# INSIGHTS FOR MICROBIOMES IN REGOLITH BASED AGRICULTURE (RBA) FOR FUTURE OFF WORLD HABITATS BASED ON SIMULANT GROWTH EXPERIMENTS


BIOTECH IN SPACE
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AMERICA'S FIRST COMMERCIAL SPACE BIOTECH

# Mission

**We lead SPACE-BASED biotechnology by empowering our collaborators with rapid access to microgravity, advanced subject matter expertise, and a spirit of innovation.**

# About

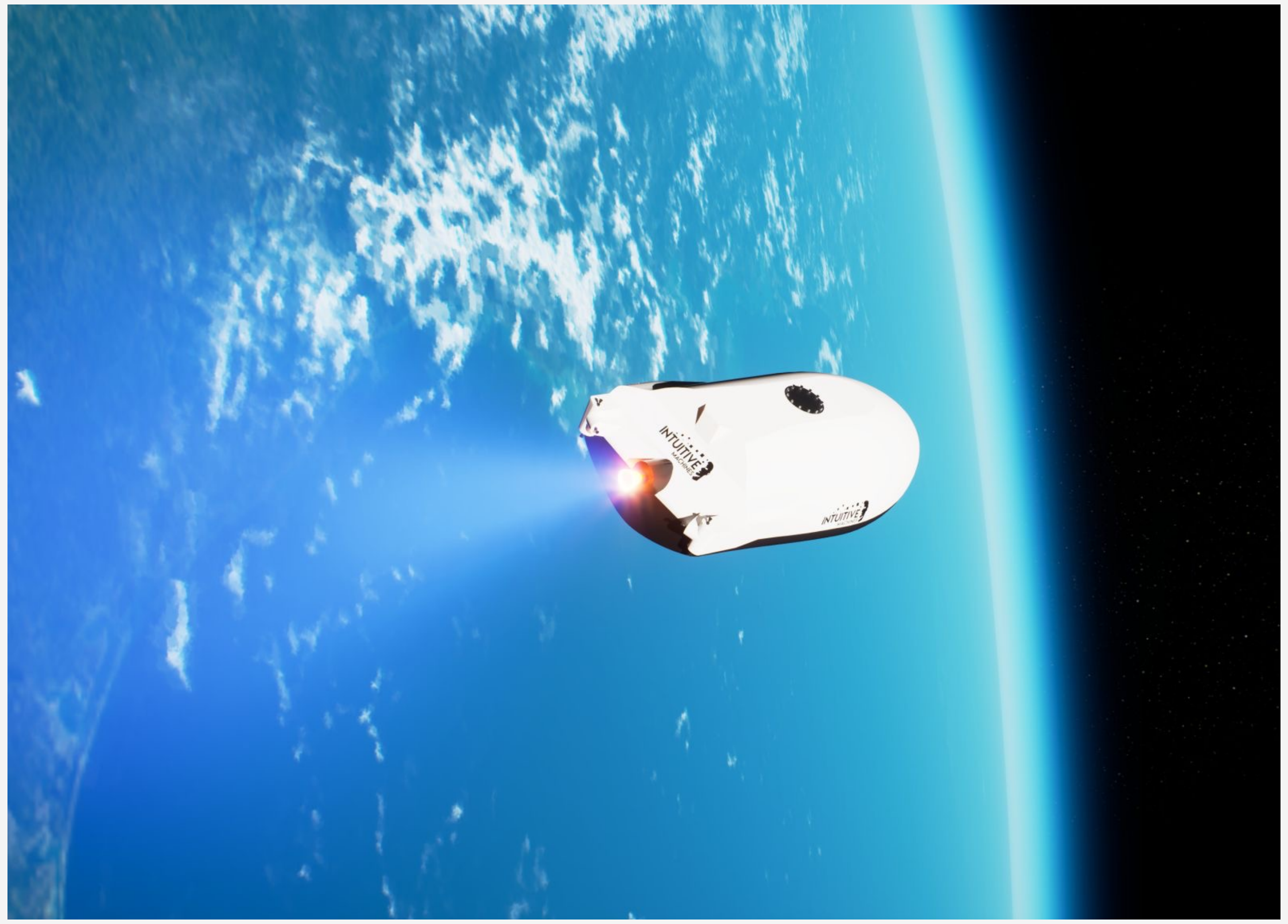
- Woman Owned & Operated with a Nation-Wide Team of Subject Matter Experts, Laboratories, and Mission Control Facilities
- Since 2020, We Have COMPLETED Over 25 missions: **Empowering our collaborators to achieve Many "1st in Space"**
- 1st Company to Implement Biotech Industry Standards into Space Processes with our “Quality, Industry Compatible (QuIC) Space Process™- VETTED & UTILIZED by DoD
- We enable **teams to conduct Space Biotech at the Speed of Business: completing** design, launch, in-orbit ops, post-flight analytics in ONE YEAR

# A Strategic Partnership With:

- Mission duration, altitude and landing location is driven by the science onboard
- Up to 100 kg payload mass
- Supports late load and rapid offloading of live biological payloads
- Low-G reentry protects science payloads (tissues, crystals, etc.)
- Enables scaling of space-based production models

# Zephyr: An Orbital-to-Earth ReEntry Vehicle

## Designed to Accommodate Biotechnology, Pharmaceutical, and Semiconductor Industries



Receive Updates and Book a 2027 Mission on Zephyr:

# Acknowledgements...


- Prof. Rafael Loureiro (Winston-Salem State)
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- The WSSU Astrobotany Lab

- The Palmer Lab
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- Steven Elsaid (Exolith Labs)
- Anca Delgado (ASU)
- Dr. Tatiana Karpova

# *In situ* food production is mission critical to successful off-world settlements

REGOLITH vs HYDROPONICS

- Improve food security to settlement
- Reduce menu fatigue
- Fresh plant material for nutrition
- Plants/Producers are a critical component of a sustainable ecosystem.
- Psychological benefits

# Regolith vs Hydroponics – Costs and Benefits

**REGOLITH**

Substrate already available on Mars & contains useful nutrients

Add microorganisms to develop native carbon, phosphate, and nitrogen cycles, improving nutrient cycling.

Can be run multiple times (improving soil)

Contains useful nutrients for plants reducing transport costs (CAN THEY BE MADE AVAILABLE?)

We don't have any Martian regolith yet

Potentially toxic elements

**HYDROPONICS**

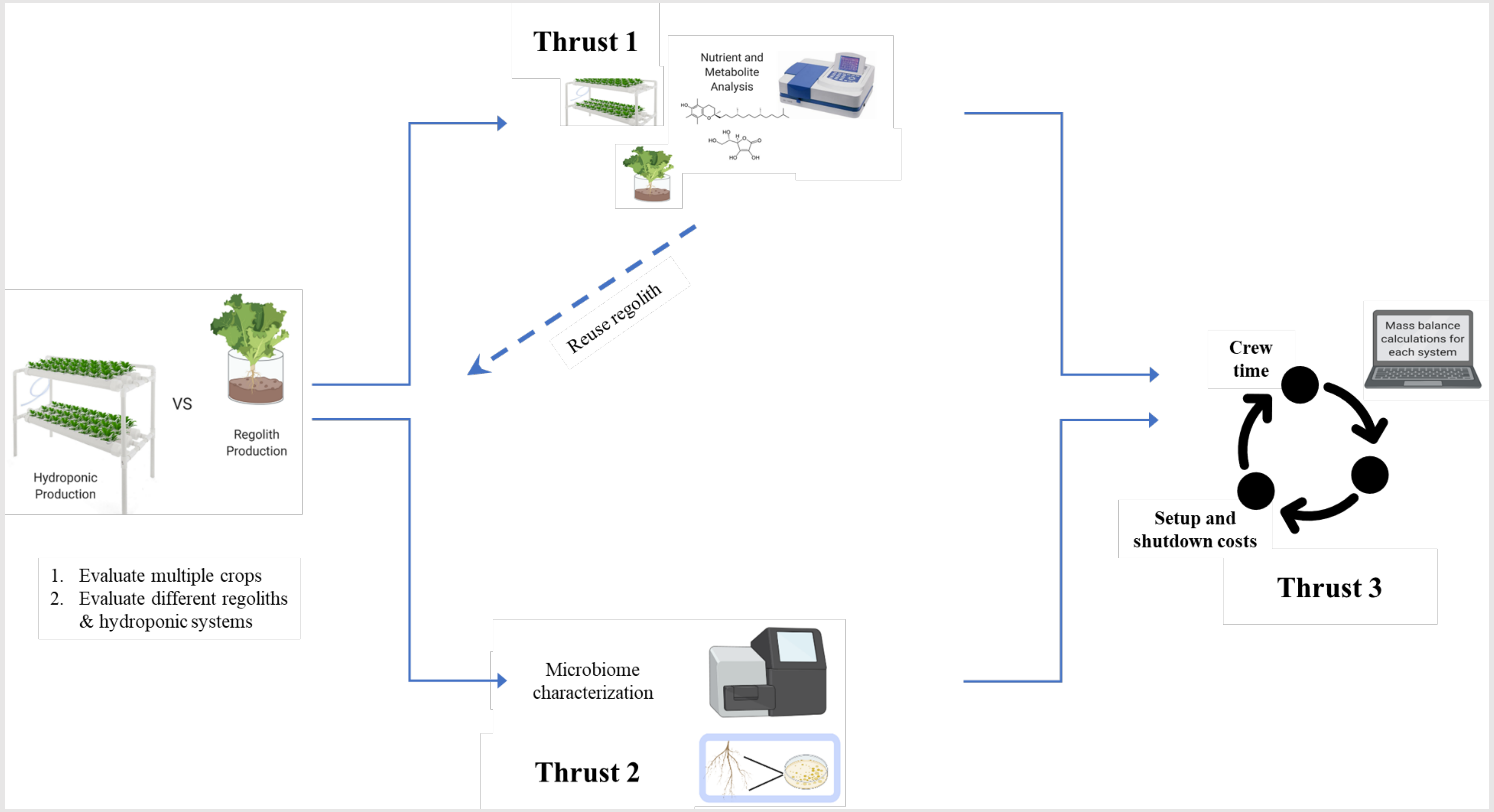
- We could do it now! High TRL
- Controlled nutrient release
- 'Better' space utilization
- Can create some microbial associations
- Disease can spread more quickly
- May require more maintenance or setup
- More parts

# Regolith AND Hydroponics

## Food security through diversity and resilience – CHRGE

- How do we decide which crops should be grown hydroponically? In regolith?
- How sustainable are these decisions?
- Do these choices change over time (multiple runs)
- How about elements beyond yield?
- A cradle-to-grave analysis
- Provide criteria for making decisions

# CHRGE Pipeline, Deliverables, & Related Projects




- Systems approach to comparing technologies
- Integrating crop production more realistically into sustainable models (waste streams, etc.)
- Evaluating Space Crops to Advance Predictive Edibility (ESCAPE)
- Understand how the regolith microbiome evolves
- Transition from ‘regolith’ to ‘soil’

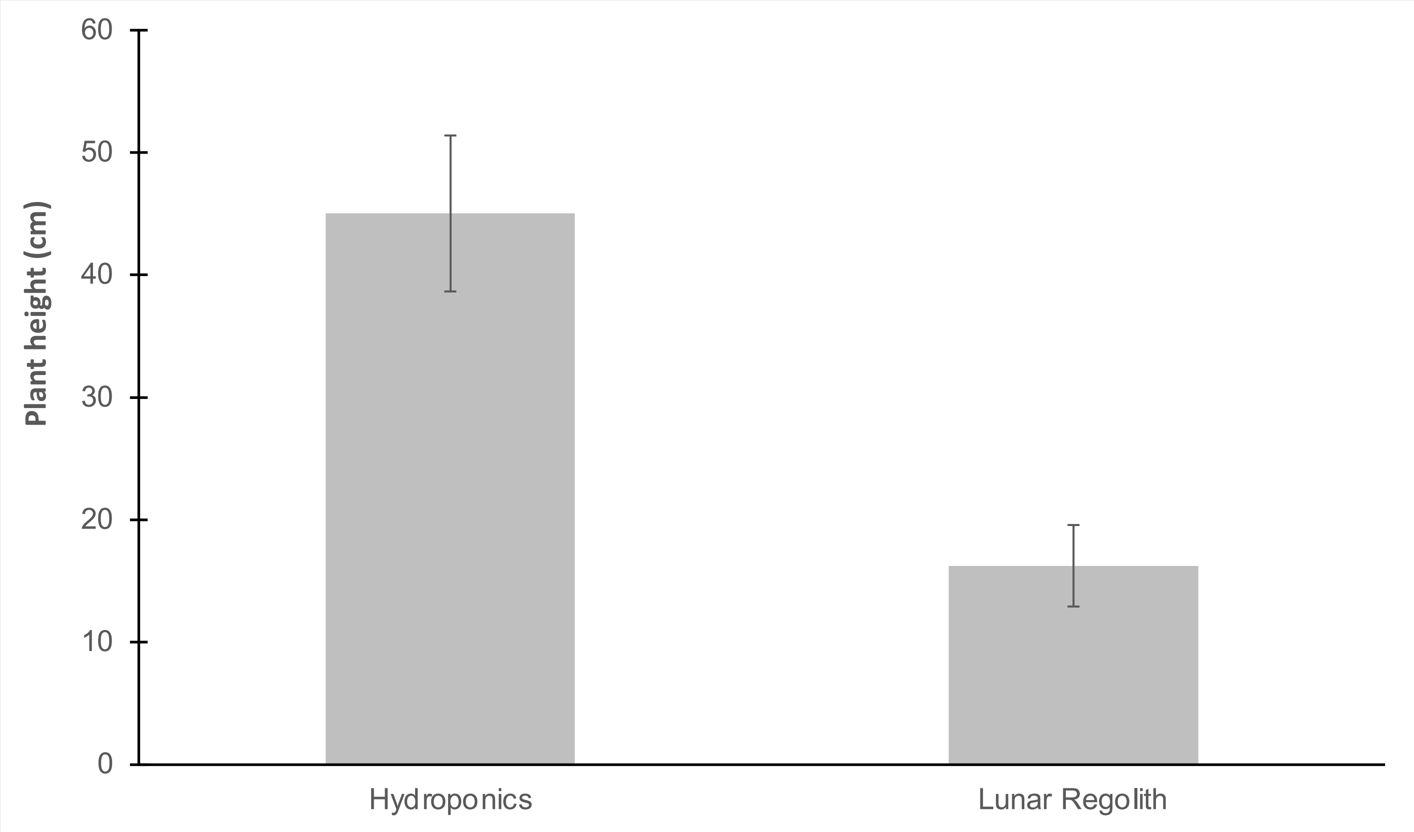
# The side-by-side (Fodder King vs Hydroponics) N=15

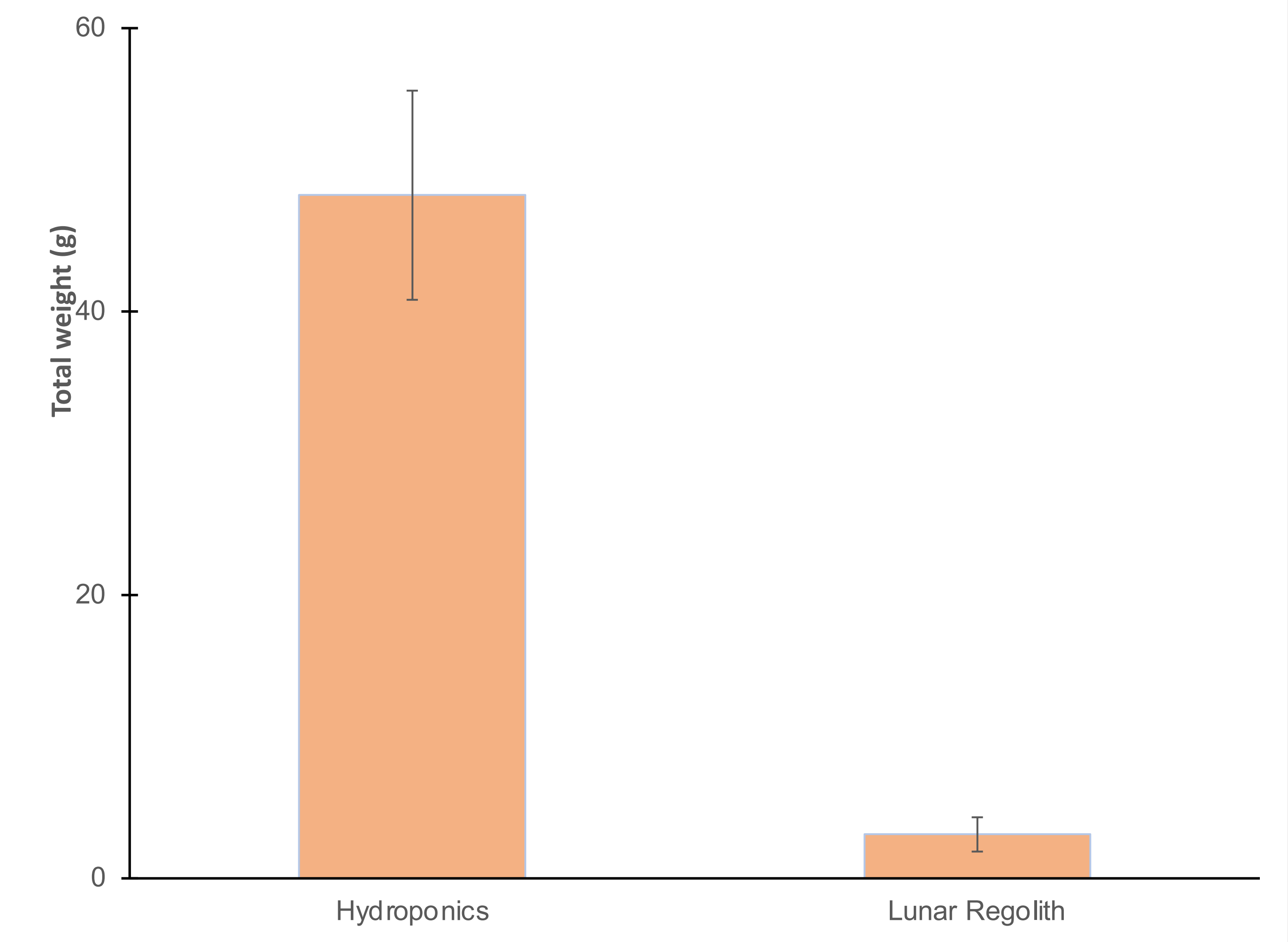
# Hydroponic lettuce is bigger, dense root system, etc.

## ‘Healthier plant’?

Hydroponics

Regolith

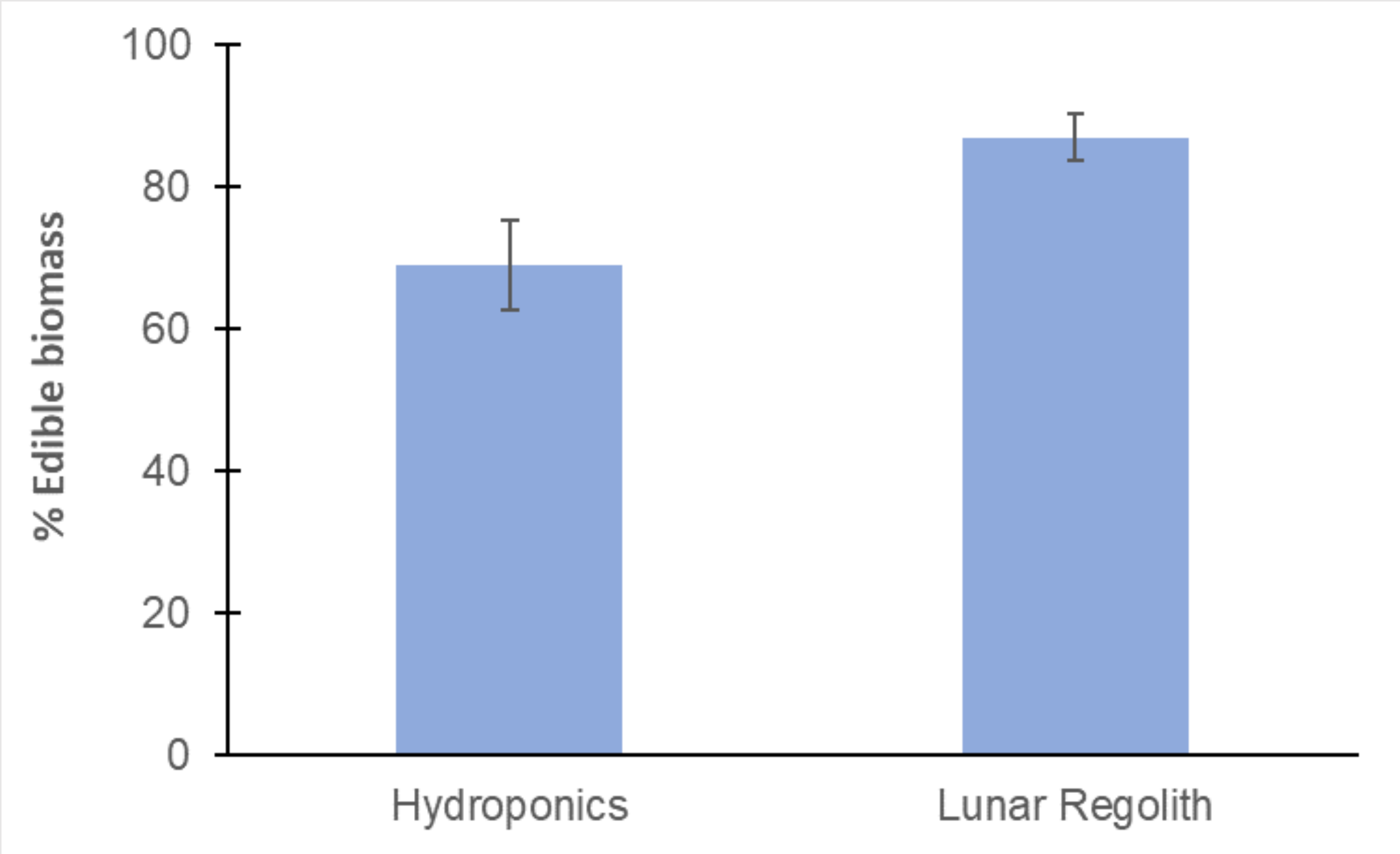


# RBA has higher % edible biomass ($p<0.05$)

Hydroponics

Regolith



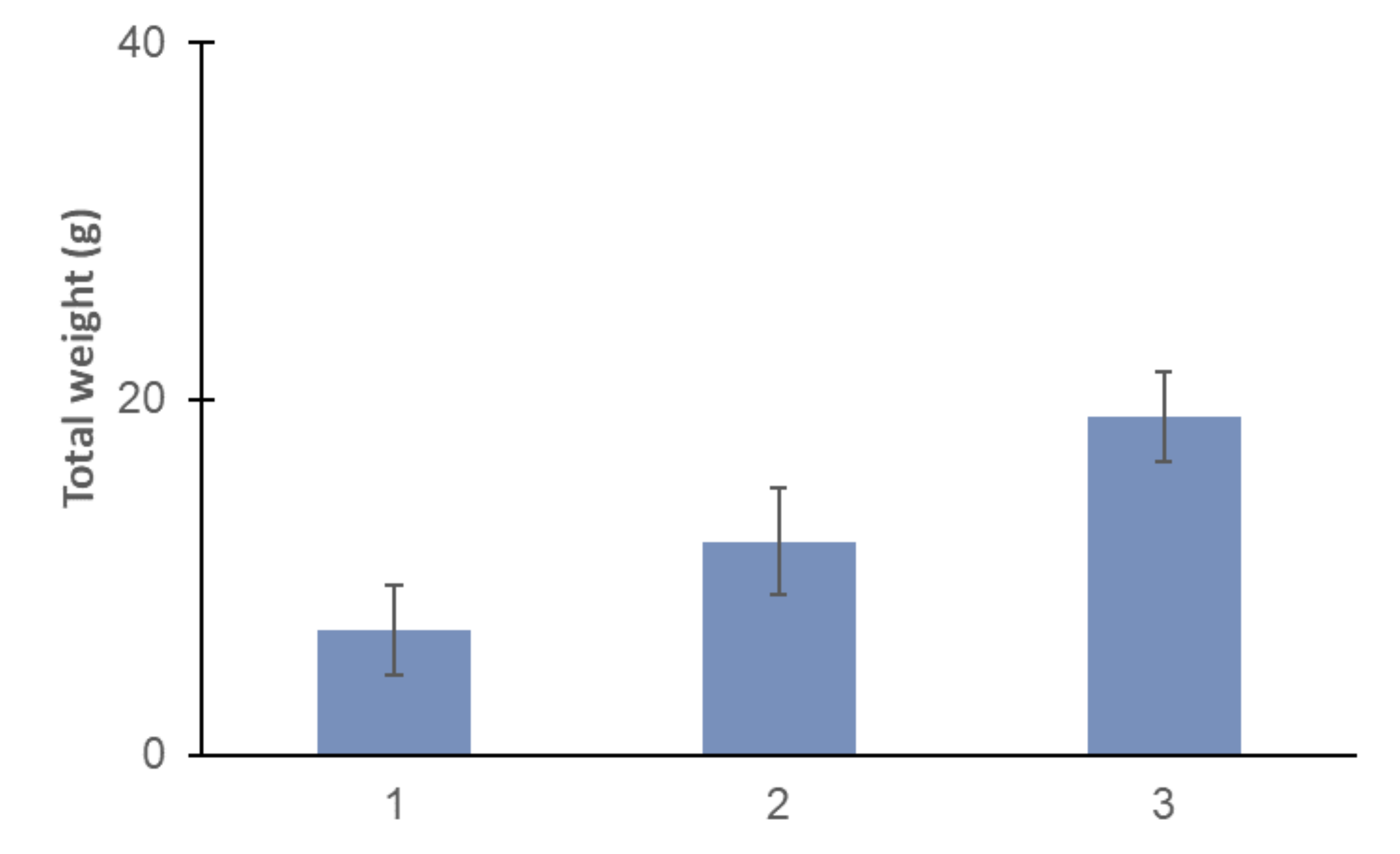

| | Kw/mo | Gallons/mo |
|---|---|---|
| Hydroponics | 2995.2 | 17.1 |
| Lunar Regolith | 2001.6 | 4.1 |

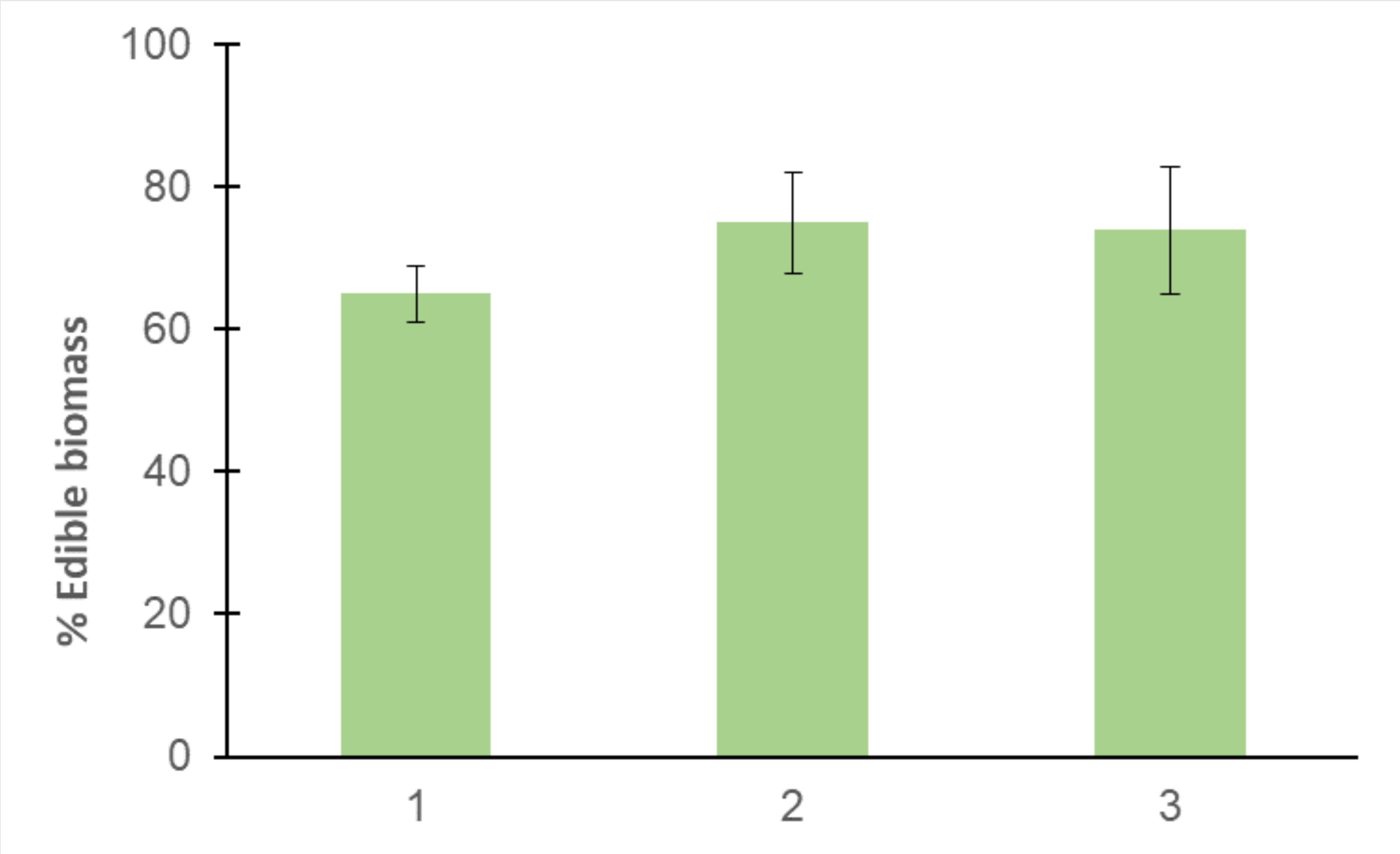
**Hydroponics system takes more electricity and water!**

**Hydroponics produced ≈50x more inedible biomass!**

# Recycling Martian Regolith Simulants...

## It does get better..... Jujuing the regolith ←

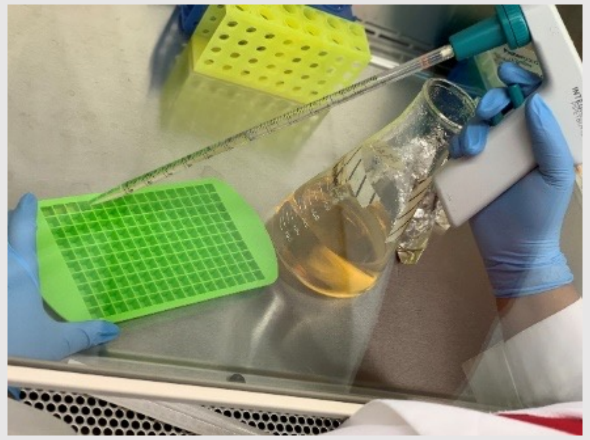


Controlled environment conditions (Photoperiod 16 light/ 8 dark; Temperature 23°C day/ 18 °C night; Relative humidity 70%; Irradiance (blue/red) ~400 µmol $m^{-2}$ $s^{-1}$ HPLED; CO2 ~ 1000 ppm) .Watering at 100 ml on the rooting area's surface (6 a.m. – 6 p.m.)

Variables Analyzed - Plant Height (cm); Number of Leaves; Number of Flowers; Stem Diameter (mm); Fruit Yield (g/plant); Fruit Size (g/fruit); Brix Level (°Bx); Seed Viability (germination rate, %)

RBA (regolith-based agriculture boxes) boxes – inoculants PEP1 recipe (*Azospirillum* spp., *Rhizobium* spp., *Azotobacter* spp., *Pseudomonas fluorescens*) using WSSU-ABL microbial cube ® technology.



WSSU-ABL microbial cube ® for regolith inoculation for the PEP1 treatment

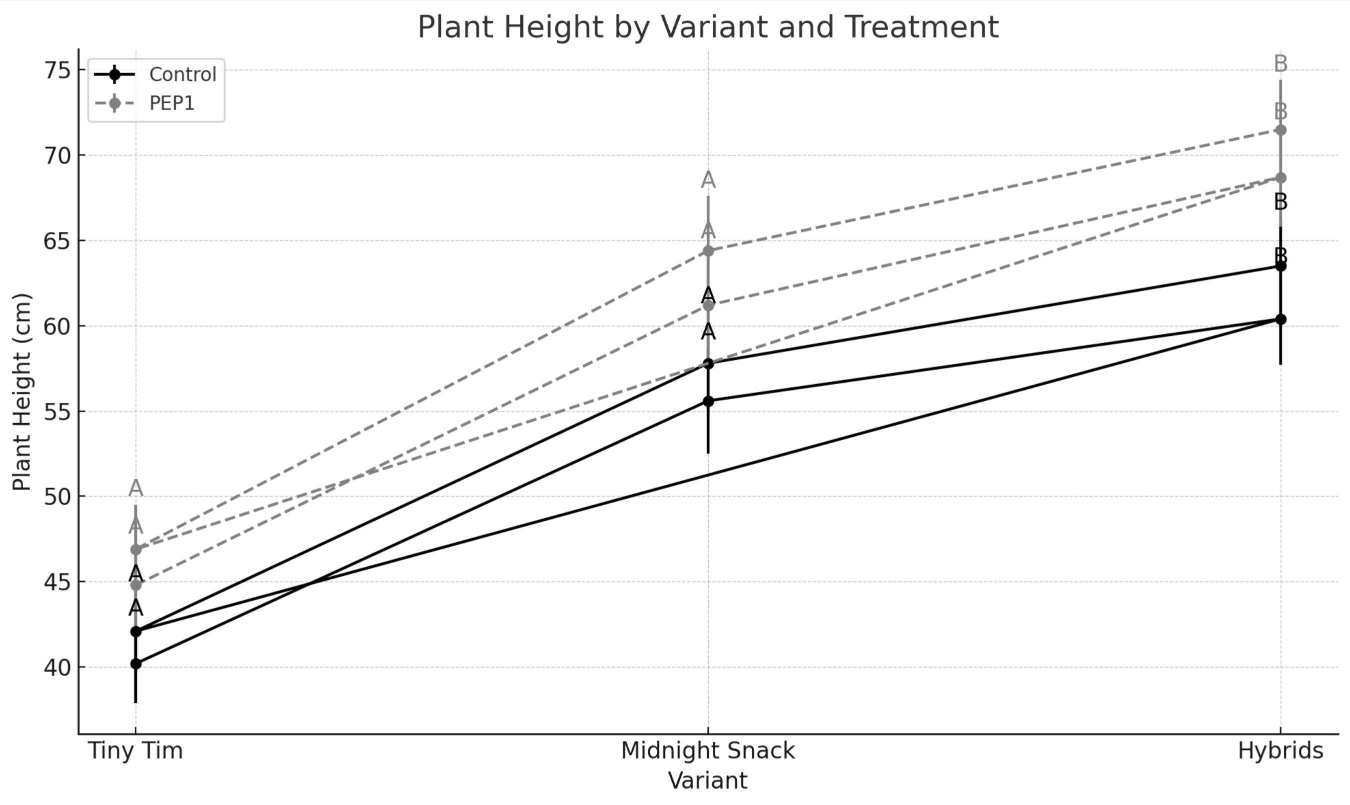
Tiny Tim - Midnight Snack – "Tiny Snack" hybrids tomato variants – 55 day grow-out (production cycle) – n=20 per variant, per treatment (n=120 plants per G)

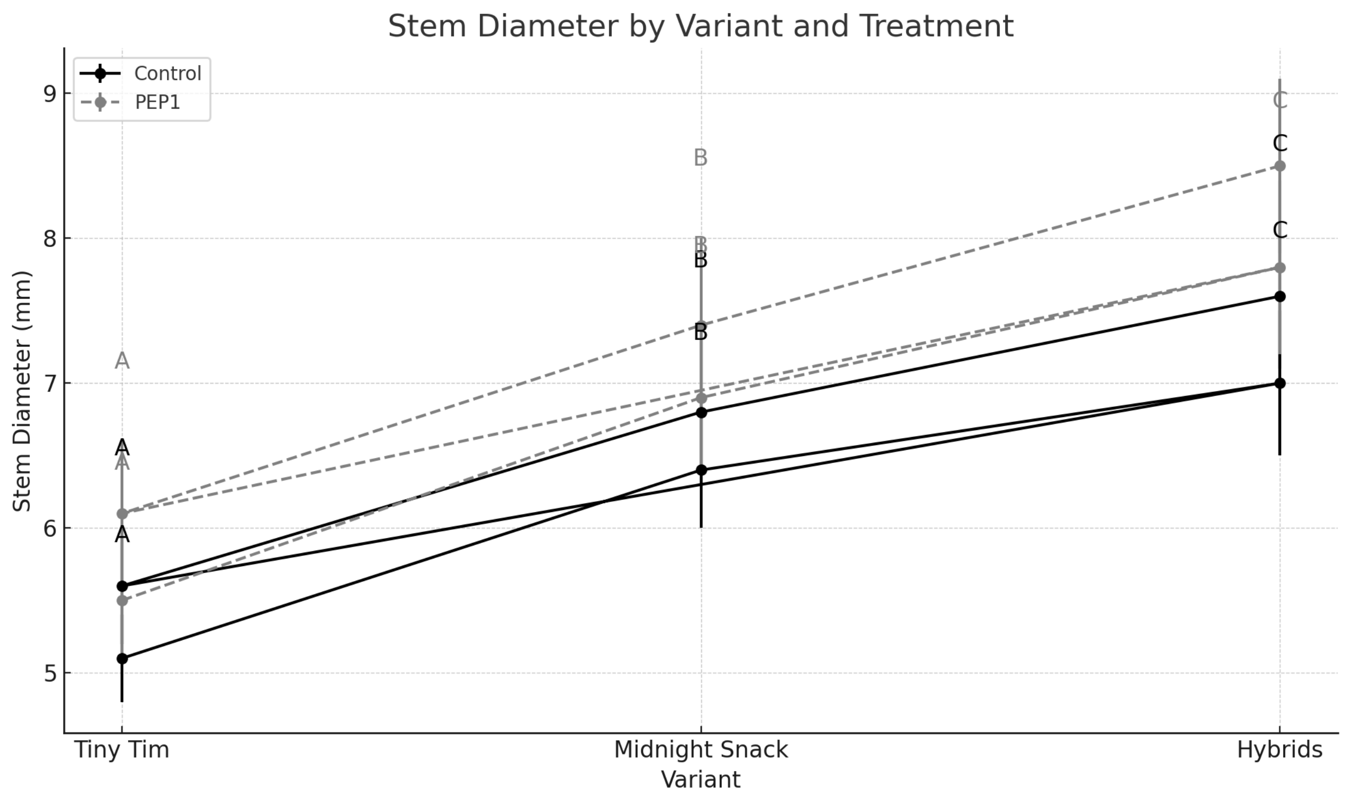
G1 – plants that grew in regolith that had never been used

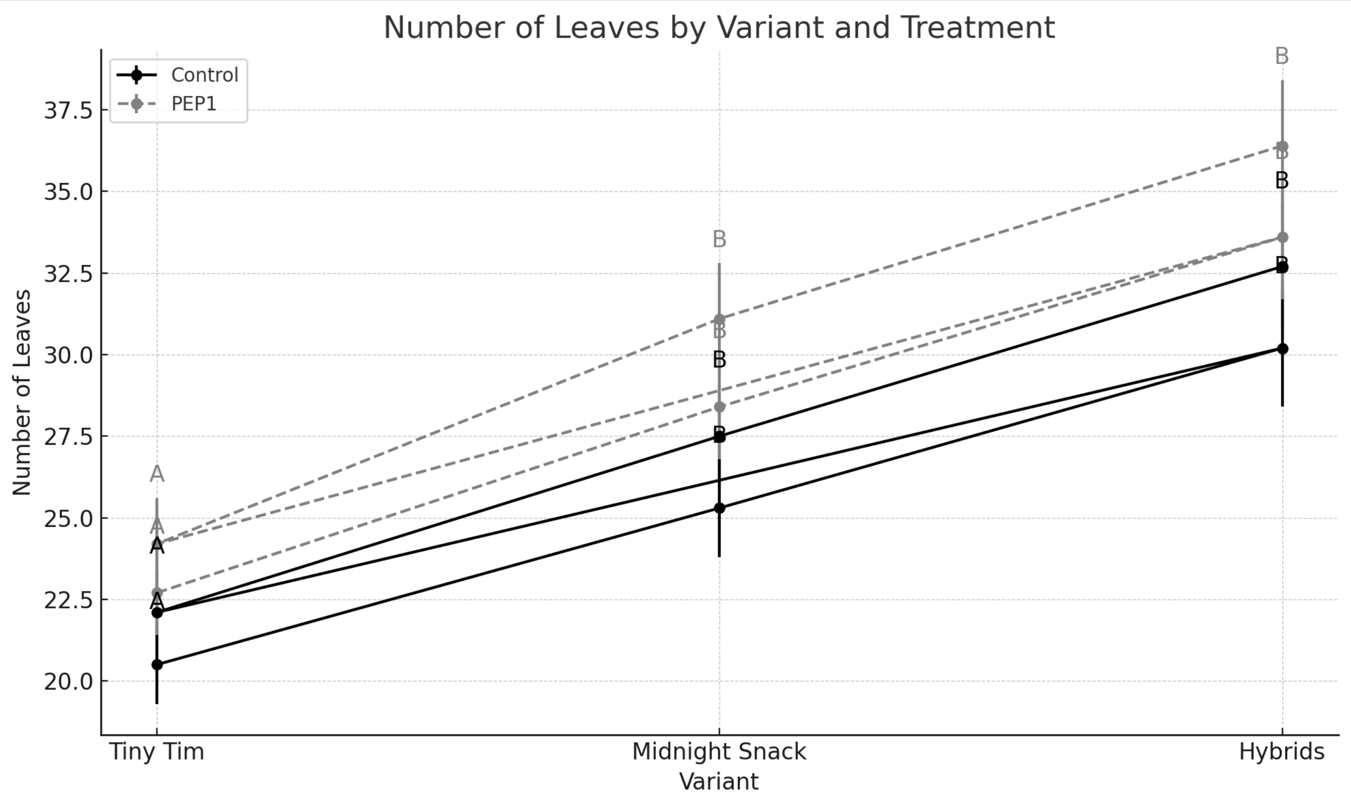
G2- plants that have been grown in regolith used in G1

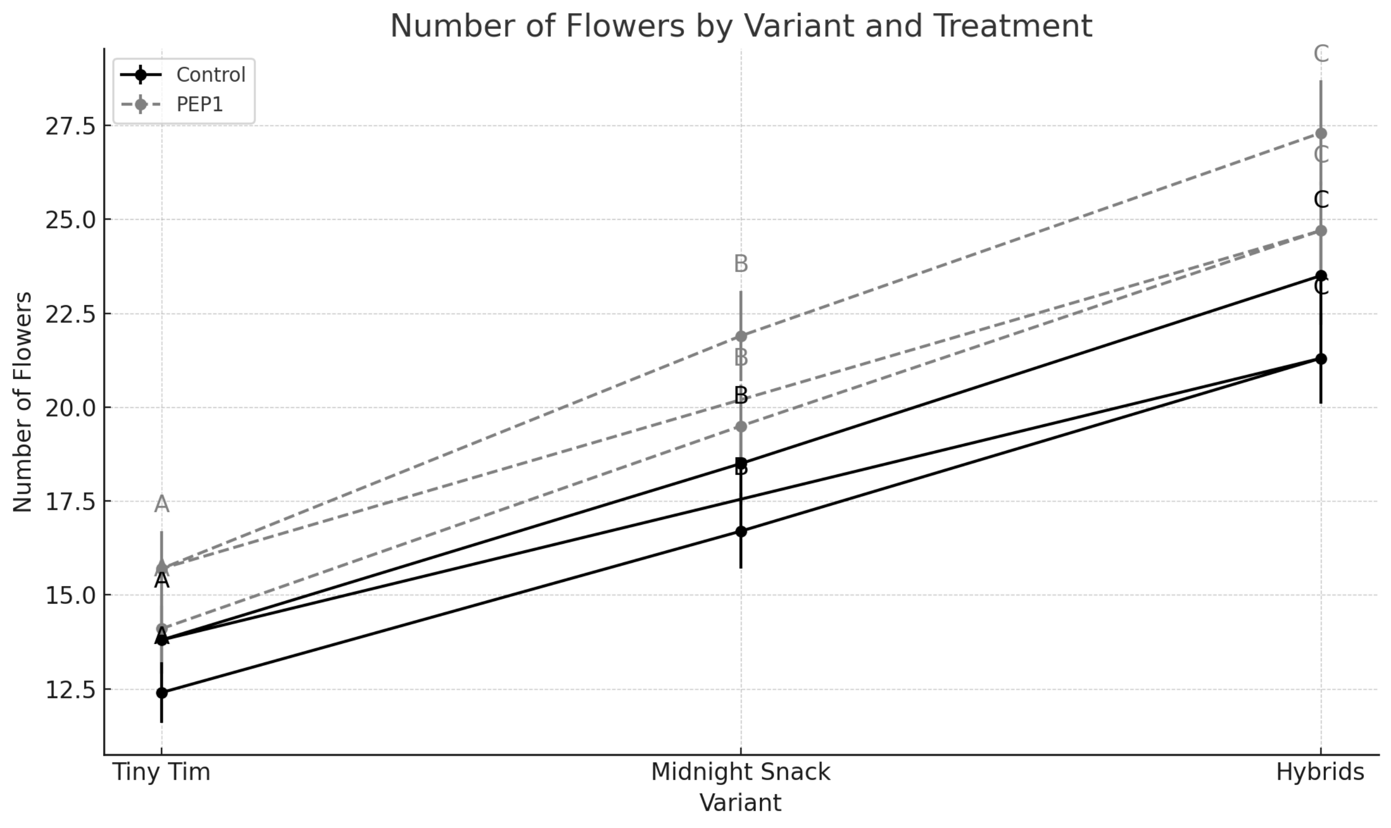
Treatments – Control (regolith with no microbiome amendment);  PEP1 ( regolith with microbiome amendment)

# Tiny-Snack plant after 55 day grow-out (production cycle)

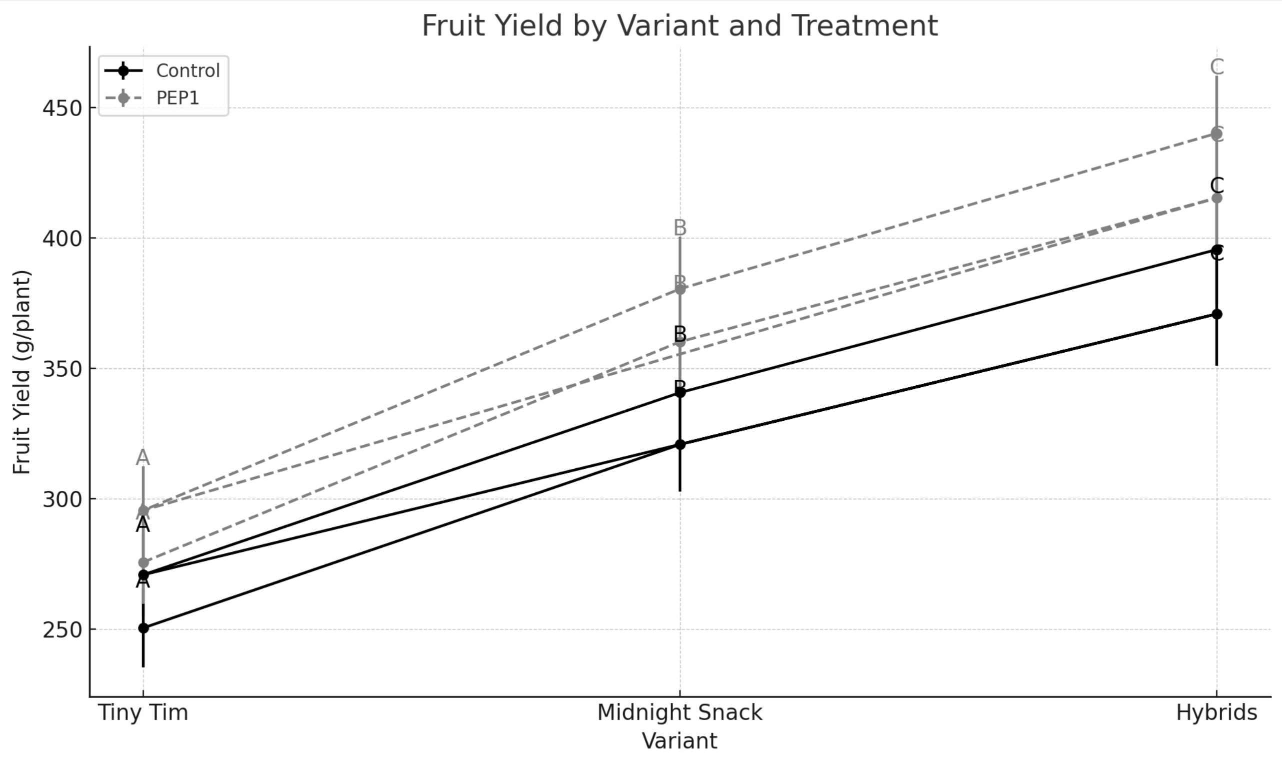


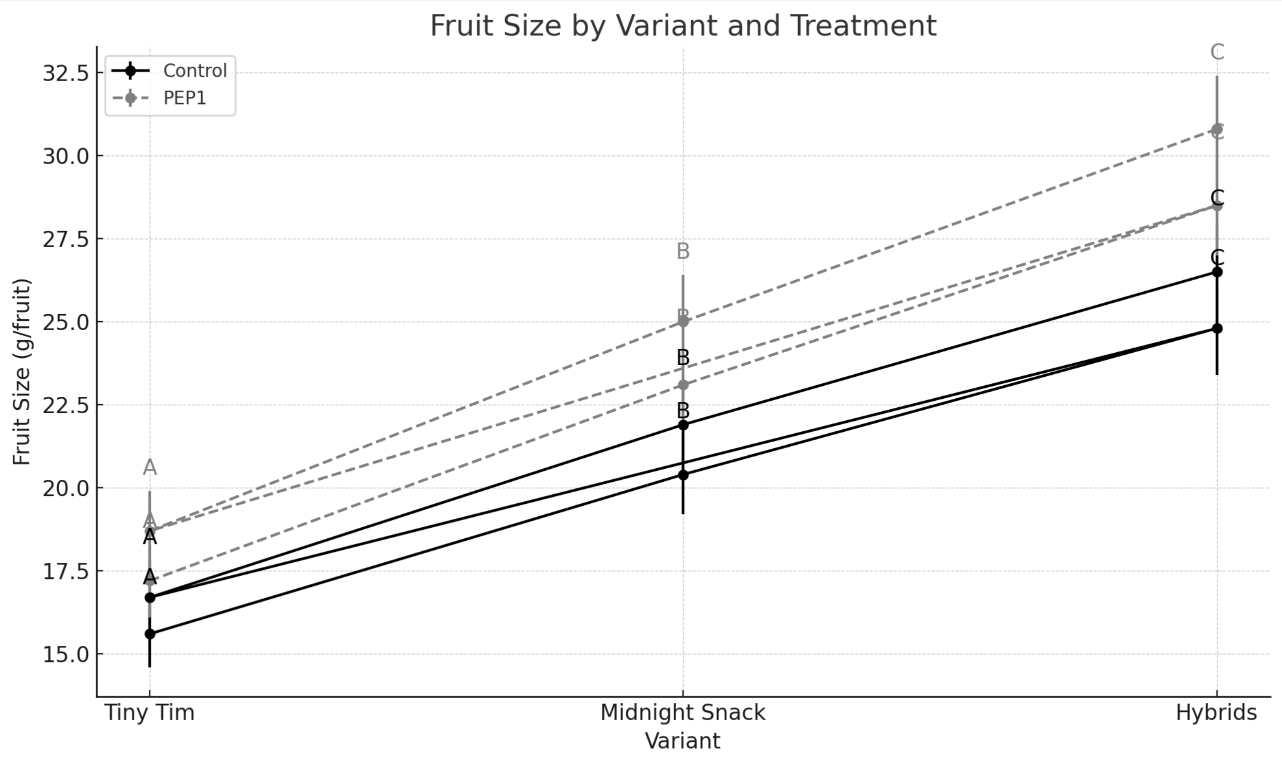



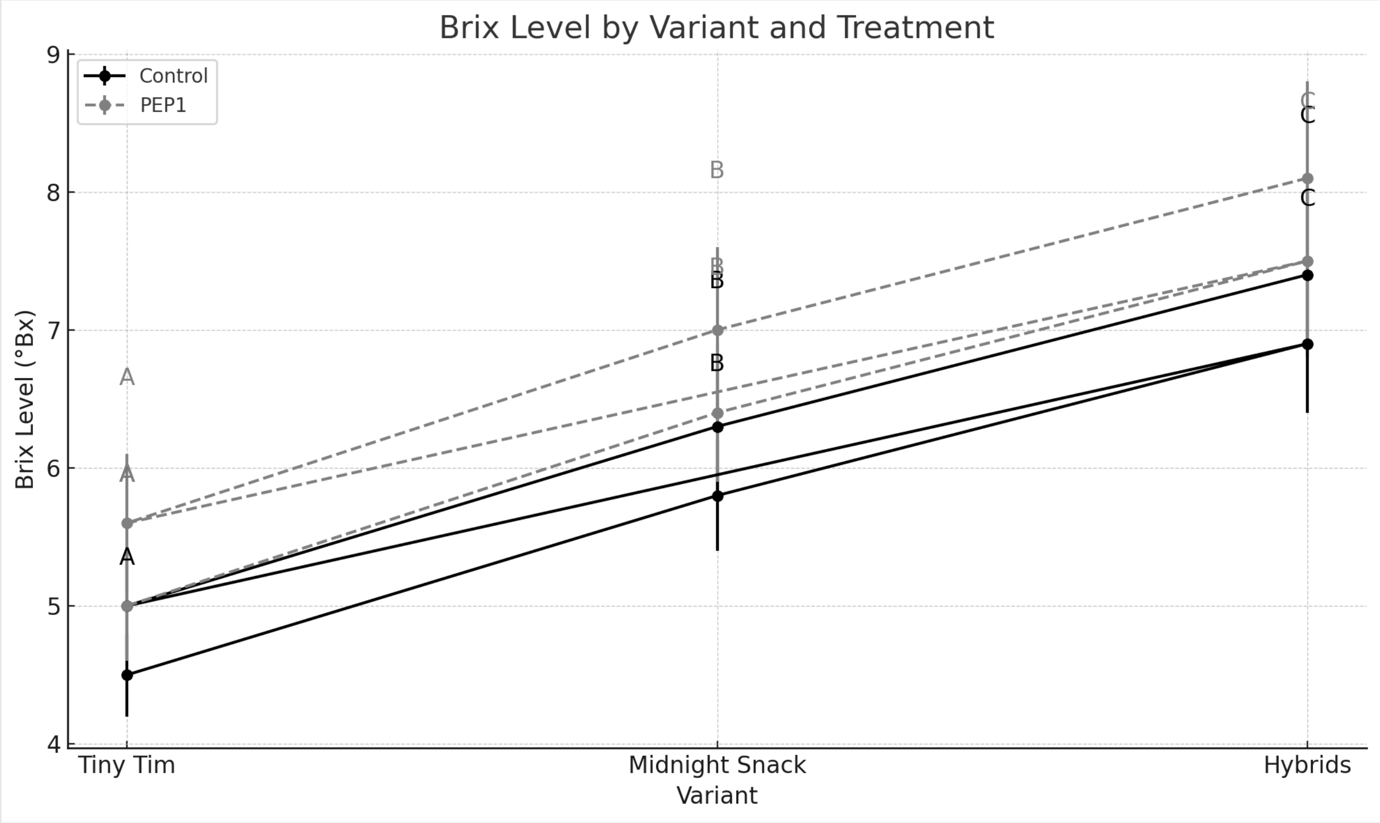



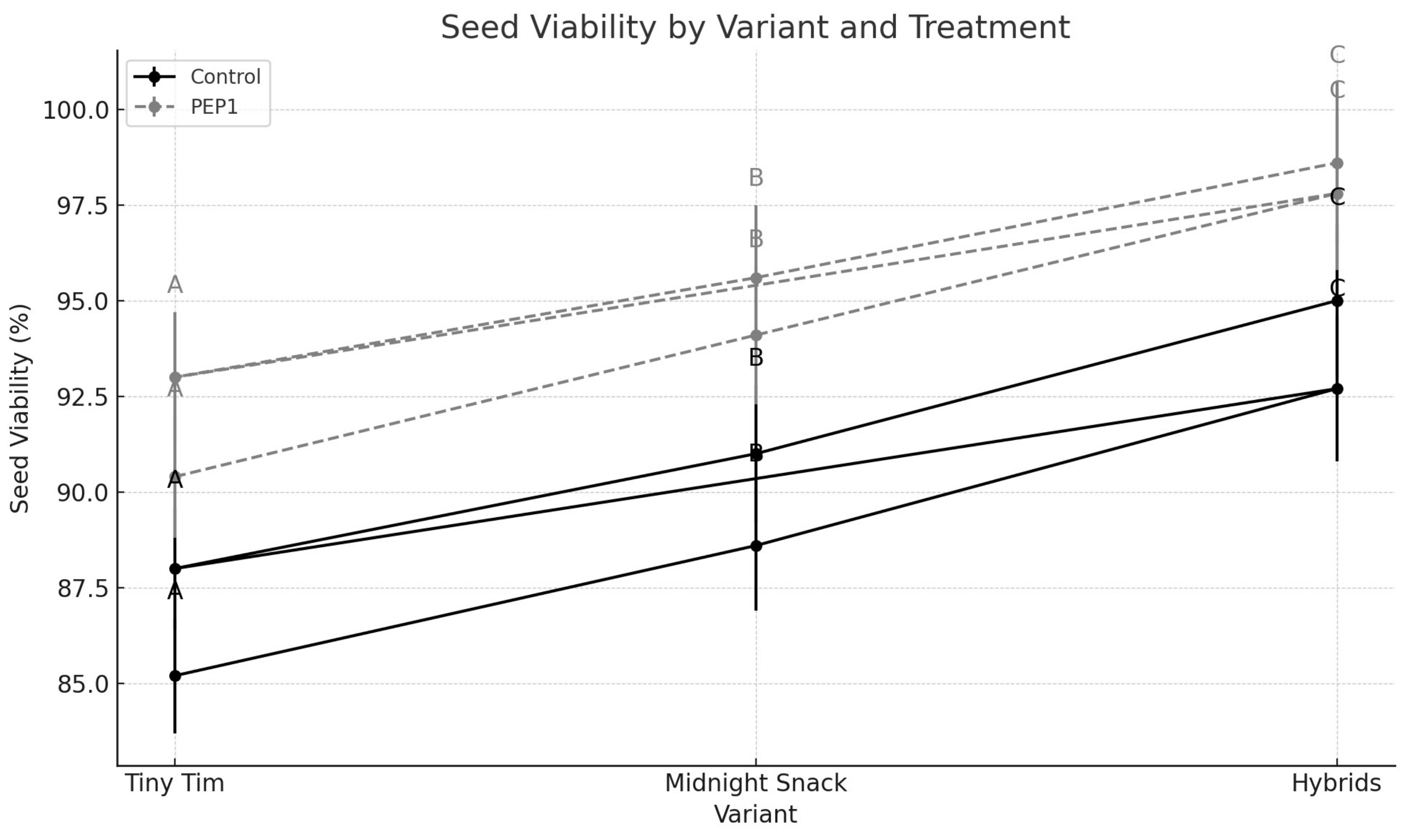

# High seed viability across variants and treatments (PEP1 and Control)

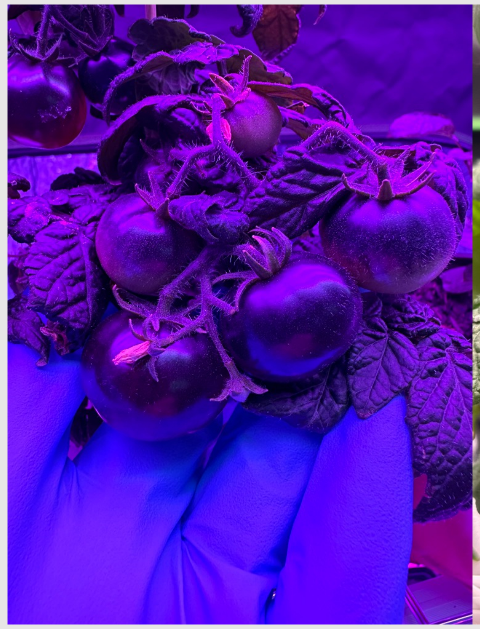



# Tiny-Snack fruits after 47 day grow-out mark.

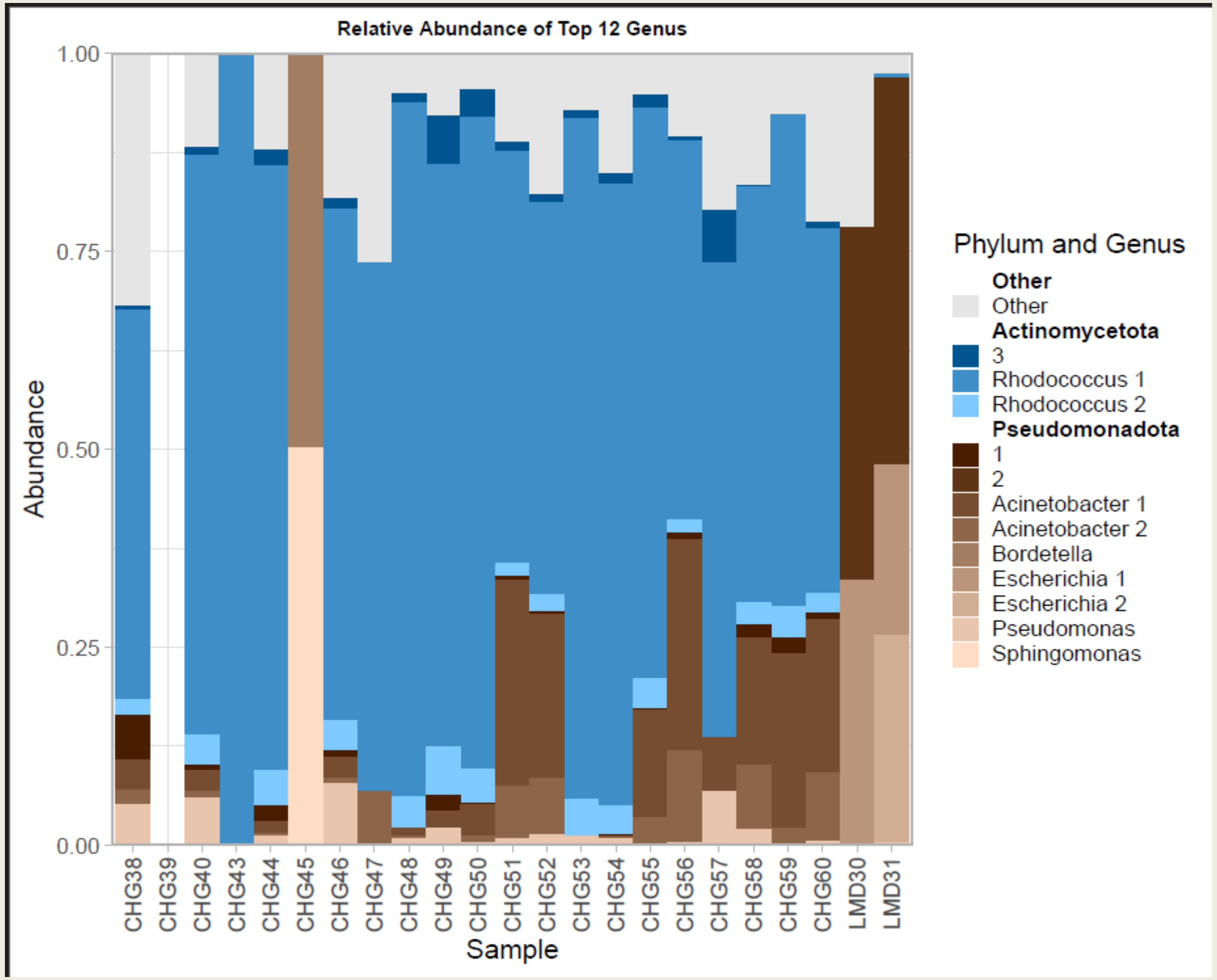


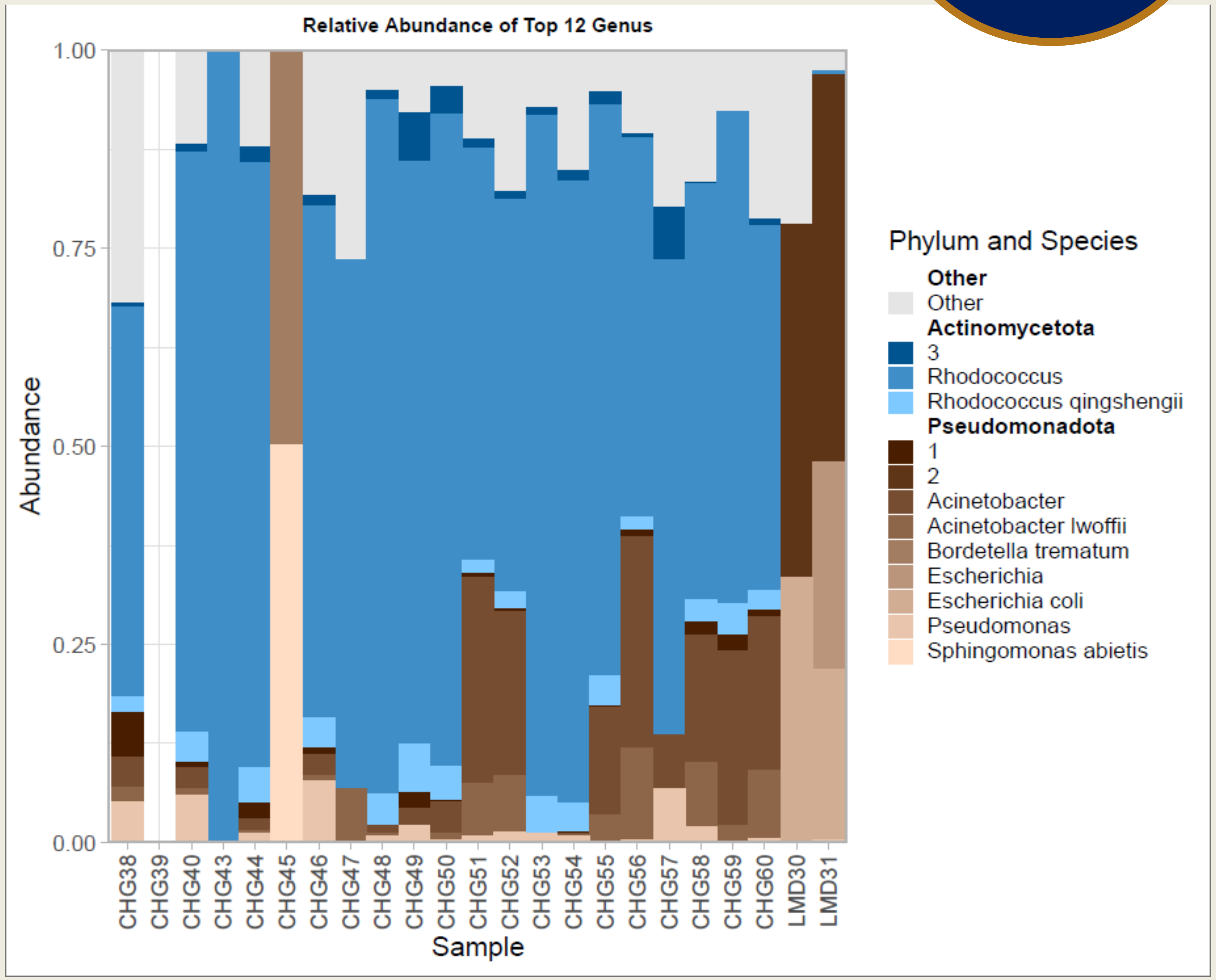



| Generation | Variant | Plant_Height (cm) | Stem Diameter (mm) | Number of Leaves | Number of Flowers | Fruit Yield (g/plant) | Fruit Size (g/fruit) | Brix Level | Seed Viability |
|---|---|---|---|---|---|---|---|---|---|
| G1 | Tiny Tim | 40.2 ± 2.1 | 5.1 ± 0.3 | 20.5 ± 1.2 | 12.4 ± 1.1 | 250.4 ± 15.4 | 12.6 ± 1.2 | 4.5 ± 0.3 | 85.2 ± 2.5 |
| G1 | Midnight Snack | 55.6 ± 3.2 | 6.4 ± 0.4 | 25.3 ± 1.4 | 16.7 ± 1.2 | 320.8 ± 20.3 | 16.4 ± 1.8 | 5.8 ± 0.4 | 88.6 ± 2.8 |
| G1 | Hybrids | 60.4 ± 2.5 | 7.0 ± 0.3 | 30.2 ± 1.3 | 21.3 ± 1.2 | 370.9 ± 18.5 | 18.8 ± 1.5 | 6.9 ± 0.4 | 92.7 ± 2.6 |
| G2 | Tiny Tim | 42.1 ± 2.2 | 5.6 ± 0.3 | 22.1 ± 1.2 | 13.8 ± 1.1 | 270.8 ± 16.2 | 16.7 ± 1.3 | 5.0 ± 0.3 | 88.0 ± 2.6 |
| G2 | Midnight Snack | 57.8 ± 3.1 | 6.8 ± 0.4 | 27.5 ± 1.5 | 18.5 ± 1.3 | 340.7 ± 19.8 | 18.9 ± 1.7 | 6.3 ± 0.4 | 91.0 ± 2.7 |
| G2 | Hybrids | 63.5 ± 2.8 | 7.6 ± 0.4 | 32.7 ± 1.4 | 23.5 ± 1.2 | 395.6 ± 18.1 | 22.5 ± 1.6 | 7.4 ± 0.4 | 95.0 ± 2.7 |

**ANOVA results indicated significant effects of generation, variant, treatment, and their interactions on all measured variables. The second generation (G2) outperformed the first generation (G1) across all variables, regardless of the variant, with even better results when inoculated with PEP1. For plant height, ANOVA showed $F = 24.3$ (generation), 22.8 (variant), 18.4 (treatment), and 5.2 (interaction), all $P < 0.001$. Tukey tests confirmed that hybrids were significantly taller than other variants ($P < 0.001$), with PEP1-treated plants also showing increased height ($P < 0.001$).**



Relative Abundance of Top 12 Genus
Abundance
1.00
0.75
0.50
0.25
0.00
CHG38
CHG39
CHG40
CHG43
CHG44
CHG45
CHG46
CHG47
CHG48
CHG49
CHG50
CHG51
CHG52
CHG53
CHG54
CHG55
CHG56
CHG57
CHG58
CHG59
CHG60
LMD30
LMD31
Sample
Phylum and Genus
Other
Other
Actinomycetota
3
Rhodococcus 1
Rhodococcus 2
Pseudomonadota
1
2
Acinetobacter 1
Acinetobacter 2
Bordetella
Escherichia 1
Escherichia 2
Pseudomonas
Sphingomonas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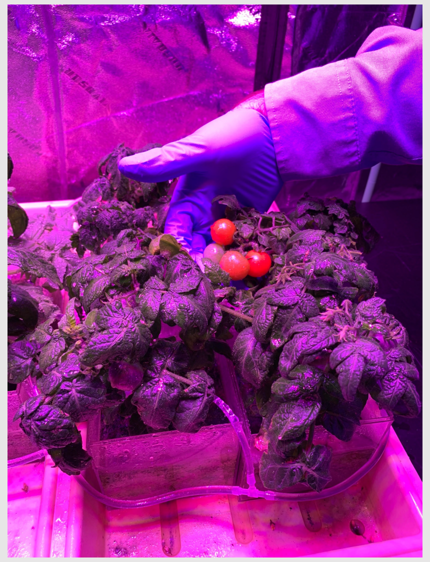
CHG51
CHG52
CHG53
CHG54
CHG55
CHG56
CHG57
CHG58
CHG59
CHG60
LMD30
LMD31
Sample
Phylum and Species
Other
Other
Actinomycetota
3
Rhodococcus
Rhodococcus qingshengii
Pseudomonadota
1
2
Acinetobacter
Acinetobacter lwoffii
Bordetella trematum
Escherichia
Escherichia coli
Pseudomonas
Sphingomonas abietis

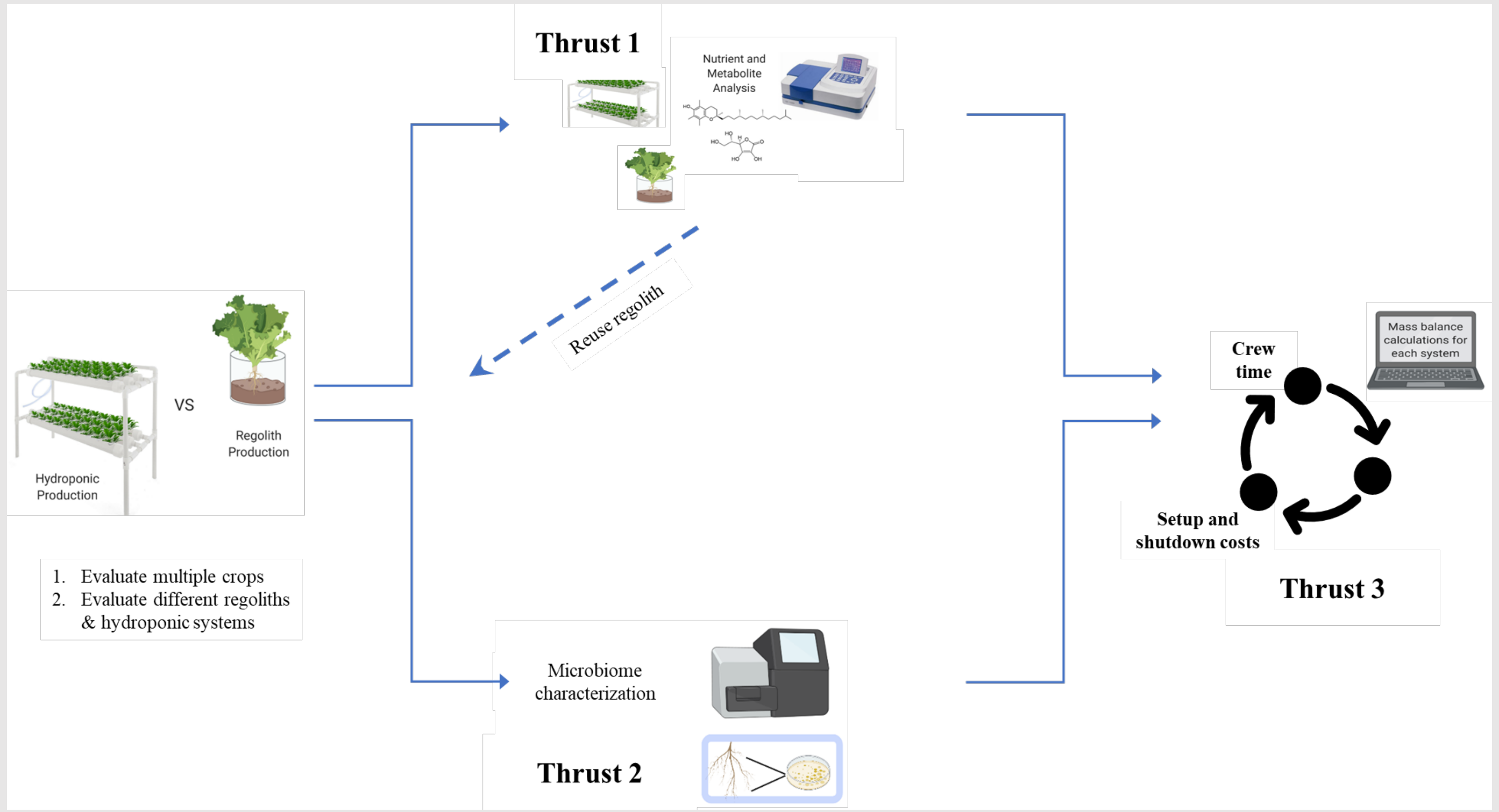
## Conclusions

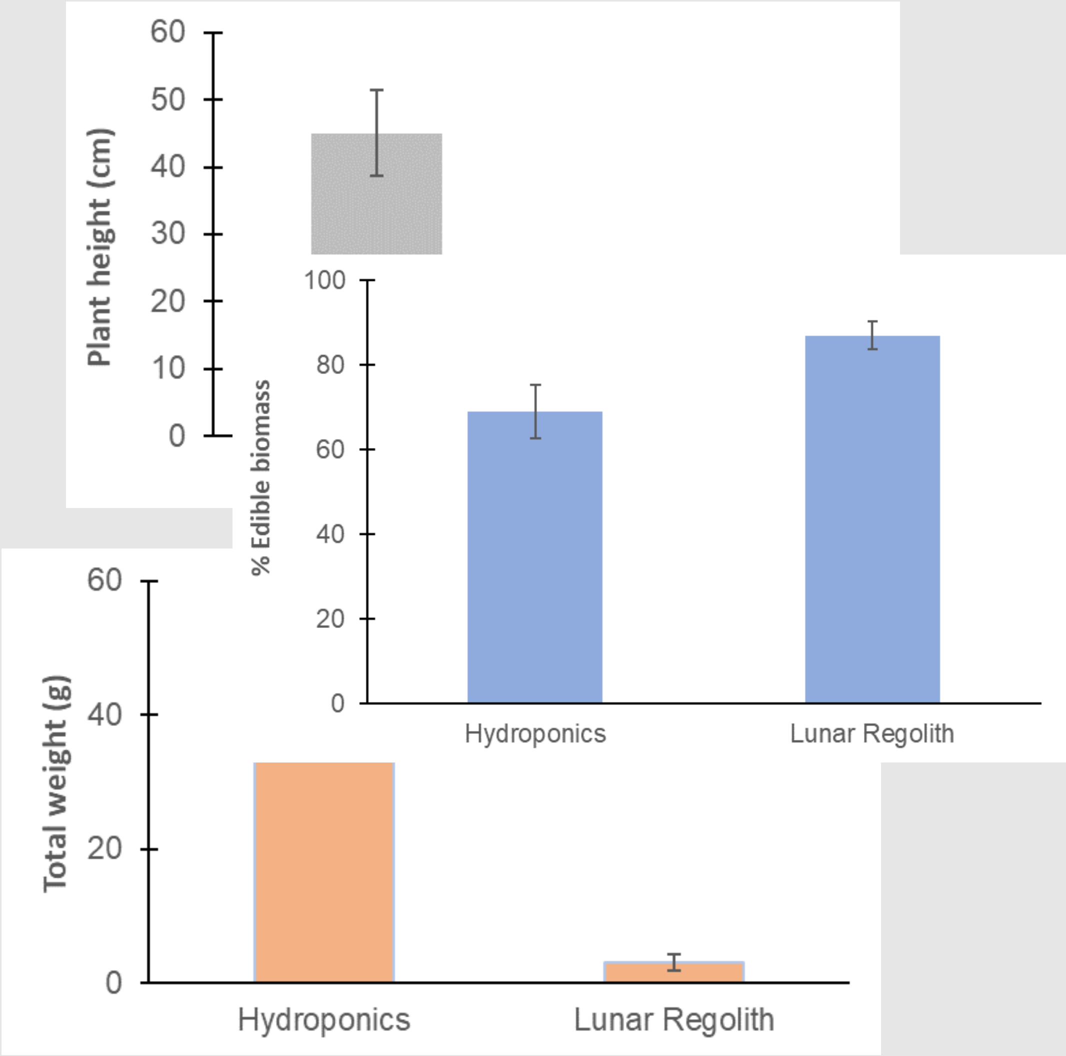
# How we define ‘success’….



Hydroponics

Regolith

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# Questions?

# Extra Slides