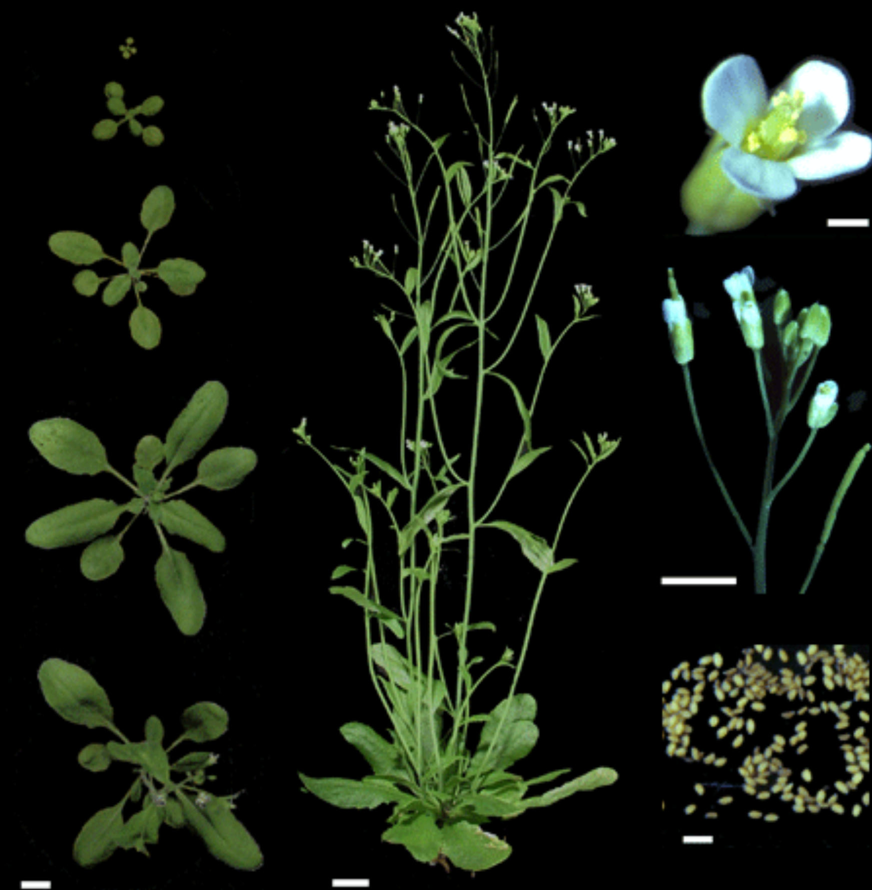
The Influence of Microgravity on Plants

Howard G. Levine, Ph.D.

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



Arabidopsis thaliana
Primary Plant Model Species

NASA ISS Research Academy and Pre-Application Meeting
South Shore Harbour Resort & Conference Center
League City, TX
August 3-5, 2010

Eventually … ➔ Moon & Mars ➔ Planetary Surface Bases ➔ Become More Complex Over Time

Launching Food Makes Less Sense as Mission Duration ↑

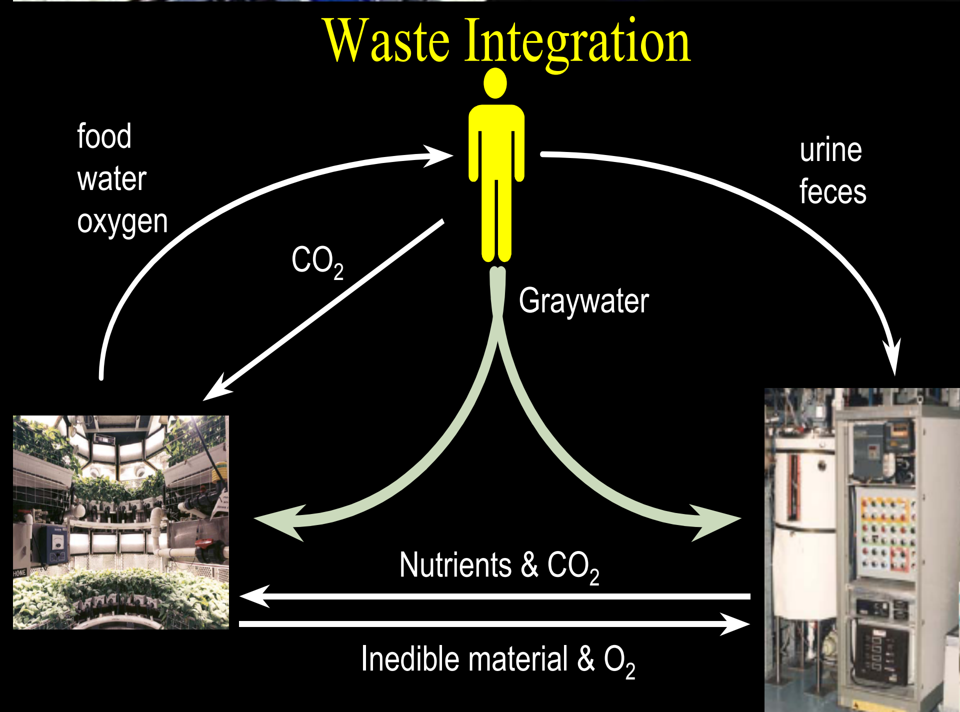
Providing Food, O_2 & H_2O in space
= costly

Currently → via stowage & resupply

As Mission Durations ↑ Costs ↑

Therefore:
Long-Term Missions
→ Plants = Key ALS Components →
recycle wastes, remove CO_2, purify water & produce O_2 & Food

Understanding **<u>BOTH</u>**
Science & Engineering Issues
→ Critical for Success



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 & CO_2 Produce High Yields

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

KSC Biomass Production Chamber (BPC)
(W. Knott, J. Sager, R. Wheeler, *et. al*)

Cultivar Selection & Development for Space Applications (B. Bugbee and others)

NASA has funded university researchers to perform cultivar comparisons (wheat, potato, soybean, lettuce, sweetpotato, tomato) and develop strains appropriate for spaceflight applications.

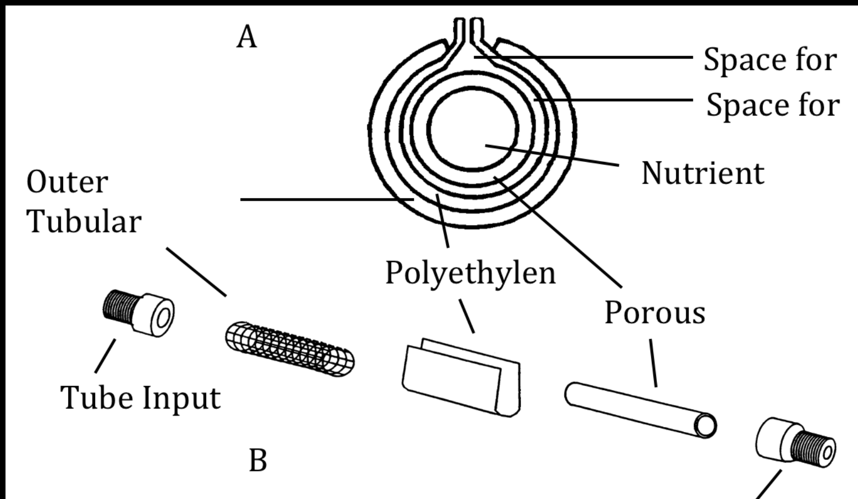
Utah State University

- Super Dwarf Wheat
- Apogee Wheat
- Perigee Wheat
- Super Dwarf Rice

Tuskegee University

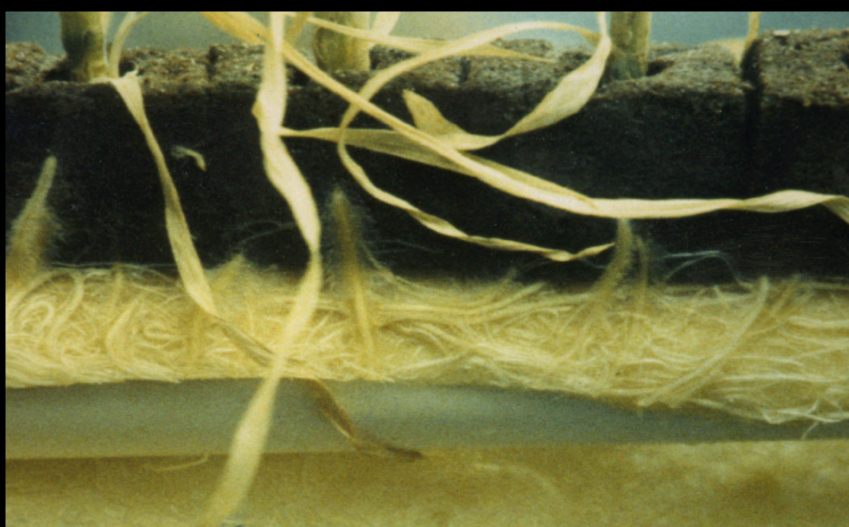
- ASP Sweetpotato

Substrate-Based & Porous Tube Nutrient Delivery Systems for Space



A. Cross section view of a porous tube (T. Dreschel).
B. Exploded View of porous tube components.

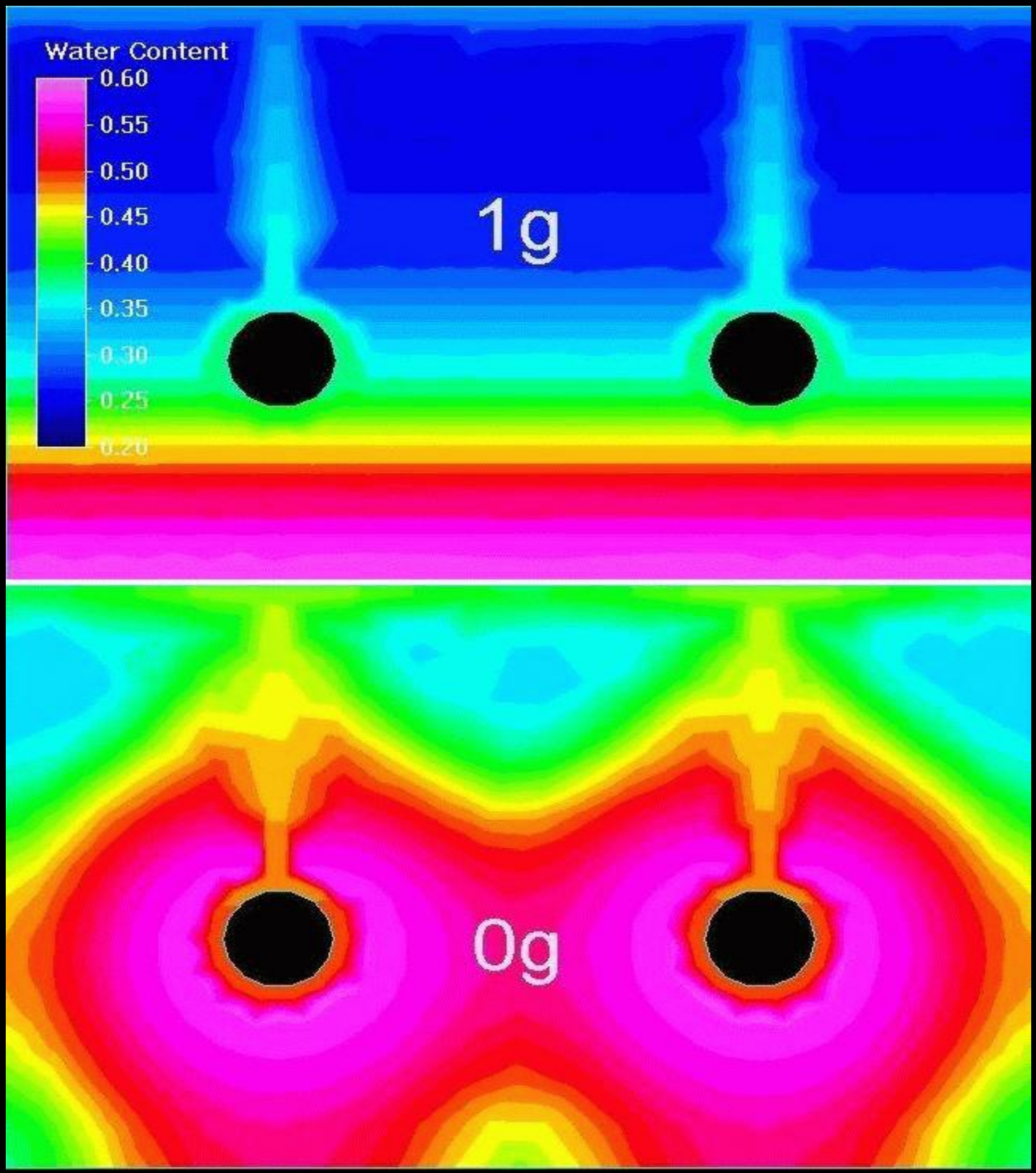
WCSAR Plant Tray with Wet (Left) and Dry (Right) Porous Tubes prior to Substrate addition.



A slice has been made along the root mass revealing the porous tube beneath (H. Levine).

Orbitec Plant Tray with Porous Tubes and Substrate containing slow release Nutrient Pellets.

Simulated Water Distribution in Svet Root Zone
(S. Jones & D. Or)



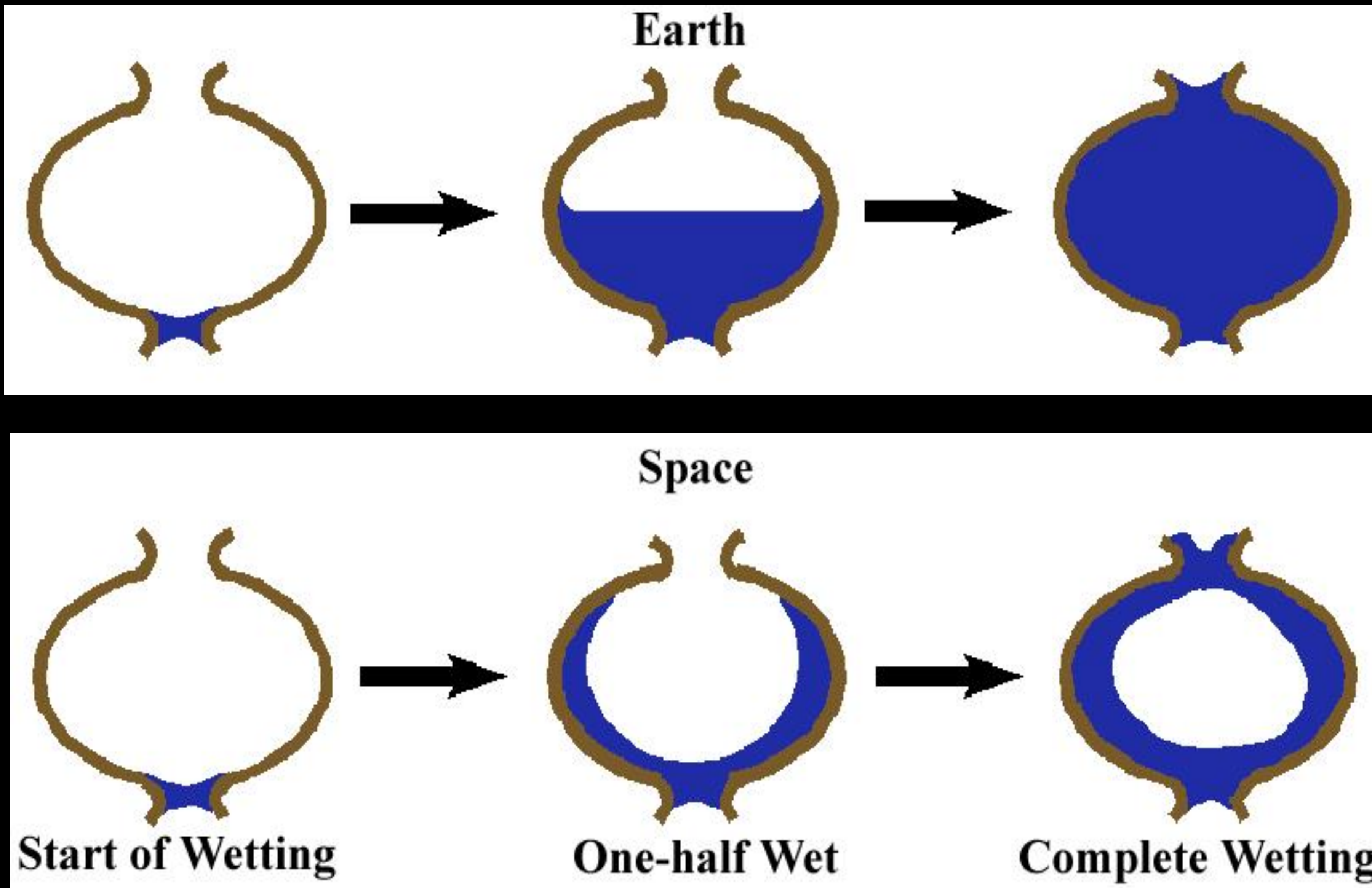
At 1G ➔ Gravity drains Water to Field Capacity

- ➔ Vertical Moisture Gradation
- ➔ Top = Dryer
- ➔ Bottom = Wetter

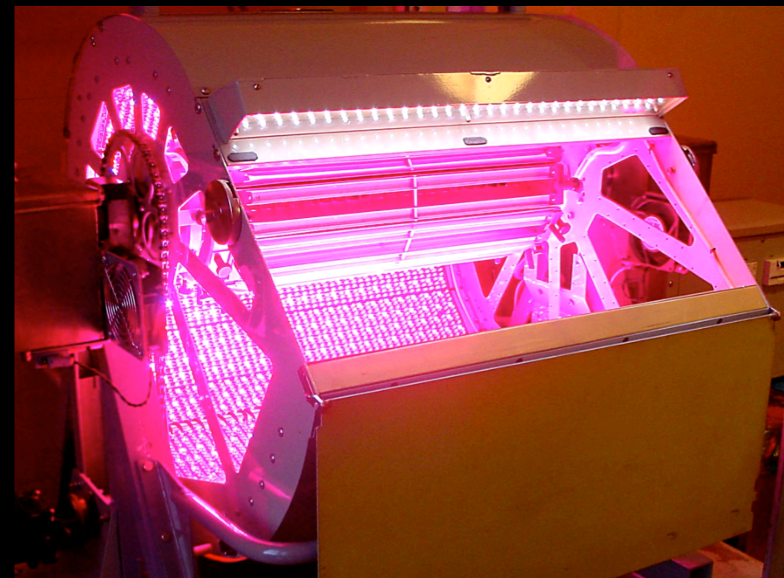
At μG ➔ Water is held near the Water Delivery Tubes by Capillary Forces

➔ Excessively Wet in Middle

Water Flow within Substrate Pores is Altered in Microgravity
(S. Jones and D. Or)



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

Ability to grow plants in space = improved

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

Introduction of Ambient Ethylene to MIR.

Ethylene (and VOC) scrubbing ➔ critical for successful seed production (SVET Studies).

Key Questions

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

G ➔ directs Root & Stem Growth

➔ requires Gravity Perception

➔ Transfer of Info

➔ Sites of Reaction

➔ Reaction to Signal by Cells

NASA-sponsored studies

➔ Identifying G-Perceiving Cells

➔ Threshold Values

➔ How G-vector is Perceived

Growing plants within the Astroculture plant chamber on ISS.

Types of Plant Tropisms

Gravitropism

Shoot Gravitropism

(Negative = Away from Gravity)

Root Gravitropism

(Positive = Toward Gravity)

Phototropism

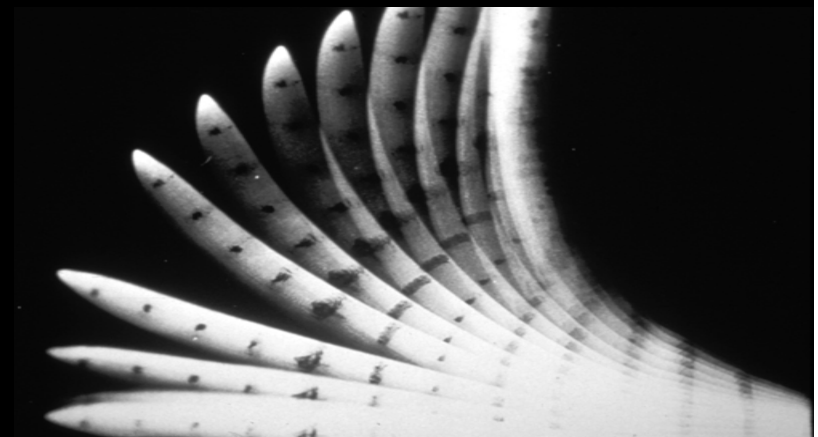
Shoot Phototropism

(Positive = Toward Light)

Hydrotropism

Root Hydrotropism

(Positive = Toward Water)



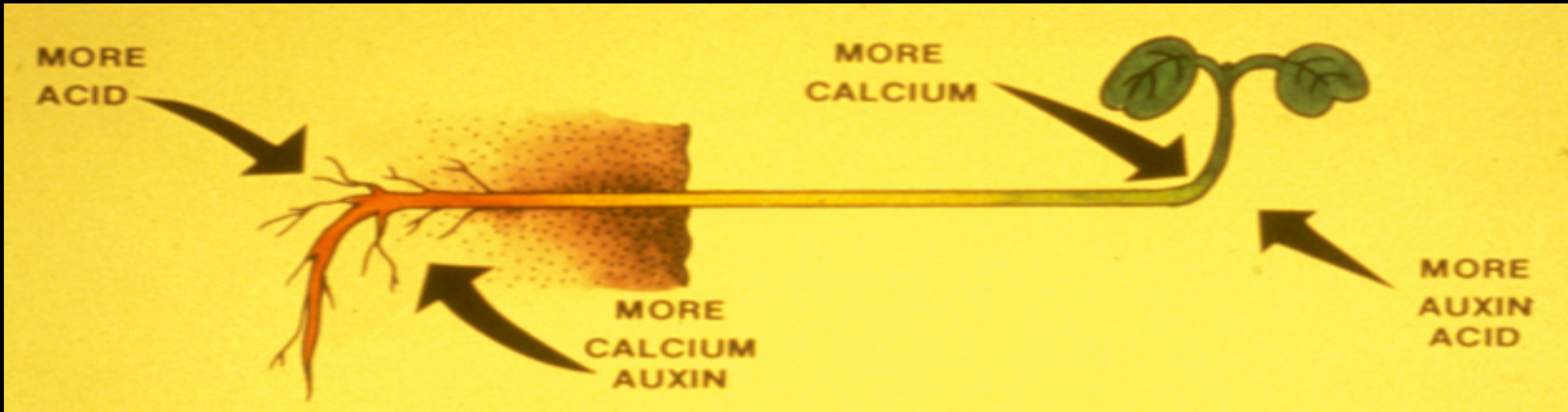
Shoot (60 minutes)

Root (90 minutes)

μG ➔ used to investigate & clarify G-obfuscated phenomena (e.g Phototropism)

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



G Stimulation → Amyloplasts Settle in Cells → Ca++ Moves → Ca++ - Calmodulin Binds Receptor Protein → Auxin Transported → Proton Gradient Develops → Selective Cell Growth → Gravitropic Response

$ Lost ➔ Wind & Rain ➔ Crops Fall Over ➔ Complicate Harvest
Plants Recovery = Gravity Response ➔ Reorient Shoot Growth ➔ Upright

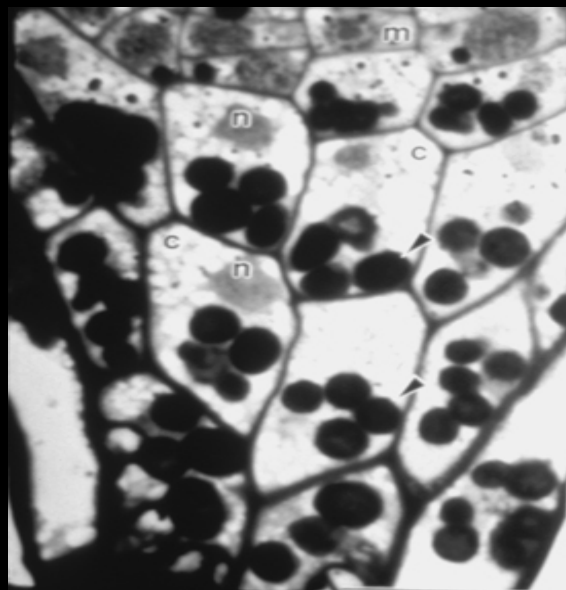
Discovering Underlying Mechanisms ➔ allow develop of crop plants + stronger & faster G-responses

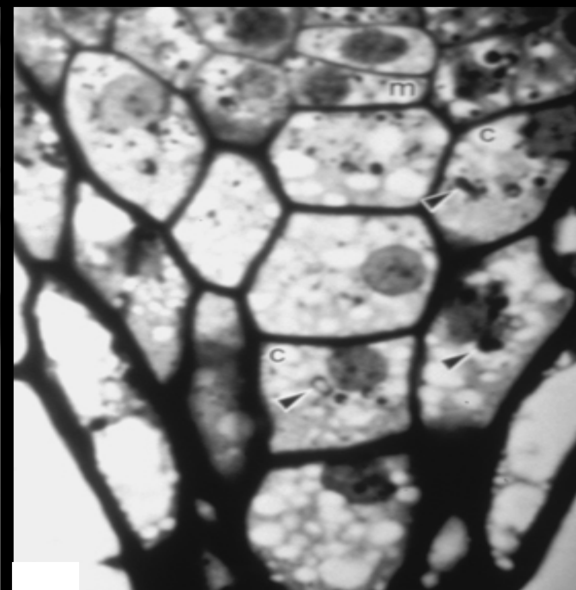
Root growth downward is key to their being able to locate and take up water.

How do Roots Perceive Gravity?

Root Tip of
Normal Plant

Root Tip of Starchless
Mutant Plant





Plants store food (starch) in Amyloplasts = involved G-Perception (left).

Amyloplasts = Denser than Cytoplasm ➔ Fall to Lower Cell Surfaces

Starchless Mutants ➔ respond sluggishly to G

➔ therefore Amyloplasts involved but not absolutely critical for G-Response

Effects of Microgravity on Plant Secondary Metabolism

Secondary Metabolism ➔affected by altered G
(both hypo- and hypergravity)

Brassica rapa ➔ *ISS* Grown & Fixed (23)

➔ Nominal Growth & Leaf Chlorophyll & Starch & Soluble Carbohydrates

➔ [Glucosinolate] ↑75% greater in μG vs GC

μG Grown Seeds

➔ Altered Biochemical make-up vs GCs

↑ [Chlorophyll] & [Starch] & [Soluble CH_2O]

↓ [Protein]

↓ Storage Reserve Deposition

μG Grown Seed Embryos

➔ Embryos at a range of developmental stages

➔ vs GC embryos = uniformly @ a single stage of development.

Therefore: Spaceflight environment influences metabolite production in ways that may affect flavor and nutritional quality of potential space produce.

Classes of Secondary Metabolites

Class	Example Compounds	Example Sources	Some Effects and Uses
NITROGEN-CONTAINING			
Alkaloids	nicotine cocaine theobromine	tobacco coca plant chocolate (cocao)	interfere with neurotransmission, block enzyme action
NITROGEN-AND SULFUR-CONTAINING			
Glucosinolates	sinigrin	cabbage, relatives	
TERPENOIDS			
Monoterpenes	menthol linalool	mint and relatives, many plants	interfere with neurotransmission, block ion transport, anesthetic
Sesquiterpenes	parthenolid	Parthenium and relatives (*Asteraceae*)	contact dermatitis
Diterpenes	gossypol	cotton	block phosphorylation; toxic
Triterpenes, cardiac glycosides	digitogenin	Digitalis (foxglove)	stimulate heart muscle, alter ion transport
Tetraterpenoids	carotene	many plants	antioxidant; orange coloring
Terpene polymers	rubber	Hevea (rubber) trees, dandelion	gum up insects; airplane tires
Sterols	spinasterol	spinach	interfere with animal hormone action
PHENOLICS			
Phenolic acids	caffeic, chlorogenic	all plants	cause oxidative damage, browning in fruits and wine
Coumarins	umbelliferone	carrots, parsnip	cross-link DNA, block cell division
Lignans	podophyllin urushiol	mayapple poison ivy	cathartic, vomiting, allergic dermatitis
Flavonoids	anthocyanin, catechin	almost all plants	flower, leaf color; inhibit enzymes, anti- and pro-oxidants, estrogenic
Tannins	gallotannin, condensed tannin	oak, hemlock trees, birdsfoot trefoil, legumes	bind to proteins, enzymes, block digestion, antioxidants
Lignin	lignin	all land plants	structure, toughness, fiber

Long-Term Space Exposure To Seeds

MIR μG Tomato Seeds (6 years) (3)

➔ Significant Differences in Growth (Yield) and Development (Fertility plus Structure of Cell Walls, Chloroplasts and Mitochondria) relative to GCs.

➔ DNA variations relative to the ground controls (4).

Short-Term Space Exposure To Seeds

Chinese Space Research (5-9)

➔ Claimed Genetic Mutants from Plants/Seeds flown in Short Duration Space Experiments.

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

Why?

The use of Spaceflight to study Endogenous Plant Movements

Additional Plant Patterns (14-15):
Circumnutation & Negative Thigmotropism

G ➔ Amplifies minute oscillatory movements
- ➔ Circumnutations (16)
- ➔ Movements still occur in μG

μG ➔ New Facets of Leaf Movements (17)
- ➔ Ultradian Patterns
- ➔ Effects of Transitions to Darkness or Light
- ➔ Several Heretofore Unknown Movements

TROPI Experiment (18)
- ➔ Gravitropism:Phototropism Interactions
- ➔ European Modular Cultivation System

This Research greatly benefits by the use of microgravity to eliminate one of the parameters (gravity) obfuscating results in earth-based experiments.

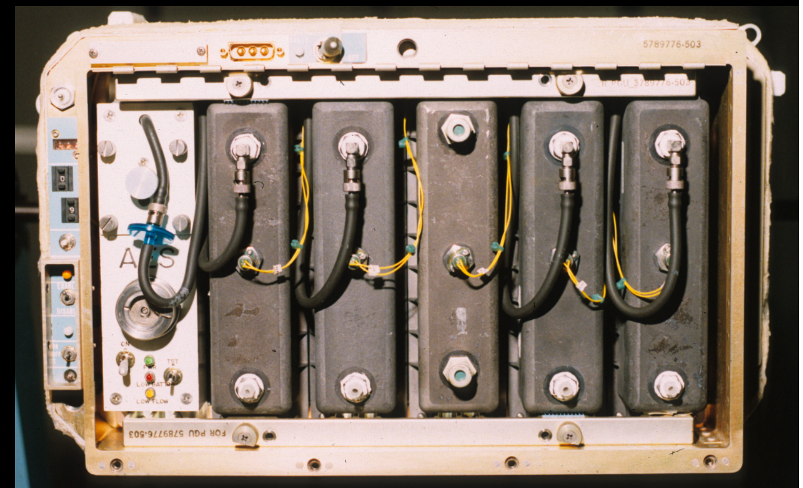
Honeysuckle Circumnutation

Pollen and Seed Development in Space (M. Musgrave *et. al.*)

Can Plants carry out normal reproductive processes in space?

Plants in µG ➔ fertilization & early seed development require CO_2 enrichment & air-exchange (19, 20)

HOWEVER:
There are still differences attributable to the spaceflight environment (21, 22).
➔ *Brassica* seeds and pollen produced in µG ➔ physiologically younger than GCs
➔ Speculation: µG limits mixing of the gaseous microenvironments inside the closed tissues and that the resulting gas composition surrounding the seeds and pollen retards their development.



Plant Growth Unit Modified for Air Flow.

Forced Air Flow Negated Lack of Convection.

Root Length Enhancement

μG ➔ Enhanced Root Production vs GCs (24-28)

Why?

➔ Physiological Basis
➔ Spaceflight-Associated Artifact
(more even distribution of moisture in root zone?)

Arabidopsis thaliana ➔ Germinated & Fixed in μG (30)

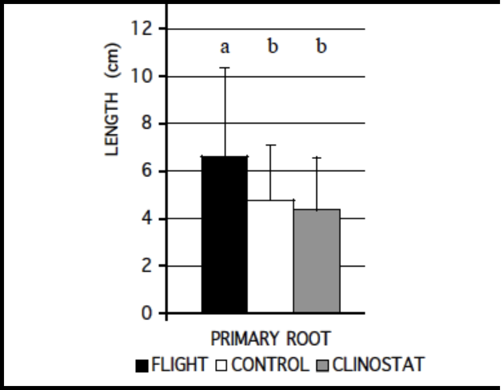
➔ Root Cortical Cells Proliferated at a Higher Rate
➔ Possibly the result of an Accelerated Cell Cycle

μG ➔ Medium Samples Extracted from the Root Zone (29)

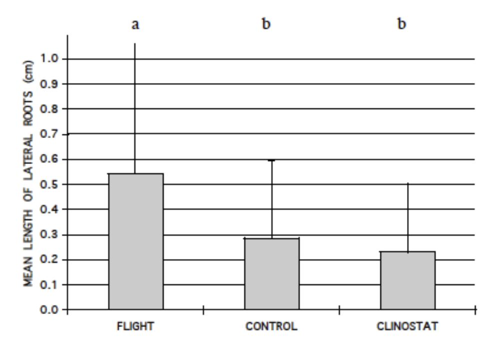
➔ 2X Difference Between the Final [K] vs GC

Why?

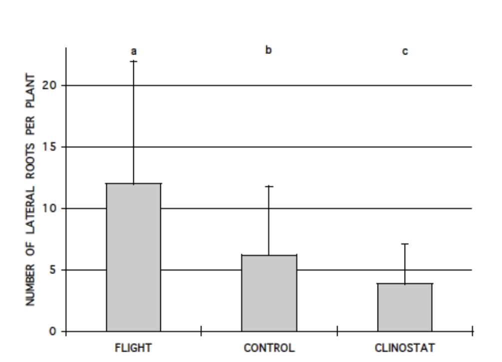
➔ Physiological Basis
➔ Spaceflight-Associated Artifact
(↑ Root Production ➔ More K Uptake)



Primary Root Lengths



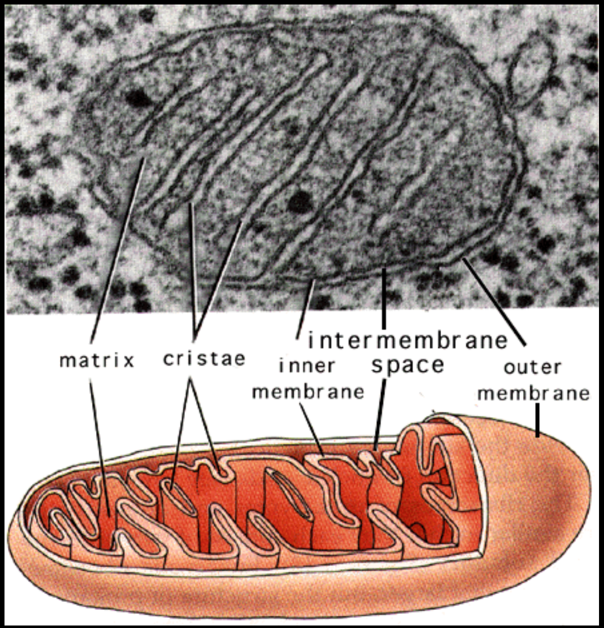
Lateral Root Lengths



Number of Lateral Roots

Ultrastructure Results

Do μG-Grown Plants exhibit alterations in ultrastructure?



Mitochondria in Root Statocytes of μG Soybean Seedlings (31)

➔ Round / Oviform ✚ Low Electron Density of Matrix

Mitochondria in Root Statocytes of GC Soybean Seedlings

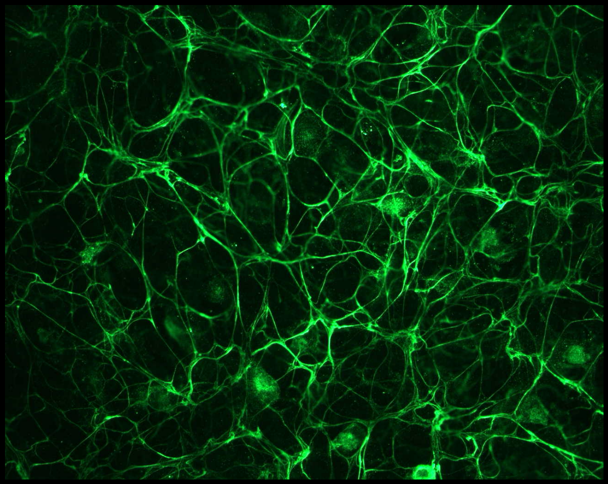
➔ Polymorphic Shape ✚ Higher Matrix Electron Density

μG Grown Soybeans (32)

➔ Changes in Vascular Structure

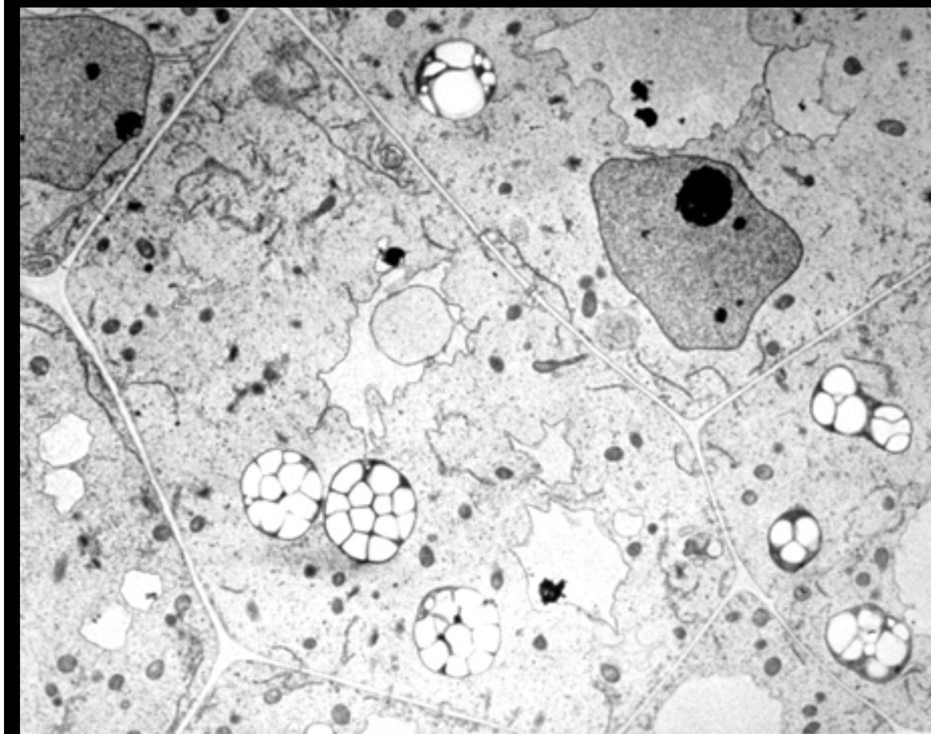
➔ Speculation

➔ Orientation of microfibrils and their assembly in developing vessels are perturbed by μG 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.



How does the Microgravity Environment affect Root Cell Structure? (R. Moore *et. al.*)

TEM of Corn Cortical Root Cell on Earth



Starch Storage in Amyloplasts Dominates

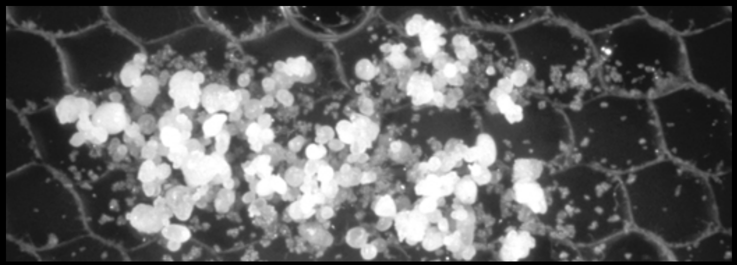
TEM of Corn Cortical Root Cell from μG

Amyloplasts contain less Starch and an abundance of Oil Droplets.

The space environment somehow disrupts normal carbohydrate metabolism.

Effects of Spaceflight on Mitosis and Chromosome Behavior
(A. Krikorian *et. al.*)

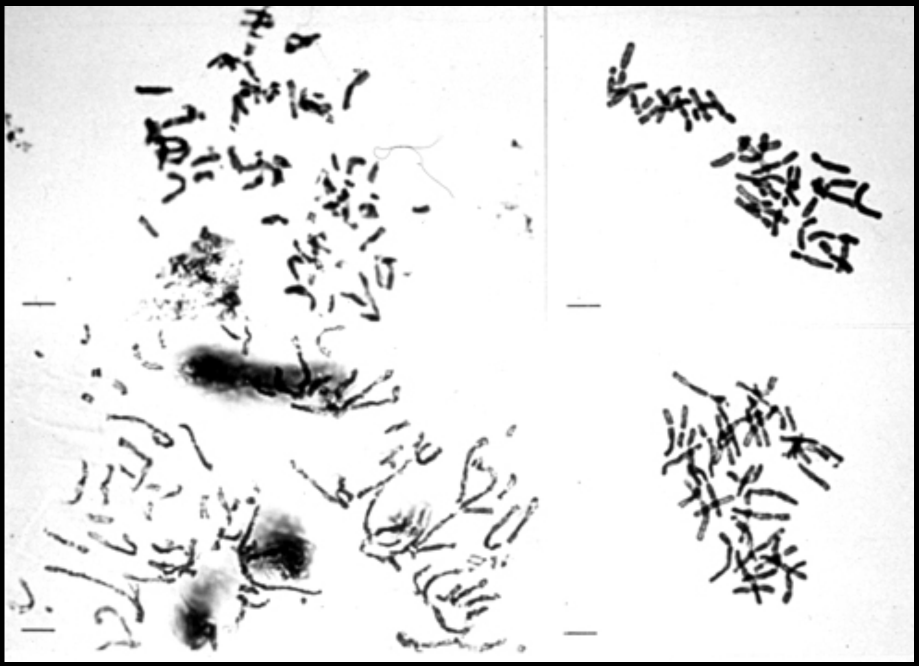
Daylily Plants flown in μG as Somatic Embryos



Left = Normal Daylily Plant
Right = Daylily derived from Space-Exposed Embryos

Unanswered Question:
Was the chromosomal damage observed due to μG or some other aspect of the space environment.

Chromosomal Aberrations (μG Grown Daylily)



Upper Left: Normal Metaphase in GC Plants

Upper Right: Structurally Perturbed μG Chromosomes

Lower Left & Right: Deteriorated/Fractured Chromosome that signify serious damage to the integrity of the cell's genetic material. Cells as badly damaged as these would not survive to divide again.

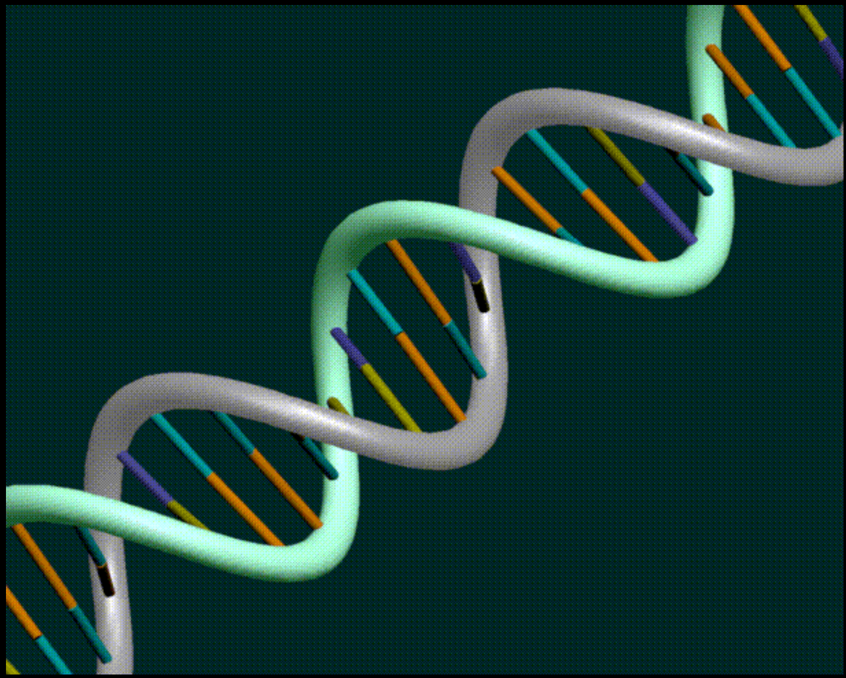
Gene Expression Results

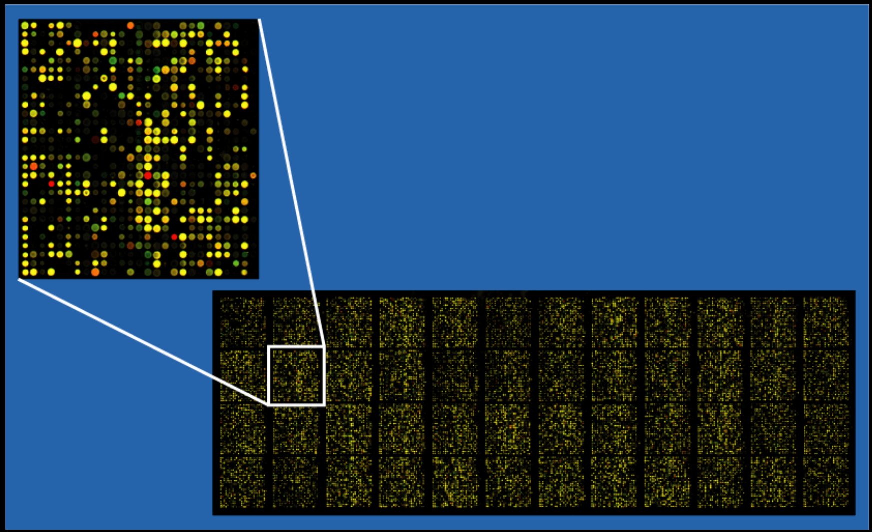
μG Grown Plants
➔ Usually ✚ Significantly Altered Gene Expression (1)

Some μG-altered genes
➔ Related to Heat Shock
➔ yet Not easily Explained by Exposure to Elevated Temperatures (2)

Some μG Grown Plants (PESTO)
➔ Not in Agreement (μG Patterns = GC Patterns)

More Work Needed
➔ Tease Apart Why the regulation of certain genes are altered by spaceflight conditions.





Plant Photosynthesis, Respiration, Transpiration in Space

μG ➔ ± Altered Photosynthesis

Whole Canopy Calculation ➔ Net Photosynthesis & Evapotranspiration Rates & Water Use Efficiency = GCs (33)

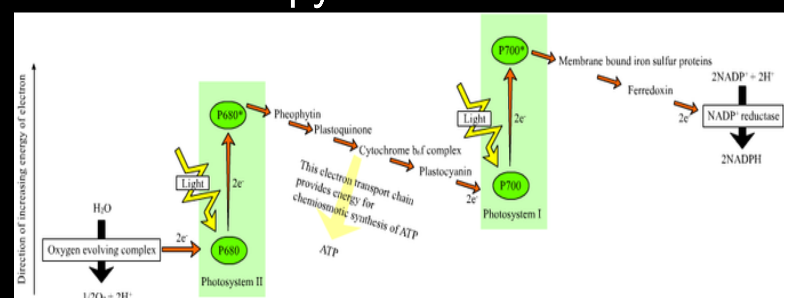
However ➔ even though single leaf measurements showed no differences in photosynthetic activity at moderate (up to 600 micromol m^{-2} s^{-1}) light levels, there was reduction in whole chain electron transport (13%), PSII (13%), and PSI (16%) activities observed under high (saturating) light & CO_2 conditions (34).

Early study (35) ➔ μG wheat plants ➔ exhibited CO_2-saturated photosynthetic rates at saturating light intensities that declined 25% relative to GCs.

Also: Using thylakoids isolated from μG-grown plants
➔ light-saturated photosynthetic electron transport rate from H_2O through photosystems II and I was ↓ 28%

Therefore:
Photosynthetic functions are affected by the space environment.

Wheat Canopy for CO2 Draw-Down



Z-Scheme in Photosynthesis

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?

References

(1) Salmi ML, Roux SJ. Gene expression changes induced by space flight in single-cells of the fern Ceratopteris richardii. Planta. 2008 Sep 20. [Epub]

(2) Paul A-L, Popp MP, Gurley WB, Guy C, Norwood KL, Ferl RJ. Arabidopsis gene expression patterns are altered during spaceflight. Adv Space Res. 2005. 36(7):1175-81.

(3) Nechitailo GS, Lu JY, Xue H, Pan Y, Tang C, Liu M. Influence of long term exposure to space flight on tomato seeds. Adv Space Res. 2005. 36(7):1329-33.

(4) Lu JY, Liu M, Xue H, Pan Y, Zhang CH, Nechitailo GS. [Random amplified polymorphic DNA analysis of tomato from seeds carried in Russian Mir space station] Space Med Med Eng (Beijing). 2005. 18(1):72-4. Chinese.

(5) Cai LT, Zheng SQ, Huang XL. A crinkly leaf and delay flowering mutant of tobacco obtained from recoverable satellite-flown seeds. Adv Space Res. 2007. 40(11):1689-93.

(6) Cheng Z, Liu M, Zhang M, Hang X, Lei C, Sun Y. Transcriptomic analyses of space-induced rice mutants with enhanced susceptibility to rice blast. Adv Space Res. 2007. 40(4):540-9.

(7) Li Y, Liu M, Cheng Z, Sun Y. Space environment induced mutations prefer to occur at polymorphic sites of rice genomes. Adv Space Res. 2007. 40(4):523-7.

(8) Ma Y, Cheng Z, Wang W, Sun Y. Proteomic analysis of high yield rice variety mutated from spaceflight. Adv Space Res. 2007. 40 (4):535-9.

(9) Yu X, Wu H, Wei LJ, Cheng ZL, Xin P, Huang CL, Zhang KP, Sun YQ. Characteristics of phenotype and genetic mutations in rice after spaceflight. Adv Space Res. 2007;. 40(4):528-34.

(10) Gostimsky SA, Levinskikh MA, Sychev VN, Kokaeva ZG, Dribnokhodova OP, Khartina GA, Bingham G. The study of the genetic effects in generation of pea plants cultivated during the whole cycle of ontogenesis on the board of RS ISS. Russ J Genet 2007. 43(8):869-74.

(11) Levinskikh MA, Sychev VN, Derendiaeva TA, Signalova OB, Podol'skii IG, Gostimskii SA, Bingham G. [Growth, development and genetic status of pea plants cultivated in space greenhouse "LADA"] Aviakosm Ekolog Med. 2005. 39(6):38-43. Russian.

(12) Visscher AM, Paul AL, Kirst M, Alling AK, Silverstone S, Nechitailo G, Nelson M, Dempster WF, Van Thillo M, Allen JP, Ferl RJ. 2009. Effects of a spaceflight environment on heritable changes in wheat gene expression. Astrobiology 2009. 9(4):359-67.

(13) Sychev VN, Levinskikh MA, Gostimsky SA, Bingham GE, Podolsky IG. Spaceflight effects on consecutive generations of peas grown onboard the Russian segment of the International Space Station. Acta Astronaut. 2007. 60(4-7):426-32.

(14) Migliaccio F, Fortunati A, Tassone P. Arabidopsis root growth movements and their symmetry: Progress and problems arising from recent work. Plant Signal Behav. 2009 Mar;4(3):183-90

(15) Oliva M, Dunand C. Waving and skewing: how gravity and the surface of growth media affect root development in Arabidopsis. New Phytol. 2007;176(1):37-43).

(16) Johnsson A, Solheim BG, Iversen TH. Gravity amplifies and microgravity decreases circumnutations in Arabidopsis thaliana stems: Results from a space experiment. New Phytol. 2009;182(3):621-9. Epub 2009 Mar 6.

(17) Solheim BG, Johnsson A, Iversen TH. Ultradian rhythms in Arabidopsis thaliana leaves in microgravity. New Phytol. 2009;183 (4):1043-52.

(18) Kiss JZ, Kumar P, Millar KD, Edelmann RE, Correll MJ. Operations of a spaceflight experiment to investigate plant tropisms. Adv Space Res. 2009 Oct 15;44(8):879-86).

(19) Musgrave ME, Kuang A, Matthews SW: Plant reproduction during spaceflight: importance of the gaseous environment. Planta 1997, 203:S177-S184

(20) Sychev VN, Levinskikh MA, Gostimsky SA, Bingham GE, Podolsky IG. Spaceflight effects on consecutive generations of peas grown onboard the Russian segment of the International Space Station. Acta Astronaut. 2007 Feb-Apr;60(4-7):426-32.

(21) Kuang A, Popova A, McClure G, Musgrave ME. Dynamics of storage reserve deposition during Brassica rapa L. pollen and seed development in microgravity. Int J Plant Sci. 2005 Jan;166(1):85-96.

(22) Popova AF, Musgrave M, Kuang A. The development of embryos in Brassica rapa L. in microgravity. Cytol Genet. 2009 Apr;43 (2):89-93.

(23) Musgrave ME, Kuang A, Tuominen LK, Levine LH, Morrow RC. Seed storage reserves and glucosinolates in Brassica rapa L. grown on the International Space Station. J Am Soc Hortic Sci. 2005 Nov;130(6):848-56.

(24) Levine, H.G., R.P. Kann and A.D. Krikorian. 1990. Plant Development in Space: Observations on Root Formation and Growth. In: *Proceedings of the Fourth European Symposium on Life Sciences Research in Space,* Trieste, Italy, 28 May to 1 June l990, pp. 503-08. ESA SP-307.

(25) Levine, H.G., and A.D. Krikorian. 1996. Enhanced Root Production in *Haplopappus gracilis* Grown Under Spaceflight Conditions. *J. Gravitational Physiology* 3(1): 17-27.

(26) Levine, H.G., J.A. Sharek, K.M. Johnson, E.C. Stryjewski, V. Prima, O. Martynenko and W.C. Piastuch. 2000. Growth Protocols for Etiolated Soybeans Germinated within BRIC-60 Canisters Under Spaceflight Conditions. Advances in Space Research 26(2): 311-314.

(27) Levine, H.G., K. Anderson, A. Boody, D. Cox, O.A. Kuznetsov and K.H. Hasenstein. 2003. Germination and Elongation of Flax in Microgravity. Advances in Space Research 31(10): 2261-2268.

(28) Levine HG, Piastuch WC. Growth patterns for etiolated soybeans germinated under spaceflight conditions. Adv Space Res. 2005;36(7):1237-43. And references cited within.

(29) Levine HG, Krikorian AD. Changes in plant medium composition after a spaceflight experiment: Potassium levels are of special interest. Adv Space Res. 2008 Sep 15;42(6):1060-5.

(30) Mati´a I, González-Camacho F, Marco R, Kiss JZ, Gasset G, Medina F-J. Nucleolar structure and proliferation activity of Arabidopsis root cells from seedlings germinated on the International Space Station. Adv Space Res. 2005; 36(7):1244-53.

(31) Klimchuk DO. Structural and functional features of mitochondria in statocytes of soybean root under microgravity conditions. Cytol Genet. 2007 Feb;41(1):25-9.

(32) De Micco V, Aronne G, Joseleau JP, Ruel K. Xylem development and cell wall changes of soybean seedlings grown in space. Ann Bot (Lond). 2008 Feb 5; [Epub ahead of print]

(33) Monje O, Stutte G, Chapman D. Microgravity does not alter plant stand gas exchange of wheat at moderate light levels and saturating CO2 concentration. Planta. 2005 Jun 21; Epub ahead of print.

(34) Stutte GW, Monje O, Goins GD, Tripathy BC. Microgravity effects on thylakoid, single leaf, and whole canopy photosynthesis of dwarf wheat. Planta. 2005 Sep 14:1-11.

(35) Tripathy, B.C., C.S. Brown, H.G. Levine, and A.D. Krikorian. 1996. Growth and Photosynthetic Responses of Wheat Plants Grown in Space. *Plant Physiol*. 110: 801-806.