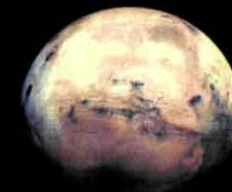




The Influence of Microgravity on Plants

Howard G. Levine, Ph.D.

NASA Sustainable Systems Office
Space Life Sciences Laboratory
Mail Code NE-S-1
Kennedy Space Center, FL 32899



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We will eventually go back to the Moon and Mars and there will be Moon and Mars bases that will grow and become more complex over time.



Launching food makes less sense as mission duration lengthens

Providing a continuous supply of food, oxygen, and clean water for humans in space is a costly proposition.

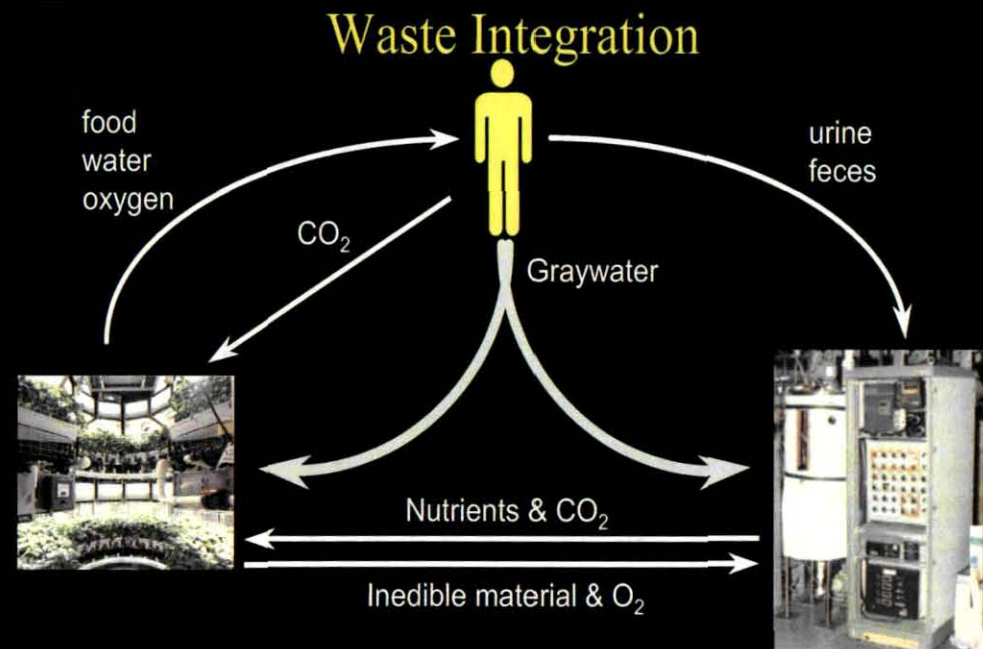
To date these needs have been met largely through stowage and resupply.

As the durations of missions increase, the costs associated with this approach become prohibitive.

For this reason, virtually all scenarios for long-term space missions involve plants as key components of the life support environment.

Plants will be used to recycle wastes, remove carbon dioxide, purify water and produce oxygen and food for astronauts.

The understanding of both the science and engineering issues necessary to build a reliable system where plants represent a key component is critical to successful operation.



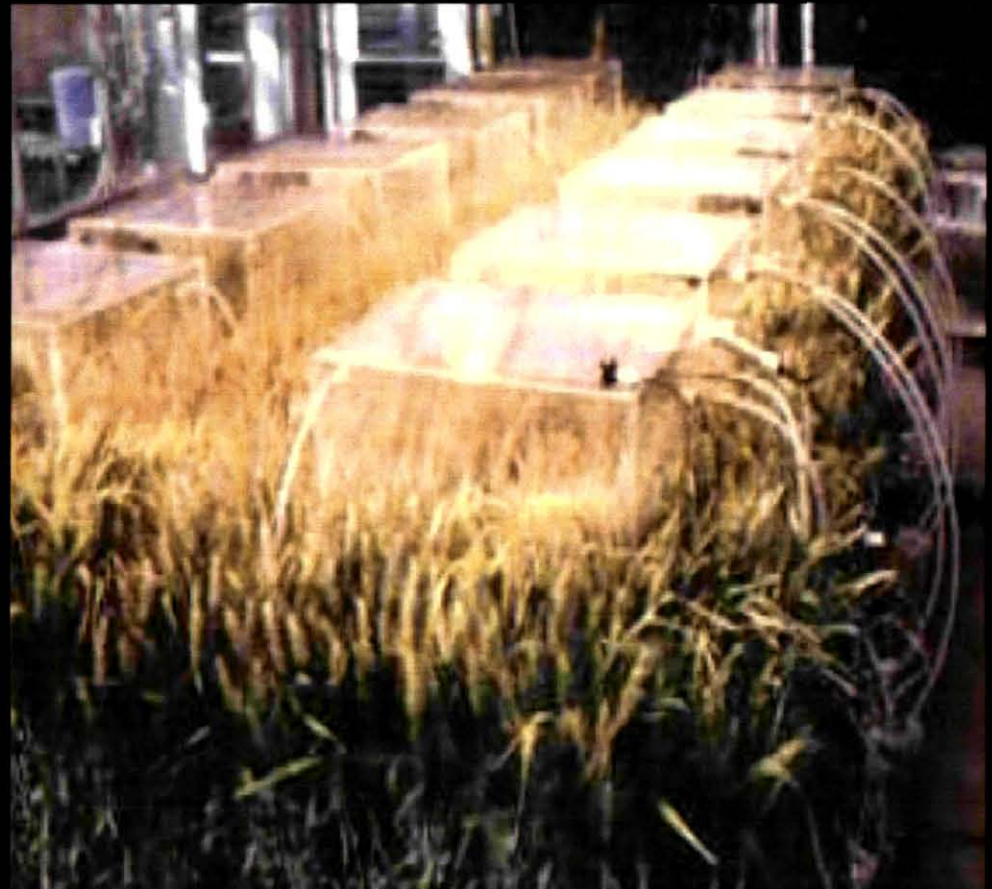
Some findings from NASA Ground Testing

Recirculating Hydroponics



- 1) Conserve Water & Nutrients
- 2) Eliminate Water Stress
- 3) Optimize Mineral Nutrition
- 4) Facilitate Harvesting

High Light & CO₂ Produce High Yields

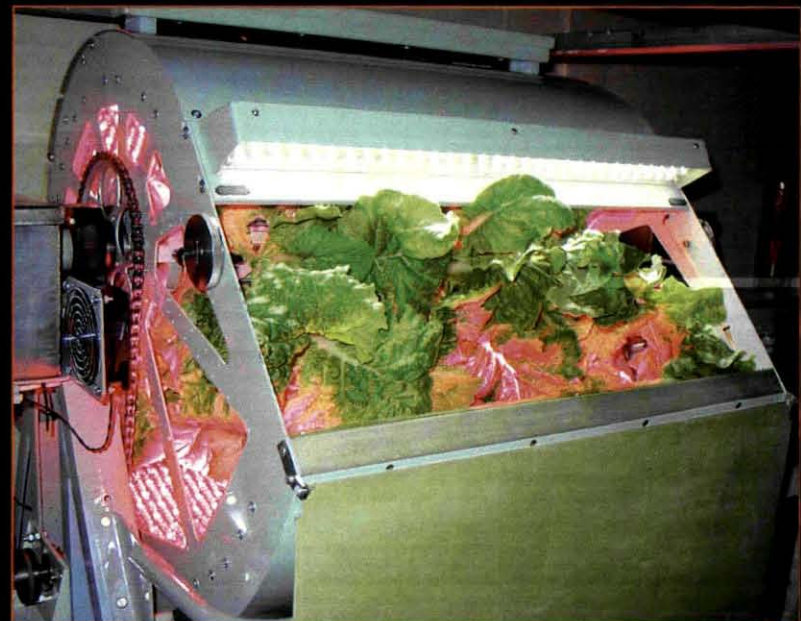
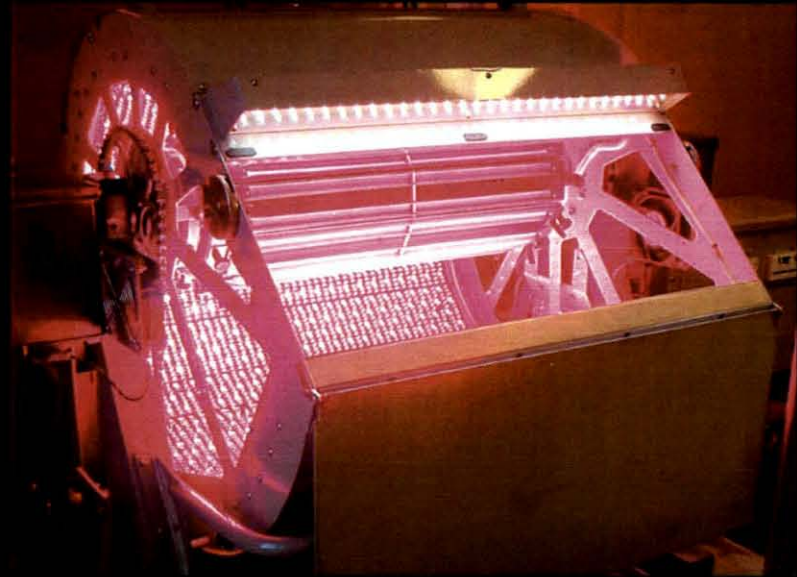


- 1) Wheat: 3-4x World Record
- 2) Potato: 2x World Record
- 3) Lettuce: Exceeded Commercial Yields

KSC Biomass Production Chamber (BPC)



Psychological Value of "Salad Machines" (Vegetable Production Units)



Targets for Plant-Related Life Support Applications

Mission	Plant Contribution	Comments
ISS	- Dietary Supplement	Salad Machine Electric Lighting
Transit Vehicles	- Dietary Supplement - Water Processing?	Salad Machine Electric or Direct Lighting ?
Planetary Surface (Near- Term)	- ~5-10% Food Prod. - ~100% Water Processing	Large Garden System Electric Lighting or Small Greenhouses
Planetary Surface (Mid-Term)	- ~50% Food Prod - ~100% O ₂ Production - ~100% Water Processing	Intermediate Greenhouse Suplmt. Electric Lighting
Planetary Surface (Far-term)	- 90% Food Prod. - ~100% O ₂ Production - ~100% Water Processing	Large Greenhouse Suplmt. Electric Lighting Nuclear Power ?

“Direct” vs “Indirect” Effects of Spaceflight on Plants

Our ability to grow plants in space has improved greatly

- but many fundamental processes of plant adaptations to spaceflight environments are only beginning to be understood.
- These issues relate to both the ***direct effects*** of microgravity on plant development and physiology
- and ***indirect effects*** of space environments
 - tightly closed atmospheres that can accumulate volatile organics
 - poor water and air movement through rooting media
 - elevated radiation levels
 - spectral effects of electric lighting systems, etc.



Ethylene (and VOC) scrubbing was found to be critical for successful seed production (SVET Studies).

Key Questions

How does the gravity environment shape the way plants grow and reproduce?

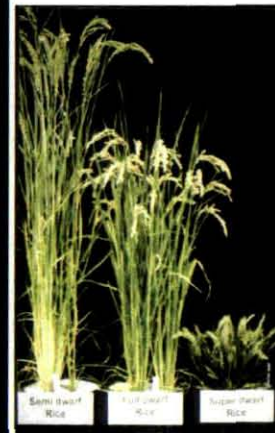
How will the microgravity of space alter the way plants grow and reproduce?

- Plants use gravity to direct the growth of roots and stems, which requires gravity perception, the transfer of this information to the sites of reaction, and reaction to the signal by the responding cells.
- NASA-sponsored studies of graviperception in plants have concentrated on identifying the cells that perceive gravity, determining the threshold values for graviperception, and investigating how the direction of the gravity vector is perceived.



Growing plants within the Astroculture plant chamber on ISS.

→ Cultivar Selection and Development :



Several Universities:
Cultivar Comparisons
(wheat, potato, soybean,
lettuce, sweetpotato, tomato)

Utah State:
Super Dwarf Wheat
Apogee Wheat
Perigee Wheat
Super Dwarf Rice

Tuskegee:
ASP Sweetpotato



Types of Plant Tropisms

Gravitropism

Shoot Gravitropism
(Negative = Away from Gravity)

Root Gravitropism
(Positive = Toward Gravity)

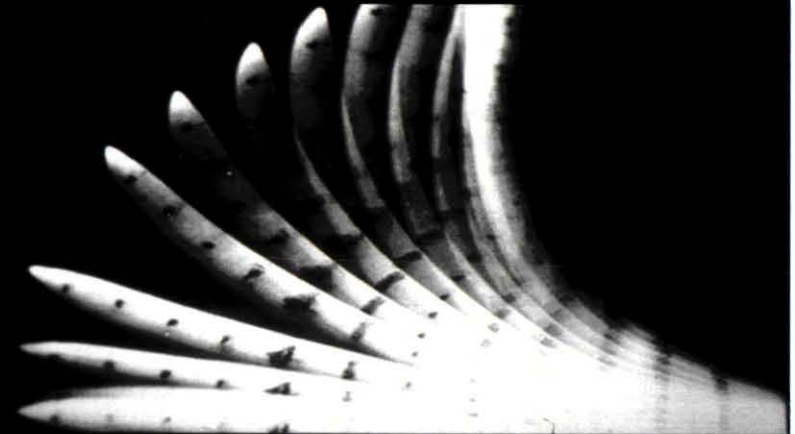
Phototropism

Shoot Phototropism
(Positive = Toward Light)

Root Phototropism
(????????)

Hydrotropism

Root Hydrotropism
(Positive = Toward Water)



Corn Shoot (60 minutes)

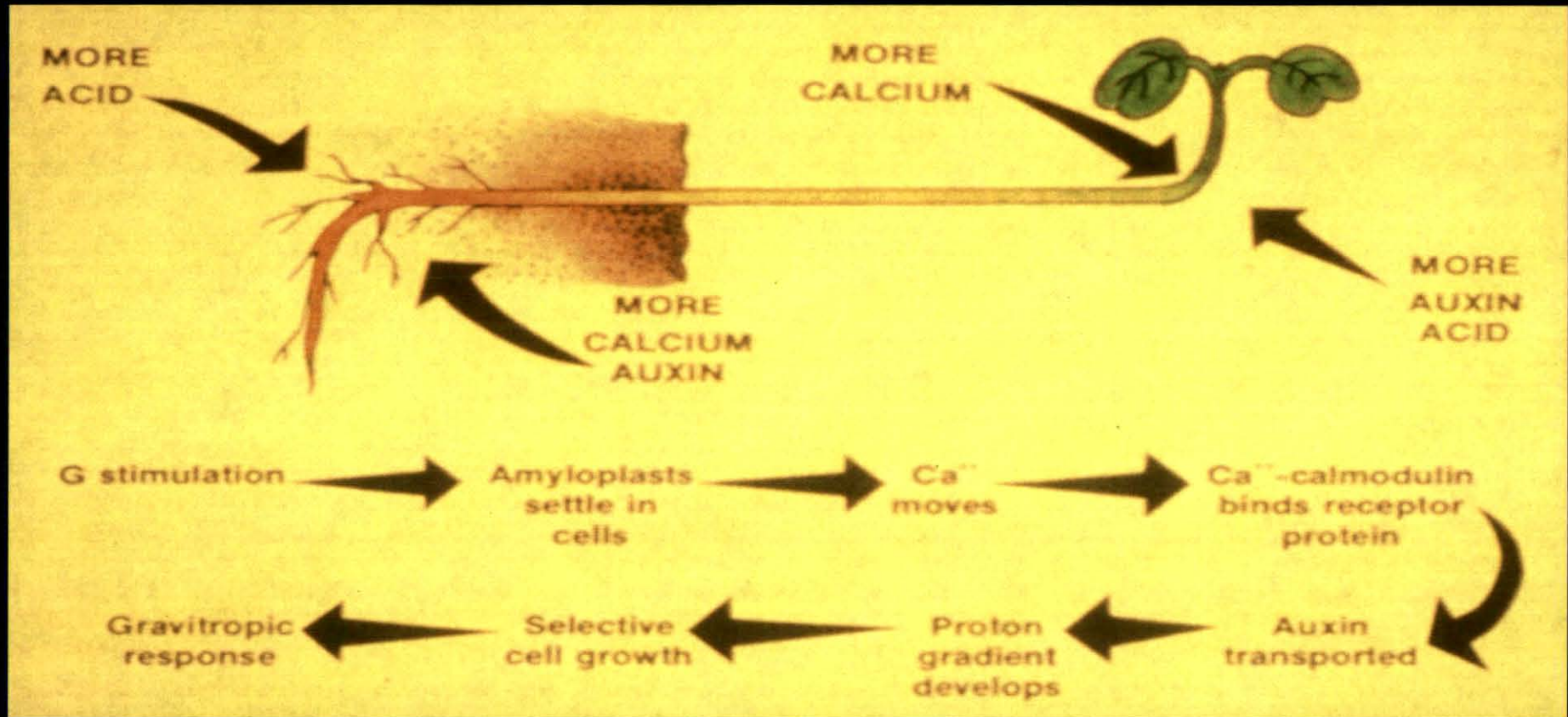


Root (90 minutes)

The microgravity of space has also been used to investigate and clarify phenomena (such as phototropism) that are obfuscated in the presence of gravity.

The fundamental knowledge gained through these investigations aids in our ability to better control plant use on earth in agriculture (and other) applications.

Model of Plant Gravity Perception



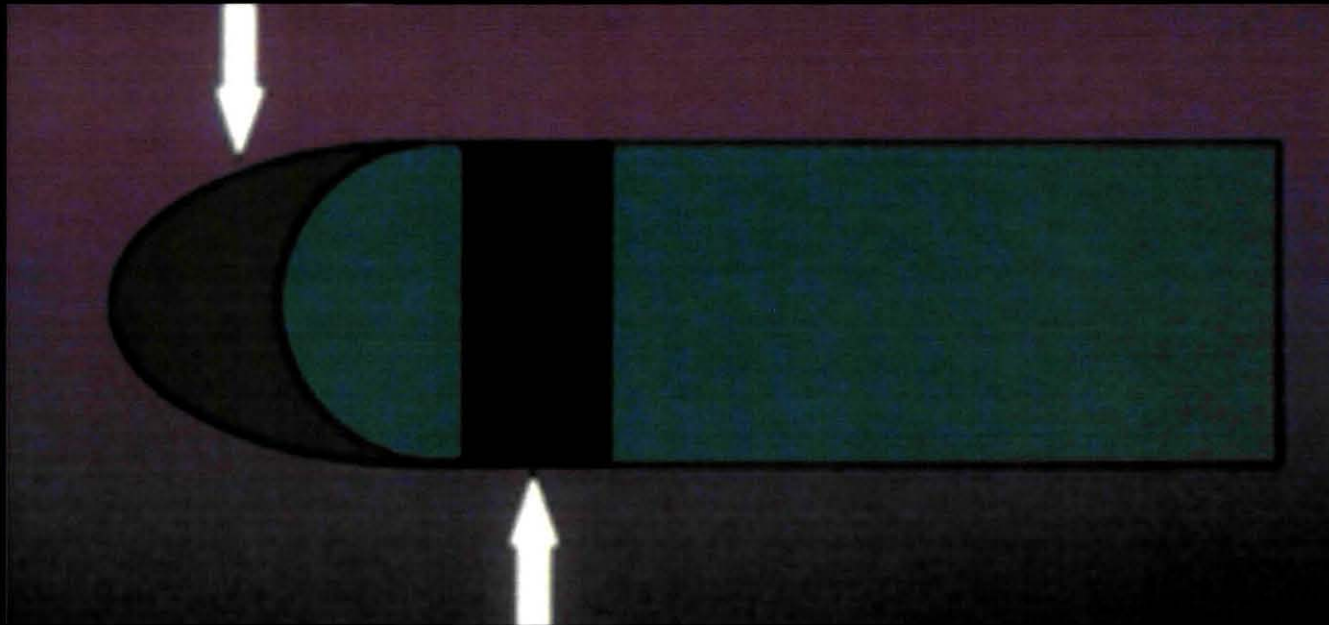
Millions of dollars are lost each year because wind & rain cause crops to fall over making them difficult/impossible to harvest. Plants can recover from this by a gravity response that allows them to reorient their shoot growth in an upright direction. Discovering the mechanisms by which this happens can allow scientists to develop crop plants that have stronger and faster gravity responses and are less susceptible to this.

Root growth downward is key to their being able to locate and take up water.

Root Gravitropism

There is a spatial separation of where it is believed gravity is perceived (the root cap) and where the growth response occurs (further back along the root).

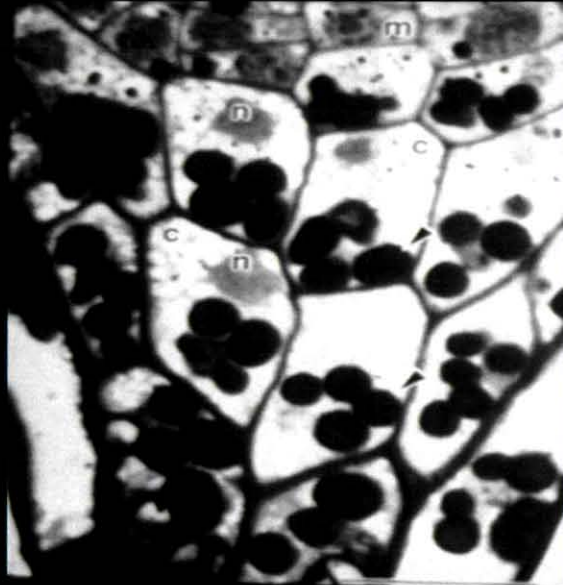
Root Cap



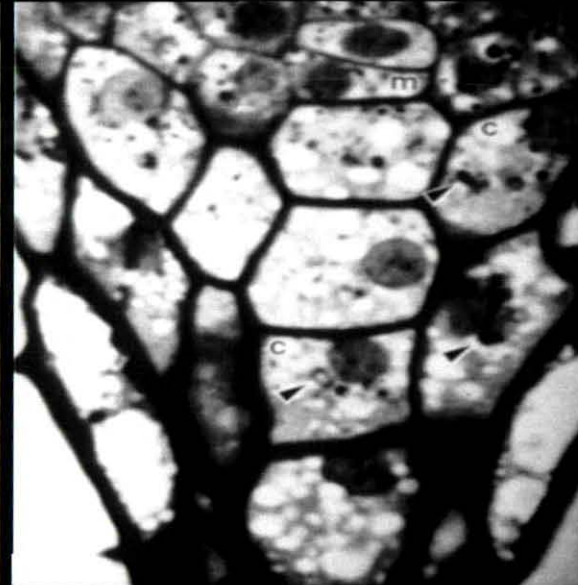
Area of Growth Response

How do Roots Perceive Gravity?

Root Tip of
Normal Plant



Root Tip of Starchless
Mutant Plant



Plants store food mainly as starch in organelles called amyloplasts that are involved in the perception of gravity (left). They are denser than the cytoplasm and therefore fall to the lower parts of the cells.

Starchless mutants respond much more sluggishly to gravity, and amyloplasts are therefore involved but not absolutely critical to the gravity response.

Positive Gravitropism in Roots

Top = 0 minutes after placing root horizontally

Bottom = 90 minutes after placing root horizontally



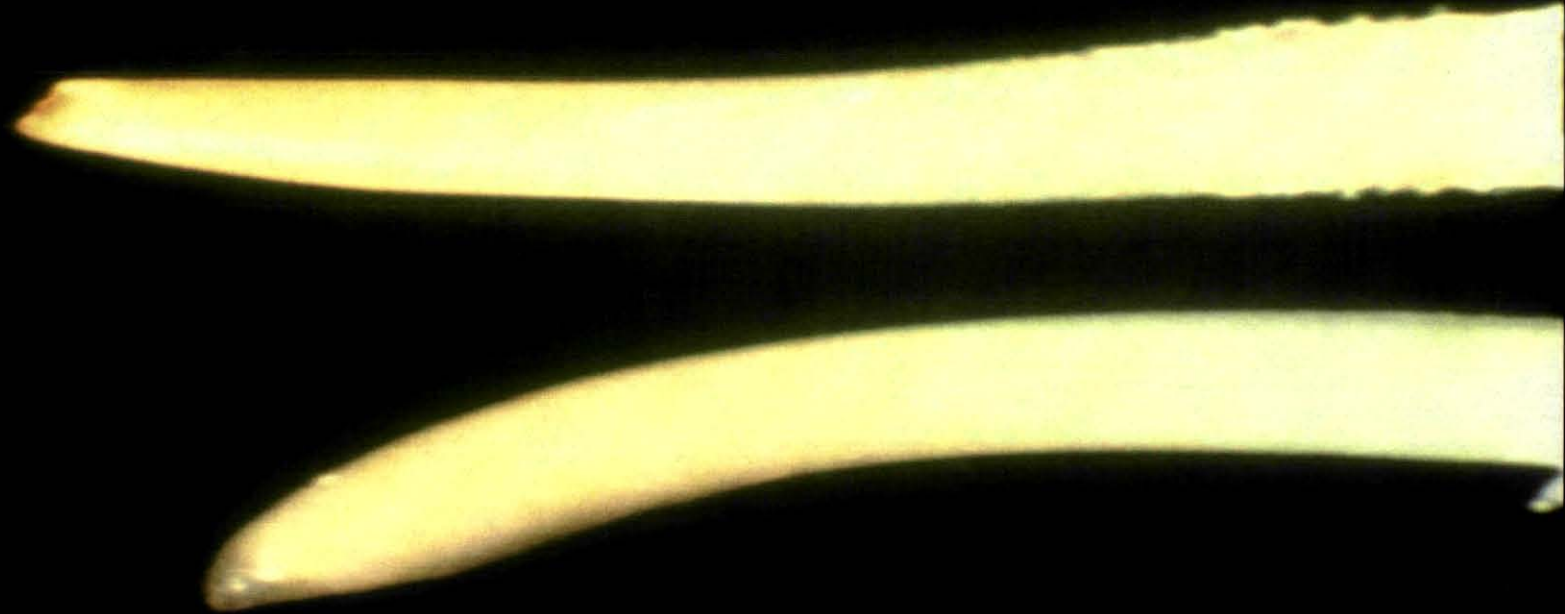
When oriented horizontally, roots adjust their growth pattern.

The growth rate along the top of the root exceeds that along the bottom causing the root to bend downward.

Effect of Root Cap Removal

Root Cap removed from top root.

Root Cap not removed from bottom root.



Both roots placed horizontally and photographed 90 minutes later.

Growth continued in both roots, but the gravity response only occurred in the root with its root cap intact.

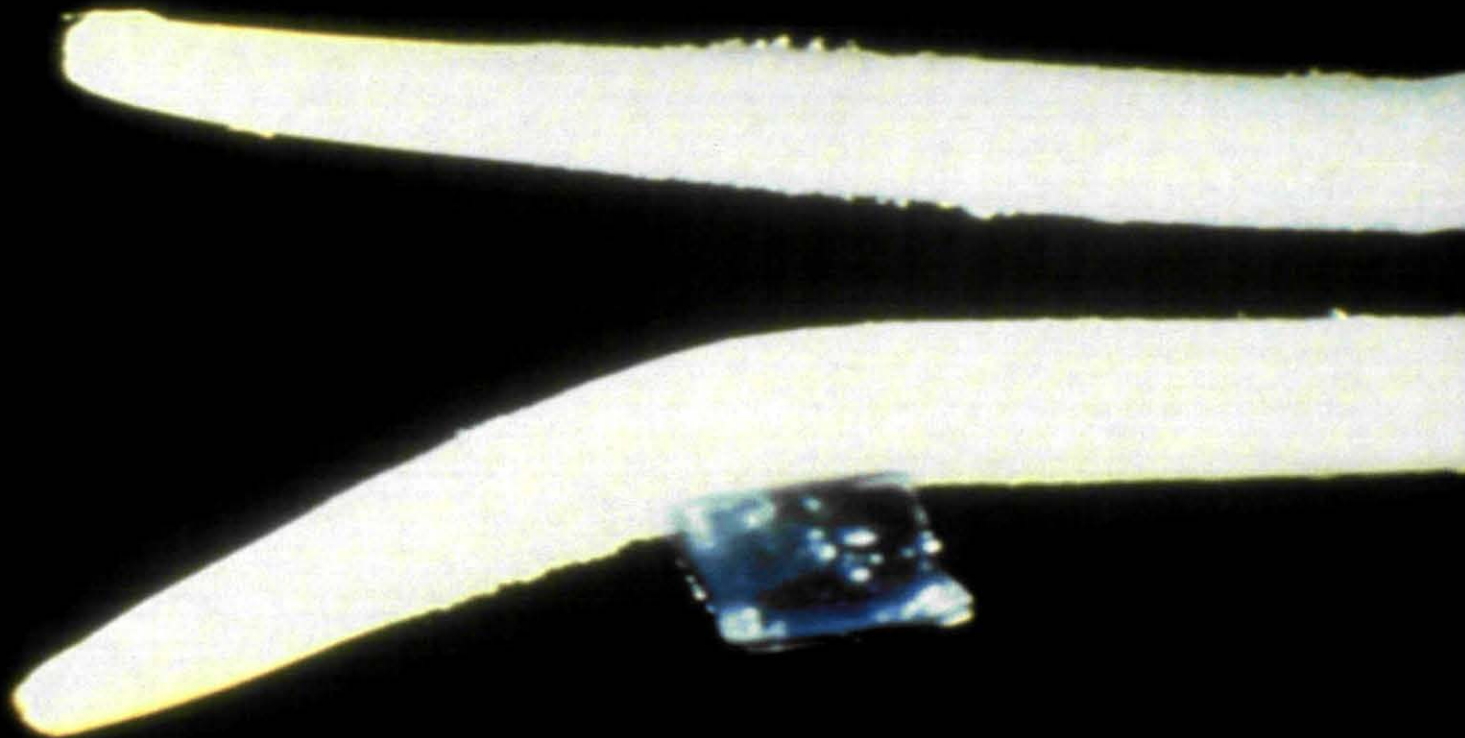
Theory: The root cap produces a plant hormone = auxin that inhibits root growth on the lower side of the root.

Test of Auxin Gravitropism Theory

Root Cap removed from both roots \Rightarrow placed horizontally.

No auxin application to top root.

Auxin applied to bottom side of bottom root.

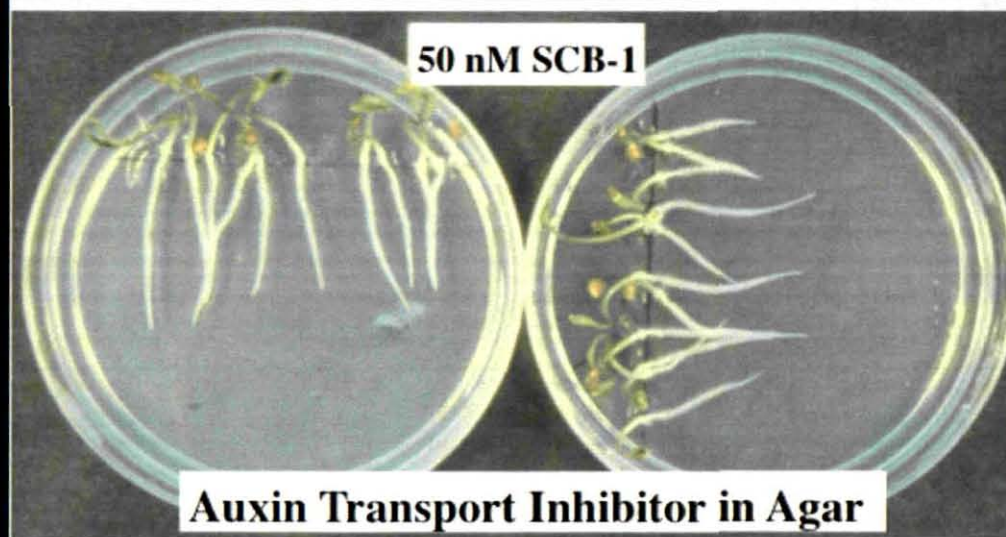


This experiment supports the theory that in normal intact roots, gravitropic curvature might result from accumulation of auxin along the lower side of the root.

Test of Auxin Gravitropism Theory

Seedlings grown 48 Hrs
Same Orientation

Seedlings grown 24 Hrs
⇒ Rotated 90°



Effects of Microgravity on Plant Secondary Metabolism

Secondary metabolism has been found to be affected by conditions of altered gravity (both hypo- and hypergravity).

Even though plants of *B. rapa* grown and fixed on ISS exhibited levels of growth, leaf chlorophyll, starch and soluble carbohydrates comparable to ground control treatments, levels of glucosinolate production (specifically 3-butenylglucosinolate) was on average 75% greater in flight samples than in ground control samples.

Similarly, the biochemical make-up of immature seeds produced during spaceflight and fixed or frozen while in orbit was significantly different from the ground controls.

The immature seeds from the spaceflight treatment had higher concentrations of chlorophyll, starch, and soluble carbohydrates than the ground controls.

Seed protein was significantly lower in the spaceflight material.

Microscopy of immature seeds fixed in flight showed embryos to be at a range of developmental stages, while the ground control embryos had all reached the premature stage of development.

Storage reserve deposition was more advanced in the ground control seeds.

The spaceflight environment thus influences *B. rapa* metabolite production in ways that may affect flavor and nutritional quality of potential space produce (23).

Long-Term Space Exposure To Seeds

Long-term (MIR for 6 years) exposure of dormant tomato seeds to space resulted in significant differences in growth and development relative to ground controls in terms of plant fertility and yield variability. Also reported were TEM-observed differences in cell walls, chloroplasts and mitochondria (3).

In addition, these tomato seeds were reported to have DNA variations relative to the ground controls (4).

It is believable that seeds exposed to space for such long intervals would experience detrimental effects relative to ground controls. The results for short duration exposures, however, are not quite so clear (see below).

Short-Term Space Exposure Results

There are a number of publications coming out of the Chinese space research efforts that make claims of obtaining genetic mutants from plants/seeds flown in short duration space experiments (5-9).

This conflicts with results obtained by American and Russian investigators (10-13).

Why?

The use of Spaceflight to study Endogenous Plant Movement

Mechanisms that dictate plant growth patterns include gravitropism, circumnutation and negative thigmotropism (14-15).

Studies with *Arabidopsis* flown on ISS have shown that gravity is important in amplifying minute oscillatory movements in microgravity into high-amplitude circumnutations (16), but movements still occur in the absence of gravity.

Investigations in space have revealed new facets of leaf movements that occur in the absence of gravity, with ultradian patterns, effects of transitions to darkness or light, and several heretofore unknown types and frequencies of movements (17).

The interaction between gravitropism and phototropism in *Arabidopsis thaliana* has been studied on ISS in the TROPI experiment using the European Modular Cultivation System (EMCS) in 2006 (18). I have not yet seen any published results teasing apart gravitropism and phototropism, but this project has the potential to do so.

This area of plant tropisms and endogenous movements can benefit greatly by the use of microgravity to eliminate one of the parameters (gravity) obfuscating results in earth-based experiments.

Plant Ontogenesis and Reproductive Functions (Viable Seed Production) in Space

In studies conducted within hardware possessing rather limited environmental control, plants frequently required special environmental conditions to permit fertilization and early seed development during spaceflight such as carbon dioxide enrichment and an air-exchange system (19).

In contrast with these earlier studies, many plant experiments in recent years have reported plant growth, development, reproductive functions and production of viable seeds in space was comparable to ground controls (20).

This is often due to improvements in hardware for the culture of plants in space.

Yet there are still reports of differences attributable to the spaceflight environment. For instance, *Brassica* seeds and pollen produced in microgravity were found to be physiologically younger than those produced in 1 g. It was speculated that microgravity limits mixing of the gaseous microenvironments inside the closed tissues and that the resulting gas composition surrounding the seeds and pollen retards their development (21).

Similarly, abnormalities in the process of embryo formation and acceleration in development of the endosperm were revealed at the early stages of embryogenesis in microgravity (22).

Root Length Enhancement

Plants grown under conditions of microgravity have been found to exhibit enhanced root production relative to their ground control counterparts (24-28).

Why is this? Is there a physiological basis or is it due to a spaceflight-associated artifact (more even distribution of moisture in the root zone?).

In a post-flight examination of medium samples extracted from the root zone of plants grown in space, there was a significant two-fold difference between the final concentrations of potassium when the Earth-based and microgravity experiments were contrasted (29).

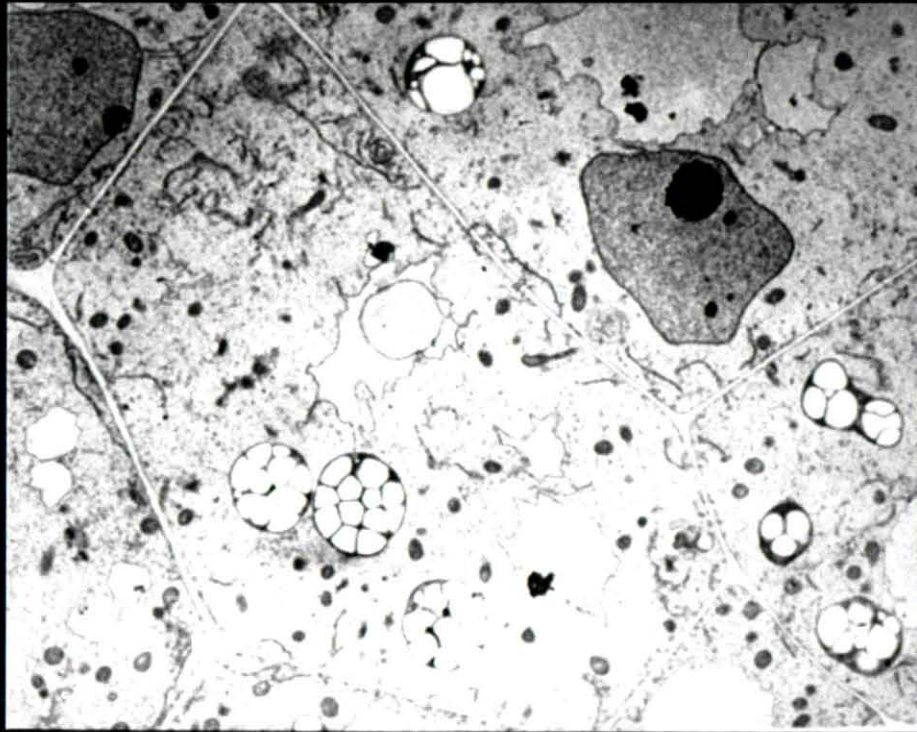
Why is this? Is there a physiological basis or is it due to a spaceflight-associated artifact (more root production in space resulting in more potassium uptake)?

One study pertinent to this question germinated and fixed *Arabidopsis thaliana* in space and also reported that the length of seedlings grown at 1g were conspicuously shorter than parallel samples grown under microgravity. These investigators found that the root cortical cells proliferated at a higher rate and their nucleoli were more active than those of stele cells. While the stele showed longer cells with larger nucleoli in the flight samples, cortical cells from space-grown seedlings were shorter, more numerous and more densely packed than ground controls. However, nucleoli were smaller and less active in fast proliferating flight cells than in the ground controls. It was speculated that the reduced level of ribosome synthesis in the flight samples was probably the result of an accelerated cell cycle (30).

How does the Microgravity Environment affect Root Cell Structure?

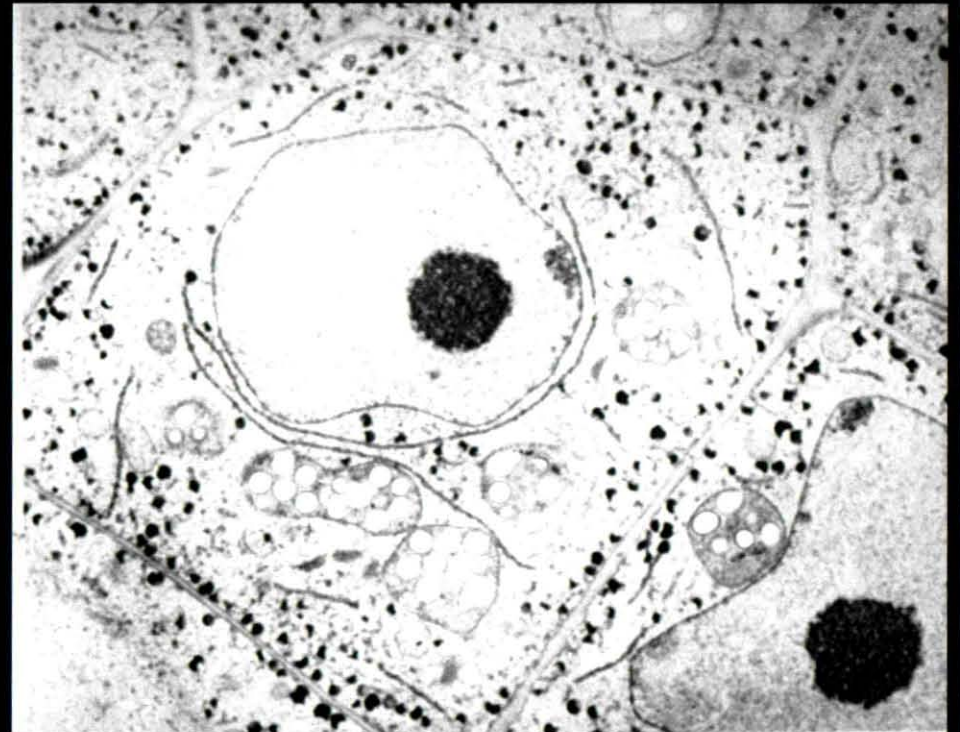
EM of root cell normal on Earth

TEM of a corn cortical root cell. Plant cells store food mainly as starch in organelles called amyloplasts. Some is stored as oil. Note that in this slide the amyloplasts each contain many granules of starch, the white structures inside the amyloplasts, and there are relatively few droplets of oil.



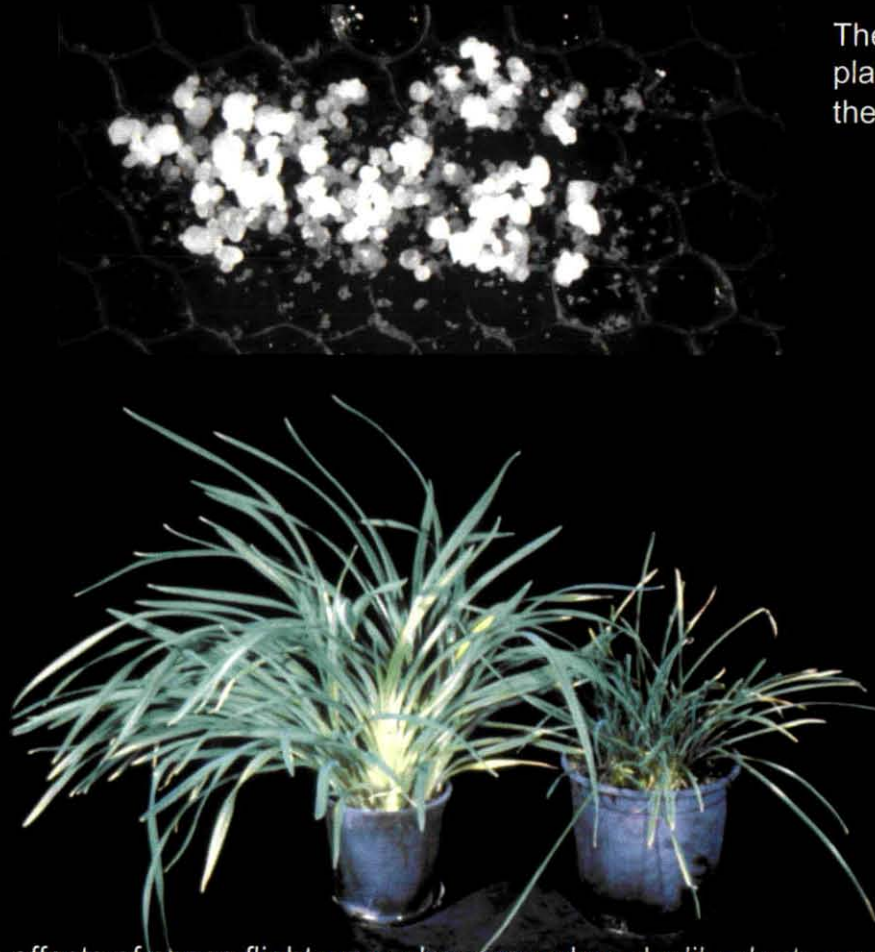
EM of root cell from micro-g

TEM of a corn cortical root cell from a plant grown in microgravity. Note that the amyloplasts contain less starch and that there is an abundance of oil droplets. Biologists believe that these kind of cytological changes are indications that the space environment somehow disrupts normal carbohydrate metabolism.

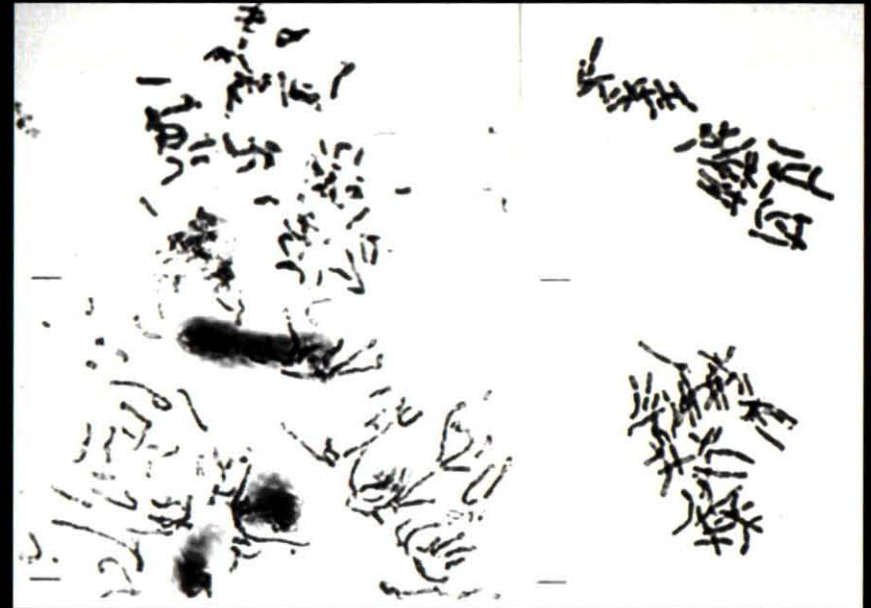


Effects of Spaceflight on Mitosis and Chromosome Behavior

The effects of space flight were also seen when daylily plants were grown from the somatic embryos exposed to the space environment (left).



The effects of space flight were also seen when daylily plants were grown from the somatic embryos exposed to the space environment. The plant on the left is a normal daylily. The plant on the right grown from space-exposed cells is clearly different. All the indications are that a mutational event or events occurred in the space-flown material. A key unanswered question in these daylily experiments is whether the chromosomal damage observed was due to microgravity or due to some other aspect of the space environment, for example higher ionizing radiation.



Chromosomal karyotypes showed evidence of progressive damage in developing somatic embryos of daylily. In the upper left-hand corner, you can see metaphase division figure showing morphology of control or undamaged cells. In the upper right, a division figure shows perturbations in structure of the chromosomes. Lower left and lower right pictures show chromosome structural deterioration and fracturing that signifies serious damage to the integrity of the cell's genetic material. Cells as badly damaged as these would not survive to divide again.

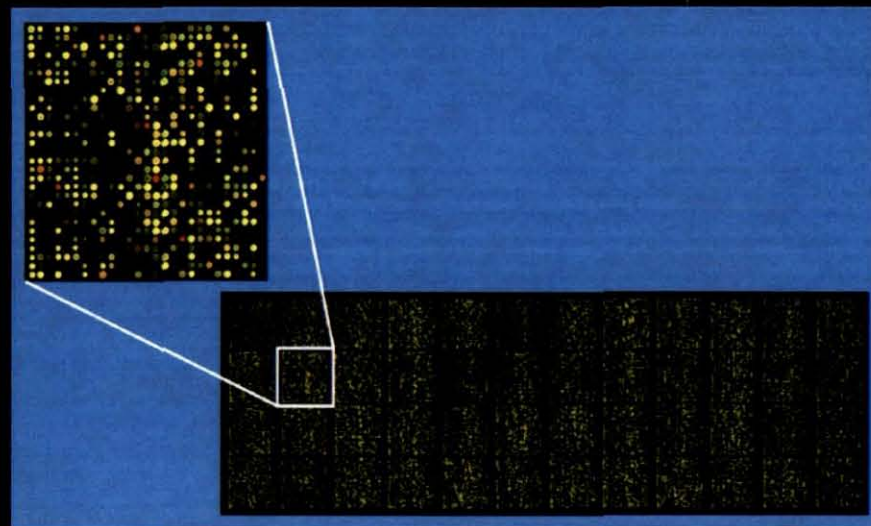
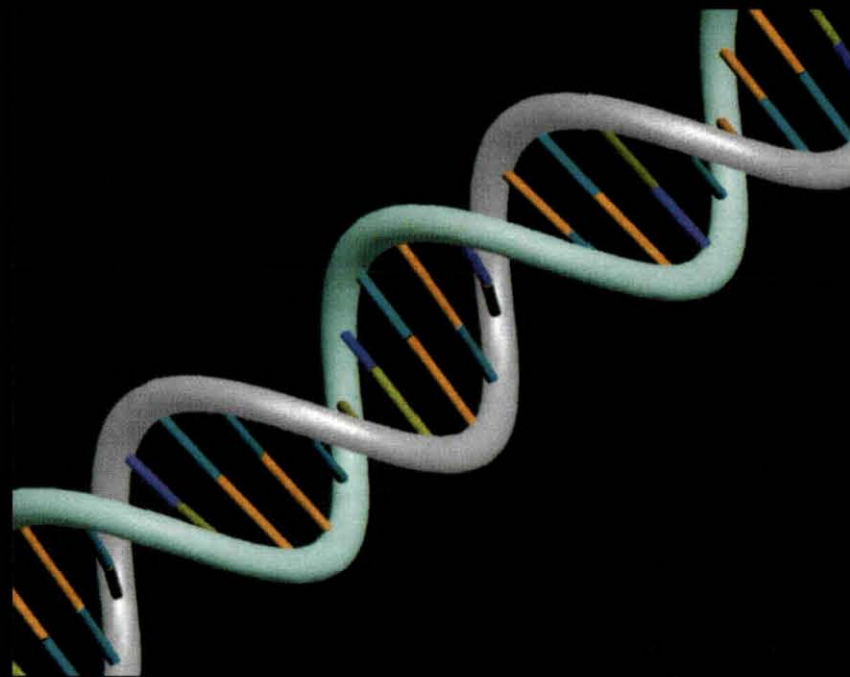
Gene Expression Results

Plants processed at recovery after spaceflight have usually shown significant levels of altered gene expression (1).

These investigators found that some genes related to heat shock were dramatically induced – but in a pattern and under growth conditions that were not easily explained by elevated temperatures (2).

Why? Also, why were the PESTO post-flight gene expression results (based on tissues placed into RNAlater on orbit) not in agreement (eg the flight and ground control gene expression patterns were similar)?

More work should be done teasing apart why the regulation of certain genes are altered by spaceflight conditions.

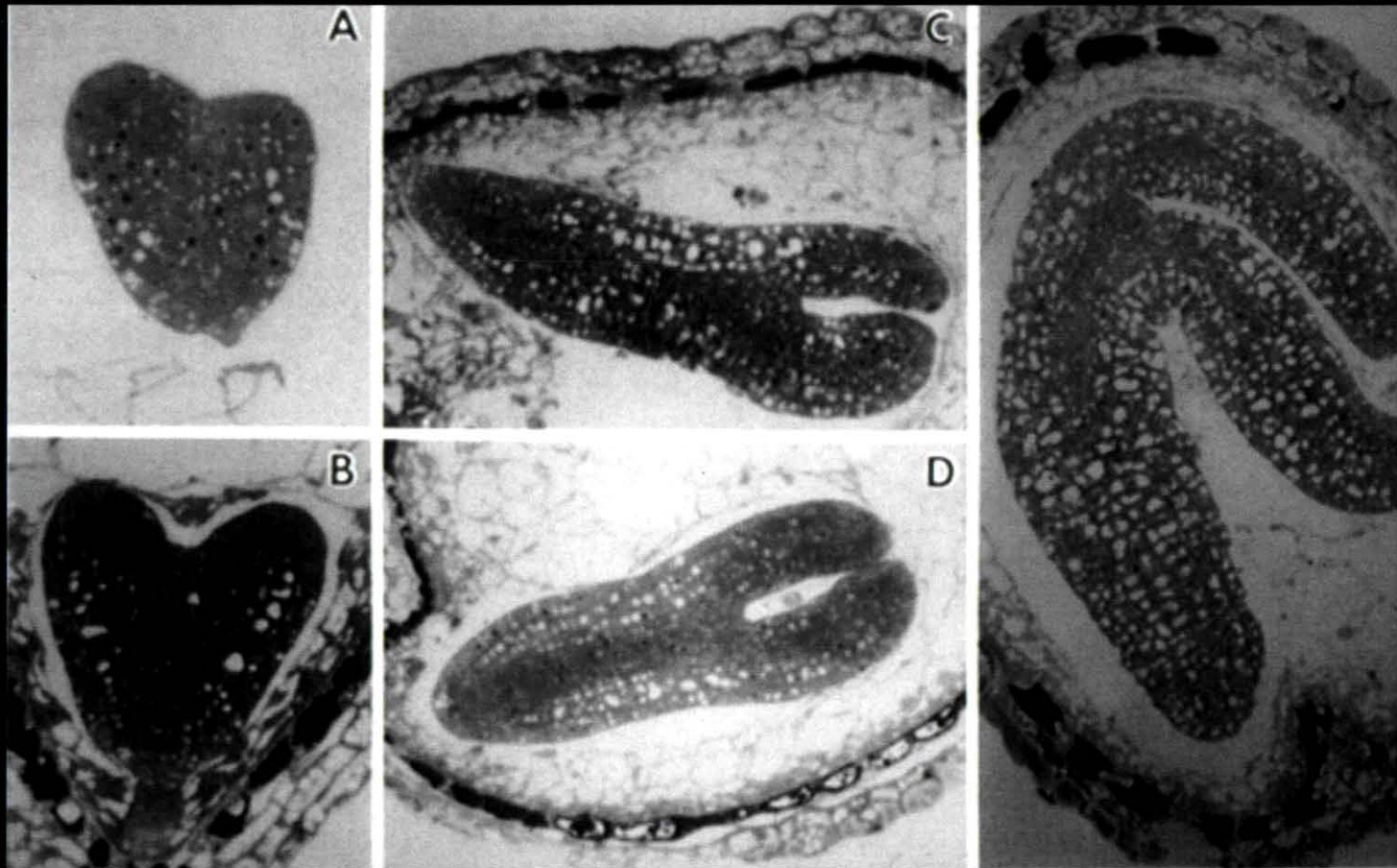


Pollen and Seed Development (Musgrave et. al.)

Can Arabidopsis carry out normal reproductive processes in space?



Plant Embryogenesis Under Microgravity Conditions



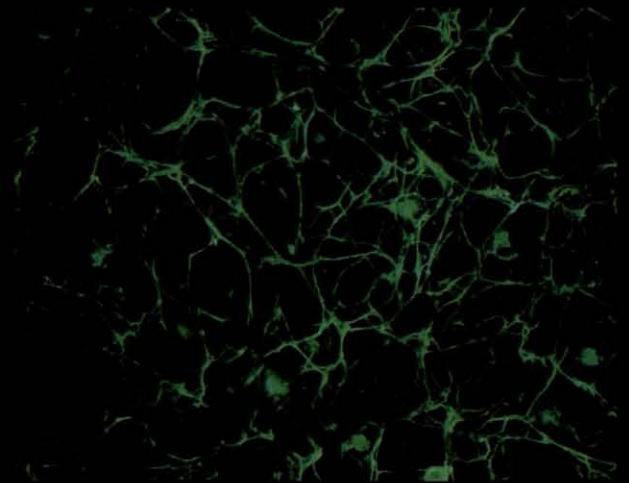
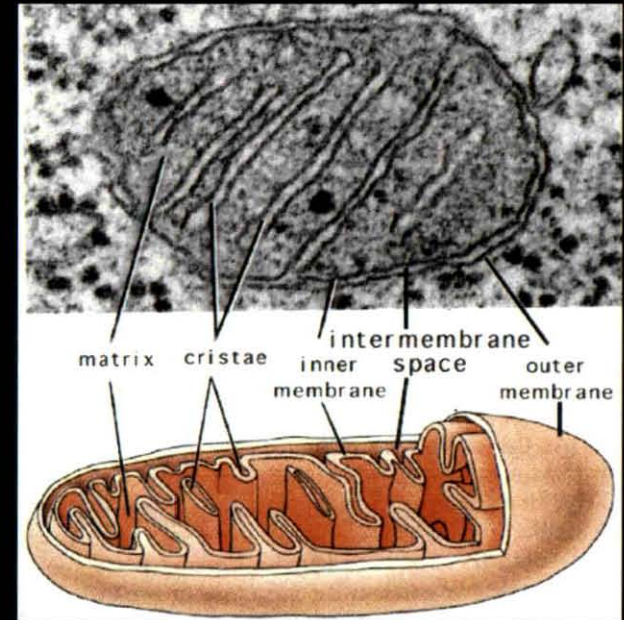
A, C, E = Embryogenesis in seeds of plants grown in microgravity (STS-68) were similar to that of control plants on Earth (B, D), representing the first report of successful plant reproduction on the Space Shuttle. Earlier failures point to the importance of enhancing carbohydrate nutrition and gas exchange for plants growing in microgravity. The absence of buoyance-driven convective air movement at microgravity probably results in a limitation on the rate of movement of metabolic gases, and this in turn, negatively affects reproductive development.

Ultrastructure Results

Another category of unsolved questions relates to differences seen in the ultrastructural organization of plant tissues flown in space. For example, mitochondria in root statocytes of soybean seedlings grown in space were characterized as round or oviform and with low electron density of organelle matrix, whereas the organelles in the ground controls were polymorphic in shape and had higher electron density of matrix (31).

Soybeans grown in space have also exhibited changes in vascular development and cell wall deposition.

Differences were conspicuous in developing vessels at the ultrastructural level, leading to speculation that the orientation of microfibrils and their assembly in developing vessels are perturbed by microgravity at the beginning of wall deposition, while they are still able to orient and arrange in thicker and ordered structures at later stages of secondary wall deposition (32).



Plant Photosynthesis, Respiration, Transpiration in Space

Although not considered to be show stoppers in terms of the potential for plants to function in Bioregenerative Life Support Systems (BLSS) in space, differences have been found the intricacies of photosynthesis in space.

For instance, despite observing a total leaf area that was significantly reduced in microgravity-grown wheat plants once closure of the canopy was achieved, rates of evapotranspiration, net photosynthesis and water use efficiency were not significantly different than ground controls, i.e., all of the available light was still absorbed by the flight plant stand (33).

While these authors found that single leaf measurements showed no differences in photosynthetic activity at moderate (up to $600 \text{ micromol m}^{-2} \text{ s}^{-1}$) light levels, there was reduction in whole chain electron transport (13%), PSII (13%), and PSI (16%) activities observed under saturating light conditions ($>2,000 \text{ micromol m}^{-2} \text{ s}^{-1}$) and CO_2 ($4000 \text{ micromol mol}^{-1}$) conditions, suggesting that microgravity-induced responses at the canopy level may occur at higher PPF intensity (34).

Similarly, an earlier study (35) found that wheat plants grown in space exhibited CO_2 -saturated photosynthetic rates at saturating light intensities that declined 25% relative to the ground control plants. The light compensation point of the spaceflight leaves increased by 33%, which likely was due to an increase (27%) in leaf dark-respiration rates. Related experiments with thylakoids isolated from space-grown plants showed that the light-saturated photosynthetic electron transport rate from H_2O through photosystems II and I was reduced by 28%, demonstrating that photosynthetic functions are affected by the microgravity environment.

CONCLUSIONS

- The use of plants for space-based life support presents multiple challenges, and there are numerous aspects of plant adaptation to spaceflight and closed environments that are not yet fully understood.
- The ISS provides the opportunity to solve many of these issues, especially given the availability of new hardware that can provide more precise environmental control and sustain larger plants for multiple production cycles.
- The solving of these challenges will be critical for the establishment of long-term extraterrestrial colonies that will become practical only when plant-based bioregeneration is utilized.



Thank you for your attention.

Questions?

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NASA ISS Research Academy and Pre-application Meeting

August 3-5, 2010

South Shore Harbour Resort & Conference Center

2500 South Shore Blvd.

League City, TX 77573

(Preliminary Agenda)

COURSE DESCRIPTION

The objectives of the NASA ISS Research Academy are:

1. To provide new principal investigators and payload developers an overview of the capabilities of the ISS for research and the unique advantages of ISS for doing groundbreaking research.
2. To educate the participants about available ISS research opportunities and the process to follow to apply for these opportunities from the following agencies: NASA, the National Institutes of Health (NIH) and the National Science Foundation (NSF).
3. To introduce new principal investigators to the various implementation partners that can provide hardware for different disciplines of research. The PI's and IP's will have time to evaluate the ability of different types of hardware to accomplish specific research objectives of the PI.
4. To provide new ISS Principal Investigators (PIs), Project Scientists (PSs) and Payload Developers (PDs) a good understanding of the NASA ISS Payload Planning, Integration and Operation Process.
5. To inform PIs, PSs and PDs about several services that the NASA ISS Payloads Office has available as part of the overall Payload Integration Process to help them during their payload integration process for a successful vehicle-payload integration and on-orbit operations.

THE FOLLOWING TOPICS WILL BE COVERED:

Day One: Microgravity Science day

1.	ISS Payloads Office Overview	W. Rod Jones/JSC	8:00 am
2.	ISS On Orbit Facilities	J. Robinson/JSC	8:15 am
3.	Microgravity Influence on Biological Systems:		
	a. Cellular Biology	N. Pellis/JSC	9:00 am
	b. Macromolecular Crystal Growth	T. Miller/MSFC	9:30 am
	Break		10:00 am
	c. Plants	H. Levine/KSC	10:15 am
	d. Animals	TBD/ARC	10:45 am
	e. Impacts on Humans	J. Charles/JSC	11:15 am
	Lunch		11:45 am
4.	Microgravity Influence on Physical Systems:		
	a. Acceleration Environment and Effects	K. McPherson/GRC	12:45 pm
	b. Fluid Physics	B. Motil/GRC	1:15 pm
	c. Combustion Science	D. Urban/GRC	1:45 pm
	d. Materials Science	F. Szofran/MSFC	2:15 pm
	Break		2:45 pm
5.	External Research		
	a. Fundamental Physics	U. Israelsson/JPL	3:00 pm
	b. Earth Science	TBD/	3:30 pm
	c. Astrophysics	TBD/	4:00 pm
	d. Heliophysics	TBD/	4:30 pm
	e. Space Science	TBD/	5:00 pm

Day Two: Research Opportunities and Implementation Partners

6. ISS Research Opportunities: NASA	8:00 am
Break	9:30 am
7. ISS Research Opportunities: NIH	9:45 am
8. ISS Research Opportunities: NSF	11:00 am
Lunch	12:00 pm
9. ISS Research Opportunities Joint Discussion: NASA, NIH, NSF, and Attendees	1:00 pm
Break	1:45 pm
10. Implementation Partners: What and Who Are They and How They Can Help You	2:00 pm

Day Three: ISS Payload Integration Process Description, Process Improvement, and Best Practices (focus on NASA and Implementation Partners)

11. Research Planning Process	R. Lofton/JS	8:00 am
12. Mission Integration Process	G. Norris/JSC	8:30 am
13. Engineering Integration Process	M. Miller/JSC	9:00 am
14. Software Integration Process	A. Rice/MSFC	9:30 am
15. Lean Integration Process	A. Rice/MSFC	10:00 am
Break		10:30 am
16. Payload Safety Review Process	P. Mitchell/JSC	11:00 am
17. Payload Operations Integration Process	C. Price/MSFC	11:30 am
Lunch		12:00 pm
18. Launch and Landing Support	J. Wahlberg/KSC	1:00 pm
19. Real-Time Operations	C. Price/JSC	1:30 pm
20. Ground Facilities	A. Sledd/MSFC; T. St.Onge/GRC; M. Coats/SW	2:00 pm
Break		3:00 pm
21. Process Improvement Discussion and Best Practices		3:15 pm

REGISTRATION FEE:

There is no registration fee associated with this training. However, all expenses associated with travel, lodging, transportation and miscellaneous are the sole responsibility of the attendees. All training material will be provided in the form of a CD free of charge by NASA. For more information on this training, please click on the link below:

http://www.nasa.gov/mission_pages/station/science/nlab/nlab_conferences.html

HOW TO REGISTER:

To register for this training, please email your full name, title, affiliation, mailing address, telephone and fax numbers, email address and citizenship information to: jsc-iss-payloads-helpline@mail.nasa.gov Please put "NASA ISS Research Academy" in the subject field of your email. No paper registration will be accepted. Each applicant will receive an acknowledgement within two (2) business days as confirmation. If no confirmation is received, please call Roger Weiss at 281-244-6187 or 281-244-7716.

CONTACT:

If you need more information or have questions regarding this training, please contact:

Marybeth Edeen
Manager, ISS National Laboratory Office
marybeth.a.edeen@nasa.gov
Phone: 281-483-9122

PROCEEDINGS:

All lecture material will be distributed to attendees on the first day of training in the form of CDs.

ACCOMMODATION:

Hotel information will be sent to all attendees three weeks prior to starting of training

MEETING LOCATION:

Meeting location map will be sent to all attendees along with hotels accommodation information.

IMPLEMENTATION PARTNERS INFORMATION:

Any Implementation Partner who desires to rent a room to set-up his/her own displays should contact Kim Keen at the address below. Please inform Kim that your activity is being held in concert with the NASA-JSC event taken place on August 3-5 in the Amphitheater room. This will help in getting you a room located in close proximity to the Amphitheater room. For room rental information, please contact:

Kim Keen
South Shore Harbour and Conference Center
281-334-1000 Ext.# 2024