PLANT GROWTH EXPERIMENTS USING MARTIAN SIMULANTS: POTENTIAL AND LIMITATIONS OF AGRICULTURE ON MARS.

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Introduction: Future manned missions to Mars will require the use of in-situ resources. One important resource for consideration is use of Mars soil and other surface materials for agricultural applications [(1–4)1-4]. Though hydroponic systems will also be an essential part of Martian agriculture, coupling these with regolith-based systems can provide additional advantages that maximize the potential for success [2].

The majority of essential plant macro- and micro-nutrients have been detected on Mars but some, such as nitrogen, are likely not in sufficient quantity [1-3]. Mars soil is also a readily accessible resource that would require minimal effort in obtaining, but the pH, salinity and other characteristics of the soil may limit the feasibility of using it in its raw form [1-2]. There is a need to establish the feasibility of treating and using Mars surface materials in extraterrestrial agriculture applications. This can be accomplished with studies that use Martian simulants which accurately demonstrate agriculturally relevant Mars soil characteristics [1-2].

Experimental Approach: Plant growth experiments presented here focus on challenges specific to using Martian regolith simulants in an environmentally controlled setting and assume that extraneous conditions will be hospitable to plant growth. All plant growth experiments are being conducted in a semi-controlled chamber where light, temperature, and humidity are regulated and tracked. The current experiments focused on a drought resistant legume moth bean (Vigna aconitifolia) and relevant set of microbial inoculants (Table 1) based on responses in previous pilot studies [5-6].

The specific challenges examined here included Mars-like salinity, plants grown in Mars-like mediums, and effectiveness of microbial inoculation. The growth mediums included five Martian agriculture simulants developed in earlier work (Figure 1) [1]. The simulants were analyzed for several fertility factors including saturated-paste salinity and pH, and extractable plant nutrients. This analysis took place at the University of Georgia Agricultural and Environmental Service Labs. Seeds were surface sterilized before planting and inoculation. Two rounds of growth experiments were completed in simulants, the first without any addition of fertilizers and the second with dilute fertilization (~1/4 the recommended rate). During the growth experiments data gathered included Growth indicators (e.g., germination), stress indicators (e.g., chlorosis), plant nutrient content, plant biomass, and changes to soil fertility analyses (e.g., extractable nutrients).

Fig. 1. Martian Regolith Simulants: Images of the simulants developed in this study including A) Global Soil (MBas), B) Phyllosilicate-smectite (MPSmec), C) Phyllosilicate-illite/chlorite (MPChl), D) Sulfate-rich (MSul) and E) Carbonate-rich (MCarb) [1].

Table 1:

Inoculant Species	Function
Cowpea miscellany (*Bradyrhizobium sp.*)	N-Fixation
Rhizobium leguminosarum biovar viceae	
Rhizobium leguminosarum biovar phaseoli	
Bacillus subtilis	P-Solubilization, Enzyme stimulation
Bacillus lichenformis	
Bacillus pumilus	
Bacillus megaterium	
Glomus clarium	P-Solubilization
Glomus mosseae	
Glomus deserticola	
Glomus claroideum	
Glomus etunicatum	

Results and Discussion: The simulants used in this study represent a variety of materials that seek to simulants characteristics of materials available on the Martian surface. A more thorough examination of the simulants and comparison to data available from Mars is available from previous work [1]. The results here focus primarily on fertility analysis of these simulants and plant response to growth in the simulants.

Simulant Fertility: The saturated-paste extract measured pH range from 1.37 to 8.70 depending on the simulant (Table 2). These most extreme were the sulfate and carbonate rich simulants; this was expected based on the mineral profile of the simulants. Both pH measurements (1..37 and 8.70) may present problems for nutrient and toxin mobility, but they also present possible agricultural tools. Though as a raw material, these would not be suitable for most plants, there is potential for use as an acidifying or liming agent to adjust pH of fluids or mediums used in agricultural systems. The sulfate simulant. The remaining simulants had pH range between 7.06-7.95 which is within a tolerable to most agricultural plants.

The conductivity measurements obtained represent a very large range, especially the sulfate simulant which was extremely high. However, all simulants had conductivity results above 4.0 mmhos/cm (High Salnity) and most well above 10 mmhos/cm (very high) presenting a problematic level of salinity based on levels used to rate terrestrial soils (Table 2). The conversion of these results is based on typical salt profile of terrestrial soils, which differs from the salt profile of Mars. Thus, traditional ratings are not as readily applicable to a Martian environment, but the level of salinity measured would still likely be problematic especially for salt-sensitive crops (e.g., most leafy greens). This would require treatments such as rinsing the soil. This does also not take into account toxic salts such as perchlorate that may present additional challenges. Affective treatments that remove or reduce salinity while not wasting limited water resources are an important challenge to address in Martian agriculture for both soil and hydroponic applications.

Extractable nutrients were below the recommended levels demonstrating a need for fertilization (Table 2). However, nutrient extraction solutions are calibrated to specific terrestrial soils and the conversion of results to recommended rates on Mars should be considered carefully. It is also expected that the abundance of phosphorus in Mars soil is higher than the simulants. Taking these points into consideration, it is likely that nitrogen a is severely limiting nutrient and potassium at least a somewhat limiting nutrient in Mars soil. However, the need to understand the abundance, speciation, placement, and mobility of nutrients (especially N, P, K) is important for understanding the feasibility of relying on in-situ resources for supplying them whether needed for soil or hydroponic based systems.

***Plant Response to Simulants*:** Due to limited availability of the global soil simulant (MBas), it was not used in the final experiments. Plants showed limited growth in all other simulant's materials even with microbial inoculation. In non-fertilized experiments, the inoculated plants showed greater growth and decreased stress response. Fertilization improved overall results, however also flipped the response between inoculated and non-inoculated treatments with better response from non-inoculated plants. This may demonstrate an increase in mobility of specific phytotoxins, though this requires further experimentation to confirm and determine what is mobilized and which inoculant(s) chiefly contributed.

Table 2: Simulant Fertility

Simulant	pH	EC (mmhos/cm)	Extractable Nutrients (ppm)				
			NH4-N	P	K	Ca	Mg
MBas	7.09	35.7	1.51	<0.24	87.6	10749	2664
MPSmec	7.06	7.1	8.04	<0.24	118.6	8959	2376
MPChl	7.95	5.9	1.11	<0.24	94.3	9257	745
MSul	1.37	976.2	11.49	144.6	40.2	6022	704
MCarb	8.70	28.1	3.77	0.9	88.0	4470	4113

Future Work: Plant showed much better response to the global soil simulant compared to other simulants in previous pilot studies, but because of limited availability of materials was not used in the final experiments. Pilot studies provide support that there is indeed potential for the global soil as an agricultural tool, but the limited nature of the pilot studies makes it impossible to draw more certain conclusions on that potential. The materials needed to construct more of this simulant can be readily accessed (outside of a global pandemic) in future work that would seek to examine results from this simulant as well. The simulants will also continue to be improved and specific features adjusted to more accurately represent Mars' soil growing conditions. For example, though salinity levels are relevant to Mars, they are limited to Ca and Mg sulfate salt, so simulants can be constructed with more complex salt profile that includes sodium, chloride and chlorate/perchlorate salts. Future growth experiments will also explore the response of other agriculturally relevant plants and inoculants including the potential for providing nitrogen through legumes used as cover crops in addition to chemical fertilizer.

References: [1] Fackrell, L.E., et al. (2021) *Icarus, 354*, 114055 [2] Eichler, A., et al. (2021) *Icarus, 354*, 114022, [3] Wamelink, G.W.W., et al. (2014) PLoS One, *9(8)* [4] Wamelink, G.W.W., et al. (2019) Open Agric, *4(1)*, 509-516. [5] Fackrell, L.E. and Schroeder, P.A. (2020) ASA-CSSA-SSSA Annual Meeting, *abstract #131435* [6] Fackrell, L.E. and Schroeder, P.A. (2018) ASA-CSSA-SSSA Annual Meeting *abstract #119532*