

Elevating the Use of Genetic Engineering to Support Sustainable Plant Agriculture for Human Space Exploration

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Background

Establishing a sustained presence on the Moon, cis-lunar orbit, and on Mars, will require plant agriculture. Growing plants has both psychological and nutritional benefits (Odeh and Guy, 2017) and has long been held as pivotal to successful long-duration human space exploration. The role of plants as components of spaceflight and planetary life support systems has been specifically called out for several decades through NASA imperatives. To date, most efforts have been dedicated to engineering plant growth hardware, testing the growth of various crop species for nutrition supplementation, investigating plant molecular responses to the spaceflight environment, and investigating plant-microbial interactions (Barker et al., 2020; Basu et al., 2017; Bishop et al., 1997; Choi et al., 2019; Ferl et al., 2014; Foster et al., 2014; Johnson et al., 2017; Khodadad et al., 2020; Kwon et al., 2015; Leach et al., 2007; Paul et al., 2021, 2017; Perchonok et al., 2012; Wheeler et al., 1996; Zhou et al., 2019). While little has been done to genetically tailor crops for spaceflight applications to enhance plant vigor, increase harvest index of crops, biofortify crops, or produce feedstocks specific to spaceflight mission goals (Graham et al., 2015), genetically engineered (GE) plants have been widely used in spaceflight applications to understand the plant's molecular responses to the spaceflight environment. This molecular foundation provides key insight into the metabolic strategies plants use to adapt to spaceflight, and creates a starting point for engineering plants that are optimized for vigorous growth in this novel environment. (Califar et al., 2020; Kiss et al., 2012; Nakashima et al., 2014; Paul et al., 2001, 2017).

The past five years have seen pivotal advances in the techniques, tools, and accomplishments that open the possibilities to engineer plants for spaceflight applications. Gene-editing tools (i.e., Clustered Regularly Interspaced Short Palindromic Repeats - CRISPR) allow precision editing of genomes to evaluate gene function, improve crops, and domesticate novel crop species (Bhat et al., 2021; Razzaq et al., 2019; Wada et al., 2020; Zhang et al., 2019). Recent advances in nanotechnology improved methods to deliver CRISPR reagents to diverse plant species (Ahmar et al., 2021; Demirer et al., 2021). Current sequencing technologies coupled with machine learning for data processing and analysis produce accurate genome assemblies rapidly at low cost. The NASA GeneLab spaceflight data repository along with many other public available databases will provide comprehensive molecular datasets to identify targets for advancing plant genetic engineering and establish sustainable space agriculture in the near future.

As we enter the next decade of space exploration, it is our opinion that the community must elevate the groundwork necessary to advance the use of GE plants by combining various techniques (i.e., Genome editing, Molecular breeding, and Gene editing) to enhance spaceflight-specific mission goals.

Benefits to Human Exploration

Agriculture depends on continuous genetic improvement in plants via breeding and genetic engineering. The Green Revolution was primarily driven by changing plant architecture through traditional breeding (Pingali, 2012). While plant breeding is still the core approach for genetic improvement (Bailey-Serres et al., 2019), the past 20 years saw a massive adoption of genetically modified row crops that carry transgenic traits enabling no-till agriculture to protect

topsoil and to reduce the environmental impact of conventional agriculture (e.g., reduce pesticide use) (Duke, 2015; Tabashnik et al., 2013). Plants have been engineered to confer resistance to biotic and abiotic stresses, improve yield, fruit quality and nutritional value as well as synthesize compounds for biopharmaceuticals, bioplastics, biofuels and other raw materials for the manufacturing sector. These terrestrial applications could translate well to future spaceflight missions (Berliner et al., 2021; McNulty et al., 2021).

In addition to direct translation of terrestrial improvements to spaceflight, there is a great opportunity to specifically engineer crops for resilience and yield under spaceflight-specific stresses and growth facilities. These applications would no doubt be back compatible with emerging terrestrial agricultural systems such as vertical farms/plant factories. This bidirectional advancement in cropping systems and crop genotypes will drive innovation and food system resiliency on both sides. GeneLab is a repository for data that shows how space impacts an organism's biology, providing information on candidate genes that would benefit space agriculture. Artificial intelligence could be used to sort through all the data sets in the GeneLab repository and identify key pathways and candidate genes that could be modified. Engineering plants for spaceflight application can lead to significant breakthroughs that can be categorized into two main groups:

1. Basic Science Aspect

- GE plants can be used as tools to help us better understand the effects of spaceflight or planetary environments. Transgenics and site-directed mutant lines allow precise hypothesis testing for the impacts of individual genes and biological pathways in supporting the plant adaptation to the spaceflight environment.
- Engineered plants can confer spaceflight-related abiotic and/or biotic stress resilience. Spaceflight discoveries could revolutionize controlled environment terrestrial horticulture.
- Engineered plants could also teach us the fundamentals of dealing with genome instability or radiation impacts as we begin to consider large-scale farming in space.
- Priming plants with epigenetic modifications will give us insights into alternative modes of engineering plants for the spaceflight environment.

2. Applied Science Aspect

- GE crops can be optimized to produce food, supplement/personalize crew's nutritional needs, produce raw materials, and other resources in various spaceflight/ planetary environments.
- Engineered plants can be used for hardware designs that are not bound to terrestrial heritages, therefore maximizing the available resources relevant to spaceflight scenarios.
- Engineered plants can be optimized for low pressure non-terrestrial atmospheres to make it easier for crew operations.
- The development of molecular tools for in-space utilization will be necessary to further this field of research.

Recommended Areas of Focus

For the coming decade, it is important to apply the tools of genetic engineering to solving the problems of growing plants in and producing crops in limited volumes and altered atmospheres to meet the needs of the crew. This must be done with the concept of a multi-year Mars mission on the horizon at the end of the decade. Key focus areas are given below.

1) Engineer plants with the ideal characteristics for the spaceflight environment.

As missions develop over the next 10-20 years that look beyond LEO and towards human-rated planetary exploration activities, there will be an increasing need for robust Bioregenerative Life-Support Systems (BLSS) (Graham and Bamsey, 2016). The physical or engineering limits of BLSS have been reached, at least conceptually – with room for incremental engineering advances (e.g., better sensors, actuators, etc.). The robustness and efficiency of BLSS could be enhanced by the genetic engineering of space-specific crop lines that are tailored to the spaceflight environment. Crops must robustly and predictably meet mission requirements for providing air, water, food, and nutrition safely and reliably to maintain crew health. The potential for tailoring plants specifically to the spaceflight environment is substantial. For example (Graham et al., 2015) broke barriers for one of the most unlikely space crops - fruit trees.

Plant growth platforms for space (i.e., Veggie, Advanced Plant Habitat (APH), and Ohalo III) define a crop plant idiomorph which demands smaller plant architecture, rapid growth cycle, and a high harvest index that eliminates the production of waste while maximizing outputs. For example, the University of California, Riverside is developing a miniaturized, high harvest index tomato variety with the goal of infusing into spaceflight production (Jinkerson and Orozco-Cardenas, TRISH Grant No. NNX16AO69A-T0410). Plants can be designed to fruit without pollination (parthenogenesis or parthenocarpy) (Acciarri et al., 2002), produce substantially less of indigestible, gravity-defying compounds (i.e., lignin – potential candidate genes: HCT, CCR1, ADPG1) (Eudes et al., 2016; Gallego-Giraldo et al., 2020), or have altered morphologies that maximize photosynthesis and reduce difficulty for post-harvest processing. Genetic engineering of the plant cell wall could also improve the accessibility of cellulose in the cell wall to make more accessible feedstocks for space manufacturing and 3D printing.

Decades' worth of investigations on the plant's molecular responses to the spaceflight environment can be used to tailor plants for spaceflight missions. For example, genes associated with spaceflight stress responses (i.e., cell wall remodeling, reactive oxygen species (ROS), pathogen attacks, and hormone signaling) can be altered to enhance growth responses in space. In addition, investigations into improving spaceflight-related stress resilience with epigenetic tools (i.e., *msh1* mutant lines) (Viridi et al., 2015) could also aid in priming plants for the unique environment of spaceflight. Several recent reviews have detailed how plants use epigenetics to cope with biotic and abiotic stresses (Lämke and Bäurle, 2017; Turgut-Kara et al., 2020) and also in interacting with the microbiome (Zhu et al., 2016). Epigenetic regulations that could enhance crop vigor in the spaceflight environment. Together, these efforts will provide the blueprint for establishing spaceflight adapted plants.

2) GE crops to produce plant-based products relevant to spaceflight mission objectives.

The ISS receives frequent resupply cargo missions to support the crew allowing a two-year shelf life for food, medicine, and other essential supplies. Long-duration deep-space missions will require food and medicines to be stable for a minimum of five years. The current pre-packaged food system has only a small fraction of menu items that are acceptable beyond two years (Cooper et al., 2011). Even with longer shelf-life products, essential nutrients and active ingredients are known to degrade during storage (Blue et al., 2019; Cooper et al., 2017, 2011; Zwart et al., 2009).

These long-term storage challenges create serious risks of micronutrient malnutrition and loss of effective pharmaceuticals for crewed missions to Mars. Consequently, fresh foods and medicines will need to be produced during deep space missions. Due to the limited volume and mass that is likely to be devoted to plant production, biofortified crops that provide high levels of nutrients that are prone to degradation are desirable to provide a supplement to packaged food supplies.

Biofortification is possible through conventional breeding and has been applied to fortifying staple crops grown throughout the developing world (Van Der Straeten et al., 2020). Conventional breeding efforts require pre-existing genetic variation to enable fortification and so far have only been able to target 1-2 essential nutrients. Moreover, breeding targets for the developing world include minerals (e.g. iron and zinc) and biosynthesized micronutrients (e.g. pro-vitamin A and folate). Mineral nutrients are not likely to degrade or lose bioavailability during storage, and biofortified crops for spaceflight are likely to require a different suite of fortification traits than will be available through conventional breeding efforts. Genetic engineering allows more nutrient traits to be developed in a single crop species as well as customization to address space-specific requirements. There are multiple examples of genetic modifications increasing provitamin A as well as B and E vitamins by 100-fold or greater (reviewed in Van Der Straeten et al., 2020). Importantly, transgenic approaches to biofortification can be combined with engineered plant architecture, as well as with new molecular breeding strategies, all at a speed of development far in excess of traditional breeding methods to meet the development timelines needed for space exploration.

In addition to biofortification, genetic engineering technologies in plants can be used for molecular pharming (McNulty et al. 2020). Although biopharmaceuticals traditionally are produced in cell culture systems, transient expression vectors for plants enables production of protein therapeutics in plants (Chen and Davis, 2016). Multiple examples of antibody therapies have been developed that could treat dengue (Dent et al., 2016), West Nile virus (Sun et al., 2018), chikungunya (Hurtado et al., 2020), Ebola (Budzianowski, 2015), and COVID-19 (Ward et al., 2021). Further development of transient expression technologies would allow crop plants to produce different therapeutics on-demand (McNulty et al. 2020). One potential model of plant-based molecular pharming would develop a library of DNA constructs or plant vectors capable of expressing diverse products. Astronauts would select and express in plants the appropriate construct needed to treat an illness. It is conceivable that the production of ubiquitous crew health elements (e.g., a radiation countermeasure compound) would be incorporated directly into the food supply to ensure a consistent and compliant dose system.

Plants already have many intrinsic and introduced metabolic pathways that lead to materials that can be used as feedstocks in spaceflight scenarios: plastic monomers for plastic production (Kourtz et al., 2007), cell wall components for 3D printing (Yang et al., 2020), plant fibers for concrete reinforcement, bulk enzyme production, etc. (Farooqi and Ali, 2018; Tschofen, 2016). Using genetic manipulation tools, the offerings of such space-specific feedstocks could be greatly expanded. Plant lines can be tailored to be an integrated component of an *in situ* resource utilization process e.g., capturing CO₂ from the Martian atmosphere; converting *in situ* water into fuel (O₂/H₂) or capturing important elements (K, P, Ca, N, Fe, S, etc.) from the Martian or lunar regolith to provide a source of food or other feedstocks.

3) Using engineered plants to understand radiation impacts and how to mitigate them.

There are currently no practical, effective shielding technologies against the omnipresent cosmic ionizing radiation (cIR) (i.e., Galactic Cosmic Rays) that originate from high-energy astrophysical events or solar particle events emitted from the Sun. These highly energized particles travel in straight tracks, at relativistic speeds, and transfer energy upon collision with matter. This collision knocks electrons out from low-lying orbitals of atoms leaving “ions” that break chemical bonds and destabilize biomolecules. They also produce secondary radiation, including high energy X-rays when they strike components of the space craft, habitats or other materials. cIR induces damages directly to the DNA and other biomolecules (proteins, carbohydrates, lipids) as well as indirectly causing the radiolysis of water forming free radicals that can cause additional damage. Radiation presents a serious hazard to all life, so it is important for us to understand how to deal with or mitigate its effects.

To engineer radiation tolerant crops we need to first understand how plants prevent and/or repair radiation damage. Plant organisms (i.e., non-vascular primitive moss (bryophytes)) that are found in habitats with high UVB photon levels and at sites with elevated ionizing particle levels have shown remarkable resilience to high radiation levels. Resilience is generally associated with: 1) Hyper-resistance to DNA breaks upon exposure to high energy photons (Wang et al., 2017); 2) A unique preference for using the accurate error-free homologous recombination (HR) pathways to repair the DNA breaks as oppose to the non-homologous end-joining (NHEJ) pathways which are intrinsically mutagenic; 3) the presence of special genes (i.e., duplicated Rad51 genes, CHD7) in the genome that function in DNA repair (Rensing et al., 2008); and 4) The presence of higher levels of non-enzymatic antioxidants (flavonoids, carotenoids, xanthophylls, anthocyanins) and antioxidant enzymes (CAT, POD, APX) that quench free radicals thus mitigating indirect cIR damage (Li et al., 2019; Taofiq et al., 2020). These plants, genes and pathways could be used to investigate pro-survival mechanisms when exposed to properly simulated cIR at a relevant dosage archived at the NASA Space Radiation Laboratory located at the Brookhaven National Laboratory. Information gathered will then be used to formulate genetic engineering approaches in constructing tolerant crop lines that can be further tested in deep space.

Conclusion

The value of genetic engineering of plants for spaceflight missions is incalculable, as the envisioned utilities of these plants for future spaceflight missions have paramount importance to establishing a sustainable habitat off Earth. Many of these same attributes discussed above will be valuable to crops grown on earth in a controlled environment and can offer benefits to people living on earth in developing countries or facing impacts of climate change.

In the previous decade, understanding the basic science of how the spaceflight environment affects plant growth and development was a major NASA science objective. In this next decade, the community must significantly advance beyond the established groundwork. This can only be achieved through the use of genetic and molecular techniques to engineer plants for future mission-related goals of building a permanent presence on the Moon and Mars.

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