Researching Plant Growth in Amended Martian Regolith Simulant, Photosynthetic Rates of Plants, Seed Surface Decontamination by Plasma Methods, New Crop Development, and Porous Concrete Media

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Nomenclature

CFU=colony forming unit
C_i = intercellular carbon dioxide

HCL = hydrochloric acid

JSC= Johnson Space Center

MGS =Mars Global Simulant
MSL= Mars Science Laboratory

I. Abstract

Plant growth research for food production at Kennedy Space Center looks at how future residents of Mars and the Moon will enjoy the sight, smell, taste, and nutrition of plants. Overall, the goal is to provide a sustainable source of healthy food, on long-duration space flights, so astronauts can get the nutrition they need and produce food. The sustainable production of food will aid in the efforts of closed life support. Plants have a vital application for bio regenerative life support as demands for food and oxygen can be provided through photosynthesis, while the carbon dioxide from human respiration is removed. Transpiration is also used in life support processes as waste water that can be recycled through plant systems with the resultant humidity then condensed as clean water. Selected crops will provide the nutrient requirements needed for long duration space flight. Currently, projects in food production are investigating how plants grow in Martian regolith simulant, new crops testing with tomato and pepper cultivars, acquiring real-time photosynthetic data on crops, assessing plant growth in porous concrete media, and the use of plasma for surface decontamination of seeds.

II. Plant Growth in Amended Martian Regolith Simulant

Future plant growth experiments in amended Martian regolith simulant will be completed after the safety has been reviewed in the processing and handling of the Martian regolith simulant. The goal of the Martian regolith research

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is to perform crop studies to assess the performance of full duration crop growth in an amended Martian regolith simulant. Mars is covered with regolith which is crushed volcanic rock that is composed of a high amount of perchlorate salts. Mars consists of basaltic rock that is mainly comprised of iron on the crust and a surface that is very similar to Earth's thin crust in which we have iron, magnesium silicate igneous rocks on the crust and surface.[7] Martian regolith simulants have been generated to further analyze plant growth and aim to replicate features of the reference sample. In Martian soil simulant, the following properties are simulated: mineralogy, bulk chemistry, and particle size. The simulant is generated in the terrestrial environment, so there are structural and chemical differences between the terrestrial simulant and extraterrestrial regolith samples.[3] Last summer, Extra Dwarf Pac Choi was grown in Martian regolith simulant: JSC Mars1A. The plant growth experiment was completed with three treatments. The first treatment measured the overall performance of Extra Dwarf Pac Choi in JSC Mars 1A autoclaved regolith simulant. The second treatment measured the growth in JSC Mars-1A in regolith that had been previously used in potato experiments. An image of the Extra Dwarf Pak Choi in the second treatment is shown in *Figure 1*. The third treatment was the control as it assessed plant growth in Arcillite, a calcined clay media that aids in water retention The MGS-1 resembles the soil analyzed at the Gale crater. It was developed based on the mineralogical data collected from the MSL Curiosity rover. This simulant has a chemical composition that is similar to other basaltic soils at different landing sites, so it was denoted with the "global" title.[3] Red Romaine Lettuce will be grown in the amended MGS-1 simulant. MGS-1 will be amended with Mushroom Compost. Mushroom compost is known for being a nutrient reservoir with a high carbon to nitrogen ratio[8] Red Romaine Lettuce will be grown for 2 28-day growth periods. The following plant growth parameters will be measured: leaf area, shoot height, shoot diameter, chlorophyll quantity, anthocyanin quantity, fresh weight, and dry weight.

Figure 1 : Extra Dwarf Pac Choi on DAP 21 in 400mL of Martian Regolith Simulant JSC Mars1A (100% treatment)

III. New Crop Development

For new crop testing, 6 cultivars of peppers were used: Espanola, Pompeii, Bulgarian Carrot, Mohawk, Baby Bell, and Big Jim. In addition to peppers, there were 5 cultivars of tomatoes used: Golden Heirloom Cherry, Tomato-97, Tomato-851, Sweet n Neat, and Red Robin. Pepper and tomato cultivars were grown under the following environmental conditions:

Temperature-23 °C (constant)
RH-50% (constant)
CO_2-3000ppm

Tomatoes provide carotene which the human body converts into vitamin A. In addition to containing carotene, tomatoes provide significant amounts of potassium to the human diet as potassium can decrease blood pressure, minimize the risk of kidney stones, and prevent bone loss. Individuals who consume diets that consist of tomatoes that are rich in carotenoid lycopene were found less likely to develop stomach and rectal cancers. [4]

Peppers contain vitamins C, K, carotenoids and flavonoids. Vitamins A and C help to prevent cell damage, cancer, and improve the functioning of the immune system. Vitamin K promotes proper blood clotting and provides rigidity in the bone structure. [4]

These crops will provide the vitamins necessary for long duration space travel.

IV. Radish and Lettuce Growth for Li-6800 Portable Photosynthesis System

Radish and Lettuce were sown for the portable photosynthesis system Li-6800. The Li-6800 portable photosynthesis measures photosynthesis at the leaf-level in real-time measurements. Initially it measures the carbon dioxide uptake and transpiration of water by the leaf through infrared gas analyzers. In addition to these parameters, the instrument calculates the stomatal conductance and intercellular carbon dioxide concentration. Following the quantification of gas exchange and transpiration processes, the instrument quantifies the fluorescence yield. [6] Seedlings were sown on March 19th, 2019 with four seeds in each pot. For each pot, a perched water table was designed and used arcillite for this method. The arcillite was placed 1.5-2.0 cm from the bottom of the pot. The cultivars used in this study were Radish, Dragoon Lettuce, Waldman's Green Lettuce, and Outredgeous Lettuce. There were 5 pots consisting of radishes, 2 pots of dragoon lettuce, 2 pots of the Waldmann's Green Lettuce, and 1 pot of the Outredgeous Lettuce. The radish and lettuce seedlings were grown in a small Percival reach-in chamber with the following environmental settings: 23°C and 50% Relative Humidity.

Figure 2 : The leaf is clamped to allow for carbon dioxide, light response and light-fan response measurements to take place.

On DAP 15, light response and carbon dioxide response was measured for 5 radish plants. Fast light response data was acquired through the following set-points: 1000, 750,500,350, 200, 100, 50, and 0 µmol m^{-2} s^{-1}. The light response curves that are generated measure the plant's response to varying light intensity. The light response curves can provide information on the quantum efficiency and the light saturated photosynthetic rate. The fast light response is used to analyze how the stomata remains open at lower light values and how the intercellular carbon dioxide levels rise throughout the measurement. In this method, the leaf is exposed to a high light value such as 1000 µmol m^{-2} s^{-1} then the light values rapidly decrease after 1-2 minutes at each light value. [10] The slow light response is used to allow time for the stomata to equilibrate at each light value through holding the measurement for 15minutes. This allows for the intercellular to remain constant which is evidence for the stomata adjusting to the set light level. [10]

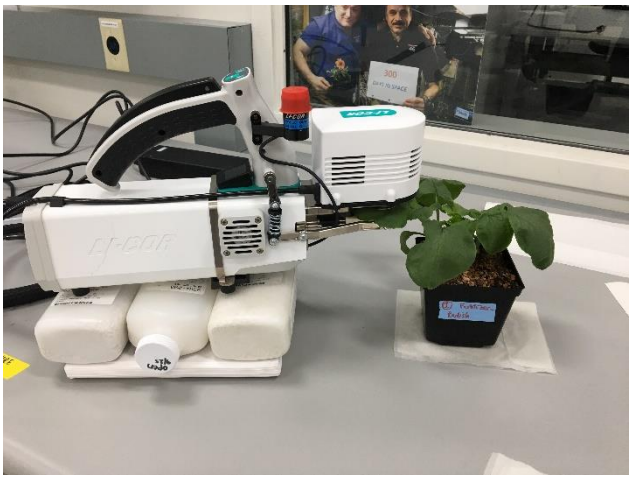

Figure 3: Licor 6800 Portable Photosynthesis System.

The carbon dioxide response allows for an analysis into the intercellular carbon dioxide concentration. Light is kept constant throughout the carbon dioxide measurements. In starting the measurements, the carbon dioxide is set to a low concentration and then measured at increasing concentrations. At the low concentrations, Rubisco is deactivated whereas at high concentrations, stomata will close. [10]

The fan light response allows for an analysis into the relationship between fan speed and carbon dioxide assimilation. Light is kept constant through the fan-light response measurements. When the fan speed is increased, the boundary layer decreases and this increases gas exchange. This results in a higher concentration of carbon dioxide being assimilated. [10] The plants were harvested on DAP 21 and the following growth parameters were observed: plant height, plant width, chlorophyll content, fresh weight, dry weight, stem length, hypocotyl length, and leaf area.

As carbon dioxide increased, the stomatal conductance decreased as shown in *Figure 5* . When the carbon dioxide levels rose, the leaf temperature increased as shown in *Figure 6*. In *Figure 7*, the carbon dioxide levels showed an inverse relationship with transpiration such that when carbon dioxide levels increased, transpiration decreased. At warmer temperatures, plants will open their stomata and release more water vapor thus resulting in higher transpiration rates. In regard to humid environments, the transpiration rates will decrease. When there is a higher concentration of carbon dioxide present, this increases the humidity and results in decreased transpiration rates. In the environment, plants in high carbon dioxide environments have adapted to have fewer stomata to further conserve water. [8]

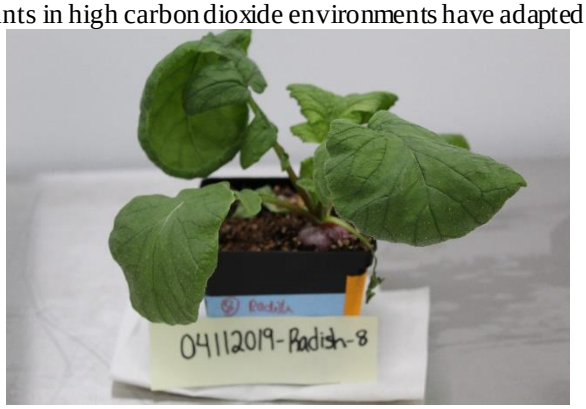

Figure 4: Radish on DAP 21.

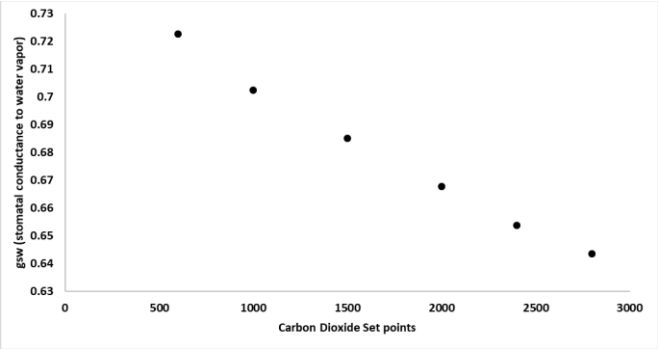

Figure 5 : Stomatal conductance to water vapor as a function of carbon dioxide set points.

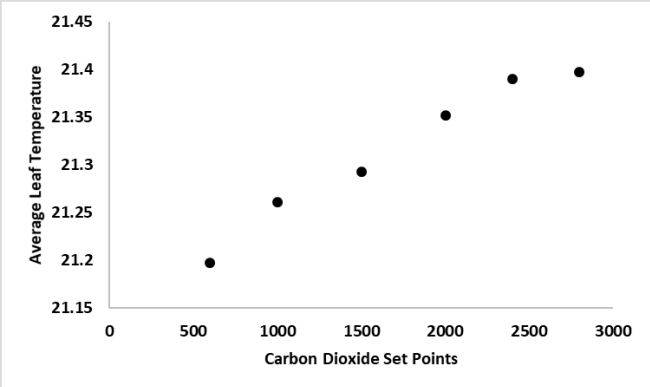

Figure 6: Average leaf temperature as a function of carbon dioxide set points.

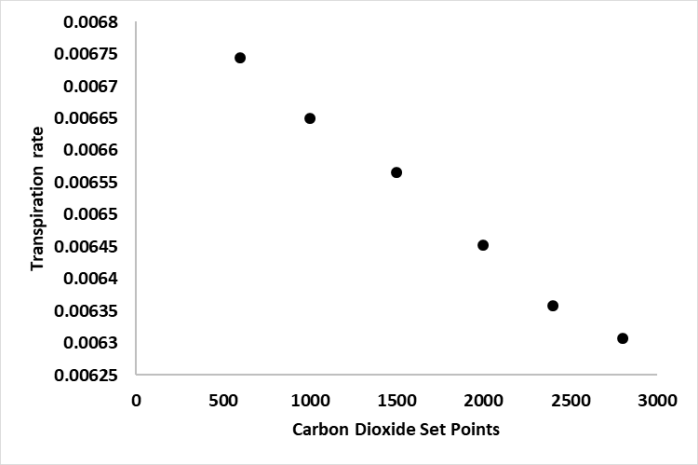

Figure 7: Transpiration rate as a function of carbon dioxide set points**.**

V. Porous Concrete Media for Assessing Plant Growth

Porous concrete cubes provide an environment that stimulates plant growth as air, water, and plant roots can navigate through the pores. [6] Porous concrete provides for a robust structure for root architecture in plants and aids in root development through the available pores [5] If there is a high percentage of connected pores, then moisture and nutrient solution can be retained and utilized as needed by plants [2]

Porous concrete cubes of 0.5m x 0.5m x 0.05m will be used to assess the growth of Outredgeous Lettuce for a 28-day growth period. The concrete cubes will be soaked in deionized water for 24 hours to allow for improved germination rates as water will be retained through the small pores present. 15 seeds will be sowed in each cube. A Hoagland's nutrient solution will be added to the growing tray where the cubes will be placed. On April 4th, Barese Swiss Chard, Babyleaf Outredgeous Lettuce, and Shungiku were germinated on petri dishes as this allows for more control on the germination environment and time-course of the germination process. The results of this experiment are in progress.

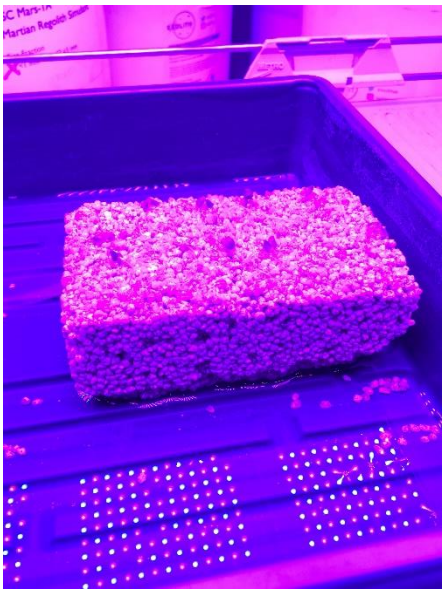

Figure 8 : Outredgeous seedlings that are DAP 7 in the porous concrete media. Prior to placement on the porous concrete cube, the seedlings were germinated on petri dishes.

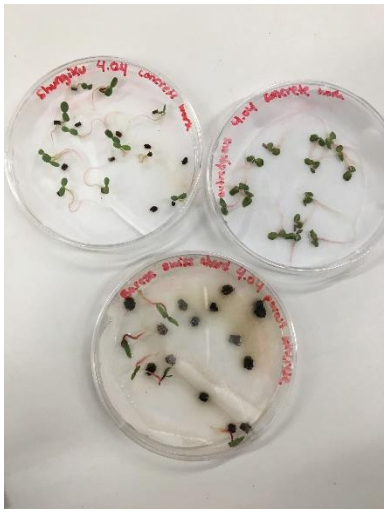

Figure 9 : Prior to sowing the seeds on the porous concrete media, the seeds were germinated in petri dishes.

VI. Seed Sanitization by Plasma Methods

Researchers at Kennedy Space Center are investigating the surface decontamination properties of plasma on seeds to compare to the current seed sanitization procedure. Plasma consists of bactericide properties that decrease microbial concentrations on surfaces as well as removing biofilm. Biofilm is a mechanism by which bacteria mobilize and colonize their host through the deposition of an EPS (extracellular polymeric substance).[13] The proposed mechanism of inactivation of the microbial organisms through plasma is a result in damaged microbial DNA and quorum sensing capabilities. In damaging the quorum sensing capabilities, microbial organisms are unable to increase their population as the plasma inhibits this mechanism of communication [1] In this study, Radish Cherry Bomb II Hybrid, Mizuna Mustard, Red Romaine Lettuce, and Barese Swiss Chard are being investigated for surface decontamination upon plasma exposure. Currently, the sanitization procedure for Red Romaine Lettuce and Mizuna Mustard consists of using hydrochloric acid (HCl) and a bleach solution to generate an oxidizing vapor that inhibits microbial colonization of the seed coat. [11] Seeds are placed in a jar and 30mL of bleach is added to each jar in addition to the 0.5mL of concentrated HCl. The dish containing the seeds is placed on top of the inverted beaker and the lid is sealed. The seeds remain in the sealed jar for an hour to allow for the reaction between the bleach and HCl to take place. [12]

Figure 10 : Radish seedlings in a sub-atmospheric plasma treatment.

Upon completion of the treatment, the seeds are transferred to a laminar flow hood to outgas overnight. Barese Swiss Chard and Radish Cherry Bomb II Hybrid seedlings have not been sanitized by the bleach and HCl method.[8] The objective of this study is to observe reduced microbial populations on the seeds treated with plasma. The seedlings were prepped in glass petri dishes prior to entry into the plasma systems. There were 45 seeds of each crop treated for 2 minutes in each treatment. The treatments consisted of an atmospheric plasma system, sub-atmospheric plasma system, and control system. Upon treatment of the plasma system, 10 seeds were sectioned off for germination tests. 35 seeds were analyzed for microbial content and to determine CFU levels on each of the seeds.

Figure 11: Sub-atmospheric plasma system

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