Monitoring root growth, analysis of cell division and confocal microscopy

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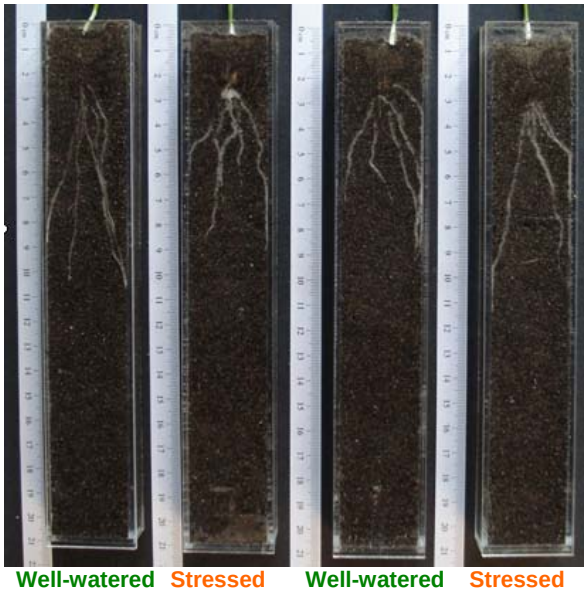
Roots are intimately associated with their environment and develop highly intricate branched networks that enable them to explore the soil. Root growth parameters such as cell division and elongation can be used to efficiently monitor the environmental stress levels. Root growth and morphology parameters can also be used to identify stress tolerant varieties.

In this demonstration we will first explore the benefits of using a "microrhizotron" which is an experimental tool to detect change of root growth parameters under various stress conditions such as elevated heat, salt stress or drought stress.

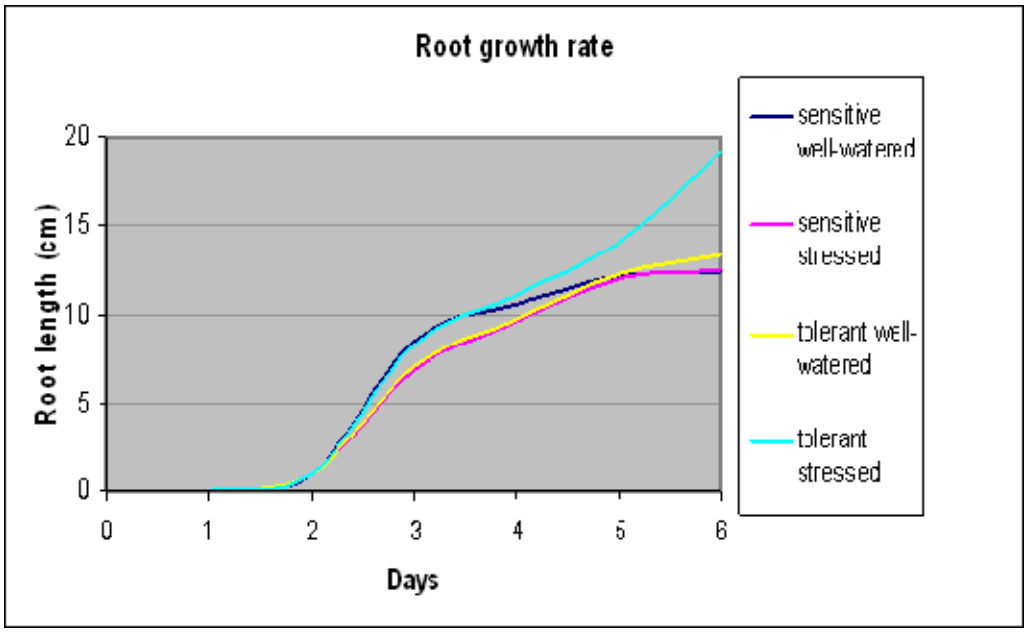
Well-watered: 60 % soil water content

Stressed: 20 % soil water content

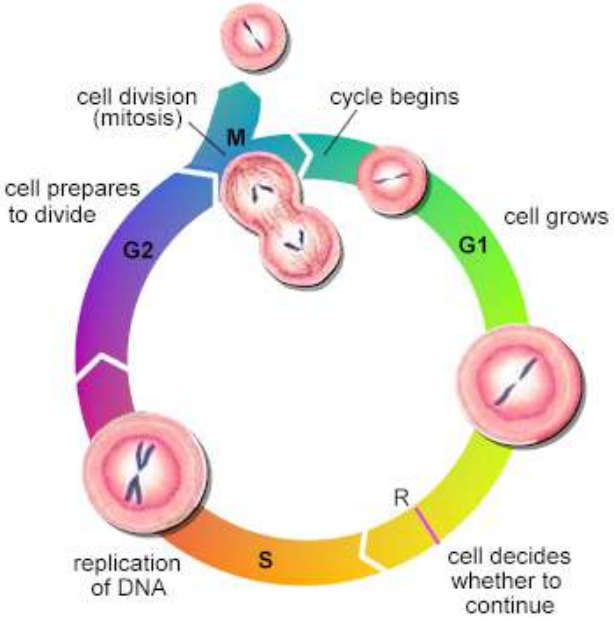
The morphology of the roots



Using this experimental system one can comparatively analyze various plant genotypes and growth conditions by measuring root growth and morphology parameters such as rate of root elongation, root thickness, root hairiness, root branching morphology, rate of lateral root formation, root curling and geotropic responses.



Apart from using above-mentioned parameters, cell division cycle in root tips can also be analyzed by monitoring the rate of DNA synthesis (during S-phase of cell cycle) or mitotic index (during mitotic phase) to assess growth rate and stress response of roots.



In this practical demonstration, we will focus our attention on DNA synthesis (S-phase) detection on wheat roots using a novel labeling method called EdU labeling. We will then visit Cellular Imaging Laboratory of Biological Research Center to analyze the labeled root tips using fluorescence stereo and confocal fluorescence microscopy techniques.

EdU (5-ethynyl-2'-deoxyuridine) analysis of S-phase cells in wheat root meristem:

1. Incubation of root tips of wheat seedlings for 2 hours with 20 µM EdU (5-ethynyl-2'-deoxyuridine) solution.

2. Fixation with formaldehyde in a detergent-containing buffer: 4 % formaldehyde, 1X PBS buffer (from 10X PBS buffer), 0.5 % detergent (Triton-X). Incubation time: 30 minutes.

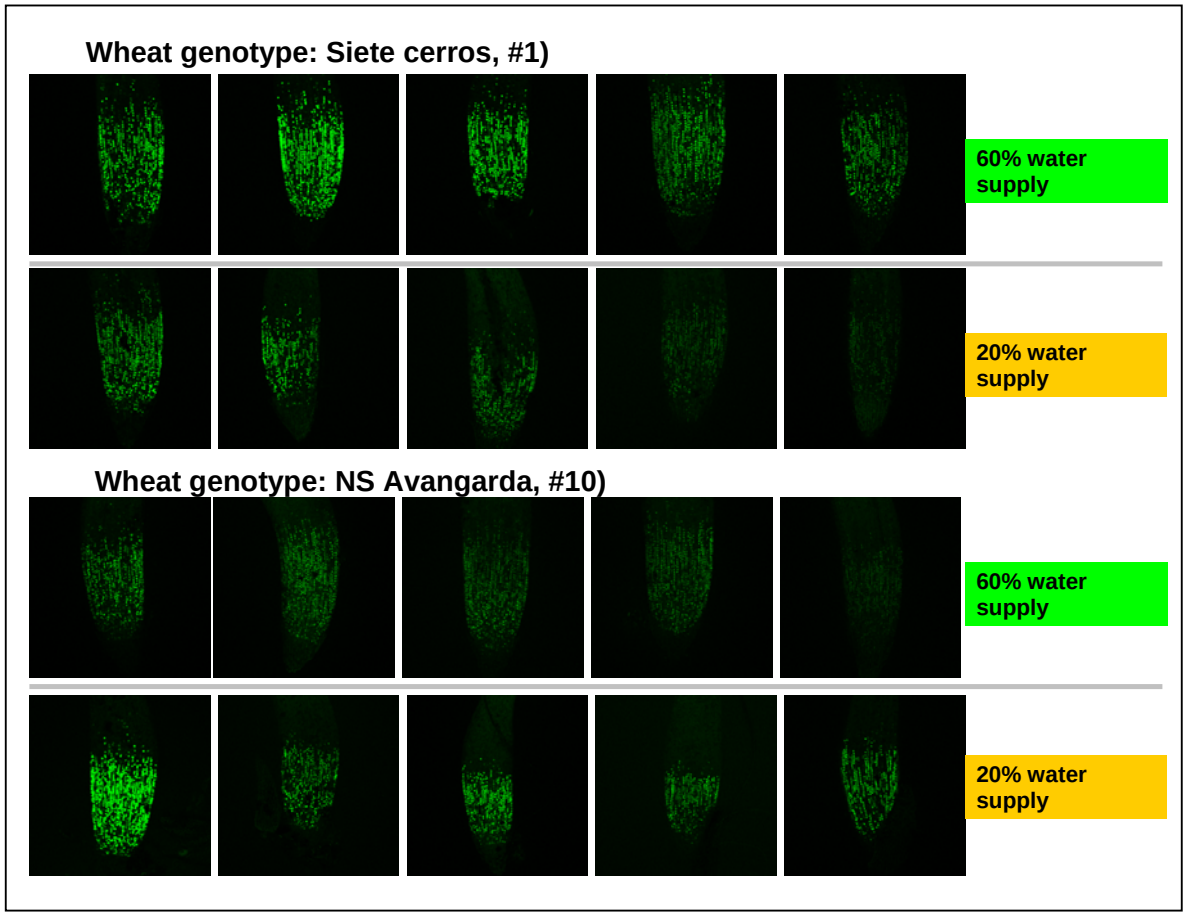
3. Washing with 1X PBS (2-3 times).

4. Incubation in EdU-detection reagent (1 hour), which contains an azide-conjugated fluorochrome (such as Alexa Fluor 488) and copper (I) as the catalyzer of the click reaction.

5. Reaction mix for 1 sample contains: 144 µl distilled water, 1.6 µl buffer additive, 14 µl reaction buffer, 6.7 µl copper(II) sulphate, 0.07 µl Alexa Fluor 488 azide (green).

6. Washing with 1X PBS (3-4 times).

7. EdU-labeled meristematic regions of roots were easily detectable with a fluorescence stereo microscope.



Confocal laser scanning and fluorescence stereo microscopy

Confocal laser scanning microscopy (CLSM) is one of the most ubiquitous research tools in basic cell biology. Mobility, dynamics, interaction and localization of living cells, organelles and even single molecules can be recorded with high precision due to properties like high resolution, sensitivity and optical sectioning capability.

In this practical demonstration, we will first analyze the EdU-labeled wheat root samples using fluorescence stereo microscope then we will analyze them in detail using Olympus FV1000 laser scanning confocal microscope. Applications of fluorescence microscopy in live

cell analysis of plant cells will also be discussed.

Olympus SZX12 fluorescence stereo microscope

Olympus FV1000 confocal laser scanning microscope.