

Maintaining Relationships in Closed Environments: Plant/Microbe Mutualisms

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5th International
Controlled Environment
Conference

AusPheno
2016



8th-12th
September 2016
CSIRO Discovery Centre
Canberra, ACT
A/NZ, Korea, Canada, EU, Japan
Russia, USA

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



Background



Ground



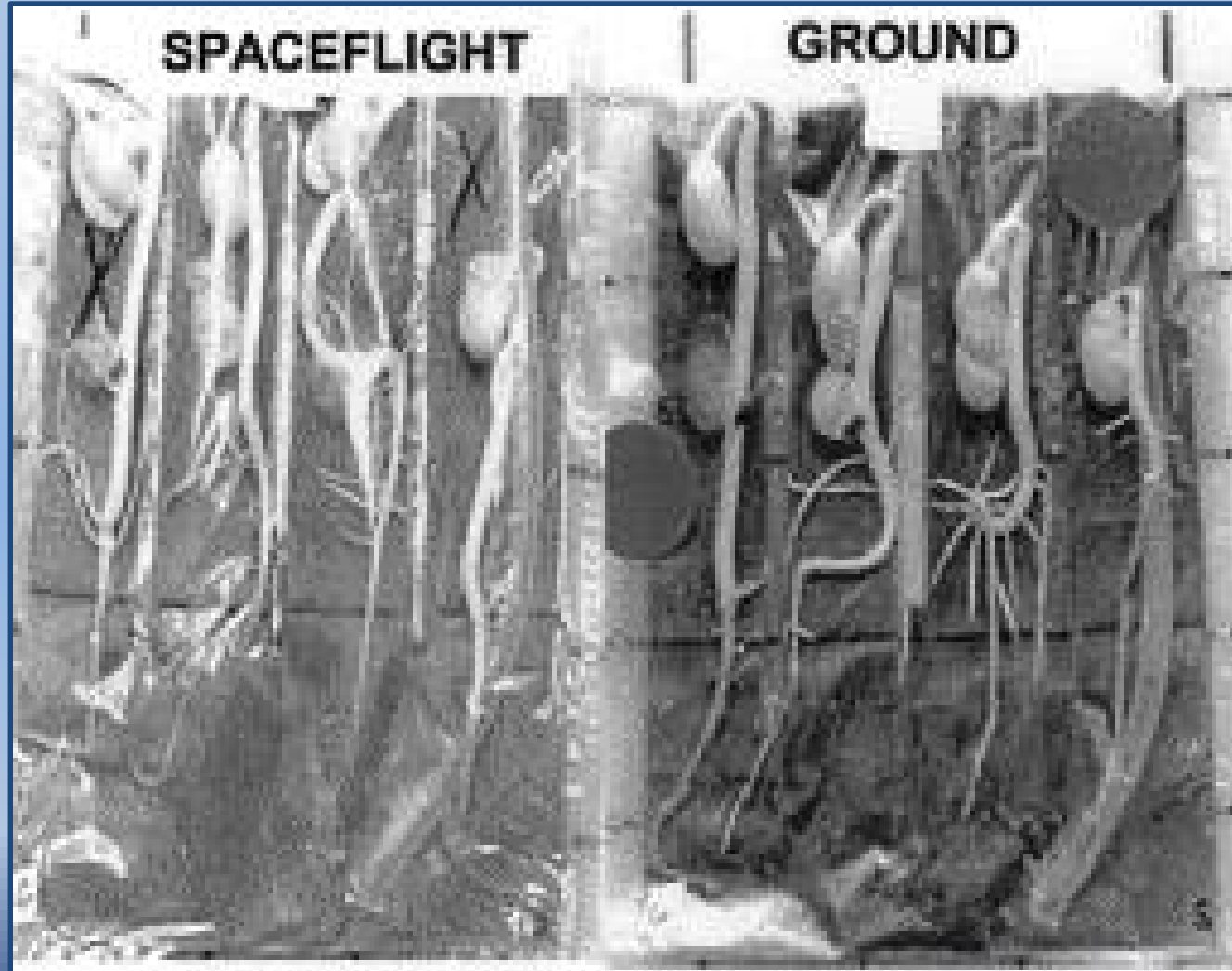
Flight



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.

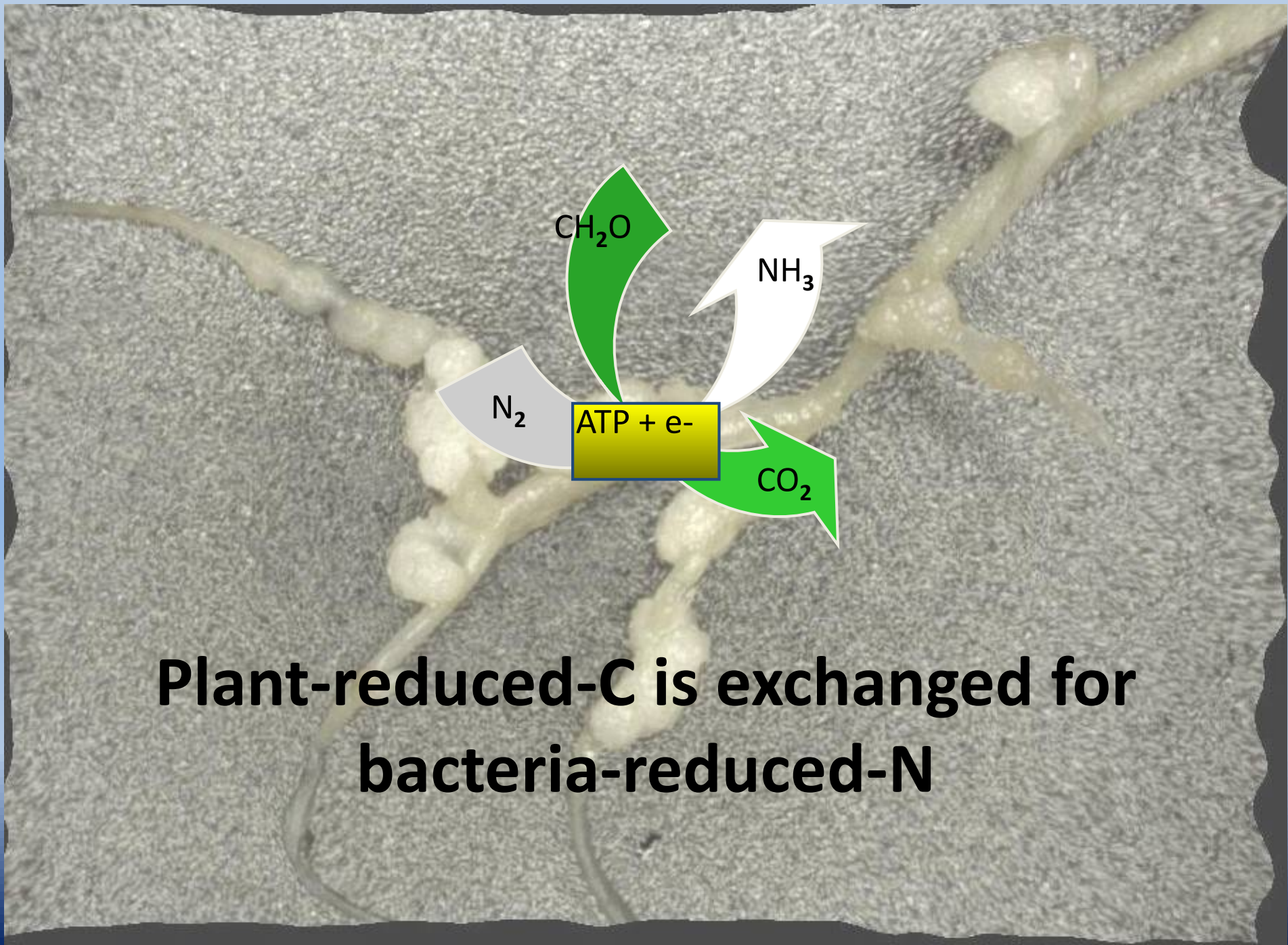




Plant/Microbe Mutualisms are critical to survival on Earth and may play similar role for long duration space missions.

- ✓ 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.





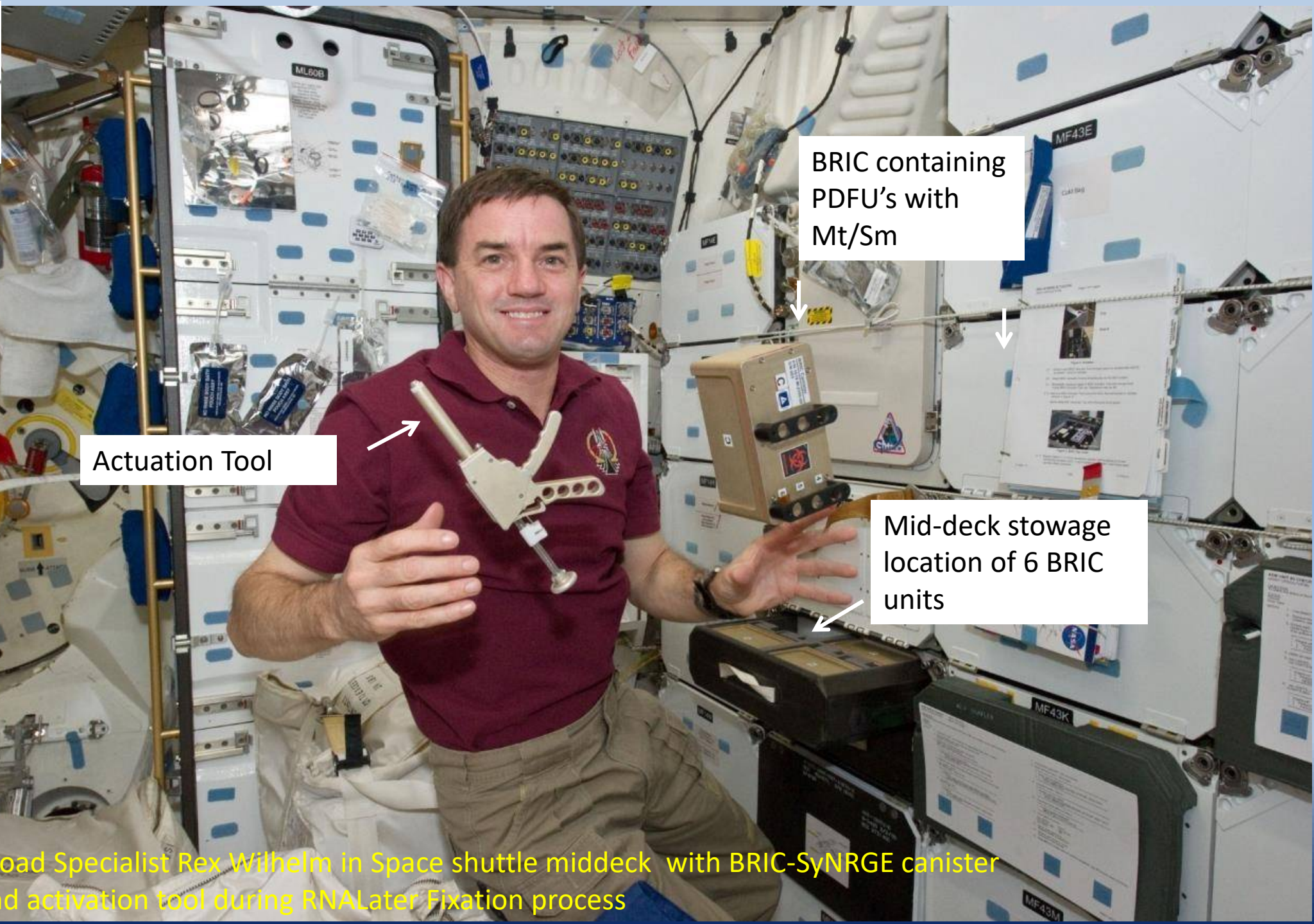
**Plant-reduced-C is exchanged for
bacteria-reduced-N**



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

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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



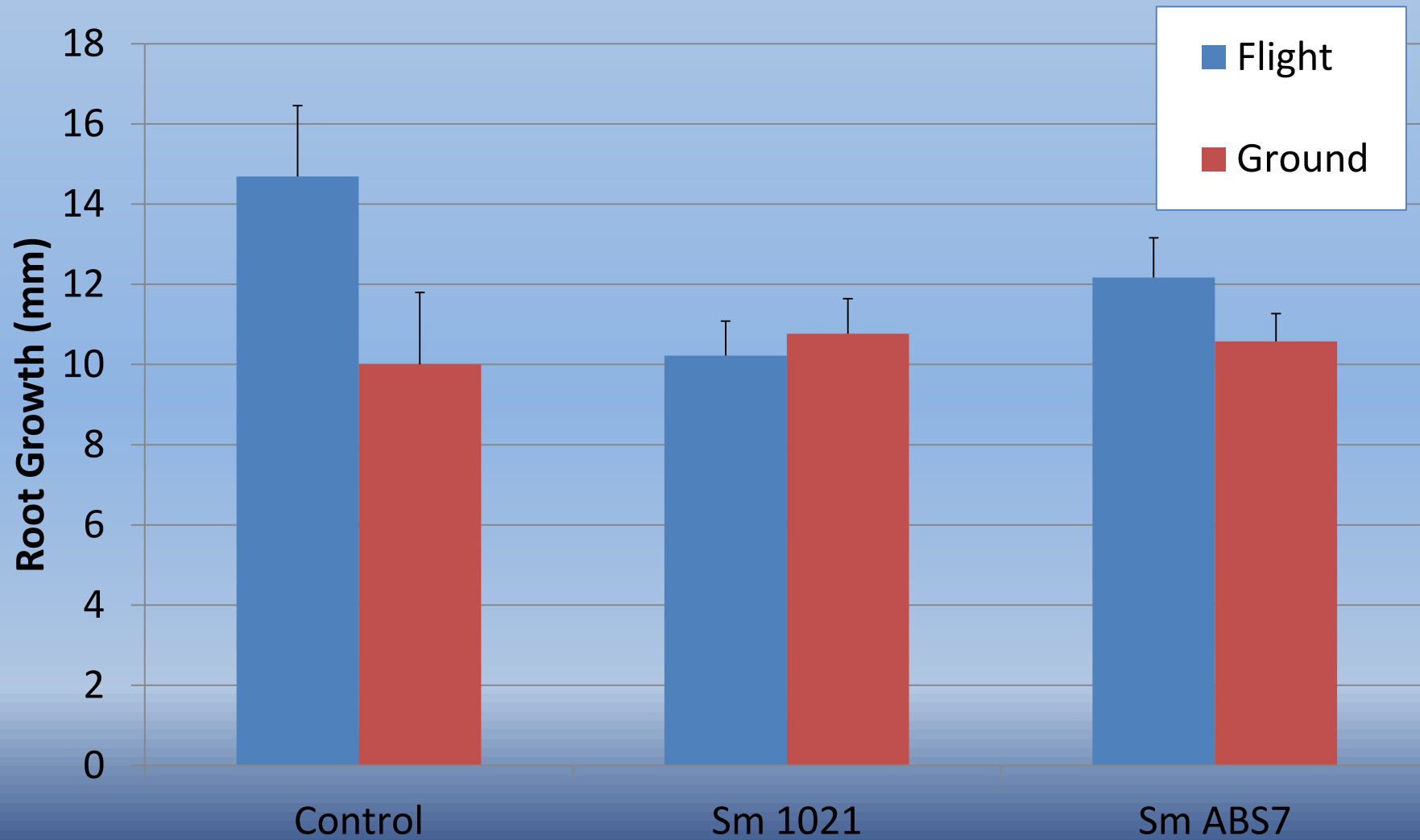
500.00µm



500.00µm

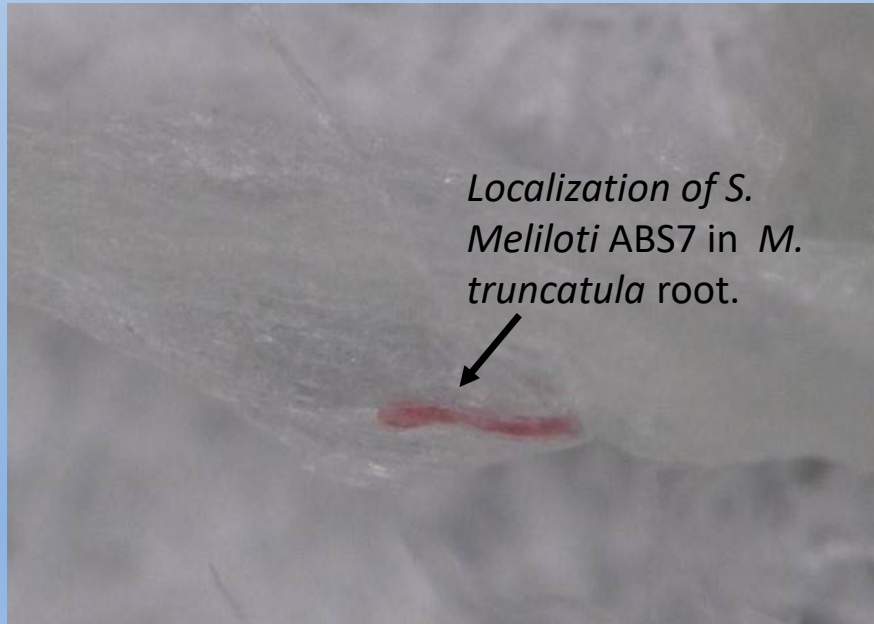


Effects of Microgravity on growth of *M. truncatula* cv. Jemalong A17 (Enod11::gus) inoculated with two strains of *S. meliloti*.

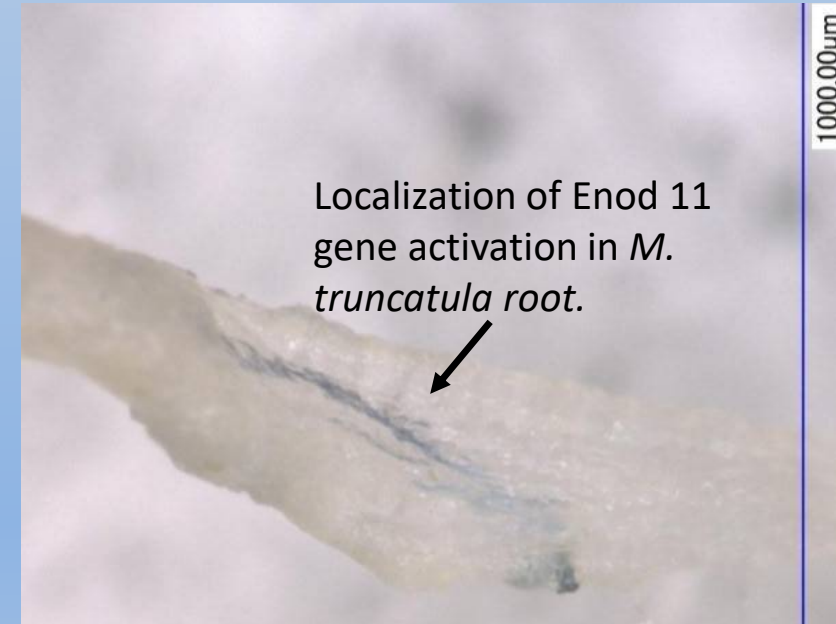




Localization of *S. meliloti* infection of *M. truncatula* roots and activation of ENOD11 gene in μg necessary for nodule formation, and subsequent biological nitrogen fixation.



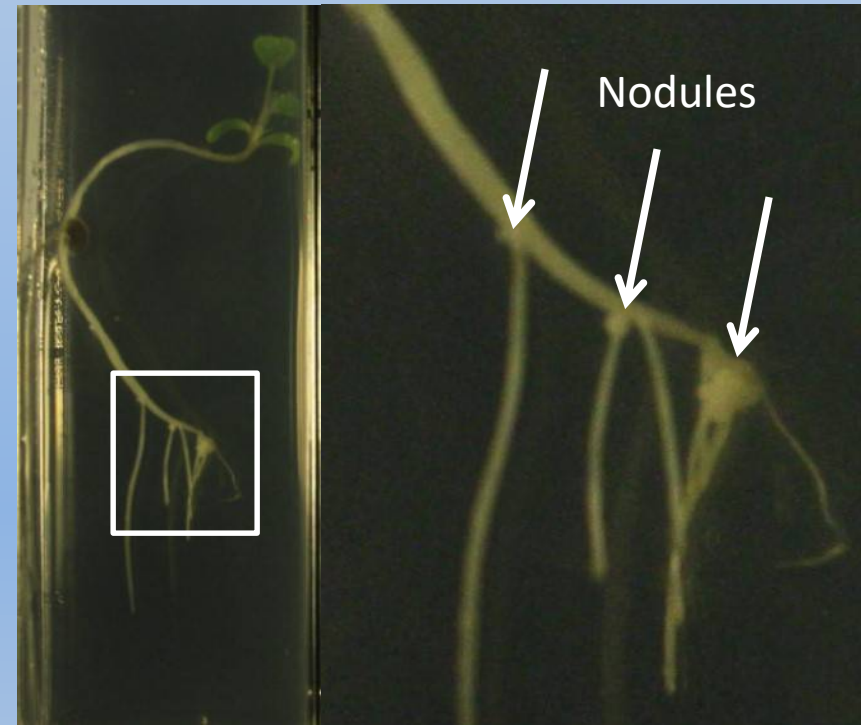
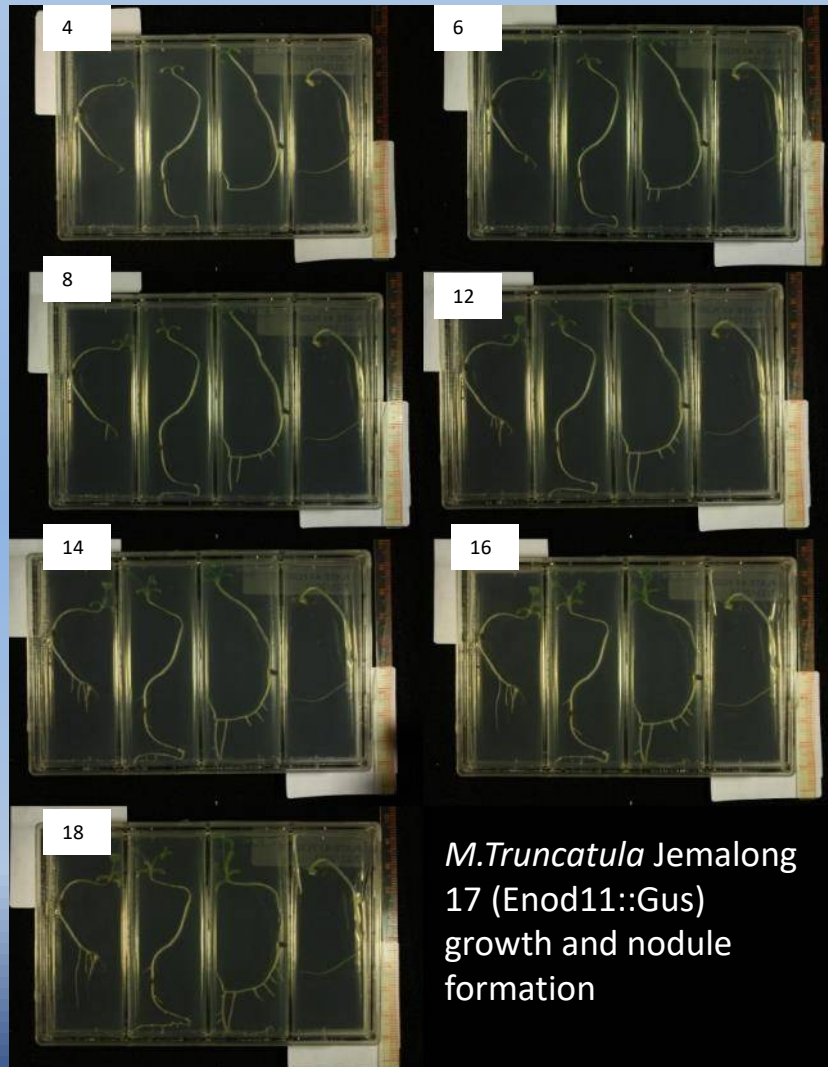
M. truncatula (Enod11::gus) inoculated with *S. meliloti* ABS7 with a hemA::LacZ marker. The stained area indicates site of *S. meliloti* infection in the etiolated *M. truncatula* root.



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.



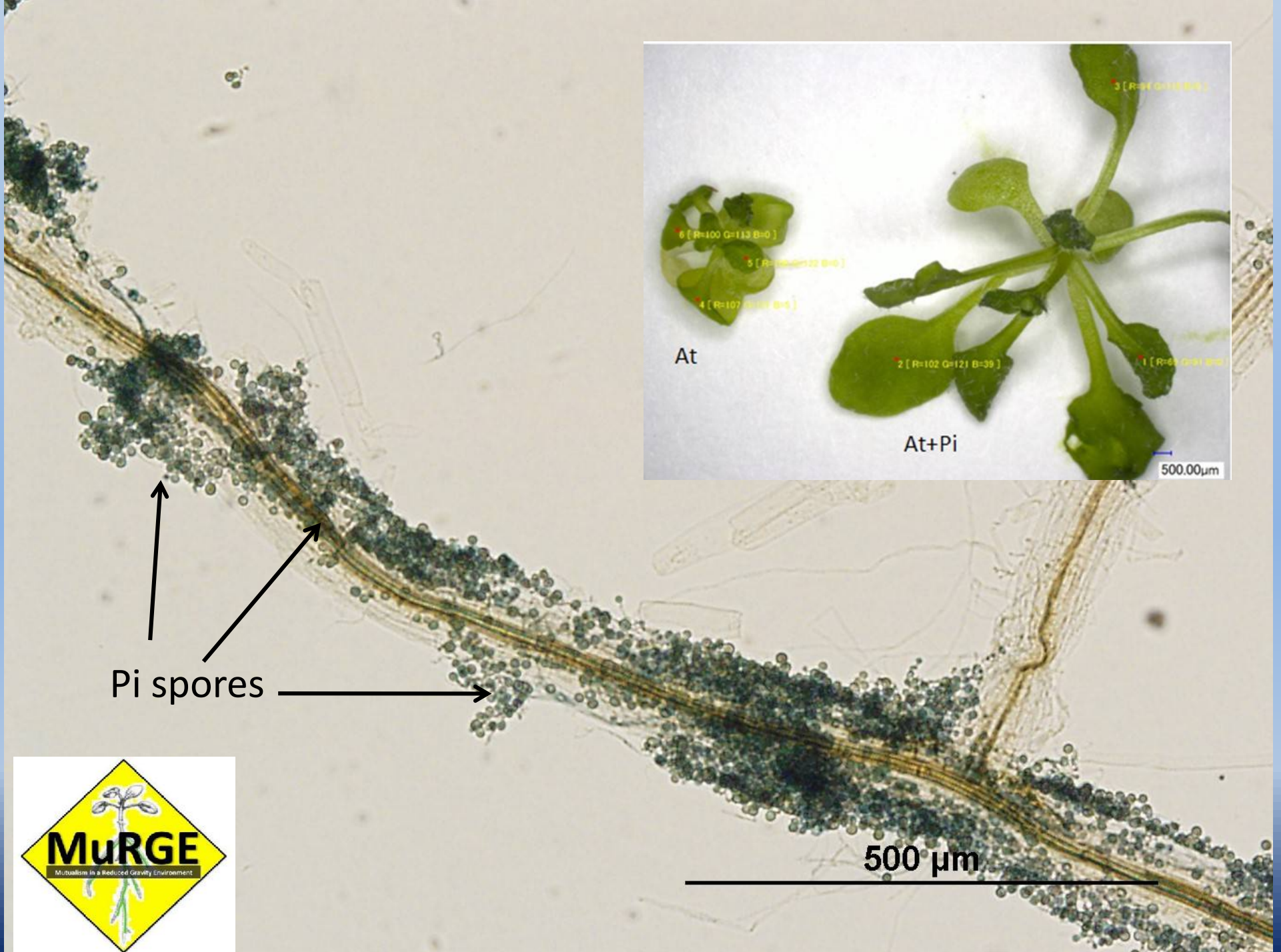
H_0 : Microgravity exposure reduces the susceptibility of the host plant (*M. truncatula*) to form nodules



M. truncatula cv Jemalong 17 (*Enod11::gus*) germinated in microgravity and inoculated with *S.meliloti* 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.

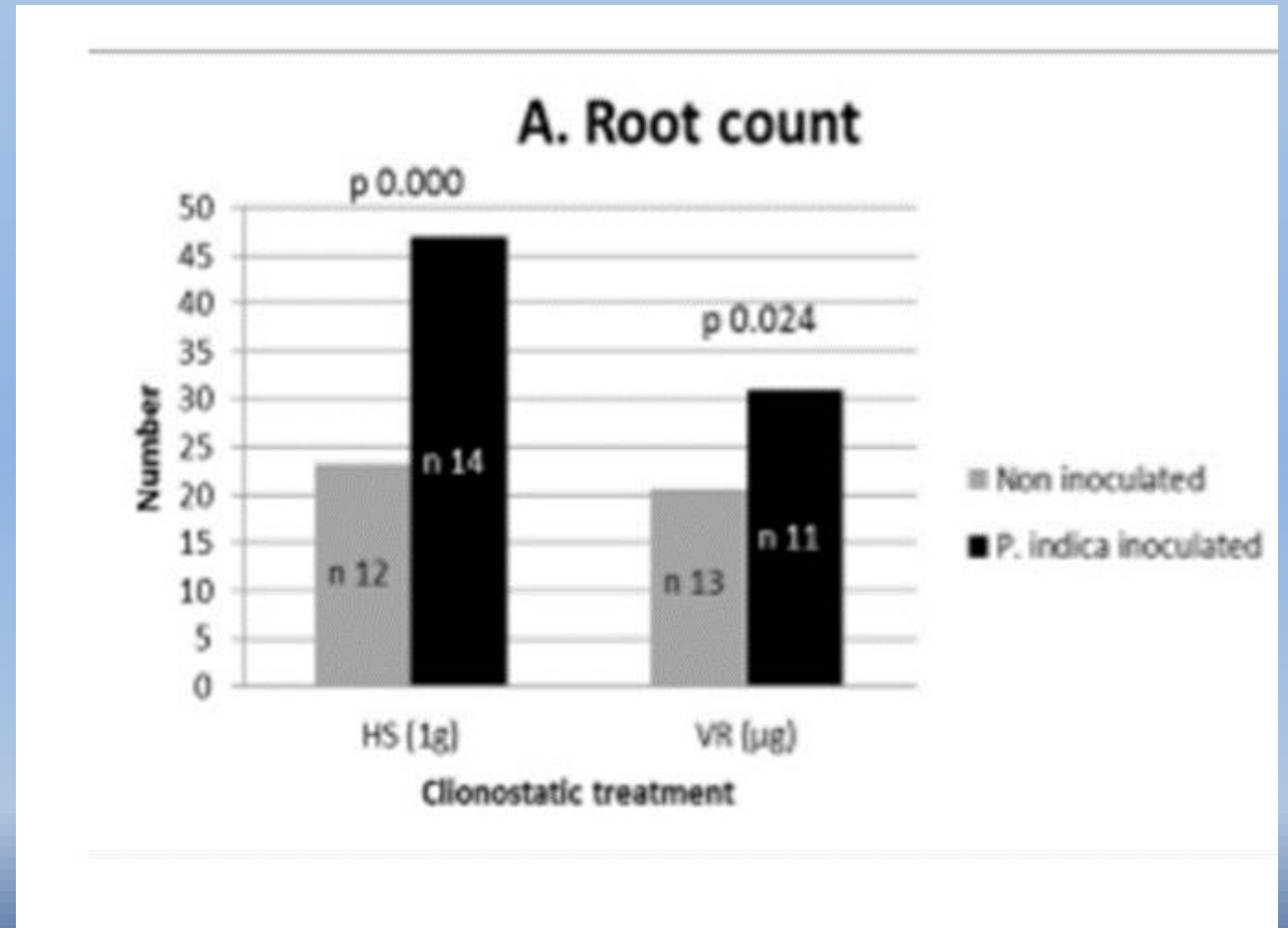
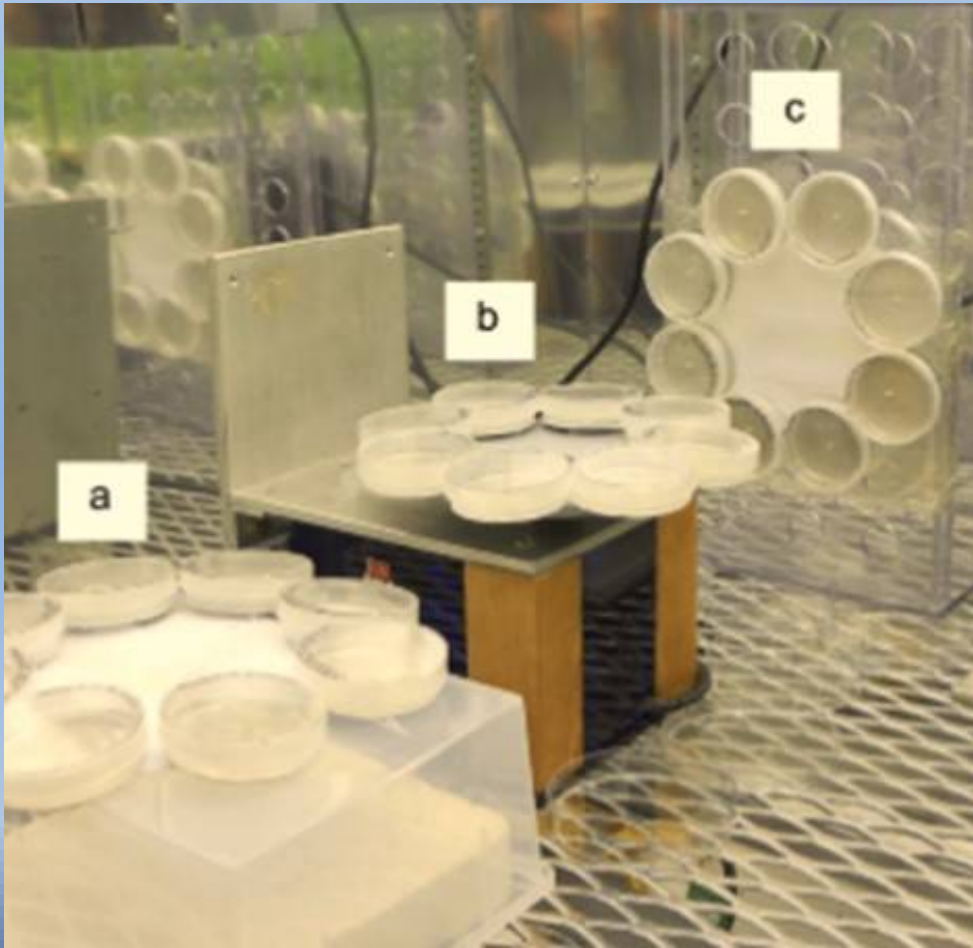


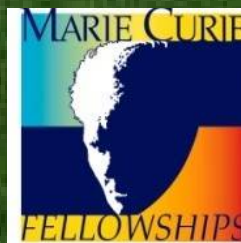
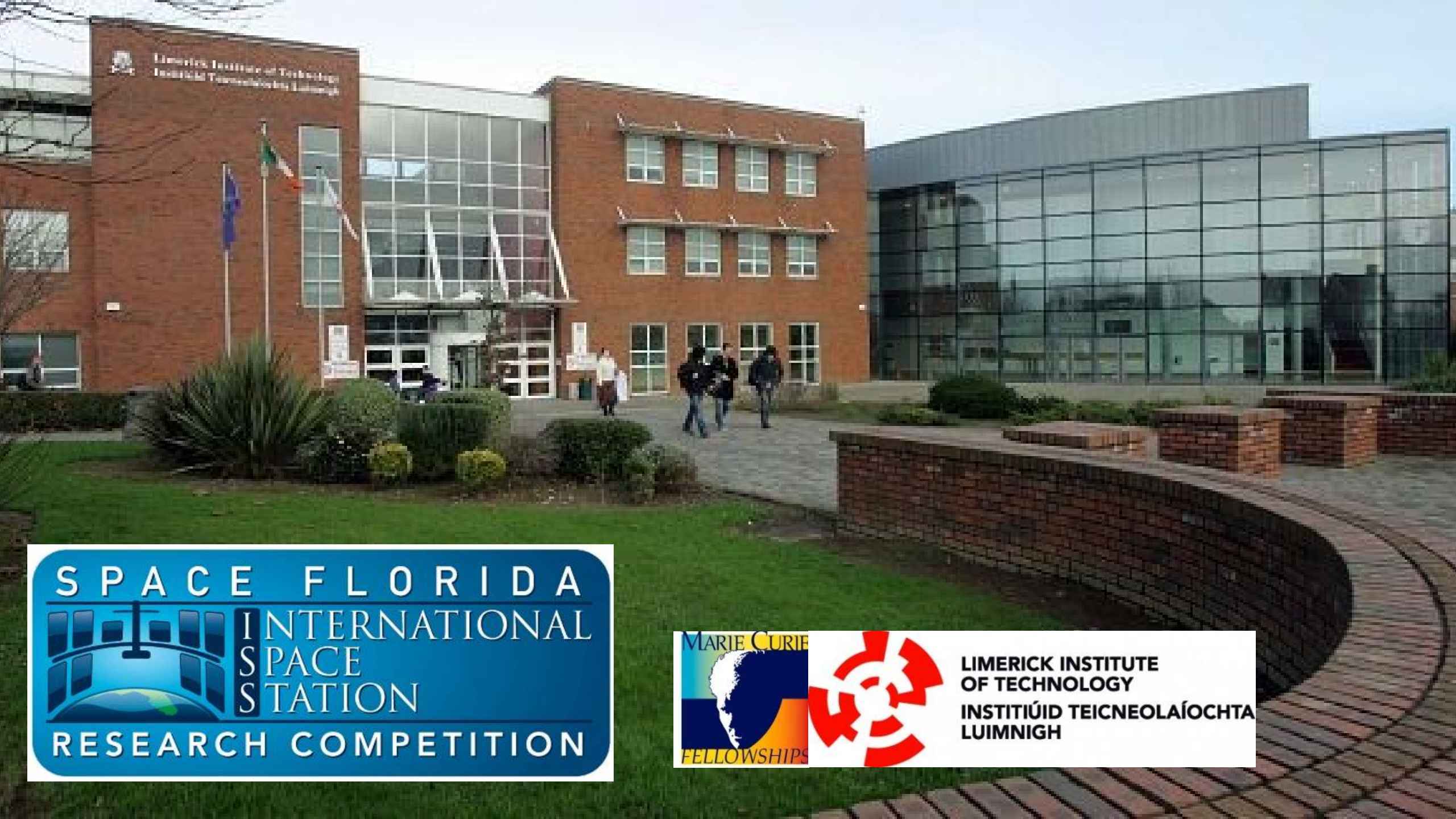
LETTUCE:

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.





LIMERICK INSTITUTE
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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 CO₂
- Fixation capabilities
- Imaging
- Data storage on board
- Periodic data download/access



SyNRGE³ 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

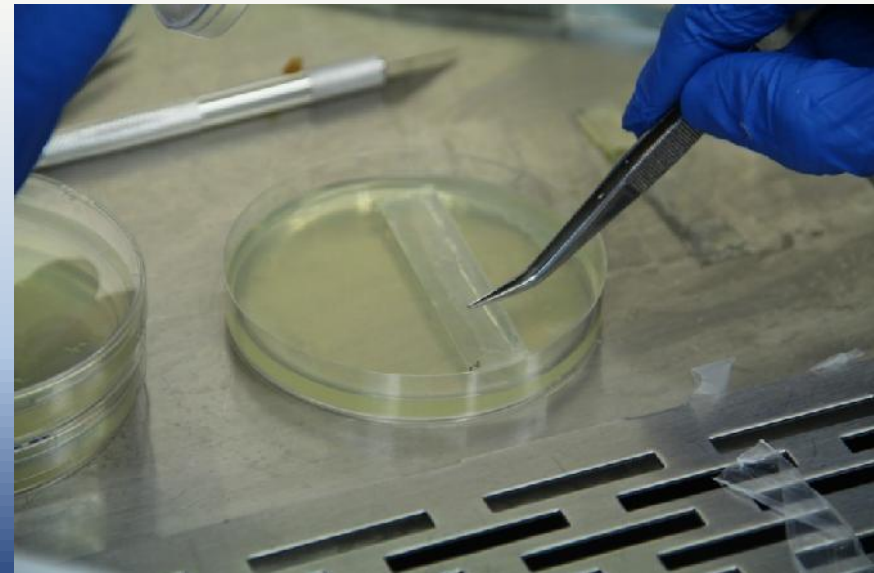
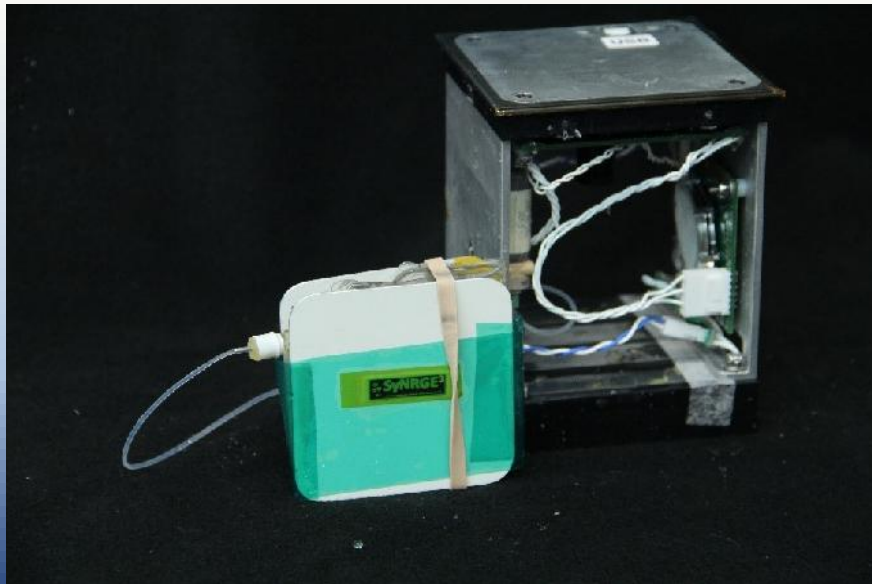
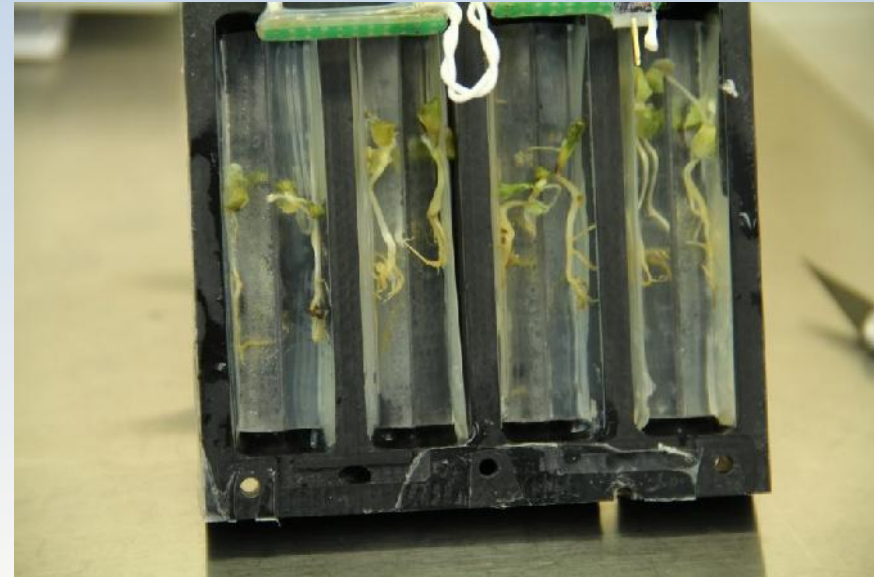
SyNRGE Plant Growth Chamber (SPGC).



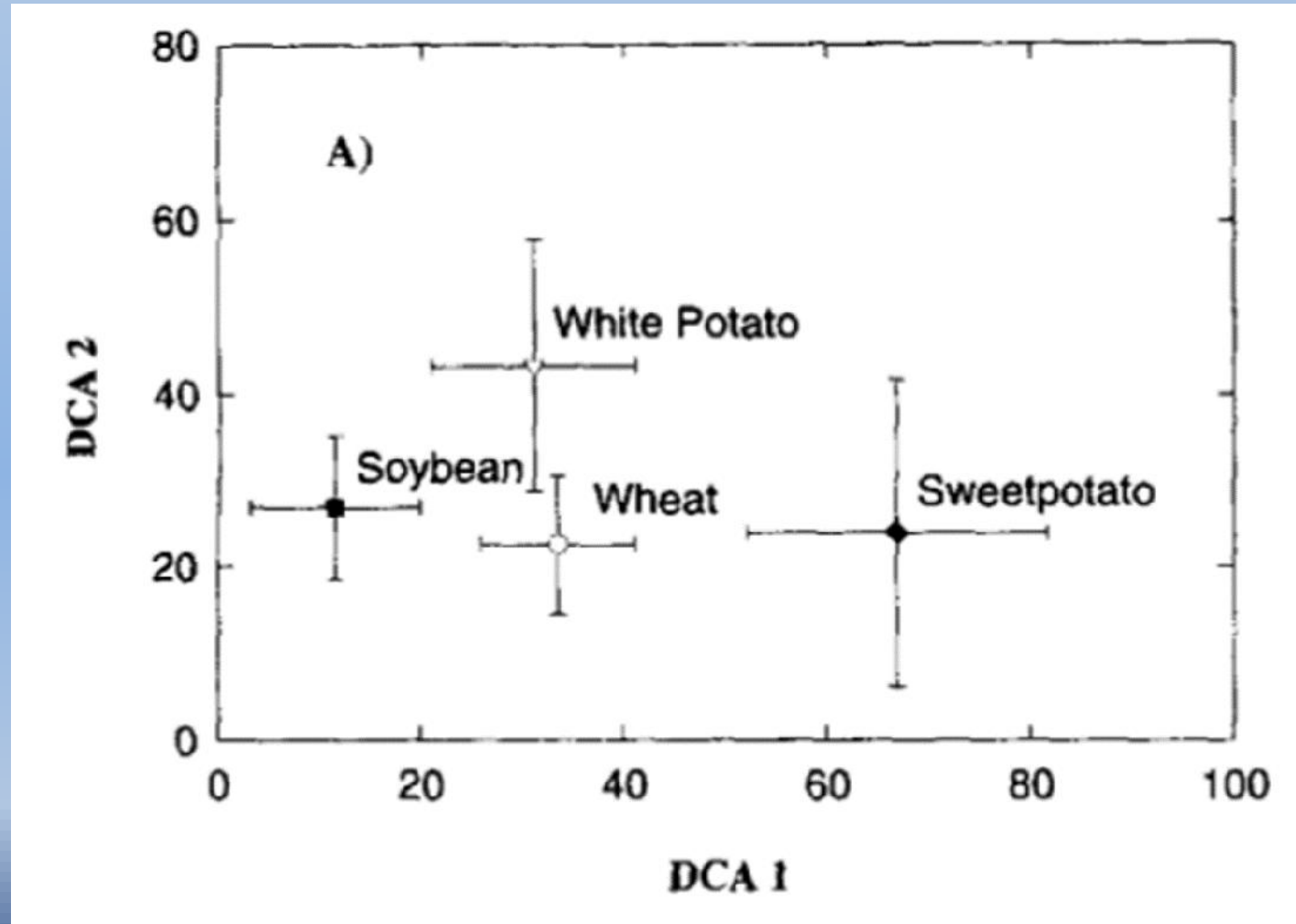
SyNRGE Plant Growth Chamber (SPGC) launched on SpaceX CRS-8 on 8 April to ISS, and it was installed in NanoLab on ISS on 11 April, 2016.



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?

