New insights into phototropism from experiments in microgravity and fractional gravity on the ISS
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Darwin: “The Power of Movement in Plants”

**TROPISMS** - directed growth in response to stimulus

- **Thigmotropism** (touch)
- **Phototropism** (light)
- **Gravitropism** (gravity)
TROPISMS - directed growth in response to stimulus

phototropism

gravitropism
British plant physiologist Malcolm Wilkins wrote that without an effective gravity vector, such as in space laboratories in low Earth orbit, the “true nature of phototropism will finally be revealed” (Wilkins, 1988).
Phototropism Studies in Space

• Heathcote (1992)
  IML-1 (STS-42); wheat coleoptiles

• Sack & Kern (1997)
  CUE (STS-87); moss protonema

• Kiss, Edelmann & Hangarter (2006)
  Increment 14 (ISS); Arabidopsis hypocotyls

• Kiss, Edelmann & Correll (2010)
  Increment 22 (ISS); Arabidopsis hypocotyls & roots
Significance--TROPI

1. Basic questions in sensory physiology
2. Bioregenerative life support
3. Exploration
TROPI: Objectives

1. Characterize phototropism without the “complications” of gravity.

2. Better understand signal transduction pathways in gravity & light in plants.
Preplastid--Biorack
STS-81 (1997)

Plastid--Biorack
STS-84 (1997)

TROPI-1-EMCS
STS-121 (2006)
STS-115
STS-116 (2007)
STS-117
STS-120

TROPI-2--EMCS
STS-130 (2010)
STS-131

BRIC-16
STS-131 (2010)
TROPI Experiments: Arabidopsis seedlings

Date of experiment
- TROPI-1: October - December 2006 (ISS Increment 14)
- TROPI-2: February - March 2010 (ISS Increment 22)

Gravity conditions studied
- TROPI-1: μg, 1g
- TROPI-2: μg, fractional-g, 1g
TROPI-1

• Basic idea: photoresponses can be studied in the absence of gravity.
European Modular Cultivation System

• We were first group to use this facility.
Experiment Unique Equipment (EUE)
Soaked with nutrient medium & hydrated on ISS

Seeds were placed on this; dark color provides better contrast for video recording

Heater prevents fogging to ensure high-quality video
Post-flight Procedures

1. Recording medium—phototropism
   - Capture still images from video
   - Analysis of germination, growth, curvature
     [Data obtained on hypocotyls—not roots]

2. Frozen samples—gene profiling
   - Analyze how various light & gravity treatments affect gene expression.
     This will involve DNA microarrays.
Phototropism

**White light:**
- **Shoot:** Positive phototropism
- **Root:** Negative phototropism

**Red light:**
- No phototropic response

**Blue light:**
- **Shoot:** Positive phototropism
- **Root:** Negative phototropism

[ phot1 & phot2 ]
Phototropism: How do plants perceive light?

Photoreceptors

Phototropins → Cryptochromes → Phytochromes

Phototropism → De-etiolation → Photoperiodism
TROPI timeline

Start of experiment: [http://www.youtube.com/watch?v=w7I3OnRdTCQ](http://www.youtube.com/watch?v=w7I3OnRdTCQ)

- Show movie
- Fractional gravity runs cancelled due to poor seed germination
Post-flight Procedures

1. Video Analysis

• Video downlinks (improved from TROPI-1)
• Analysis of germination, growth, curvature

2. Frozen samples.

• Analyze how various light & gravity treatments affect gene expression. This will involve DNA microarrays (powerful technique).
Positive phototropic curvature in hypocotyls in response to red light in microgravity

Curvature in μg is significantly different relative to the:
(1) 1-g control & (2) baseline of 0.

Moss protonemata detect the direction of red light via phytochromes

- Plants from older lineages, such as mosses and ferns, have a versatile directional response to both red and blue light.
Hypothesis: Red-light-induced phototropism, normally found in ancient plant lineages, is masked by 1-g conditions but also occurs in flowering plants.

http://www.nibb.ac.jp/~evodevo/
Microarray Studies

• Genes in plant hormone pathways are affected by microgravity: auxin, cytokinin, ethylene, & brassionsteroids.
• Genes in important developmental pathways are affected by microgravity: photo-perception, heat shock, plastid development, root hairs, among others.
• The down-regulated genes (long vs. short exposure to μg) can be representatives of early-inducible genes in gravity-related pathways in plants.
• Analysis still in progress.
• Fractional gravity studies performed.
Video Downlinks from ISS

TROPI-1

TROPI-2

- Improved seed germination & seedling growth in TROPI-2!
Comparison Table

<table>
<thead>
<tr>
<th></th>
<th>TROPI-1</th>
<th>TROPI-2</th>
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</thead>
<tbody>
<tr>
<td>Date of experiment</td>
<td>October - December 2006 (ISS Increment 14)</td>
<td>February - March 2010 (ISS Increment 22)</td>
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<tr>
<td>Storage time of seeds in spaceflight</td>
<td>6 - 9 months (3 runs)</td>
<td>2 months (2 runs)</td>
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<tr>
<td>hardware</td>
<td></td>
<td></td>
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<tr>
<td>Number of Seeds</td>
<td>1680</td>
<td>1120</td>
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<tr>
<td>Growth medium</td>
<td>Murashige-Skoog medium with 1% sucrose</td>
<td>Murashige-Skoog medium with no sucrose</td>
</tr>
<tr>
<td>Age of spaceflight hardware</td>
<td>1 year</td>
<td>5 years</td>
</tr>
<tr>
<td>Gravity conditions studied</td>
<td>$\mu g, 1g$</td>
<td>$\mu g, fractional-g, 1g$</td>
</tr>
<tr>
<td>Video data collection procedures</td>
<td>hi-8 video tapes</td>
<td>direct video downlink from ISS</td>
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<tr>
<td>Cryo-transfer in ISS</td>
<td>return 1: &gt; 3 min</td>
<td>&lt; 3 min</td>
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<tr>
<td></td>
<td>return 2, 3: &lt; 3 min</td>
<td></td>
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<tr>
<td>Cryo-storage in space shuttle</td>
<td>NASA Cold Bag (approx. -35°C )</td>
<td>GLACIER (set at -80°C )</td>
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</tbody>
</table>

***Longer storage time of seeds resulted in increased off-gassing of materials used in the circuit boards and other components found in the EUE.
Seed germination & length was greater in TROPI-2
Red-based phototropic curvature in μg in hypocotyls confirmed in TROPI-2
Positive Curvature in Response to Red Light in Microgravity
Roots: Improved system allowed for imaging in TROPI 2

Roots also exhibit positive phototropic curvature in microgravity
Hypocotyls: Response is attenuated at 0.3 g

Roots: Response is attenuated at 0.1 g
Phytochromes

- Absorb red light
- Molecular switching mechanism
- Small gene family: *PHY-A* to *PHY-E*
**Hypocotyls:**
PHY-B attenuates the response

**Roots:**
PHY-A and PHY-B promote the response
Hypocotyls:
Little or no promotion of the response in $\mu g$

Roots:
Response is attenuated at $0.3 \, g$
Phototropism & nutation occur in microgravity

**Nutation** = oscillatory or helical growth pattern around an axis

http://youtu.be/FZYiKu8tDW4
Frozen Samples:
Good quality RNA for Microarray Analysis

MELFI on ISS

GLACIER on Shuttle

In Progress:
Microarray Analysis
Positive phototropic response to red light in μg was confirmed in hypocotyls (and roots).

Hypothesis: directional red-light-sensing, found in ancient plant lineages, is masked by normal 1g conditions in the more recently evolved lineages.

Red-light phototropism in hypocotyls and blue-light phototropism in roots is attenuated at 0.3g. In contrast, red-light phototropism in roots is attenuated at 0.1g.

Phytochromes are involved in red-light phototropism in both roots and hypocotyls.

These studies are the first to examine plant behavior in fractional gravity, and in the long term, may provide basic knowledge towards growing plants on Moon/Mars.
Thanks to: