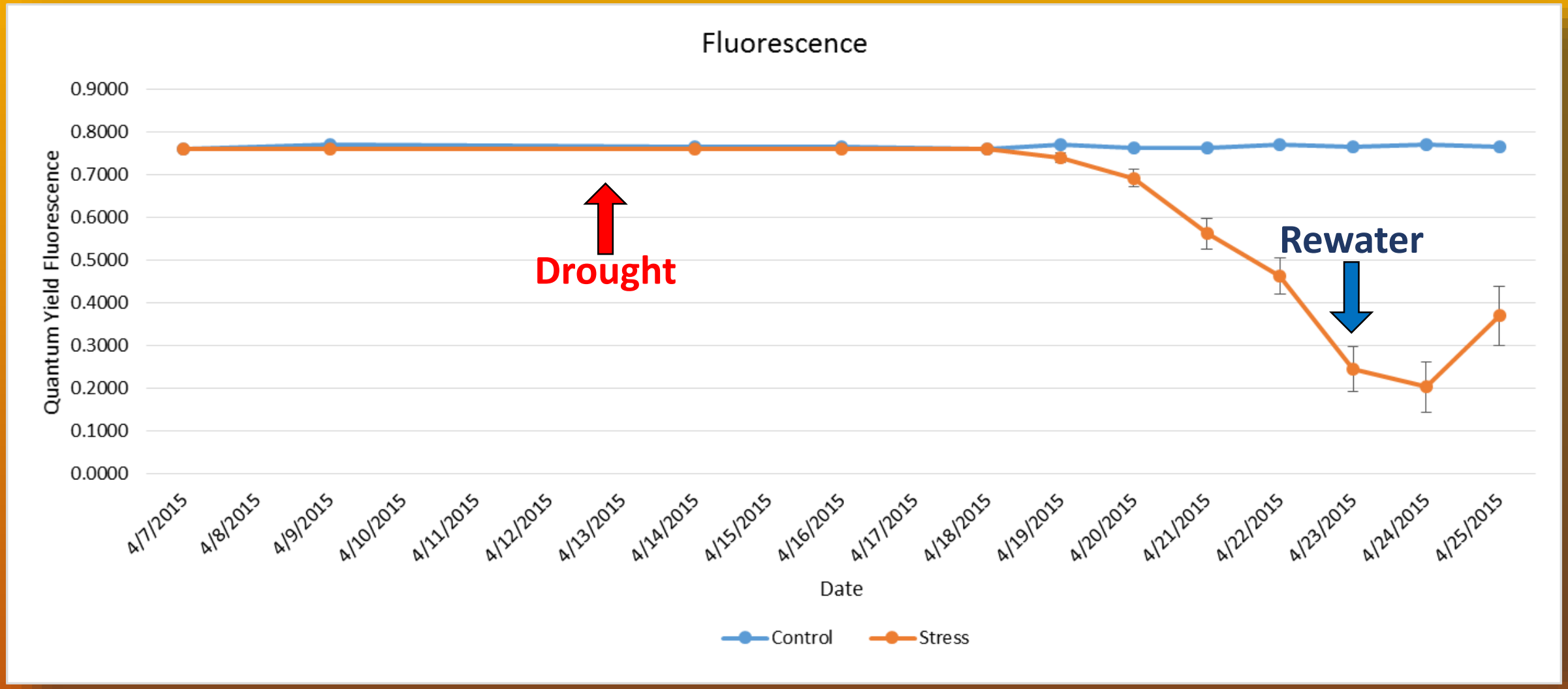
Day 37

HARVEST D1 PLANTS

Soil Moisture

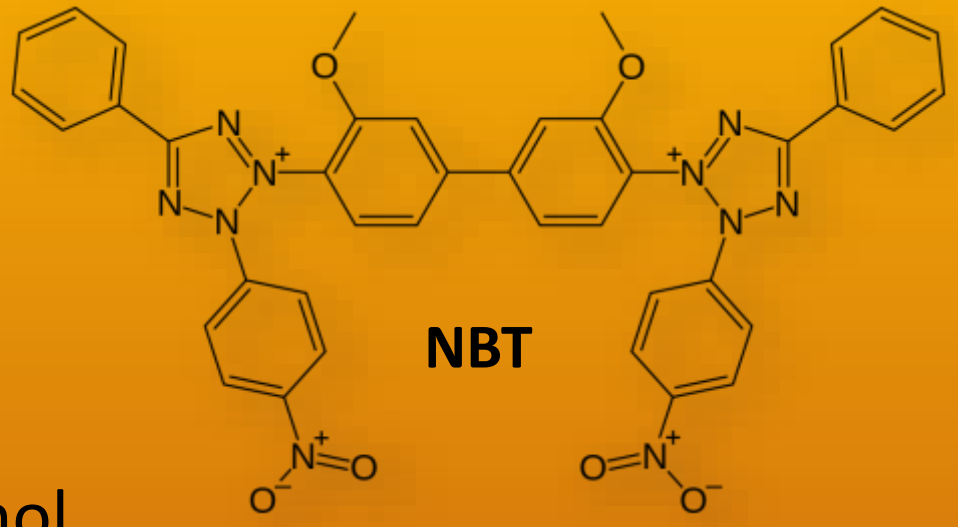


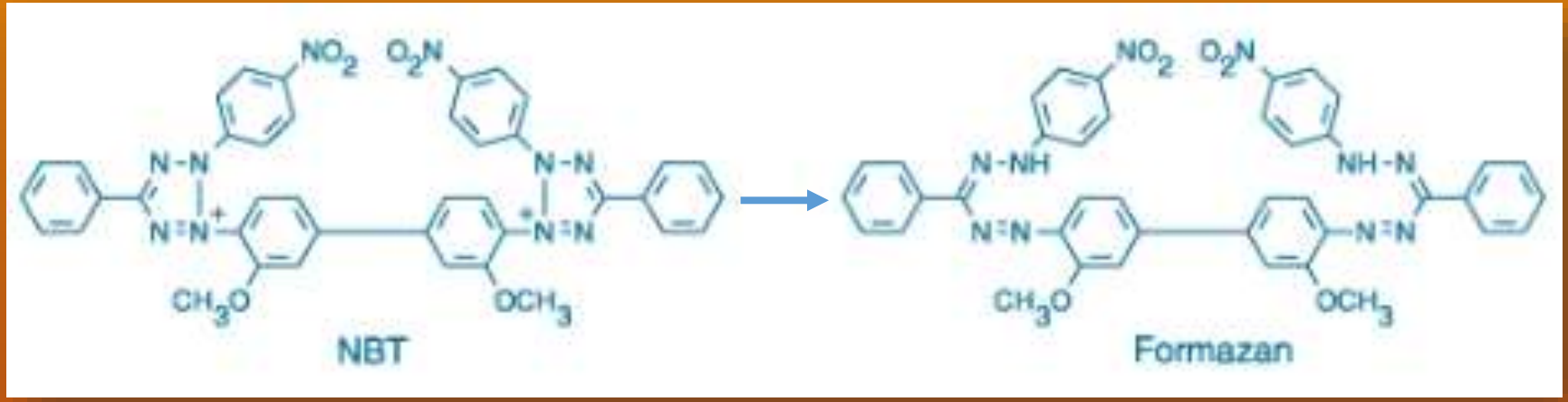
Fluorescence



NBT Staining

- Purpose: Identify “lipid peroxidation”
  - Occurs when ROS attacks lipid membranes
- NBT = “Nitro Blue Tetrazolium Chloride”
  - Reacts with ROS-damaged membranes to produce formazan
  - Formazan appears purple!
- Remove green chlorophyll with 80% ethanol





NBT Staining

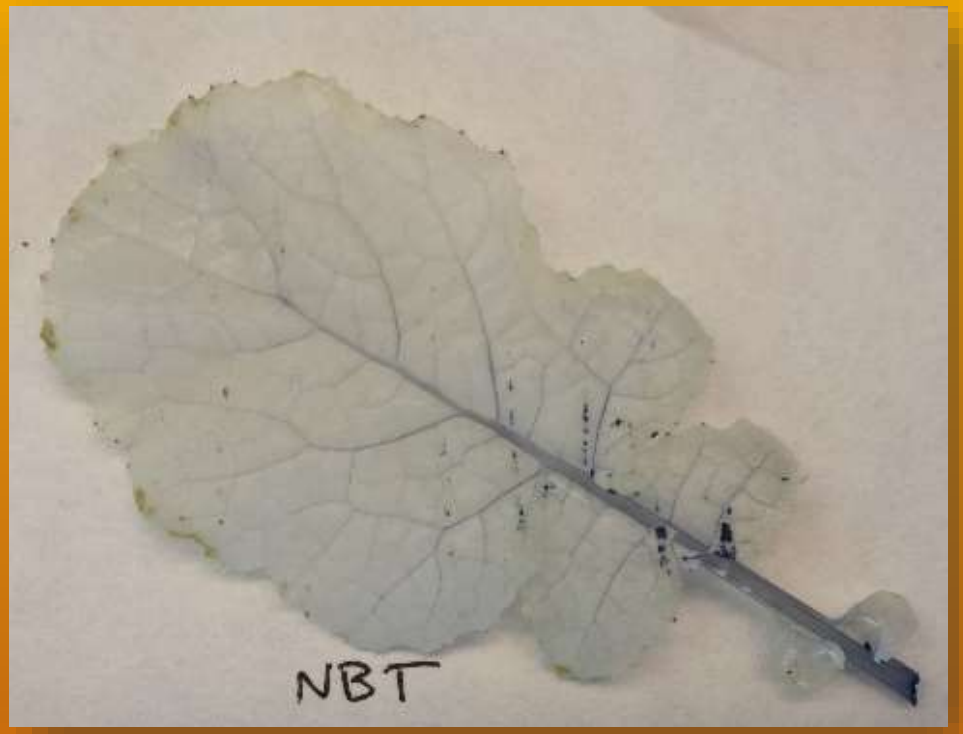
80%
Ethanol

1 Day Drought

Extra Stress Plant

NBT Staining

80%
Ethanol



6 Days Drought

Extra Stress Plant

NBT Staining

80%
Ethanol

Staining Failed-

The stress was too prolonged and leaves could no longer conduct the NBT solution.

11 Days Drought

Harvested Stress Plant

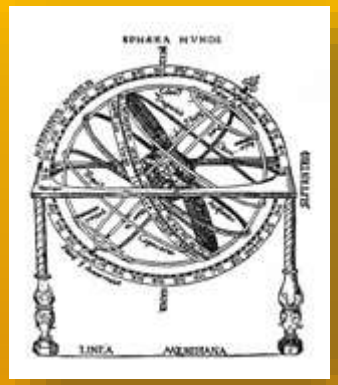
Experimental adjustments upon preliminary results

- The drought stress of 11 days resulted too strong for NBT staining application
- Experimental adjustments:
  - Re-water droughted plants to determine recovery rate
  - Dry down Control plants to see how far into drought the NBT staining is reliable
- Run a new experiment using obtained information

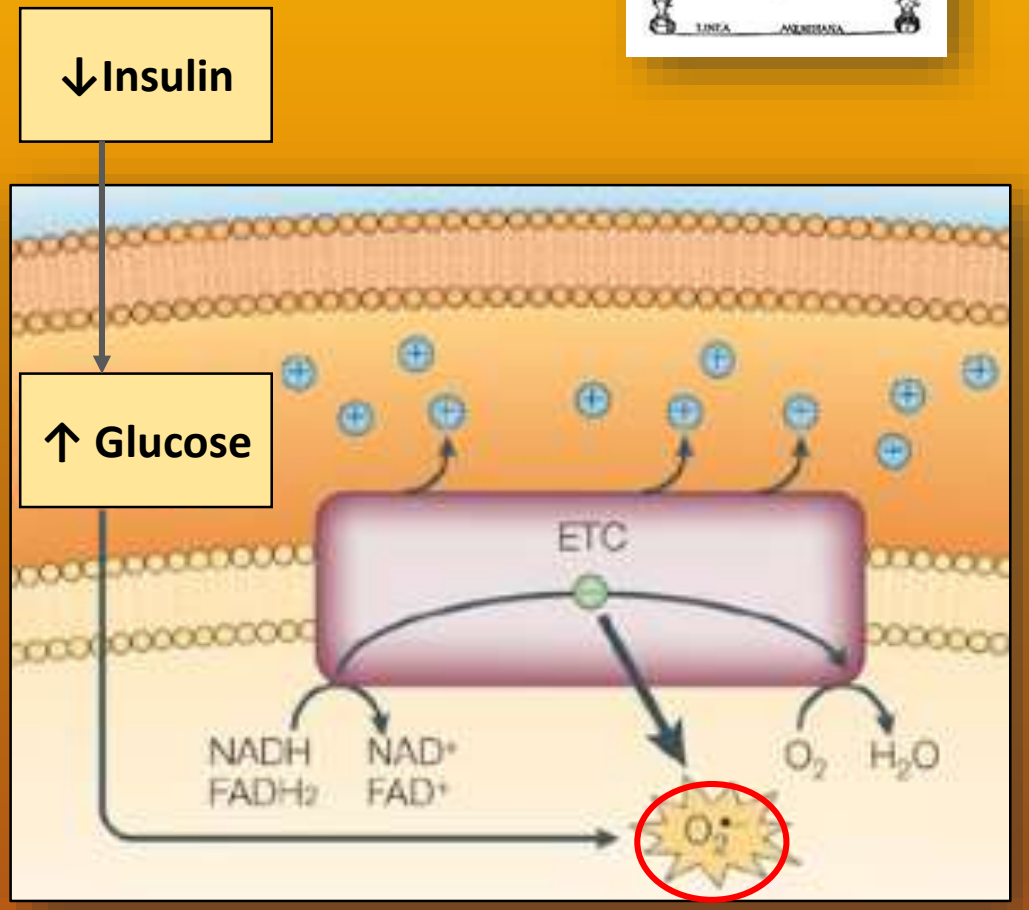
Day 6: Rewatered Stress

ROS in Humans

UW Honors Program
Senior Project

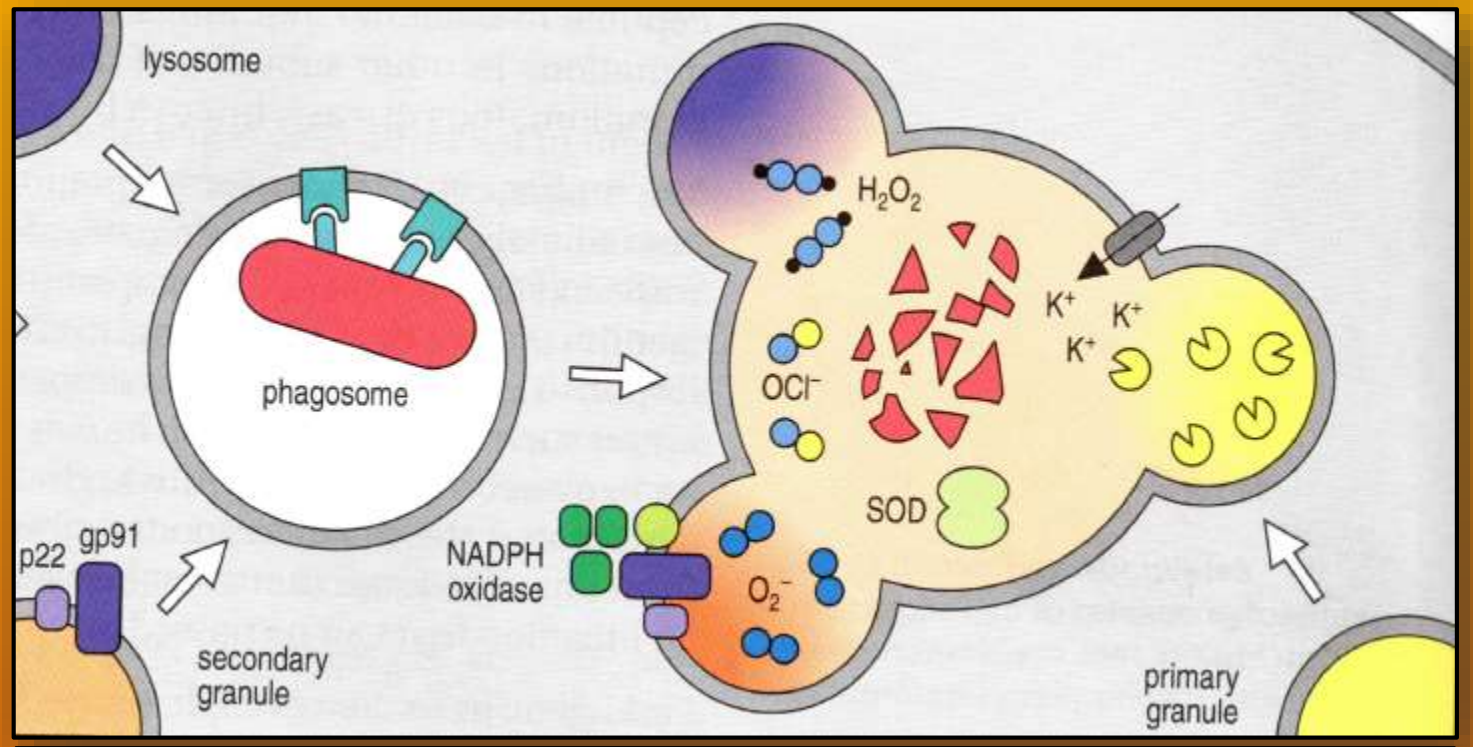


- DNA, protein, lipid, & carbohydrate damage
- ROS implicated in human diseases
- Example: Diabetes Mellitus (Types I & II)
  - High levels of glucose → ROS generation
  - Downstream consequences:
    - Diabetic retinopathy (retina)
    - Diabetic neuropathy (neurons)
    - Diabetic nephropathy (kidneys)
    - Glaucoma
    - Atherosclerosis



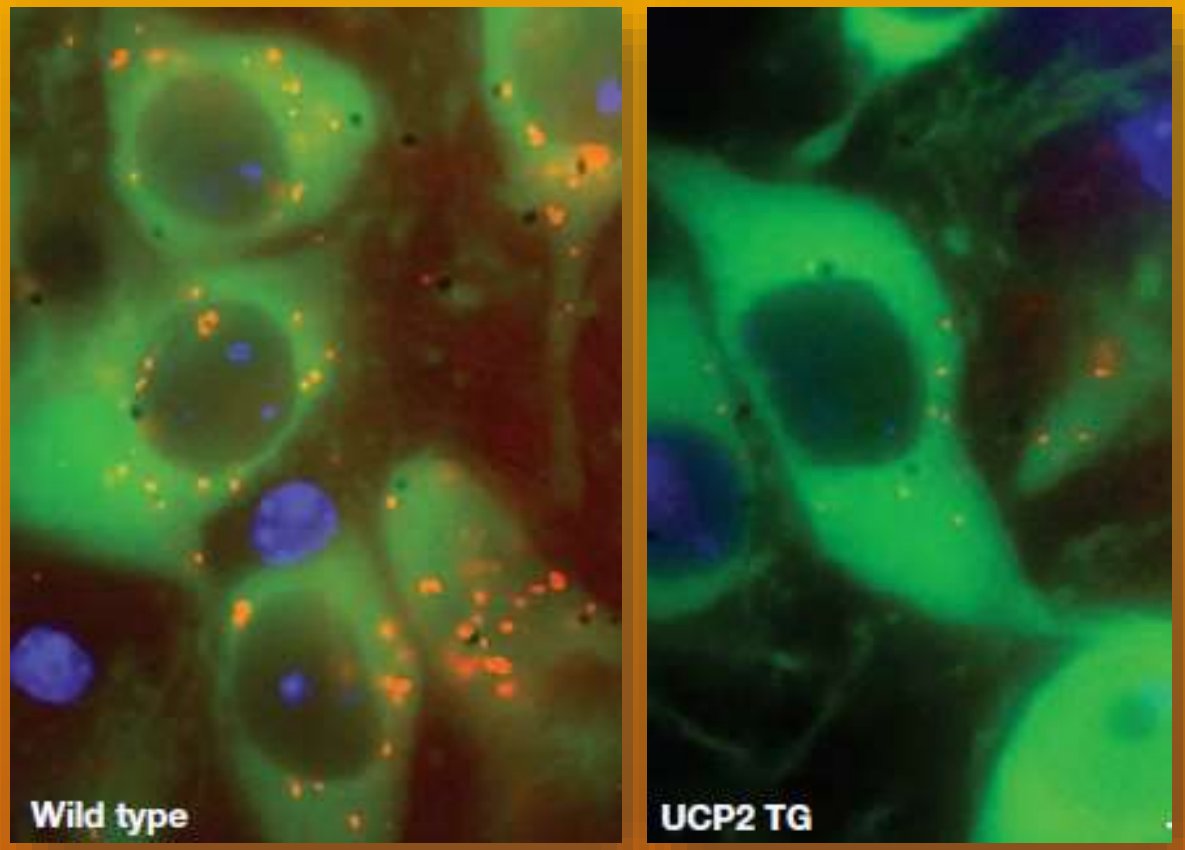
ROS in Humans

- ROS used in normal physiological processes
- Examples:
  - Thyroid synthesis
  - Macrophage activity
  - Pathogen defense in lung mucosae



ROS in Macrophage Activity
(Fig 3.5, *Janeway Immunobiology, 8th ed.*)

ROS in Humans



ROS in cells without (left) vs with (right) UCPs

Orange: ROS

- ROS can be signaling molecules in signaling pathways
- Examples:
  - Vascular smooth muscle remodeling
  - Inducing DNA & protein repair mechanisms
  - Inducing proteasome activity to degrade ROS-oxidized proteins
  - Upregulating uncoupling proteins (UCPs) to decrease ROS production in mitochondria
- Other functions under investigation, and yet to be discovered

Acknowledgements

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Questions?