

RICE CAN GROW AND SURVIVE IN THE MARTIAN REGOLITH WITH CHALLENGES THAT COULD BE OVERCOME THROUGH CONTROL OF STRESS-RELATED GENES. Peter James Icalia Gann^{1,2}, Abhilash Ramachandran³, Yheni Dwiningsih², Dominic Dharwadker³, and Vibha Srivastava^{1,2,5}, ¹Cell and Molecular Biology Program, ²Department of Crop, Soil and Environmental Sciences, University of Arkansas System Division of Agriculture, Fayetteville, Arkansas 72701, ³Arkansas Center for Space and Planetary Sciences, ⁴Department of Horticulture, University of Arkansas System Division of Agriculture, Fayetteville, Arkansas 72701; av034@uark.edu

Introduction: In-Situ Resource Utilization (ISRU) will become increasingly important as human space exploration progresses. Resupply missions can be expensive, so we need practical and affordable ways to use resources on other planetary bodies. On Mars, there are resources such as water, regolith, light, CO₂ which can be used to introduce new biomass for food production [1,2]. There have been studies reported to grow *Arabidopsis thaliana*, *Lactuca sativa*, bean plants in Martian regolith simulants such as JSC-1 and MMS-1 [1,3]. In this study we present results of rice plants growth in MMS1.

Result and Discussion: Plants have been grown in Martian regolith showing signs of stress response and few underlying associated mechanisms have been described, yet there were no efforts to regulate genes associated to these stress responses to increase survivability and growth in this condition. *OsSnRK1a* and *OsTOR* encodes for signaling molecules that regulate the reaction of rice plant to stress including sugar starvation, salinity, drought and scarcity of soil nutrients. Here, we demonstrate the performance of rice in Martian regolith (MMS1), describe differentially expressed stress-related genes and show that perturbation of *OsSnRK1a* and *OsTOR* can influence the ability of rice to grow from the challenges in Martian regolith.

We initially challenged the germination and growth of rice in MMS1, potting mix (PM) and combination of both. Plants grown in MMS1 have stunted growth, poor root morphology, and lower photosynthetic activity (Figures 1a-g) which corroborates to the report of [4] that MMS1 can be challenging for plant growth due to poor nutrients, small amount of nitrogen and high pH. However, addition of any proportion of PM in MMS1 showed better growth and root characteristics compared to 100% MMS1.

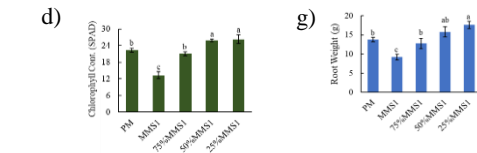
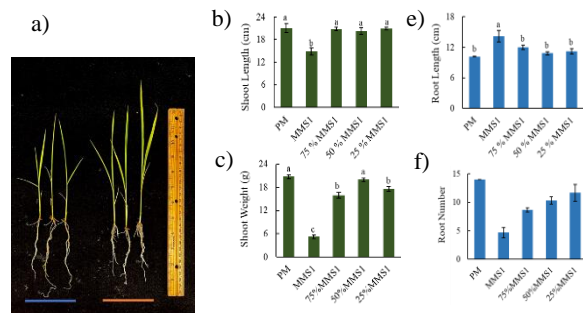


Figure 1. Shoot and root characteristics of rice seedlings in the different media 1 month after germination (MAG). (a) Photograph of seedlings grown in Potting Mix (PM) and Mars Soil Simulant (MMS1) showing differences in shoots. PM grown seedlings have a blue underline and orange underline for the seedlings from MMS1. A 12-foot ruler is provided as reference. (b-d) Bar graphs showing the shoot characteristics of the seedlings including shoot length, shoot weight and chlorophyll content from the different growing media used. (e-g) Bar graph showing the root characteristics of the seedlings including root length, root number and root weight from the different growing media used. Error bars represent standard error of 3 biological replicates consisting of 3 seedlings each. Significance in Tukey's multiple comparison at $\alpha = 0.05$ within a parameter is shown by small letters.

Despite plants presenting signs of stress under MMS1, the germination and growth observed suggests that the water holding capacity, mineral compositions, soil pH, particle size, other physical and chemical characteristics of MMS1 can support the development of the rice plants. Moreover, shoot and root growth, and also chlorophyll content in MMS1 with PM (75% MMS1, 50% MMS1, and 25% MMS1) significantly improved and even comparable to the control media (100% PM). Based on the development of the rice plants, all essential minerals, such as N, P, and K content for the growth of the rice plants appear to be present in sufficient quantities in 100% MMS1 and MMS1 with PM.

With the germination and growth observed in MMS1, we incorporated magnesium chloride ($\text{Mg}(\text{ClO}_4)_2$) in two different concentrations (0.1 and 0.3%) to closely mimic to the actual Martian soil and challenged wild type rice plant (WT) and two mutant lines (pNs73, and ΔTOR) for germination and growth (Figure 2a-c).

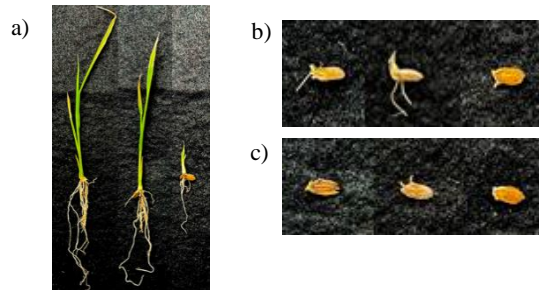


Figure 2. Shoot and root characteristics of rice seedlings in the different media 2 weeks after germination (WAG). (a) Photograph of seedlings/seeds germinated/grown in (PM), (b) MMS1 + 0.1 % ($\text{Mg}(\text{ClO}_4)_2$) and (c) MMS1 + 0.3 % ($\text{Mg}(\text{ClO}_4)_2$). From left to right are WT, pNs73, and ΔTOR .

All the lines germinated within two days and grow in the PM where the WT and pNs73 showed similar shoot and root characteristics while ΔTOR was significantly shorter (Figure 2a). Incorporation of 0.1% ($\text{Mg}(\text{ClO}_4)_2$) impacted germination and growth. Germination took approximately five days. Interestingly, pNs73 developed a shoot and root structures in 0.1% ($\text{Mg}(\text{ClO}_4)_2$), a root was only observed in the WT and no signs of any germination or growth in the ΔTOR . The ability of the pNs73 to advance in germination and growth compared to the WT suggests that through editing the *SnRK1a* gene, a cascade of stress-related mechanisms may have changed and improved its response in the MMS1 soil with ($\text{Mg}(\text{ClO}_4)_2$). However, all the three lines did not germinate in 0.3% ($\text{Mg}(\text{ClO}_4)_2$) which indicates that higher levels of ($\text{Mg}(\text{ClO}_4)_2$) are detrimental to germination in rice.

Our results show that in principle it is possible to grow rice plants in MMS1 but levels of ($\text{Mg}(\text{ClO}_4)_2$) can be critical to both germination and growth. Moreover, we presented that editing *SnRK1a* could potentially provide an approach to develop as table rice line that can germinate and grow in MMS1 with ($\text{Mg}(\text{ClO}_4)_2$).

Methods:

Regolith: Mojave Martian simulant (MMS1) was used as Mars regolith simulant. We incorporated magnesium chloride ($\text{Mg}(\text{ClO}_4)_2$) in two different concentrations (0.1 and 0.3%) to closely mimic to the

actual Martian soil. As a control, we used potting mix. We overmedicalized water to mimic water from Mars and to prevent contamination with nutrients.

Plants: The first experiment on testing germination and growth characteristics in PM, MMS1 and combinations of PM and MMS1 used ZHE 733. The second setup which included testing of three different lines on MMS1 with ($\text{Mg}(\text{ClO}_4)_2$) used Kitaake.

Experimental Designs and Observation: Small pots were filled with 5g of soil media and demineralized water was added to each pot. A filter was placed on the bottom of each pot to prevent soil from leaking. For each soil type there were 3 replicate pots used. In each pot, we positioned 3 seeds. The pots were placed in a growth chamber in a completely randomized design. The pots were placed on a large tray in the growth chamber. During the experimental period, the average temperature was 33°C during daytime and 23 °C at night and air humidity was. Mean day time lasted for 12 hours. The plants were watered with demineralized water once or twice a day depending on the evaporation rate.

Seeds were scored on germination rate and time for first leaf production. At the end of the experiment, when the rice plants in control media were reaching V3 developmental stage with three leaves; shoot length, shoot weight, chlorophyll content, root length, root number, and root weight were measured. And also leaves sampling were performed for genotypic characteristics.

Statistical Analysis. Data were subjected to arcsine transformation and one-way ANOVA. To determine the significant differences, Tukey's multiple comparison test was used. All statistical analyses were performed in SAS statistical software (version 9.4, SAS Institute Inc.).

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References:

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