**GROWING PLANTS ON MARS: THE DEVELOPMENT OF SUSTAINABLE CROPS FOR FUTURE HUMAN HABITATS.** P. Pantha[1], G. Wang[1], K. Tran[1], and M. Dassanayake[1], [1]Louisiana State University, Department of Biological Sciences, 202 Life Sciences Building, Baton Rouge, LA 70803 (maheshid@lsu.edu).

**Introduction:** Human habitation beyond earth has been a collective goal of humanity for decades. Human exploration missions to mars planned within the decade brings us closer to the next phase of planning for future habitable systems[1]. Designing sustainable plant growth systems is imperative to sustainable human habitation on Mars and other planetary surfaces[2]. Crop systems currently used today are designed to use the best agricultural soils on earth with a high demand for water. Therefore, our current crop systems will fall short in Martian soil analogs or hydroponic growth systems that may not have the best elemental profiles needed to successfully grow our current crop cultivars[3,4].

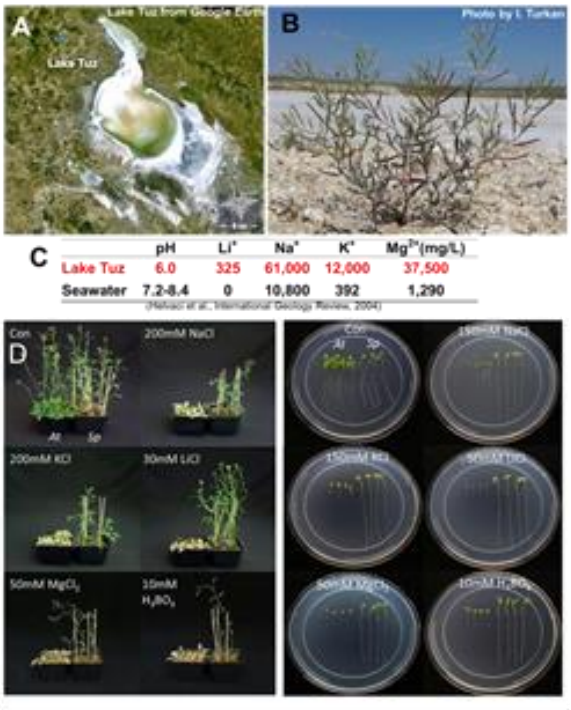


Figure 1. [A] Google image of Lake Tuz. [B] *Schrenkiella parvula* (*Sp*) in its natural habitat. [C] Comparison of lake Tuz and sea water ion compositions. [D] Treatment of *S. parvula* and *Arabidopsis thaliana* (*At*) with high concentrations of various salts. Four-week-old *A. thaliana* and *S. parvula* plants were treated with the indicated concentrations of salts for 2 weeks. *S. parvula* can completes its life cycle while *A. thaliana* cannot. [E] Two-week-old seedlings of *A. thaliana* and *S. parvula*, grown on modified Murashige and Skoog medium were transferred to the same medium supplemented with the indicated concentrations of various salts. Photographs were taken after 10 days of treatment. *A. thaliana* shows reduction in the root growth while *S. parvula* does not.

Crop domestication and breeding typically takes a wild plant from a low resource environment and breeds for higher yield or quality of the product under high energy input systems, agriculturally feasible on earth. However, we have a different goal when designing new crops adapted to Martian soils enriched in salts at levels toxic to most plants. Extremophytes (extremophile plants) that thrive in extreme environments offer an underexplored genetic resource to mine for genes and genetic mechanisms that could make our crop plants acquire stress adapted traits faster than conventional breeding methods[5]. Extremophytes reflect the evolutionary trajectory in their genomes that enabled growth in stressful environments. These were unavailable as genetic resources for crop improvement until recently. The new DNA sequencing platforms have revolutionized the rate of acquiring genetic information and enabled exploring naturally stress adapted species to ask: what are the naturally selected genes that bring about the significant changes in stress adaptation; are these genes shared with crop species; what are the differences in these genes between crops and stress adapted wild species; and how best we can adapt the functions of stress adapted genes into our crops to better prepare for future Martian habitats and beyond.

We have been using the extremophyte model, *Schrenkiella parvula*, in the family of mustard-cabbage family of plants to investigate novel genes and pathways naturally selected for harsh environments. *S. parvula* is native to the saline lakes in the western Irano-Turanian floristic region. It is naturally found near hypersaline lakes, in soils rich in $Na^+$, $Li^+$, $K^+$, $Mg^{++}$, together with sulfate, borate, and chloride salts at levels toxic to most plants[6] (Figure 1). In addition, it tolerates high heat, cold, fluctuating diurnal and nocturnal temperatures, low water, low N and P soils, and high light/UV conditions extreme to most plants. It can withstand saline water similar to seawater strengths[7]. *S. parvula* is the first extremophyte genome sequenced and continues to be among the top five best contiguously assembled land plant genomes available to date[8,9]. *S. parvula* is closely related to the most studied model plant, *Arabidopsis thaliana* among all land plants, at a level where interpretation of basic gene functions established for *A. thaliana* can be used to identify genetic pathways differently regulated in *S. parvula*. *A. thaliana* also serves as a comparator spe-

cies sensitive to environmental stresses in the comparative design of molecular genetic studies.

**Methods: Plant material and growth conditions.** *S. parvula* and *A. thaliana* plants were grown hydroponically with 1/5-strength Hoagland's solution in a growth chamber under a 14-h-day/10-h-night cycle, 100-150 µmol m-2 s-1 light intensity, and 22°C to 24°C temperature. 4-week-old plants for each species were subjected to different treatments. **Ionome profiling.** Tissue samples were dried at 37°C for one week to yield 5 to 60mg of tissues. Elemental profiling was accomplished by ICP-MS at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Plant Genetics Facility at the Donald Danforth Plant Science Center. Elemental analysis was performed for Li, B, Na, Mg, Al, P, S, K, Ca, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, and Cd. All samples were normalized to calculated dry weights. Significant differences between samples were determined by one-way ANOVA followed by Tukey's post-hoc tests in R. **Transcriptome profiling.** Root and shoot samples were harvested separately for each species, 3 and 24 hours after 150 mM NaCl treatment for A. thaliana and 3 and 24 hours after 150 and 250 mM NaCl treatment for S. parvula. Total RNA was extracted from root and shoot separately using the RNeasy Plant Mini kit (Qiagen, Hilden, Germany). The RNA sequencing libraries were prepared with TruSeq Stranded mRNAseq Sample Prep kit (Illumina, San Diego, CA, USA). Libraries from control and treated samples, with three biological replicates for each species, were barcoded and sequenced on three lanes of the HiSeq4000 platform (Illumina), generating > 15 million high-quality 50-nucleotide (nt) single-end RNA-seq reads per sample.

**Results and Discussion.** *S. parvula* accumulated less $Na^+$ and maintained higher $K^+$ in both shoots and roots compared to *A. thaliana* under salt stress (Figure 2). In addition to ionic stress caused by $Na^+$, most plants including crop plants show $Na^+$ toxicity due to their failure to uptake and maintain $K^+$ under salt stresses. $K^+$ is a macronutrient for all plants and therefore it is important for growth an survival during salt stress. The extremophyte, *S. parvula* successfully manages to grow and maintain $K^+$ when A. thaliana fails to maintain essential nutrient uptake. We wanted to find out the significant differences at the genomic and transcriptomic level that would enable nutrient homeostasis under salt stress in the two test plant systems. *A. thaliana* showed a greater response to salt stress in shoots than in roots, with a significant decrease in amino acids, sugars, fatty acids. In the *A. thaliana* transcriptome, genes involved in amino acid and fatty acid biosynthesis were significantly down regulated during salt stress, while *S. parvula* transcriptomes showed fewer changes among them were dominated by salt-stress responsive genes including antioxidant biosynthesis. The *S. parvula* transcriptome showed a remarkable level of genes that uniquely responded to salt stress and among them was *CBL10*. *CBL10* is known for its regulation in compartmentalizing excess $Na^+$ to vacuoles and alleviating salt stress effects. We expressed one of the *S. parvula CBL10* genes in *A. thaliana* as a preliminary effort to investigate the functional significance of selected extremophyte genes in a stress sensitive system. The transgenic *A. thaliana* plants with overexpressed *SpCBL10* was significantly salt tolerant than the wildtype *A. thaliana* plants (Figure 3).

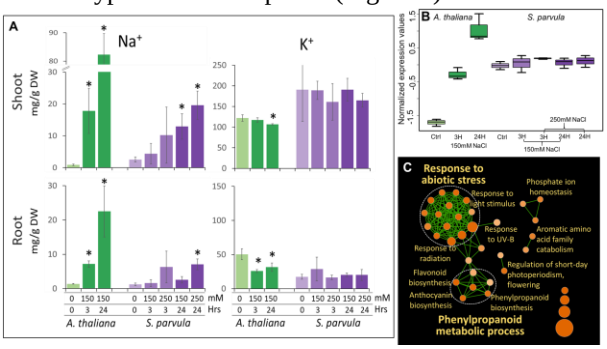


Figure 2. [A] *S. parvula* shows lower accumulation of $Na^+$ on both root and shoot upon NaCl treatment for 3 and 24 hrs compared to *A. thaliana* while maintaining the $K^+/Na^+$ ratio. [B] *S. parvula* show a stress-preparedness cluster by regulating a small number of genes in selected pathways compared to *A. thaliana*. [C] Gene ontology enrichment analysis for the stress-preparedness cluster.

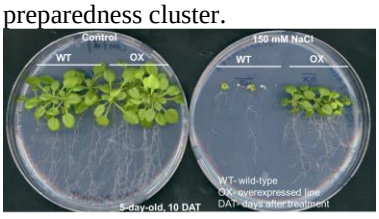


Figure 3. *SpCBL10.3* overexpression in Arabidopsis wild-type induced tolerance to NaCl.

**Conclusion:** This preliminary study highlights the importance in the use of extremophytes to develop future crops adapted to Martian soils and other plant growth conditions suboptimal for normal plant growth.

**References:** 1.NASA. NASA's Journey To Mars: Pioneering Next Steps in SpaceExploration. (2015). 2.Oh, D.-H. et al. Genome Structures and Transcriptomes Signify Niche Adaptation for the Multiple-Ion-Tolerant Extremophyte Schrenkiella parvula. Plant Physiol 164, 2123–2138 (2014). 3.Dassanayake, M. et al. The genome of the extremophile crucifer Thellungiella parvula. Nat Genet 43, 913–918 (2011).