

# Maintaining Relationships in Closed Environments: Plant/Microbe Mutualisms

Gary W. Stutte  
Space Life Science Laboratory  
Exploration Park, Florida

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AusPheno  
2016

8th-12th  
September 2016  
CSIRO Discovery Centre  
Canberra ACT  
ANU Kildea Centre  
Sturt St NSW

18-22 September, 2016, Canberra, ACT, Australia



Background



Ground



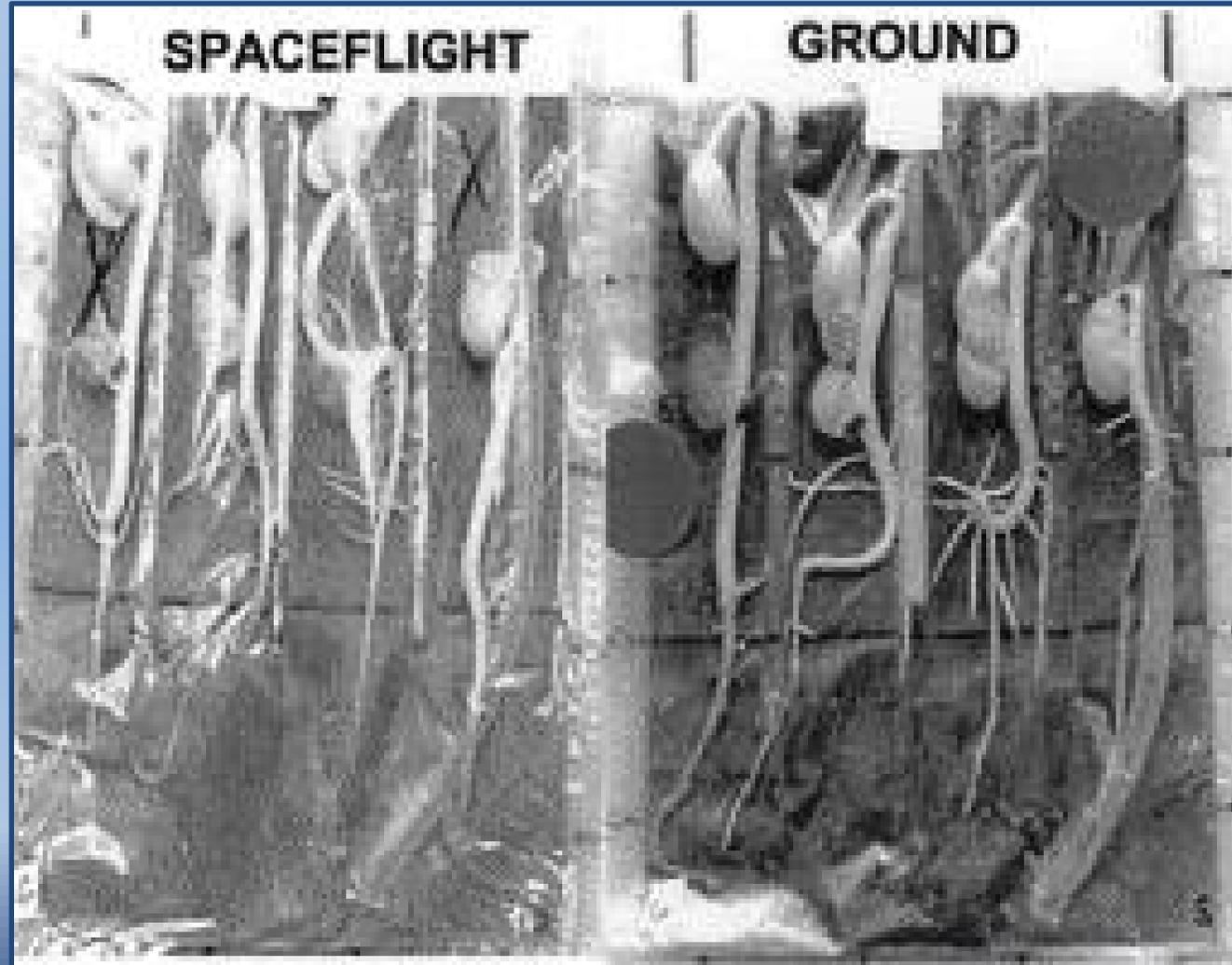
Flight



Speculation



**Background:** Microgravity environment is conducive to preferential growth of microorganisms and potential pathogens



*Soybean seedlings from day 7 harvest on STS-97* From Ryba-White, et al.2001. *Plant Cell Physiol.* 42;657-664.



**Background:** Microbial growth occurs on sanitized seed/rooting materials when exposed to ISS ambient conditions.



( NASA ISS image of zinnia plants grown in VEGGIE, 2016).



Wheat grown in non-sterile conditions had diverse rhizosphere, high germination, and no pathogenicity.



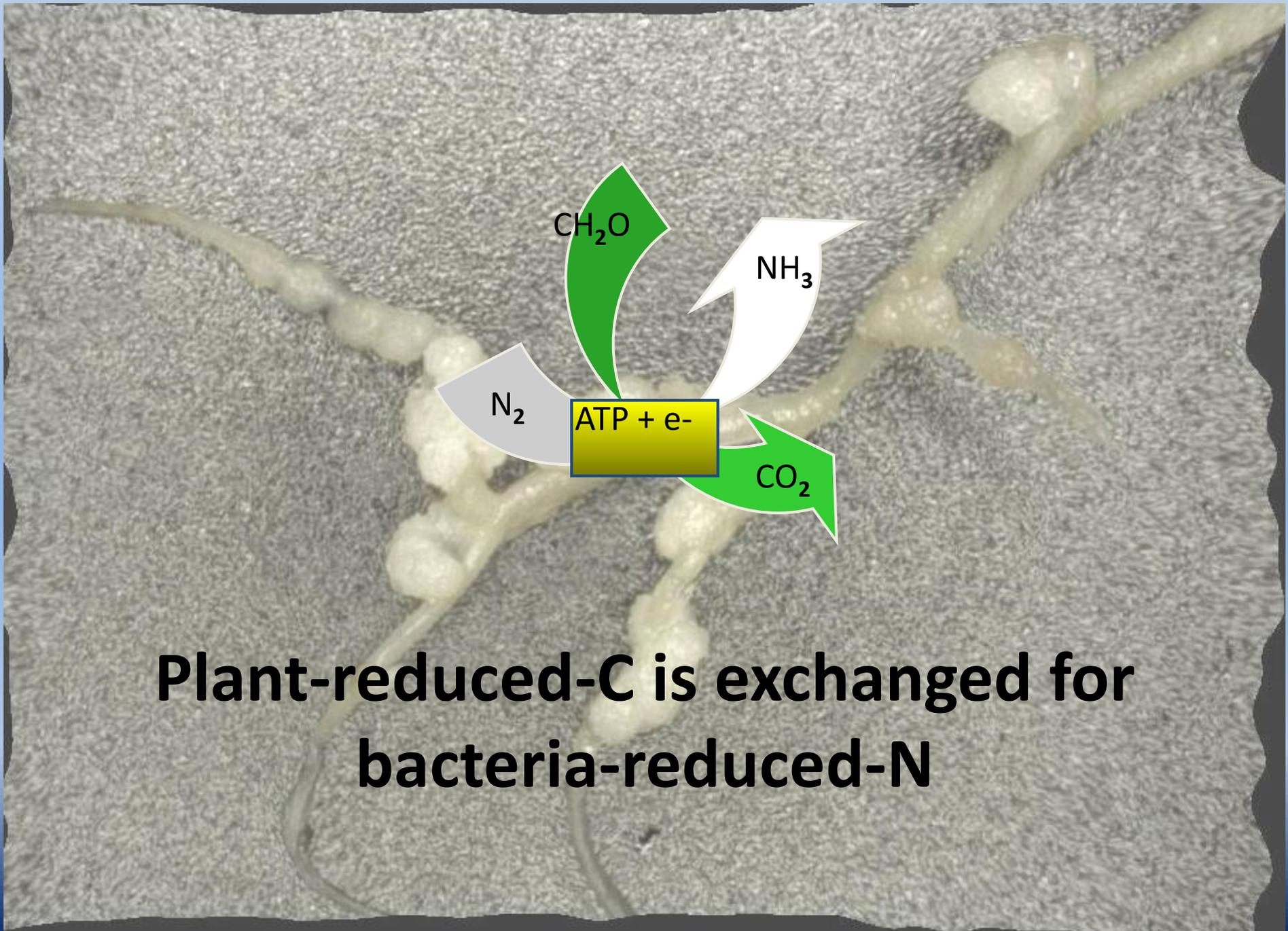
Frazier et al., 2003; Stutte et al., 2004)

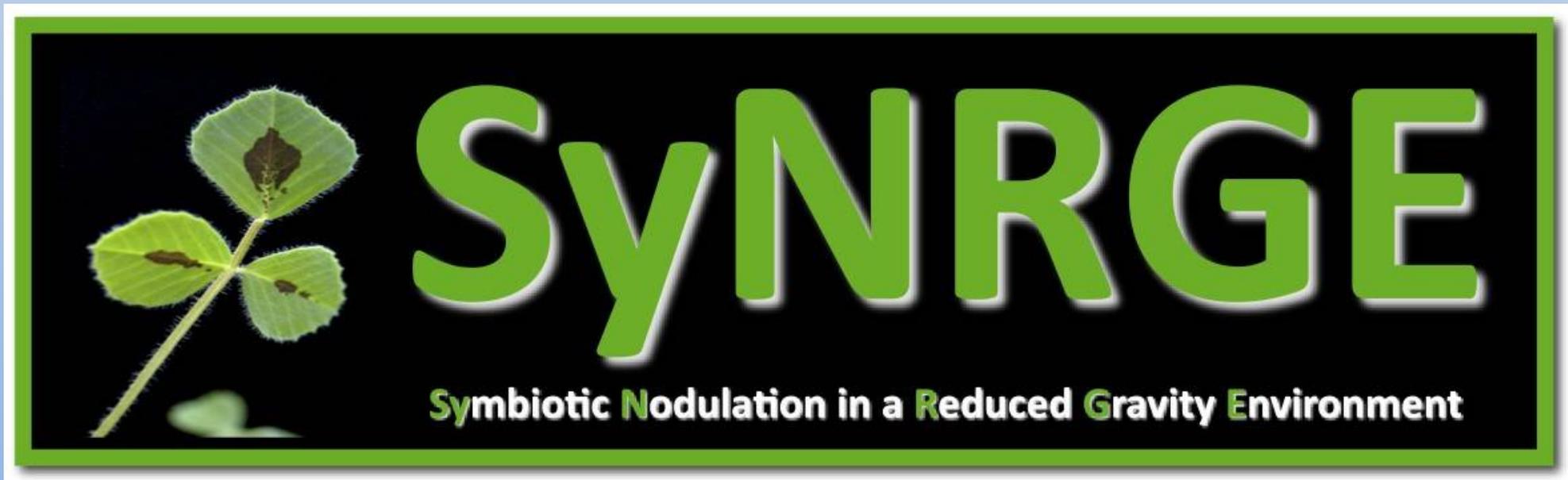


## Plant/Microbe Mutualisms are critical to survival on Earth and may play similar role for long duration space missions.

- ✓ Legumes to 20% of the protein in our diets though direct or are important crops and provide up indirect consumption.
- ✓ Understanding the nodulation process and its genetic machinery may have broad implications for decreasing resupply costs on long duration space missions in improving agriculture , reducing dependence on chemical nitrogen fertilizers.
- ✓ Little research on plant/microbe interactions in microgravity exists.



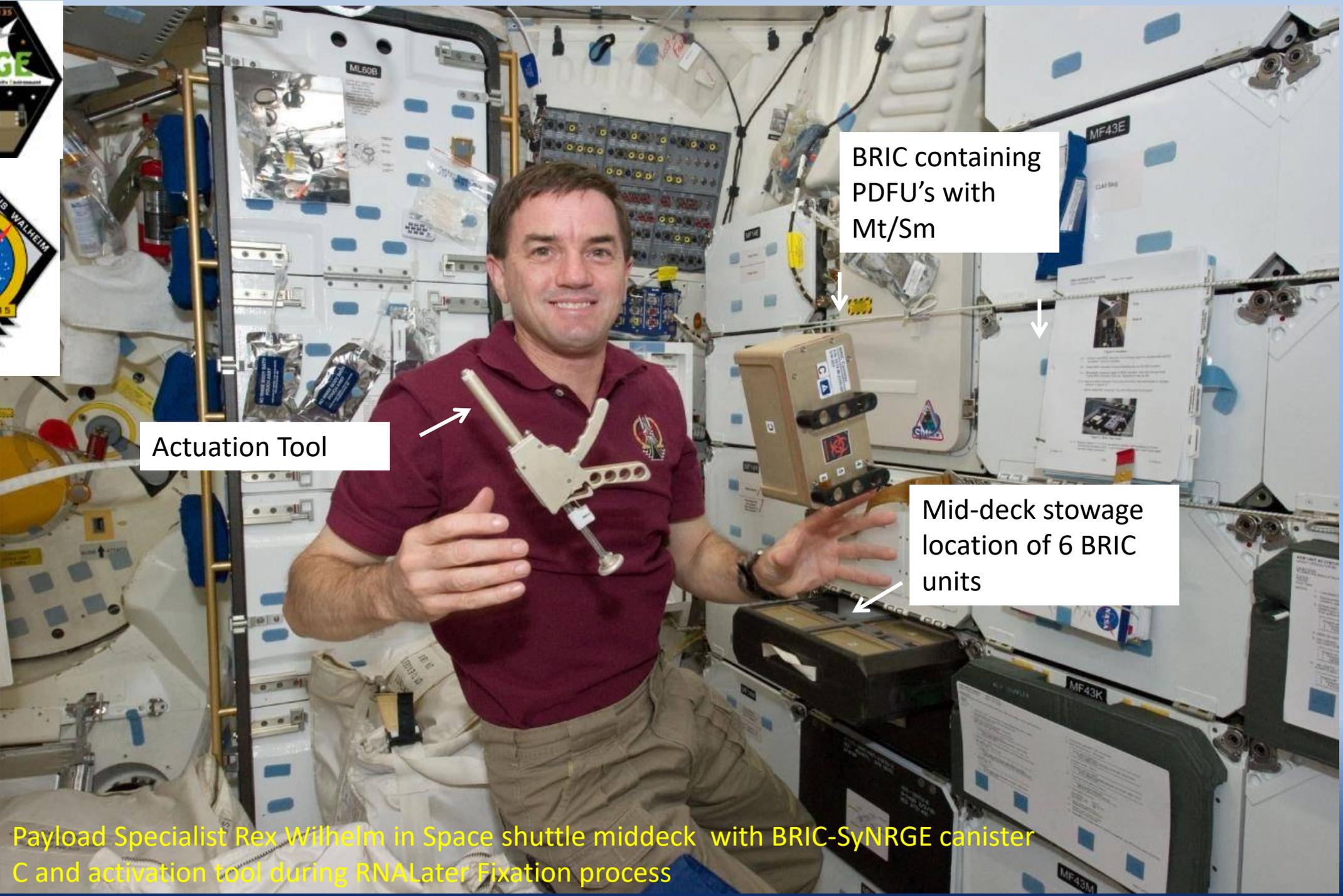




***Effect of Microgravity on Early Events of Biological Nitrogen Fixation in *Medicago truncatula****

Gary W. Stutte, Ph.D<sup>1</sup> and Michael Roberts, Ph.D  
QinetiQ-NA, Space Life Science Laboratory  
Kennedy Space Center, FL 32899

International Life Sciences Research Announcement: Research Opportunities for Flight Experiments in Space Life Sciences on the ISS (ILSRA-2009) Cooperative Agreement Number: NNX10AR090A



Actuation Tool

BRIC containing PDFU's with Mt/Sm

Mid-deck storage location of 6 BRIC units

Payload Specialist Rex Wilhelm in Space shuttle middeck with BRIC-SyNRGE canister C and activation tool during RNALater Fixation process



*Medicago truncatula*

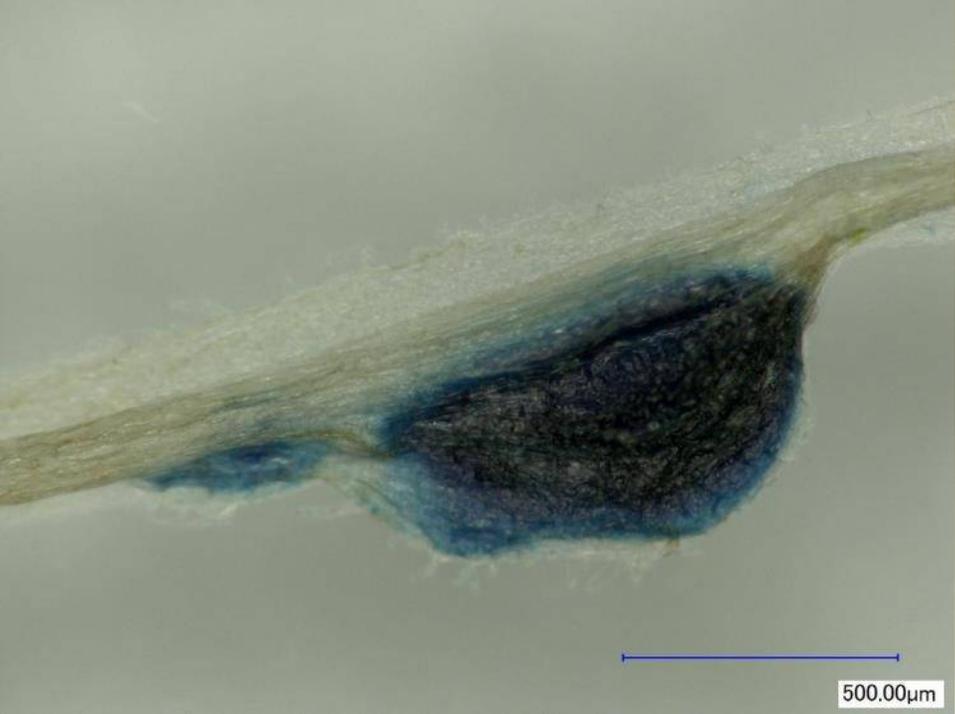
500.00µm



*Sinorhizobium meliloti*



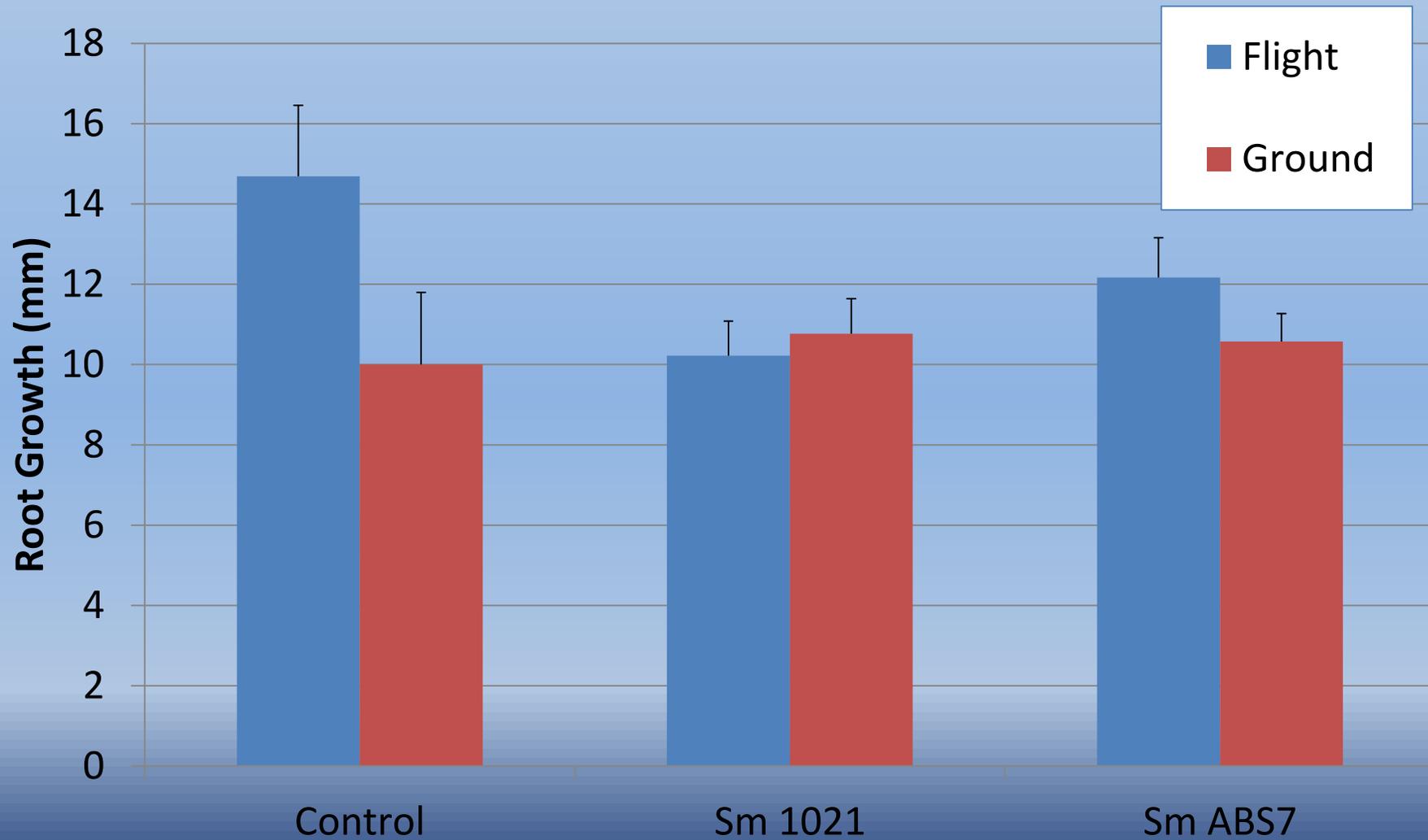
500.00µm



500.00µm

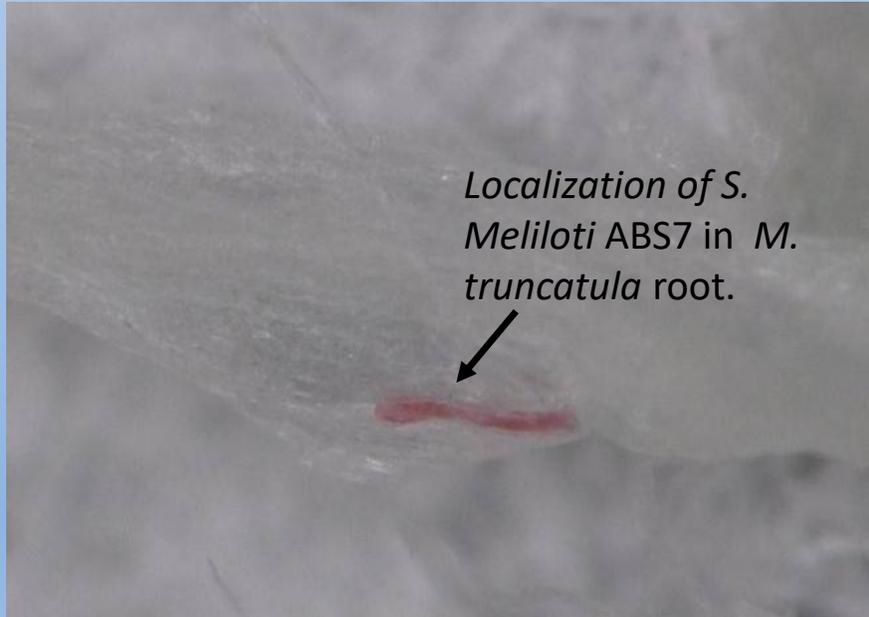


# Effects of Microgravity on growth of *M. truncatula* cv. Jemalong A17 (Enod11::gus) inoculated with two strains of *S. meliloti*.

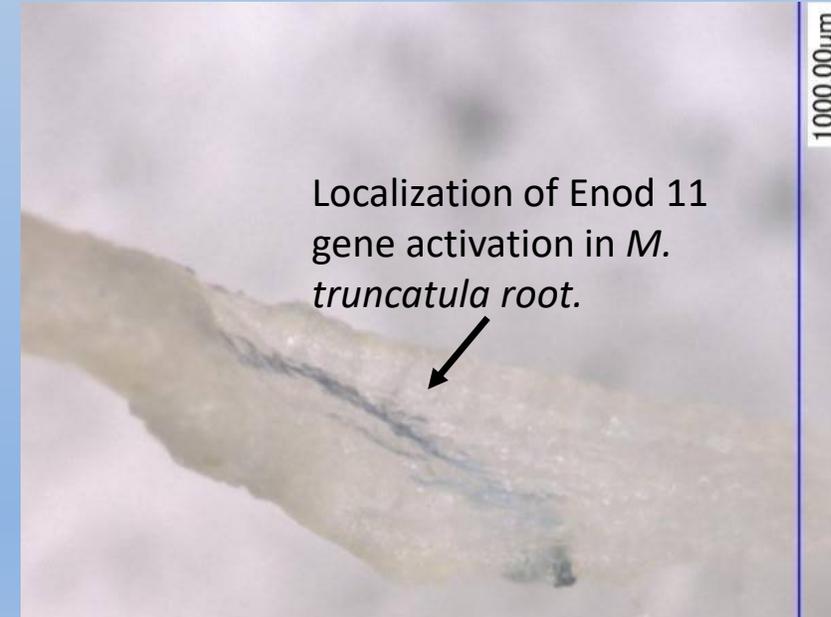




# Localization of *S. meliloti* infection of *M. truncatula* roots and activation of ENOD11 gene in $\mu g$ necessary for nodule formation, and subsequent biological nitrogen fixation.



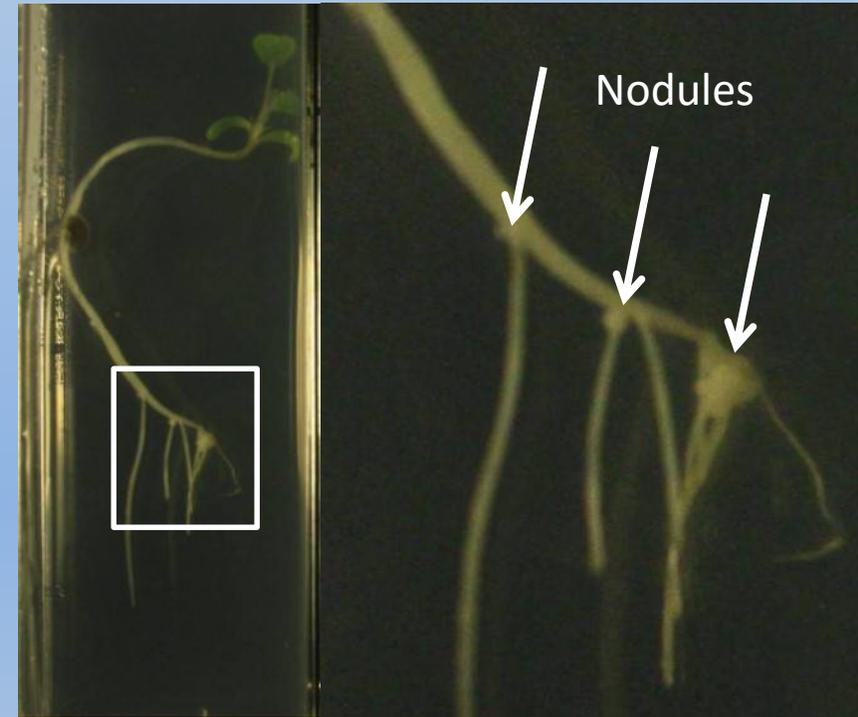
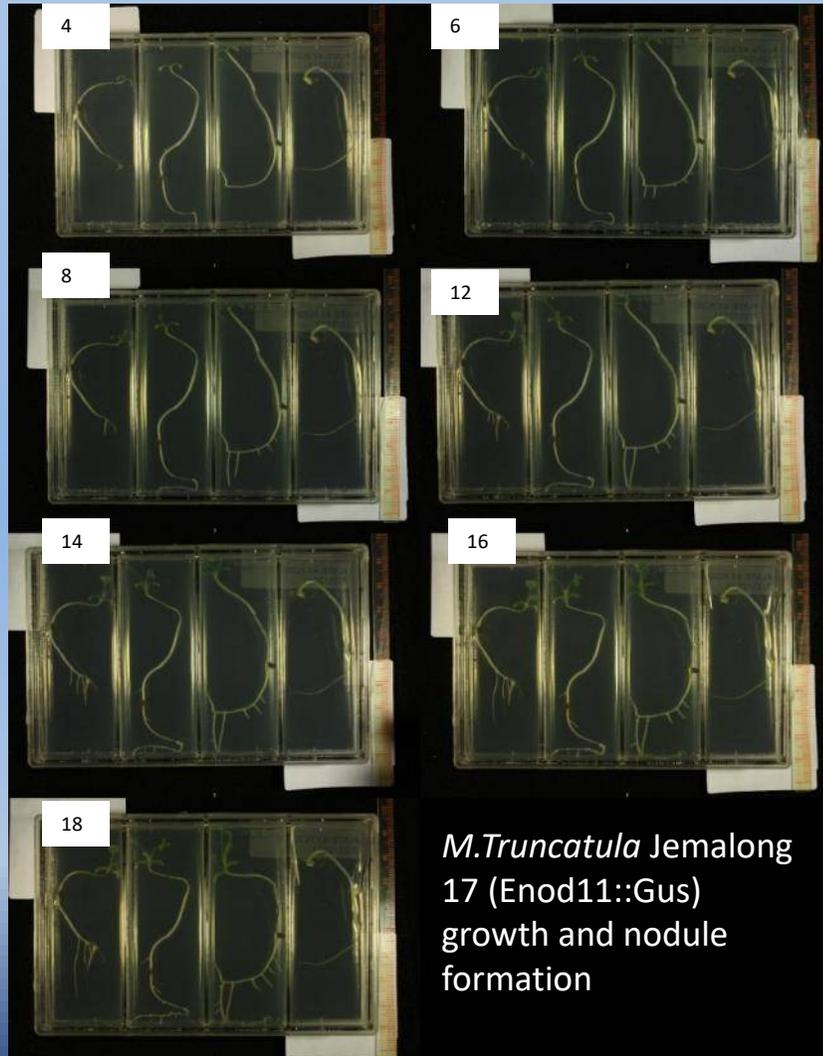
*M. truncatula* (Enod11::gus) inoculated with *S. meliloti* ABS7 with a hemA::LacZ marker. The stained area indicates site of *S. meliloti* infection in the etiolated *M. truncatula* root.



*M. truncatula* (Enod1::Gus) inoculated with *S. meliloti* ABS7 with a hemi::LacZ marker. The stained area indicates site of *Enod11:gus* gene activation in the etiolated *M. truncatula* root.



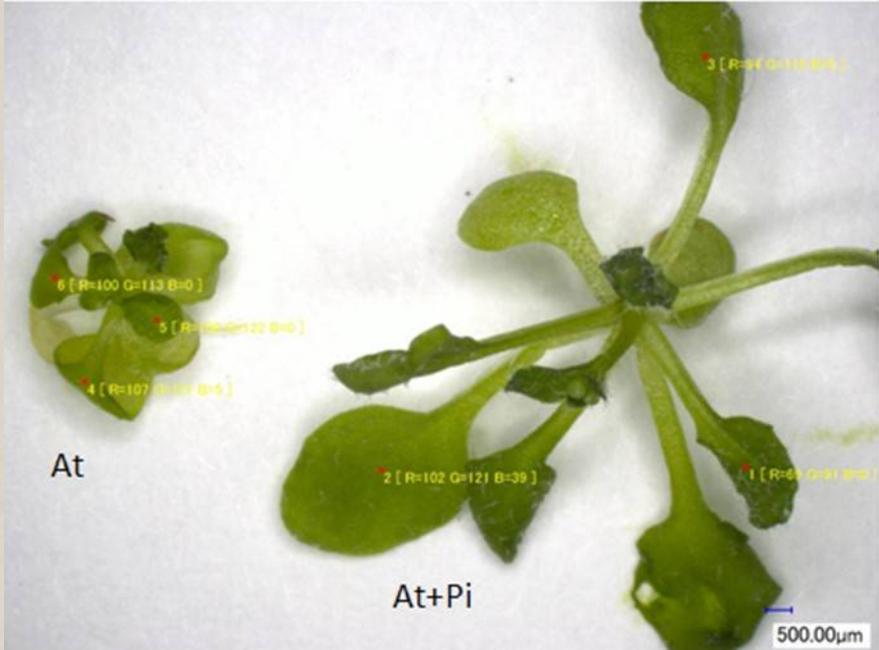
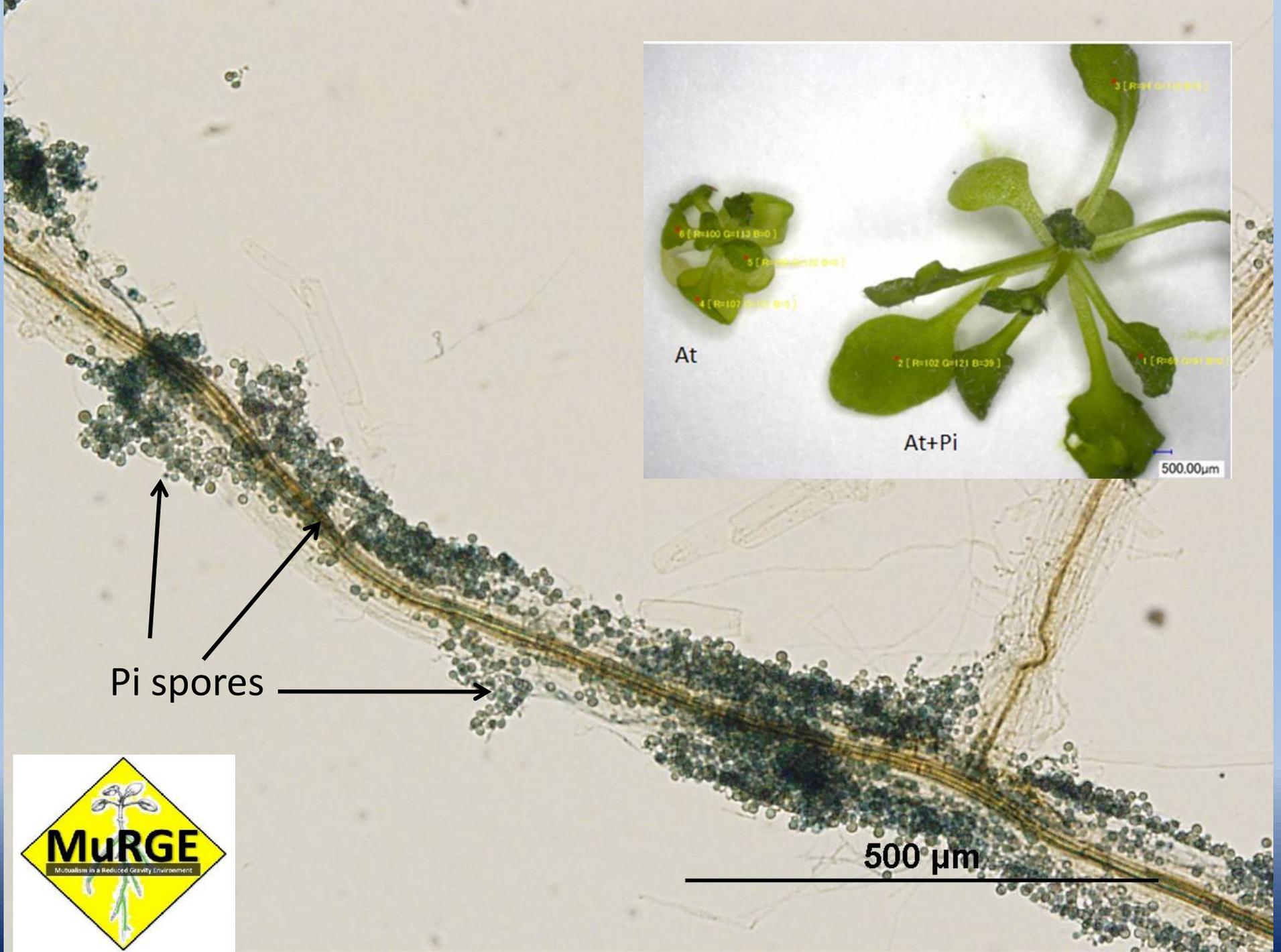
# H<sub>0</sub>: Microgravity exposure reduces the susceptibility of the host plant (*M. truncatula*) to form nodules



*M. truncatula* cv Jemalong 17 (*Enod11::gus*) germinated in microgravity and inoculated with *S.melliloti* ABS7 cultured in microgravity at 18 days after inoculation. Roots of *M. truncatula* were inoculated within 8 hours of landing, and cultured on buffered nodulation media (BNM), which contains no carbon or nitrogen source in Nunc™ 4-well plates.

# MUTUALISM IN A REDUCED GRAVITY ENVIRONMENT (MURGE): PIRIFORMOSPORA INDICA: ARABIDOPSIS THALIANA INTERACTIONS IN MICROGRAVITY



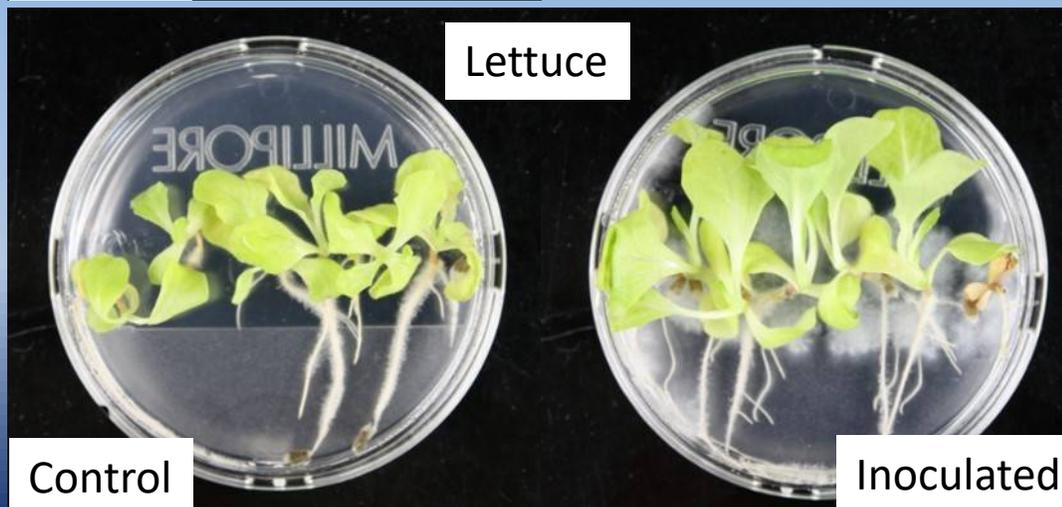


# *P. indica* shows strong biostimulatory effect on a number of species



## **TOMATO:**

More uniform germination, increased root branching, denser root hairs, and 15% increased in seedling biomass observed.

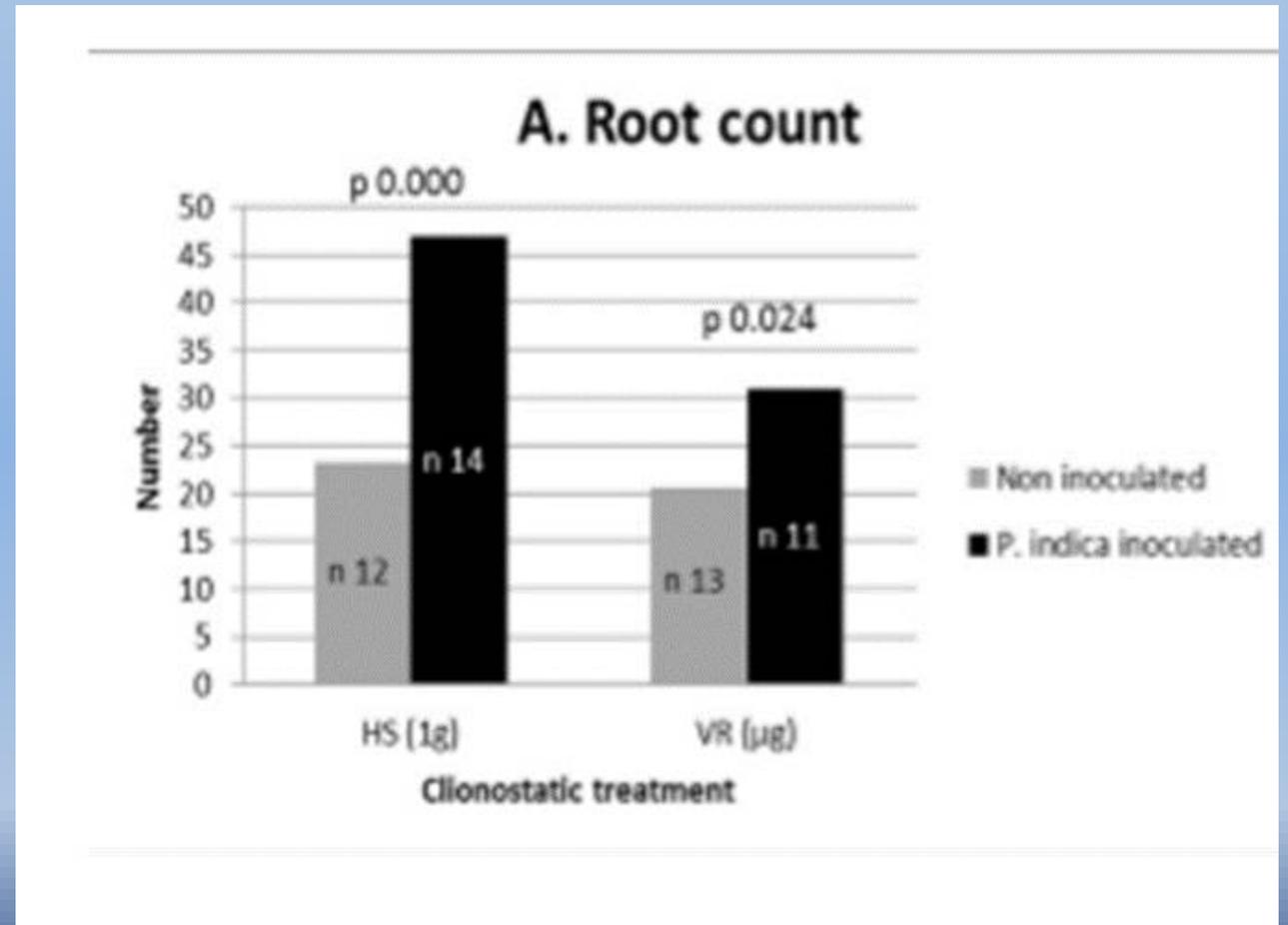
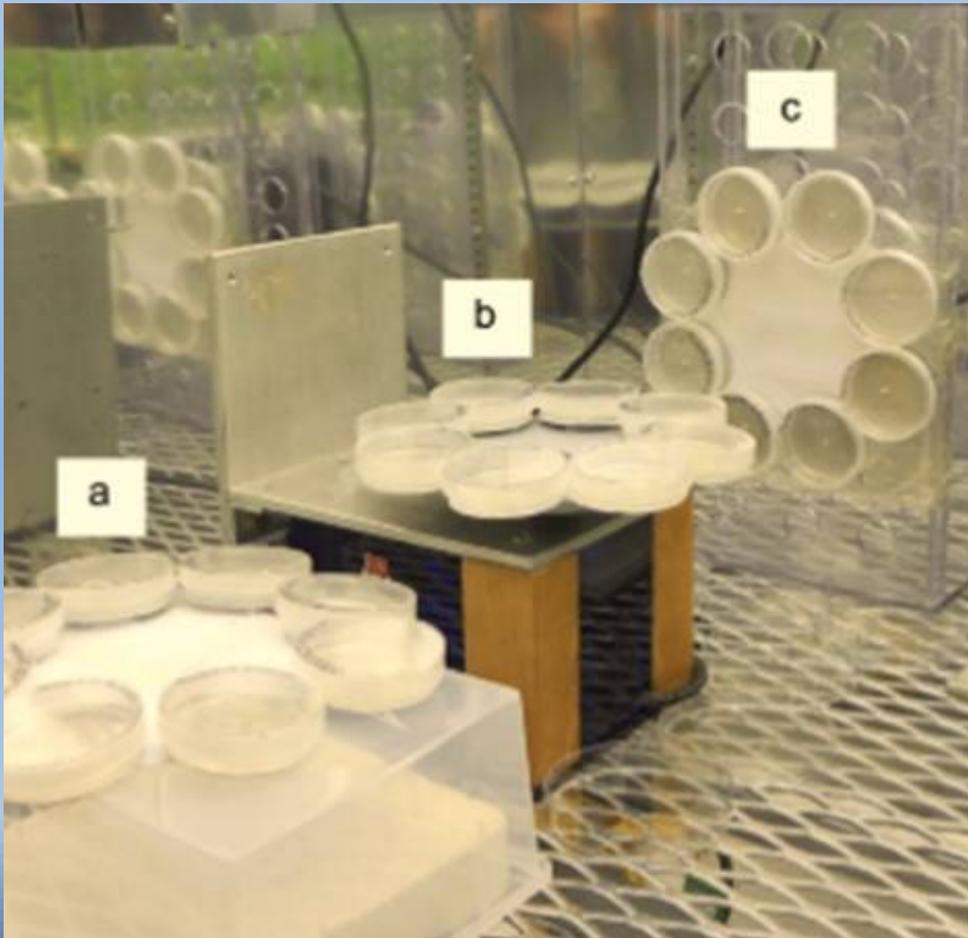


## **LETTUCE:**

More uniform germination, increased root branching, larger leaves, and 55% increased in seedling biomass observed.



# Biostimulatory effect of *P. indica* retained, but reduced in magnitude under simulated microgravity conditions.





SPACE FLORIDA  
INTERNATIONAL  
SPACE  
STATION  
RESEARCH COMPETITION

The logo for the Space Florida International Space Station Research Competition. It features a blue background with a white and blue illustration of the International Space Station in orbit above a globe. The text is arranged in a grid-like fashion around the illustration.

MARIE CURIE  
FELLOWSHIPS

The Marie Curie Fellowships logo, featuring a stylized profile of Marie Curie's head in white and blue, with the text 'MARIE CURIE' above and 'FELLOWSHIPS' below.

LIMERICK INSTITUTE  
OF TECHNOLOGY  
INSTITIÚID TEICNEOLAÍOCHTA  
LUIMNIGH



# NanoCube Plant Growth Chamber

- 10 cm x 10 cm x 15 cm
- Power to NanoLab via USB port (3.2 W)
- 4 white LED's (15  $\mu\text{mol m}^2 \text{s}^{-1}$  PAR)
- 4 growth channels (2 plants/channel)
- Monitor temp and CO<sub>2</sub>
- Fixation capabilities
- Imaging
- Data storage on board
- Periodic data download/access



# SyNRGE<sup>3</sup> launched on SpaceX in September, 2014.



- Lights failed to turn on on-orbit resulting in etiolation of plants.
- Tissue was returned after ~ 2 weeks after landing and microbes recovered.
- Viability of Sm and Pi to develop mutualism retained.

Eight *M. truncatula* plants were launched that had been inoculated with either *S. meliloti* or *P. indica*

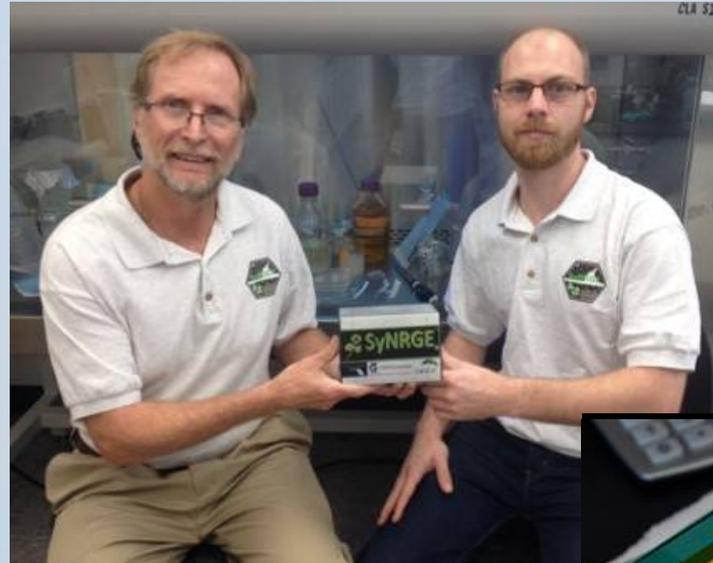


SyNRGE3 sponsored by Space Florida/Nanoracks ISS Research Competition

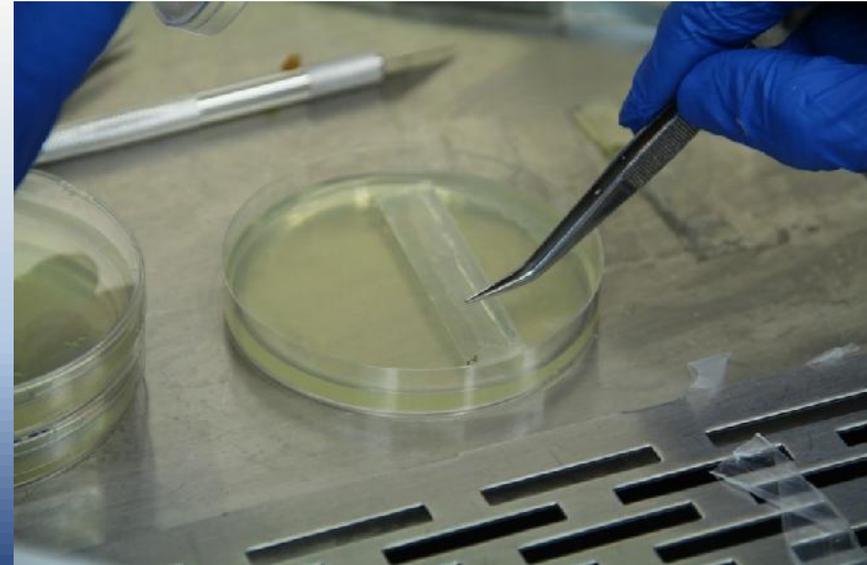
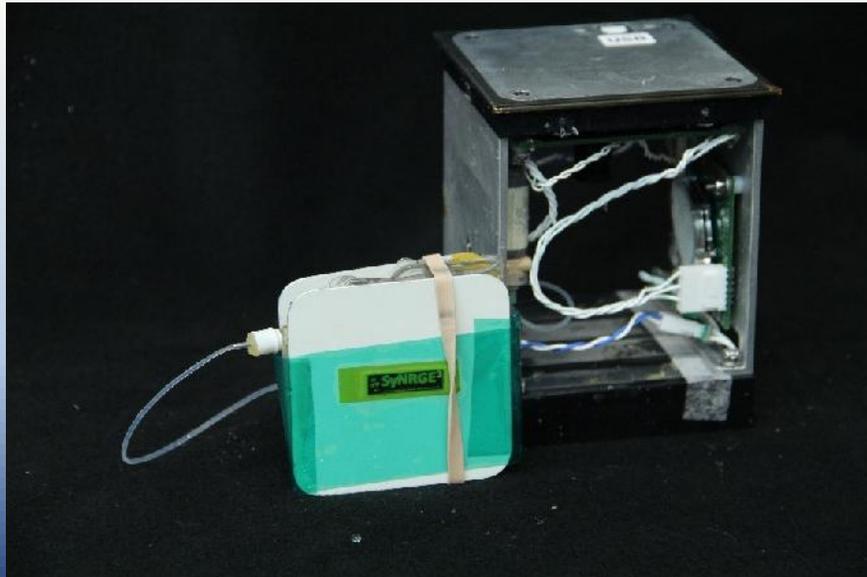
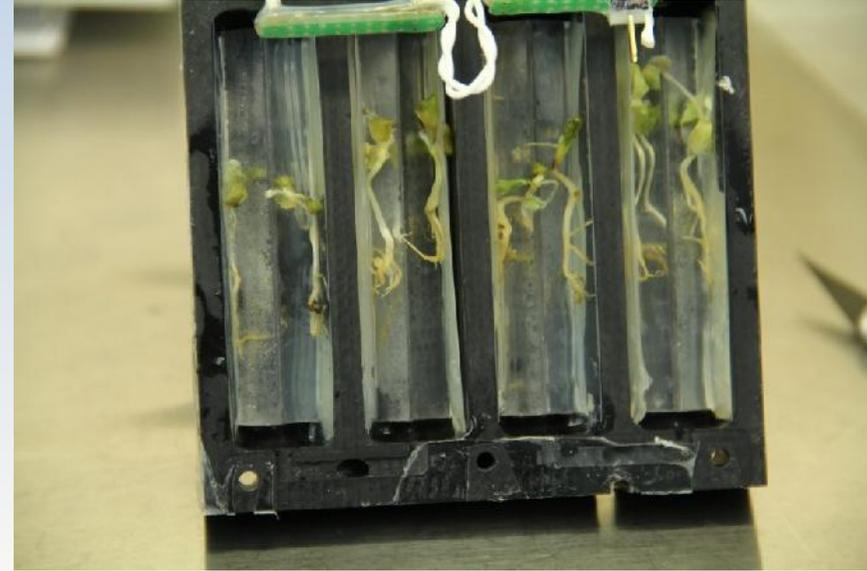
# SyNRGE Plant Growth Chamber (SPGC).



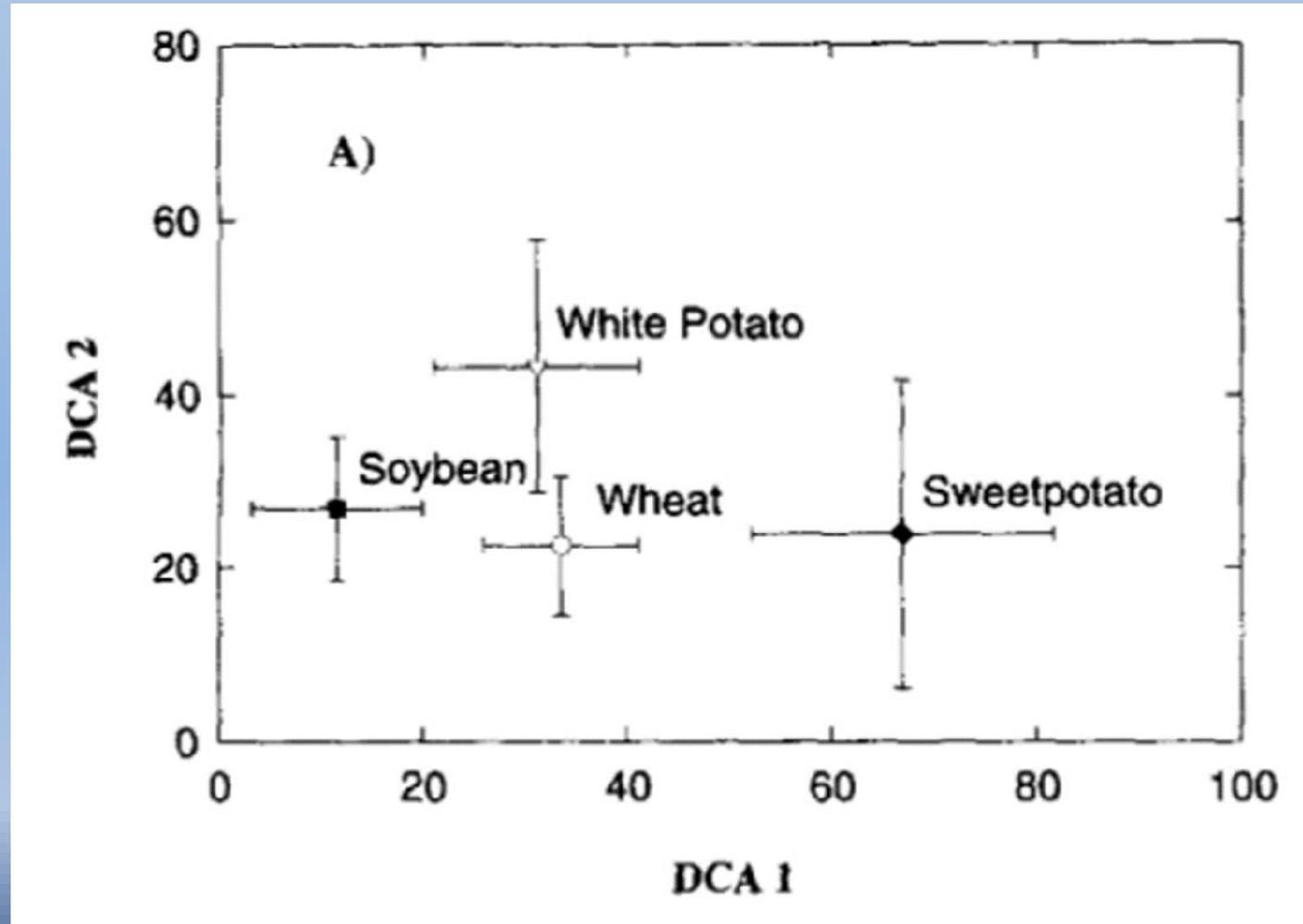
SyNRGE Plant Growth Chamber (SPGC) launched on SpaceX CRS-8 on 8 April to ISS, and it was installed in NanoLab on ISS on 11 April, 2016.



# SyNRGE PGC Experiment Return, May, 2016



# Will the plant/microbe relationship improve when we stop meddling?



(Garland, 1996; Morales, et al., 1996 ;Jenkins, et al, 2000; Frazier et al., 2013; Roberts, et al, 2004)



Thank You!



# Questions?

