WHITEPAPER FOR CONSIDERATION IN THE 2023-2032 NASA
DECADAL SURVEY ON BIOLOGICAL AND PHYSICAL SCIENCES
RESEARCH IN SPACE

Plants as Chemical Factories for Supporting Human Exploration in Space

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Executive Summary

Plants, as higher eukaryotic photosynthetic organisms, are an ideal host platform for production of recombinant proteins, biochemicals, and biomaterials for deep space exploration due to their minimal resource and infrastructure requirements, genetic programmability, and recyclability of biomass waste. In this white paper we argue that plants can serve a much broader purpose than just for food, nutrition, and general life support in space exploration. Here we describe the different ways in which plants can be used as chemical factories, current state-of-the art approaches in plant molecular farming, and we identify a research campaign to establish plant molecular foundries in space.

RESEARCH CAMPAIGN
ESTIMATED COST: $10,000,000/year ; $100,000,000 Total
PROJECT DURATION: 2023-2032; 10 years

Introduction

Spaceflight presents both anticipated and unexpected situations during spaceflight and in space environments in which the crew will need food, medicine, and materials that they did not bring with them. This can be to supplement flown supplies in either a replacement (for expended or expired supplies) or augmentation (for unanticipated or otherwise not-flown supplies) function. Plants have the potential to make biological materials (enzymes, metabolites, therapeutics, biopolymers, etc.) rapidly on-planet or within a spacecraft with minimal infrastructure requirements in response to both of these situations, serving as a versatile tool to de-risk space exploration missions.

Plants will be essential for any deep space mission as a food source, but also for life-support (carbon dioxide consumption, oxygen generation and pure water generation through transpiration), and psychological benefits[1]. Eukaryotic cellular machinery in plant cells offers tremendous potential as chemical factories in spaceflight and space environments to produce high-value chemicals, which can be used for critical utilities including human therapeutics, diagnostics, enzymes, construction materials, and chemical precursors, in spaceflight and space environments (Figure 1)[2, 3].The ability to provide light and carbon dioxide (e.g., in transit or for example on Mars) makes growing plants using *in situ* resources much simpler than other biological production hosts for biomanufacturing, such as microbial cell cultures or cell-free protein synthesis that require complex (and sterile) bioreactor systems, growth medium, and reagents. Additionally, plant production has advantages of linear scalability[4], inherent safety in its low pathogen load and inability to harbor and replicate human pathogens[5], and useful biomass side streams[6].

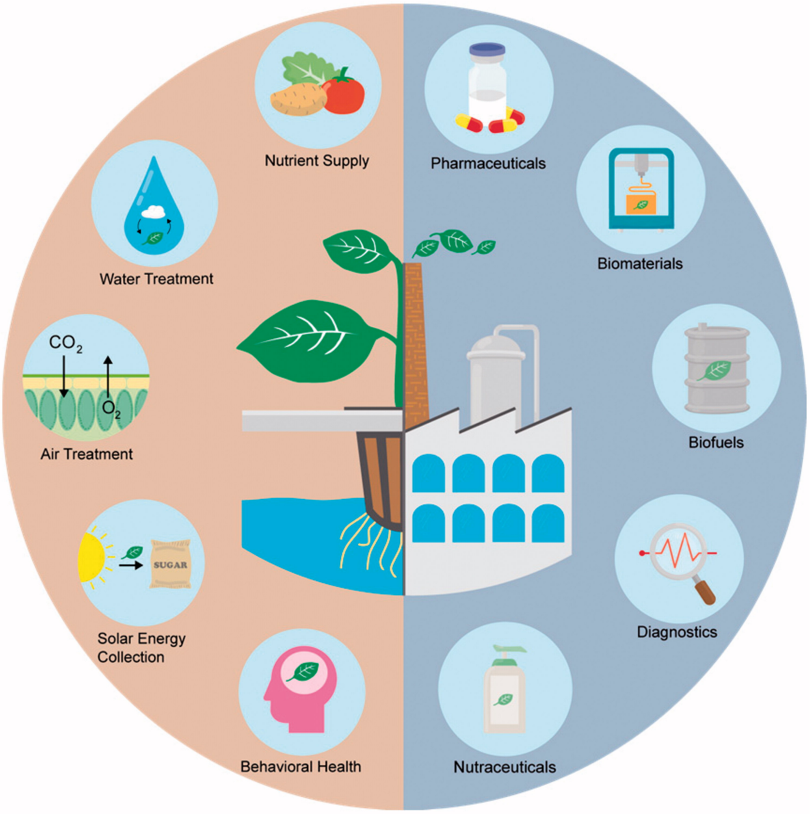

Figure 1: Molecular farming embodies the perspective that plants are chemical factories. Viewing plants as factories vastly expands the bioregenerative life support capabilities of plants in space. Figure from McNulty et al., 2021.

The infrastructure for growing plants will be available for any deep space mission. Host plant production will not require additional mass, volume and power required to transport equipment. Plants, as a biological system, are self-replicating; a limited amount of seed needs to be transported as additional seed can be generated on site. Plant biomass such as plant waste and/or inedible plant biomass can be used for transient production of valuable compounds. Plant cells can serve as natural bioencapsulants for oral delivery of protein-based drugs[7], protecting these biologics from the acidic stomach environment and enabling release and transport across the epithelial lining in the intestine into the circulatory system[8].

The development of plants as chemical factories directly benefits humans on earth since it is an inherently "green", sustainable, and environmentally friendly bioproduction technology. Furthermore, it simplifies biomanufacturing by replacing expensive bioreactors that must be maintained sterilely, minimizing requirements of a highly trained/skilled workforce (given the prevalence of basic agricultural skills), and lowering global supply chain complexity for media, instrumentation, valves, and consumables, with plants and lower cost agricultural technologies[9]. Plants as chemical factories reduce costs for global production[10–12], product development time to market, and ensures resiliency by enabling more globally distributed industrial and biopharmaceutical manufacturing[13].

Stable production of high-value chemicals using dedicated transgenic plant lines

Using the tools of synthetic biology, many academic and commercial entities have edited the genes of plants to generate transgenic or transfected plants capable of producing a wide range of high-value chemicals including different materials, enzymes, and medicines.

Research areas, concepts, and methods

Considering the resource constraints (equipment, reagents, human resources, scientific skills, etc.) in the space environment it may be worth to develop the transgenic homozygous seeds on earth and growing those seeds/plants in space for which NASA has existing experience. Generally, in a transgenic line, recombinant molecules are expressed in specific plant parts/tissues (e.g., seed, leaf, flower, root etc.) via tissue specific expression system while rest of the plant can be utilized to produce various other products as mentioned above. Characteristically, a transgenic plant has been shown not to produce/accumulate any recombinant molecule when a strong tissue specific promoter has been used (see Fig. 4 of Nandi et al., 2002 and Figs. 2 and 6 of Huang et al., 2001).

Recombinant plant growth systems can now be developed for space flight and for sustainable extraterrestrial habitats. These next steps need to focus on the integration of systems for life support and food production including the use and modification of local resources. Plants as flexible bioproduction systems can be manipulated in space to address known and unknown issues including medical and structural challenges. The ability to provide the space traveler with a rapid, safe, and product diverse production system helps insure sustainability. Using these techniques we can develop a robust and more versatile toolbox for production of food, pharmaceuticals, and other high value chemicals.

Level of existing information and inputs required to fully evaluate the idea/project?

In best of our knowledge NASA has ample experience growing food plants in space like situation (in contained environment) and that should be enough to grow a stable transgenic line/plant. Because a carefully selected stable transgenic line grows as normal as it's native counterpart while the specific tissue accumulate/produce the target molecule(s), so the rest of the biomass can certainly be used for other purpose (See Figs. 1 and 3 in Buyel, 2019). Such as bioplastic[14], industrial proteins[10], chemical precursors[15], etc. Using transgenic approach has advantage of scalability and stability of the target molecule. In CUBES, we are also developing homozygous transgenic lettuce lines which stably express the human parathyroid hormone Fc fusion protein [PTH-Fc, 1-34 amino acid] in leaves, or PTH-Fc. The production of PTH-Fc in these lines are under characterization of PTH-Fc per unit of plant biomass/leaves (unpublished data). These lettuce leaves expressing PTH-Fc can serve as natural bioencapsulants for mucosal delivery of protein-based drugs as mentioned above, and Fc can give further protection of this small biologics (1 – 34 amino acid) from the acidic stomach environment and enabling release and transport across the epithelial lining in the intestine into the circulatory system.

Intermittent production of high-value chemicals using transient production of high-value chemicals in non-dedicated plant lines

Transient expression of recombinant proteins in plants offers rapid production of human therapeutics, diagnostics, enzymes, metabolites, and chemical precursors. Transient expression does not require the long lead times of developing stable transgenic lines, which limit the number of products to be "on demand." Current transient expression systems require the use of well-developed vectors, containing cassettes of genetic information to produce specific proteins or intermediary metabolites. These cassettes are generic and can be easily modified to incorporate a wide variety of genes to produce specific products. Once introduced into the plant, the vector hijacks the protein synthesis machinery of the plant and focuses biosynthesis on the gene of insertion, which is transcribed and translated into the product of interest within several days. The plant biomass is then extracted to recover and purify (if needed) the target product. There is no need to integrate DNA into the plant genome (i.e., no need to generate a stable transgenic plant). Often this process results in a burst of gene expression that leads to higher levels of production than a corresponding stable transgenic line.

Research areas, concepts, and methods

Research into adapting recombinant methods for efficiently and rapidly introducing DNA/RNA into plant tissues (e.g., engineered plant viruses[16], DNA/RNA coated nanoparticles[17], designed DNA origami nanostructures[18], biolistics[19], agrobacterium[20], etc.) under the resource constraints (equipment, reagents, etc.) of the space environment would ensure a flexible manufacturing system. Engineering of generic plant production hosts that can maximize production of specific targets by eliminating proteases, amplifying gene copy number, providing unique post-translational processing, reducing inherent gene silencing mechanisms and/or making them more resistant to effects of galactic cosmic radiation would also ensure maximum utility and resilience. Maximizing the speed of transcription and translational processes, enabling cell-to-cell movement of the genetic instructions, and/or understanding the process from a multi-omics perspective would make this process even more productive and flexible.

Level of existing information and inputs required to fully evaluate the idea/project?

NASA has a lot of experience and success growing plants in space, but to our knowledge there is very little, if any, information on transient expression of recombinant proteins in a space environment. Robust techniques for generating genetic constructs and using delivery mechanisms such as viral vectors and biolistics exist on earth. The opportunity to build compact and flexible systems to build nucleotides in space to generate new and different genetic instructions for transient expression plant chemical factories in response to spaceflight or space environment needs is very doable and builds on a thirty-year base of research[21]. This key technological step unlocks the maximum flexibility of the plant production system in space.

So while there is quite a bit of information on the plant inputs required, there is not much information on how DNA/RNA could be synthesized in space, different inputs that would be needed for delivery of DNA/RNA to plants that would strongly depend on the delivery mechanisms used (e.g., engineered plant viruses, DNA/RNA coated nanoparticles or biolistics, etc.), use of harvested plant tissues for production, and production kinetics and product quality obtained under space conditions. In addition to understanding the implications of transient plant-based production technologies in space it would also be important to assess the resources needed (mass, volume, power, cooling, crew time, etc.) and training required of crew members to implement transient production processes.

Establishing space-relevant bioprocessing techniques for purifying high-value chemicals from plants

Some form of purification of the high-value chemical from the host plant tissue will be required for most products of interest. This is true of any biologically-produced high-value chemical. The processing is typically accomplished by a step-wise purification flow path beginning with homogenization of the tissue, extraction of the product, clarification of the extract, secondary purification by product characteristic (e.g., ion exchange chromatography to exploit electrostatic differences), and formulation[22]. The ability to modify the product of interest with molecular tags for ease of separation (e.g., lipophilic adducts[23]) is a well-known approach to shorten and simplify the purification path.

Research areas, concepts, and methods

Development of innovative bioprocessing methodologies and technologies for the extraction and purification of accumulated bioproducts (e.g., plastics, enzymes, chemical precursors, medicines) from the plant host into a form acceptable for their specific use application is crucial for unlocking the potential of plants as versatile mission elements.

Novel crude product formulations or genetically controlled intracellular storage could either leverage the structural benefits of plant host impurities (e.g., bioplastics formulations with plant wall cellulose supplement[24]) or exploit the advantageous classification of most plants as Generally Recognized as Safe (GRAS) per the U.S. FDA (e.g., oral delivery of plant-made medicine[7]).

Novel processing technologies can address the unique limited resource constraints of spaceflight and space environments. One promising avenue for this is biologically-derived processing technology (e.g., plant virus-based immunosorbent nanoparticles[25, 26]) whose bioregenerability and synergy with plant-based production (e.g., production of the purification reagent uses similar resources as that of the high-value chemical) builds mission resilience without significant infrastructure costs. A second avenue here would be the development of novel processing technology with limited space-relevant cost penalties (e.g., mass, volume, power), such as the established approach of molecular tags or the nascent methodology of apoplast wash fluid recovery[27], in which the product is secreted into the cell wall and recovered via vacuum infiltration and centrifugation without needing to homogenize the plant tissue.

The ultimate goal of this research area should be proof of concept bioprocessing of a high-priority plant-product bioproduct with a prototype system capable of spaceflight and space environment operation.

Level of existing information and inputs required to fully evaluate the idea/project?

The opportunities cited in the above section have already been applied in commercial development of plant-made therapeutics. This research base provides a solid foundation for moving these concepts into systems suitable for space travel and habitation while maintaining the flexibility to deal with both predictable and unpredictable circumstances. The biotechnology business is constantly improving recovery and purification of materials. The challenge is to develop systems that are easy to adapt to new opportunities. The most promising of which is the ability to rapidly introduce new functional genes into plants[28, 29], whose function minimized complication downstream purification processes. Building on existing procedures and data, an evaluation framework can be established for assessing the current and projected future landscape of space bioprocessing methodology. The analytical requirements for bioproduct (processing) characterization are also an essential component of this landscape analysis. Critical prerequisites include a detailed design premise (i.e., reference mission architecture) for proof-of-concept that includes a specific plant host organism, growth chamber, bioproduct (including a demand profile), and system design constraints (e.g., mass, volume, power, crew time availability).

Establishing plant waste recycling for nutrient recapture or amending Martian regolith

Ideally, recycling of expended resources should be accomplished in a short timeframe and with minimal supplementary inputs. All unused plant nutrients and structural components are theoretically recoverable, but the length of recovery and minimal extent of supplementary resources required to promote recovery are yet undetermined.

Research areas, concepts, and methods

Investigation into plant waste recycling (i.e., the decomposition of plant wastes and the uptake of plant waste resources) is key for space-relevant efficiencies in the use of plants in longer-duration exploration missions. Decomposition will need to be done with little to no power (composting) to be efficient. Identification of microorganisms capable of rapid degradation of organic wastes in a controlled environment will decrease processing time. Aerobic vs. anaerobic processes will need to be weighed against each other to optimize recovery[30]. There are opportunities here for novel transgenic plant development to aid in the rate and extent of decomposition. For example, development of transgenic lines that produce cellulases in response to a critical thermal threshold being met would be an auto-catalytical reaction for degrading the plant cell wall[31]. The uptake of plant waste resources can be generally classified as either nutrient recapture or new resource generation. Research into nutrient capture would include integration of plant waste streams with existing mission elements (e.g., plant waste serving as a feedstock for microbial systems that in turn provide critical mission resources). New resource generation, a less common utility of waste products, could be an emergent utility critical for longer-duration habitation of Mars through regolith amendment. If plant waste will be amended into the regolith, how will this be done? Manual and automated amendment, as well as frequency of amendment must be determined. Volume of plant waste generated per unit time will need to be modelled to understand scale of disposal requirements. We will need to determine the timeframe over which plant wastes will be applied in a certain area before it will be considered totally amended.

Level of existing information and inputs required to fully evaluate the idea/project?

We need to know the composition and quantity of crew member diets to determine the volume and composition of wastes. NASA provides expected diets for long-term missions, which are available in the baseline values and assumption document[32]. Using these values, the mass of unusable plant material (wastes) can be calculated (by knowing harvest index) to obtain an estimate for the mass of plant wastes that will need to be composted during a given day. Once the inputs are estimated, the outputs can be predicted using known microbial composting models for a first-order approximation[33].

We also need to know the efficiency of current microorganisms performed decomposition to model and/or test speed and efficiency. This is much more difficult to narrow down as a wide range of organisms are capable of decomposition and reactors are often inhabited my multiple species. Hyperthemophilic composting has been shown to be a very efficient process and has potential suitability for inclusion in a closed life support system[34, 35].

Recalcitrant material from which nutrients can no longer be easily extracted can be amended into the Martian regolith to increase the amount of organic matter[36]. Elevated levels of perchlorates in Martian regolith present potential toxicities that may be reduced if organic matter becomes incorporated [37].

Campaign roadmap

We argue that such groundwork requires multidisciplinary centers that can build long term partnerships and understanding; train the workforce in this unique application space; and perform the large-scale, long-term science necessary to succeed. Thus, our proposed campaign roadmap will be based on expanding the mandate and resources of NASA's 2017 Space Technology Research Institute (STRI) program – specifically an evolution of Center for the Utilization of Biological Engineering in Space (CUBES, `https://cubes.space/`) into a new paradigm (STRI-II). After 4.5/5 years of operation and with a budget of ∼3 million/year, the CUBES STRI has established a foundational center for driving SBE through 50+ publications, presentations to congress, open dialogue between NASA and other governmental research stakeholders, and the training of ∼90 students ranging in career stages from undergraduates to postdoctoral scholars from across 5 university partners. One of the primary lessons learned from CUBES is that integrated, multidisciplinary centers are critical in their integration of solutions for specific mission profiles. For plants, this means understanding the relationship between *in situ* resource utilization and manufacturing as up- and down-stream connections to food and pharmaceutical synthesis, and expanding to biopolymers, enzymes, metabolites, and biochemical precursors. While early efforts in CUBES have paved the way for understanding and integrating many of the critical aspects of agriculture-driven chemical factories for supporting human exploration on Mars, we propose that the new paradigm expand the scope for plants for use on Lunar systems such that important testing of subsystems can be carried out prior to a human Martian exploration mission. Initial efforts to establish the timeline for integrating plant-based systems for the proposed STRI-II have been carried out and published[38]. The innovations necessary to meet the challenges of low-cost, energy and mass efficient, closed-loop, and regenerable biomanufacturing – especially as it is driven by plants – for space will undoubtedly yield important contributions to forwarding sustainable biomanufacturing on Earth.

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