PLANT-CENTERED BIOSYSTEMS IN SPACE ENVIRONMENTS: TECHNOLOGICAL CONCEPTS FOR DEVELOPING A PLANT GENETIC ASSESSMENT AND CONTROL SYSTEM

Terri L. Lomax[1,2,3], Kirk A. Findlay[1], T. J. White[1,2] and William E. Winner[1]
[1]Department of Botany and Plant Pathology, [2]Center for Gene Research and Biotechnology
Oregon State University, Corvallis, OR

ABSTRACT

Plants will play an essential role in providing life support for any long-term space exploration or habitation. We are evaluating the feasibility of an adaptable system for measuring the response of plants to any unique space condition and optimizing plant performance under those conditions. The proposed system is based on a unique combination of systems including the rapid advances in the field of plant genomics, microarray technology for measuring gene expression, bioinformatics, gene pathways and networks, physiological measurements in controlled environments, and advances in automation and robotics. The resulting flexible module for monitoring and optimizing plant responses will be able to be inserted as a cassette into a variety of platforms and missions for either experimental or life support purposes.

The results from future plant functional genomics projects have great potential to be applied to those plant species most likely to be used in space environments. Eventually, it will be possible to use the plant genetic assessment and control system to optimize the performance of any plant in any space environment. In addition to allowing the effective control of environmental parameters for enhanced plant productivity and other life support functions, the proposed module will also allow the selection or engineering of plants to thrive in specific space environments. The proposed project will advance human exploration of space in the near- and mid-term future on the International Space Station and free-flying satellites and in the far-term for longer duration missions and eventual space habitation.

INTRODUCTION

> "*To advance human exploration, use, and development of space*"
>
> Basic tenet within NASA's Strategic Plan

Resources for Life in Space

Inherent within the short-term and long-term goals of NASA's Strategic Plan is the ever-increasing presence of humans as part of extended missions and as occupants of both the International Space Station (ISS) and planetary exploration missions. Included in the physical requirements of a prolonged human presence in space are the components necessary for manageable and affordable subsistence. Current environmental control and life support systems include both physical and chemical processes as well as a continual re-supply system for the space station.

* *Correspondence to:* Terri L. Lomax, Ph.D
Department of Botany and Plant Pathology
Oregon State University
2082 Cordley, Corvallis, OR 97331-2902
Email: Terri.Lomax@oregonstate.edu
Phone: 202-494-9159; Fax: 202-358-4168

Although there is now some limited recycling of water and air on the ISS, required inputs of food, water, and air remain large and are coupled with generation of sizable quantities of waste, as illustrated in Figure 1. On the ISS, the Carbon Dioxide Removal Assembly (CDRA) and Russian Vozdukh remove CO_2 via regenerable sorbent beds, which outgas the CO_2 and other contaminants into space. Lithium hydroxide canisters are used to backup and augment system capacity. Nitrogen is re-supplied from transported tanks while oxygen is supplied from electrolysis of water, transported tanks, and solid fuel oxygen generators, in that order. Potable water is shipped up from the ground or supplied from a visiting space shuttle's fuel cells. Some non-potable water is reclaimed as condensate from air, for use in the Russian Elektron electrolysis unit. Urine is dumped or stored but may be recycled in the future (personal communication, Paul Wilson, Senior Flight Analyst, Science Applications International Corporation, Houston, TX). Without the use of a regenerative life support system, current estimates for a 3-year mission with a crew of 6 would require 572,000 lbs (216,000 kg) of consumables. Eighty-nine percent of required supplies would be water, almost 3,000 gallons or 10,680 liters. With a maximal shuttle cargo of 55,000 lbs (25,000 kg), transportation of consumables alone would require 11 shuttle flights (Henninger, 2002).

The Role for Plants

As on Earth, the most efficient and self-sustaining system to provide food, oxygen, and raw materials is a plant-centered biosystem. Plants are primary producers of sugars, fats, proteins, complex carbohydrates, and vitamins. Additional life support functions that plants provide include pharmaceuticals production, recycling CO_2 and producing O_2 via photosynthesis, recycling water and modifying humidity via transpiration, recycling solid wastes through decomposition, and providing aesthetic value and avocation for those living in space.

The harsh environments of space will provide stresses on plants that can limit their capacity to fulfill life support functions, such as combinations of microgravity, cosmic radiation, low atmospheric pressure, high CO_2, temperature, and disease. Some plant stress response mechanisms are generally understood from experiments done on Earth. For example, it is now possible to predict the effects of water stress on photosynthesis of many crop and tree species. However, space travel poses new stresses for which there is neither the capacity for experimentation on Earth nor an evolutionary history for plants. Consequently, the responses of plants to unique space environments are not known. Since plant physiological responses are typically modified by additional stresses, there is little capacity for predicting

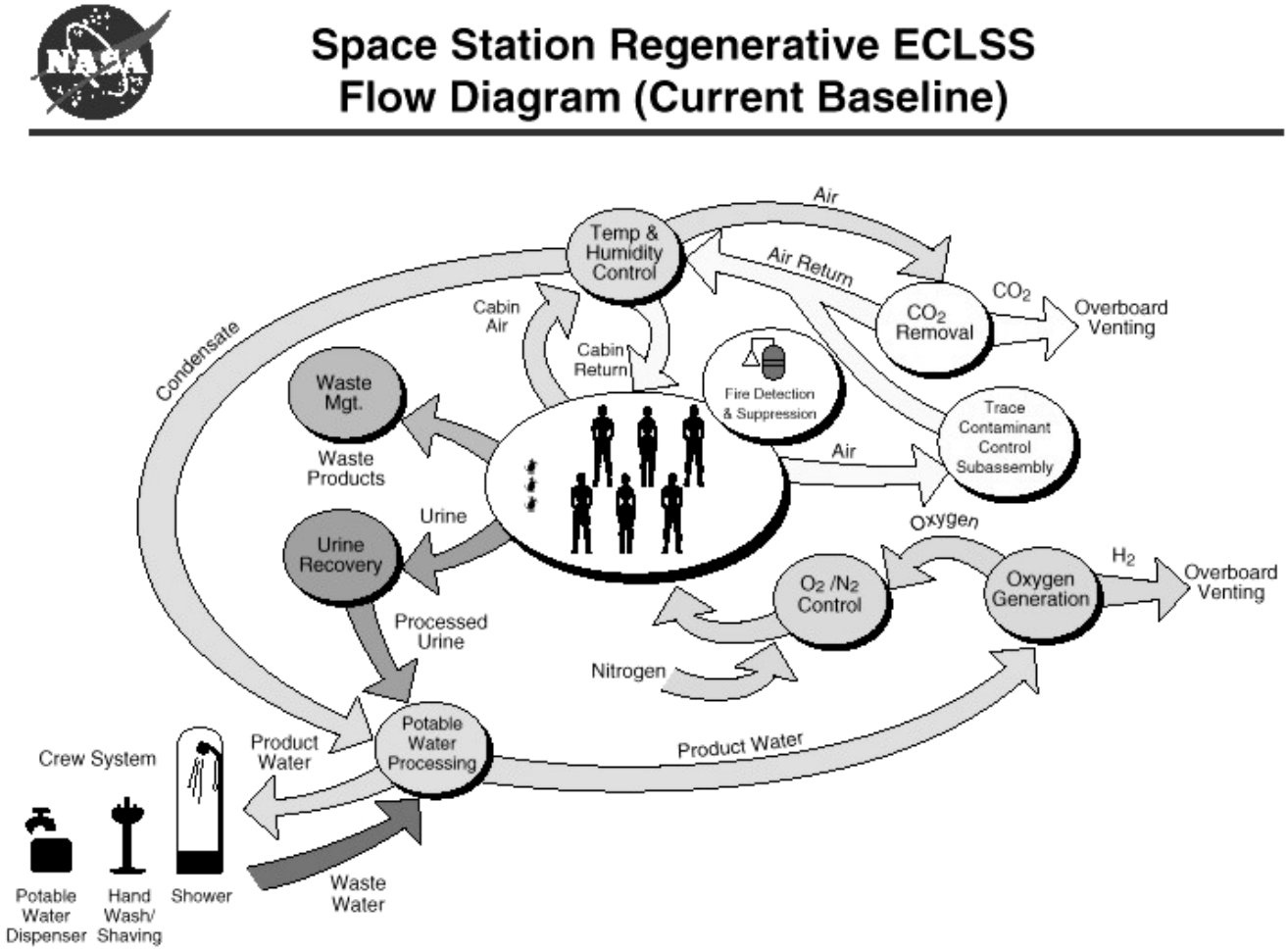

Figure 1. Space Station Regenerative Environmental Control and Life Support System. (http://spaceflight.nasa.gov/living/factsheets/breathing.html). *The current life support system used on the International Space Station utilizes physiochemical methods and frequent resupply, but longer-term missions will require plants for regenerable life support.*

how plants will function in space or how to maximize their life support functions. Further, it will be impossible to conduct Earth-bound experiments that unravel all of the impacts of space-induced environmental stresses. Figure 2 depicts an adaptable system that measures and optimizes plant responses to any unique combination of space conditions, i.e. a plant genetic assessment and control module, be included in those missions where plant-based life support systems play an integral role.

Analysis of Plant Genes

Plant genes are the units fundamentally responsible for controlling protein synthesis and, ultimately, plant functions underlying physiological processes. The expression of genes regulates metabolic functions in plant cells as well as the whole plant that will be used to support human life. To provide life support in space, plants will likely be grown either in stand-alone greenhouses, where environmental conditions are harsh or uninhabitable for humans, or as integral parts of human living quarters, and/or as vats of plants cells or algae cultured in liquid media. In any of these scenarios, monitoring the activity of many genes in the plant or cell may be the only way to determine which proteins are expressed and, by extension, to ensure the efficiency of biological functions needed for life support in space.

Technical Tools and Microarrays

A plant genetic assessment and control system can be developed based on a unique combination of existing and emerging technologies, including the rapidly developing field of plant genomics. The first complete genome sequence of a model plant, *Arabidopsis thaliana*, became available in 2000 (The *Arabidopsis* Genome Initiative, 2000) and the first draft genome sequences of a crop plant, rice, were published in 2002 (Goff *et al.*, 2002; Yu *et al.*, 2002). Additionally, government and private industry are funding researchers in efforts to complete the sequencing of the entire genomes of several species of crops, including wheat corn, tomato, potato, and soybean.

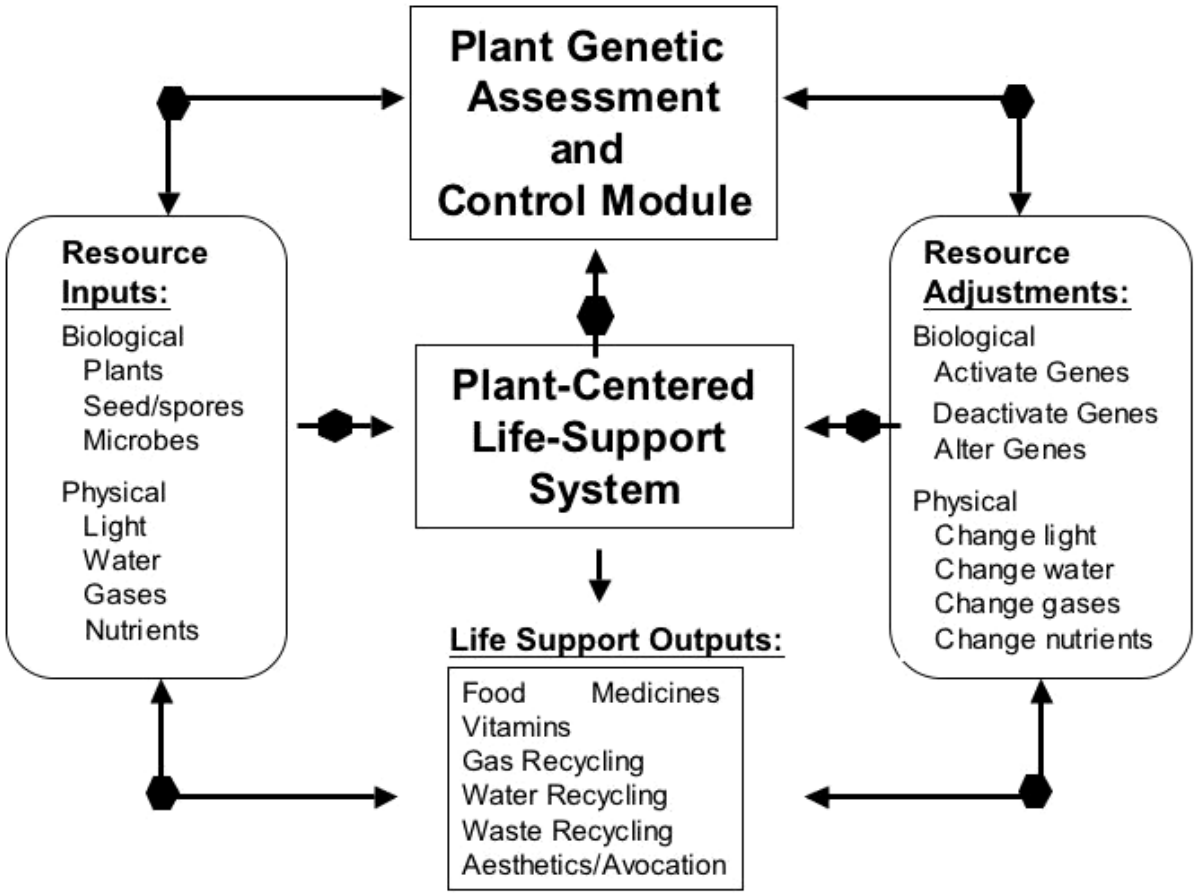

Figure 2. Role of the Plant Genetic Assessment and Control System in a Plant-Centered Life Support System. *Optimal use of any plant-centered life support system will require the ability to adjust resource inputs and outputs, as well as respond to changing environmental conditions. The Plant Genetic assessment and Control System will allow monitoring of plant status and control of inputs and outputs. Hexagons indicate control points.*

Wheat, tomato, potato, and soybean are all crops considered to be of high interest for spaceflight and habitation.

The gene sequence information determined to date provides a vast and diverse catalog of reference genes. However, in the absence of other information, a gene's sequence does not provide any clues to a gene's expression pattern, level, or function. One technology enabled by the genome sequencing projects is the use of DNA microarrays to analyze gene expression systematically on a large scale. Microarray technology allows investigators to determine activity levels of many genes simultaneously and has enormous power to measure plant genetic responses to a variety of environmental conditions. Microarrays also provide the capacity to deal with the high level of complexity needed for optimizing plant performance in difficult, rapidly changing conditions. By using the powerful microarray technique, it is possible to identify entire pathways that are turned on in response to a stimulus in a single experiment.

The basic principle behind a microarray is that the entire genome of a plant can be arrayed as separate gene identifiers and individual genes can be assayed to determine which are being expressed. The microarray technology is based on the fact that a piece of DNA will match only with the exact complementary strand, i.e. DNA comes in matched sets so you can use one part to find the other. Using sequence data generated by the genome projects, robots mass-produce gene indicator sequences on membranes, silicon chips, or glass slides. Microarrays containing from 100s to 10s of thousands of genes can help select the complementary strands of DNA generated from mRNA (the "expressed" portion of the gene that will result in the synthesis of a protein) from extracts of cells or tissues "washed" over the chip. To detect the pairing, samples are tagged with fluorescent dyes that light up under a special multi-laser scanner.

Testing the Concept with the Model Plant Arabidopsis

Since the entire *Arabidopsis* genome sequence is now known, microarrays have been constructed that represent 99.9% of the genes in the genome. *Arabidopsis,* the premier model plant species for plant genetic studies, is an ideal test species because it has a rapid generation time, is very small, and it has a well-defined gravitropic response (including numerous gravitropic mutants). In addition, *Arabidopsis* is a subject of study in the International Space Station, including seed to seed experiments (Musgrave et al., 2000). Most importantly, the genome of *Arabidopsis* is similar to that of many species of plants that will be utilized for life support in space, such as soybeans, cotton, vegetables, and oil seed crops. Therefore, information gained from this model species can be applied to other plants of interest.

Without understanding gene protein function, however, researchers have a limited ability to translate the gene sequence and expression data into information necessary to manipulate plant productivity and adaptability. By far the most promising resource currently being developed for the study of functional genomics is the Coordinated *Arabidopsis* 2010 Project and the Plant Genome Research Program. To utilize the wealth of information provided by the *Arabidopsis* genome sequencing effort, plant biologists have proposed an important and revolutionary new initiative: to determine the function of all genes within this reference species and to place genes within their cellular, organismal, and evolutionary context by the year 2010 (http://www.nsf.gov/bio/pubs/awards/2010fy01.htm). The National Science Foundation-sponsored *Arabidopsis* 2010 project involves the collaborative efforts of 49 research groups (29 funded in 2001, 20 in 2002). Each group either analyzes a specific set of related genes (e.g. nitrogen networks) or develops methodology to determine gene function (e.g. isolation and distribution of a knockout mutant for every gene in *Arabidopsis*) as the subject of their research.

All available means of creative and innovative research are being used to determine the function of gene networks within *Arabidopsis*. In the coming decades, the analysis will be expanded to other species. At the same time, research will examine how genes function together to define a plant's physiological processes. The prospect of having such complete functional genomics information concurrent with the continued design of a flexible, modular architecture illustrates the adaptability of our advanced concept to utilize information and new technology as it becomes available. As technology and information becomes more accessible, e.g. increased bioinformatics capacity and knowledge about the interaction of molecular signal cascades, those components can be added to the cyclic architecture proposed for the plant genetic assessment and control system to better design the next adaptive strategies.

TECHNOLOGIES FOR MONITORING AND CONTROLLING PLANT-CENTERED BIOSYSTEMS

The NASA Institute for Advanced Concepts (NIAC) funds studies on systems and architectures that will facilitate space exploration and other aerospace development ten to forty years from now. In our NIAC-funded study, we are investigating the potential of a future technological system for assessing the status of plant gene expression and utilizing that information to optimize production in any plant-centered life support system. The Plant Genetic Assessment and Control System (PGACS) will ultimately allow prediction of and compensation for the effects of single and multiple environmental stresses on plant physiology and growth that will be essential for sustaining human presence in space. As illustrated in Figure 3, the PGACS utilizes new and emerging technologies for evaluating gene activity, and then, based on information about plant functional genomics and knowledge based on post-genomic biology, changes environmental parameters to either sustain or alter gene expression. Only by assessing plant genes and then controlling their expression will it be possible to optimize the role of plants as a life support system. The following sections describe the enabling technologies for developing a system to monitor and control plant status in space environments.

Sample Collection and Preparation

Microarray analysis is based on measuring the levels of mRNAs being expressed in a given tissue at a given point in time. Conventional plant tissue collection for RNA recovery can be as simple as using a hole punch to acquire standardized tissue samples from select plants or more complex, e.g. using aseptic collection procedures for plants grown under sterile conditions. Either way, collected samples are usually frozen in liquid nitrogen, then ground or homogenized in a neutral buffer. Components are then separated by organic extraction followed by selective precipitation of the nucleic acid fraction or purification over a resin column. The DNA fraction is removed by enzymatic digestion and the remaining RNA fraction is purified and concentrated by ethanol precipitation.

Currently, the preparation of samples for analysis of gene expression is complex, requiring many steps of liquid handling and extensive sample manipulation. Sample preparation for microarray analysis begins with purified total RNA that is reverse-transcribed into cDNA from the poly-A tail of gene transcripts. The oligo(dT) primer is modified on its 5-end to include the priming site for a RNA polymerase, usually RNA polymerase T7. Once purified, the cDNA is used as a template to generate labeled complementary RNA (cRNA) by incorporating tagged nucleotides into the transcribed product. Product quality is determined spectrophotometrically and electrophoretically. The labeled cRNA is then fragmented for use against the probe oligonucleotide microarray sequences. More direct methods for this process are currently being developed.

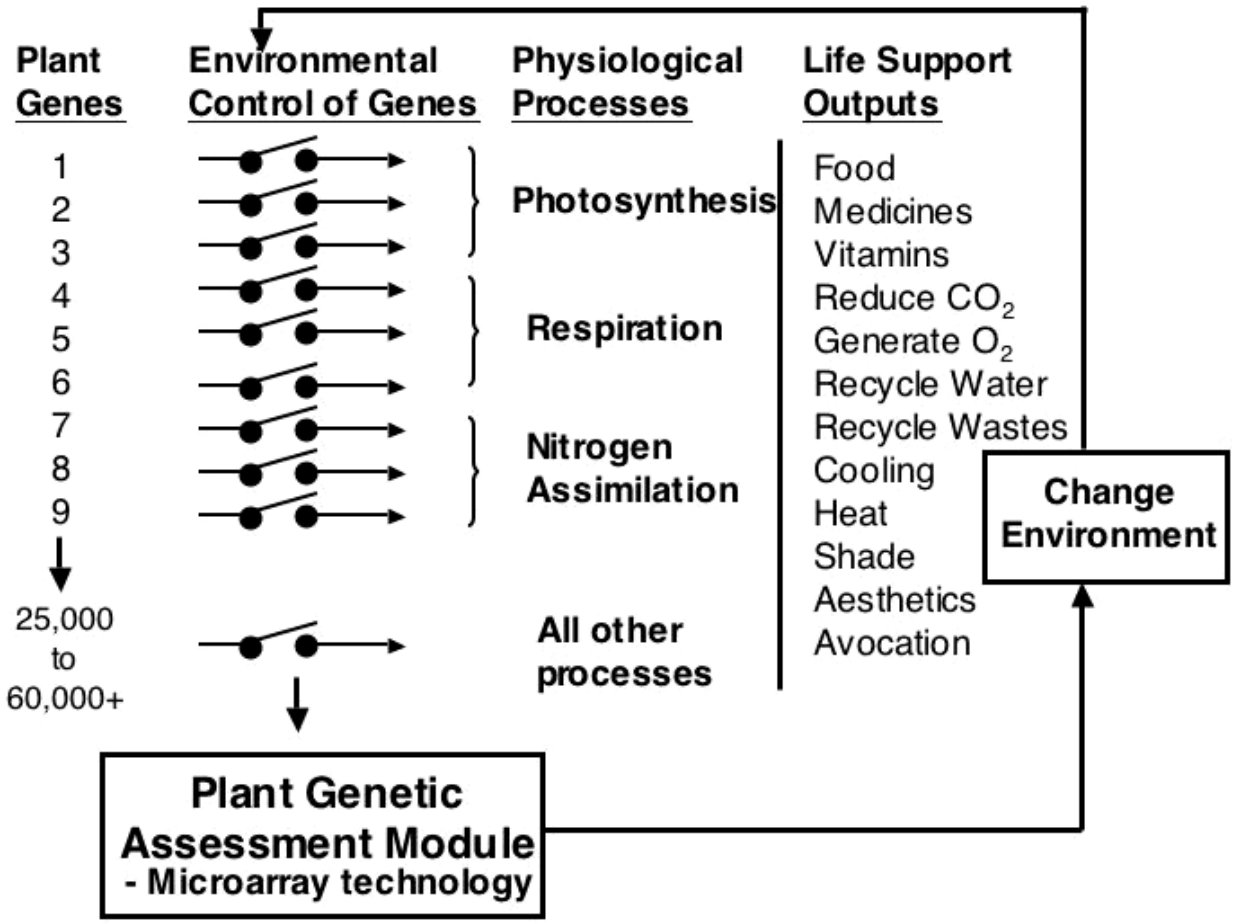

Figure 3. The Plant Genetic Assessment and Control System. *This approach uses microarray analysis of the activity of thousands of genes simultaneously to fine-tune the environment and to guide gene activation and deactivation, as needed to optimize life support systems.*

Microarray Hybridization and Analysis

Microarray analysis has the capacity to indicate which genes in plants are active or inactive, which genes are being up-regulated or down-regulated, and which genes are mutated and no longer function properly. Two basic types of microarray platforms are currently in use. One method utilizes pre-fabricated gene chips that contain all of the known genes within a sequenced genome. Currently, the only commercially available total genome array for plants is produced by Affymetrix using *Arabidopsis* (www.affymetrix.com). Additional gene chips are under construction and will represent rice, cotton, and tomato genomes. The second platform utilizes spotted arrays that can represent either an entire genome or any subset of genes within the genome. Spotted arrays enable additional flexibility by customizing studies for tissue- or organ-specific gene expression, gene members of identified pathways or networks, or subsets of differentially expressed genes previously identified by genome analysis.

At this time, all hybridization and wash steps of microarrays are performed by hand. Chips are washed following a standard post-hybridization format, i.e. low stringency washes followed by high stringency washes, each performed at hybridization temperatures. Washed microarray chips are then analyzed by a multi-laser scanner, which identifies and records the amount of fluorescence, if any, and its intensity for each probe on the array. These raw data are saved and available for further analysis and data mining to identify genes of interest.

Data Analysis and Gene Networks

One single microarray experiment can reveal the expression level of hundreds or thousands of genes. Several data analysis tools and software packages are currently available to facilitate gene expression profiling of the generated data. However, within each of these systems are various algorithms, statistical analyses, data

filters, and sorting criteria. Standardization between analysis techniques and data interpretation is now a recognized issue within the scientific community. Several peer-reviewed journals have adopted the Minimum Information About Microarray Experiments (MIAME) standards (Brazma *et al.*, 2001), in an attempt to outline the minimum information required to unambiguously interpret and potentially reproduce and verify an array-based gene expression experiment. This is an important step toward generating a standard format for data exchange. Additional information needed includes agreement on what data and annotations should be provided, development of standard vocabularies and ontologies for describing microarray experiments and samples, and developing standard protocols, controls, and data normalization methods.

Beyond initial data interpretation and gene expression profiling rests the very basic question: "What does this information mean?" For the PGACS, it will be necessary to determine how differences in gene expression profiles relate to the overall physiological state of an individual plant with respect to its health, its response to environmental stimuli or stress, and, more importantly, its ability to function at its maximal capacity. Post-genomic biology requires the interpretation of vast amounts of information to discern the patterns that underlie the biological design within each organism.

Understanding genomic information is based largely on what is already known. However, this base of knowledge lies buried within decades of published research articles that describe genetic function and biochemical activity. Methods to structure existing information into an accessible format are needed to advance the utility of gene expression profiling and to determine gene pathways and networks as they relate to gene function and plant physiology. Specifically, each scientific vernacular used to describe individual or related genes, phenotypes, or physiological conditions must be translated into a common language that can be used to deduce meaning within a biological context. The result will be the generation of a virtual plant.

Plant Physiology and Environmental Control

Elucidation of related networks and systems interconnected by specific gene expression patterns will further the scientific community's understanding of and appreciation for the underlying biological complexity inherent within living organisms. More importantly, understanding the genetic basis for biological complexity will enable correlation between plant health and performance and specific indicator gene products that represent defined processes within each plant. For example, altered gene expression in one or two specific gene products could indicate viral infection or lack of necessary nutrient.

In depth comprehension of plant physiological mechanisms and their relationship to gene expression will permit control of plants at the gene and/or environmental level to provide for specific needs. For example, in some situations, food production may be a priority. If so, environmental conditions could be adjusted to turn on genes that optimize production of proteins, sugars, complex carbohydrates, and fats needed for food. In other situations, reducing CO_2 concentrations and increasing O_2 concentrations may be a priority. In that case, environmental changes can be implemented to activate the genes necessary for optimizing photosynthesis. Alternatively, different plant species or variants bred or genetically altered to thrive under specific conditions, or, for example to produce specific pharmaceuticals, may be introduced into the biosystem to optimize life support functions in response to plant genetic response information.

THE PLANT GENETIC ASSESSMENT AND CONTROL SYSTEM

Figure 4 illustrates how integration of the PGAC system into NASA's space mission design will require a fully automated procedure for the complete process including plant tissue acquisition, sample preparation, hybridization and raw data accumulation, data normalization and analysis, and interpretation as related to whole plant physiology. Below, we present some current and enabling technologies to be considered for further investigation as components within a Plant Genetic Assessment and Control module.

Automation and Robotics

Automation and robotics will play important roles in the PGAC system for several reasons. First, we anticipate that the people using the module will not be bench-trained scientists. To optimize results, they will have to rely on an automated system to process plant tissue into a labeled sample. This is not a trivial procedure and can result in misleading results even when done on the ground. In a space environment where crew safety might depend on accurate measurements, a system that removes human error could have great utility. Second, we cannot be sure that all plant-based life support components will be accessible to the crew. It is likely that for maximum growth, plants will be kept in a separate growth chamber under environmental conditions not suitable for humans. In that case, the crew will have to rely on robotic systems to visually monitor the plants, measure conditions within the chamber, collect samples for the PGACS, and possibly even harvest food as it becomes available.

Scientists at the University of Central Florida and the Kennedy Space Center in Florida have designed the Advanced Life Support Automated Remote Manipulator (ALSARM), a three-degree-of-freedom robotic system that can operate automatically via a personal computer (http://www.nasatech.com/Briefs/Sep01/KSC12084.html). This system contains sensors to measure light intensity, air temperature, infrared temperature, relative humidity, and airflow. It also contains a horizontal, telescoping arm through which sensor cables are routed.

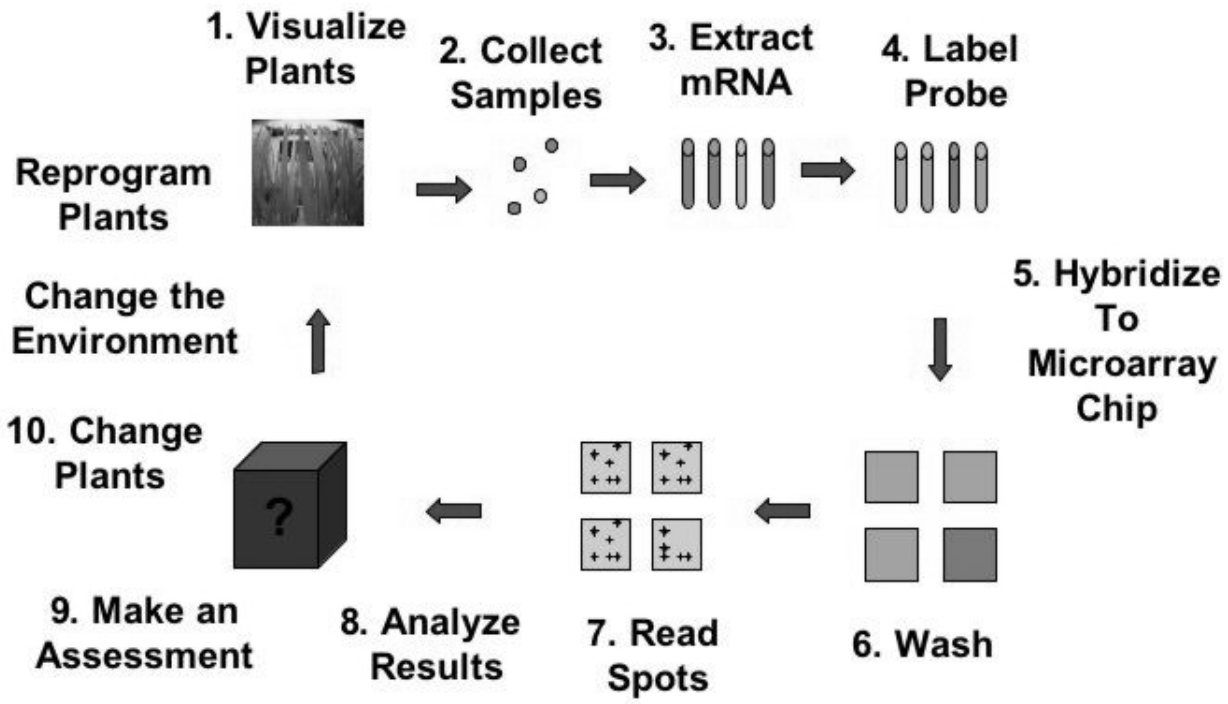

Figure 4. Steps Necessary to Evaluate Plant Gene Expression Profiles. *Automated probe labeling, hybridization, washing, and signal reading are currently possible. Important technology development will include automation of sample collection and RNA extraction, as well as the bioinformation tools necessary to correlate gene expression changes with physiological changes. Once the genetic assessment of physiological status is made, the system can be adjusted by either changing the plants or changing the environments, or potentially even reprogramming the plants.*

The Robot Systems Technology Branch at NASA's Johnson Space Center, in a collaborative effort with the Defense Advanced Research Projects Agency (DARPA), has developed a humanoid robot system, aptly named Robonaut (http://vesuvius.jsc.nasa.gov/er_er/html/robonaut/Robonaut1.htm). Its purpose is to specifically address the manipulation limitations encountered by a suited astronaut. Robonaut is an anthropomorphic robot, the size of an astronaut in a space suit, and is configured with two arms, two five-fingered hands, a head, and a torso. Its dexterous pair of arms enables dual-arm operations and its hands that can interact directly with a wide range of interfaces without special tooling. This design concept advances state of the art anthropomorphic robotic systems by including multiple use tool handling and modular robotic systems components. The control system for Robonaut includes an onboard, real time CPU with miniature data acquisition and power management. Off-board guidance uses a telepresence (remote) control station with human tracking. Further development of these or similar robotic systems can be utilized for plant sample collection and environmental monitoring.

The Wisconsin Center for Space Automation and Robotics (WCSAR http://wcsar.engr.wisc.edu/index.html) conducts experiments with plants in space and develops automation technologies. Robotics under development include a prototype being developed for Johnson Space Center's bio-mass production system that would be able to collect tissue samples, harvest crops, monitor environmental parameters (temperature, CO_2, humidity, etc), and take video images from anywhere within the chamber. Scientists at WCSAR are also collaborating with a company that makes microarrays to develop a robot that can deliver nanoliter amounts of liquids under controlled environments with the degree of precision necessary for making custom arrays. Robotic systems like these are likely to be integral parts of future applications such as the PGACS.

Microfluidic Wet Chemistry

Once collected, the tissue sample is taken through several stages of wet chemistry that starts with RNA isolation and ends with raw data acquisition from a microarray experiment. As detailed above, this process is labor intensive, time-consuming, and, at present, requires several system components that are not suitable for zero- or low-gravity environments. A novel solution to this dilemma developed by Gyros AB of Uppsala, Sweden, utilizes miniaturization and integration of multiple procedures into a single, streamlined, self-contained,

spinning microlaboratory located on a 12-centimeter diameter compact disc (http://www.gyros.com/). This microfluidic platform facilitates sample preparation and enables parallel processing of nanoliter-scale samples through a series of concentrically-arranged microchambers. Each microfabricated operational unit performs an individual step required for the application. A precision robot transfers samples and reagents from microplates or containers to the compact disc (CD) microlaboratory and liquids enter as a result of capillary action. Microchannels and controlled spinning of the CD, to create centrifugal force, ensure that liquids move at the required flow rate through each step of the application. Hydrophobic breaks prevent liquid from moving further into the microstructure until a higher g-force is applied. In essence, the CD microlaboratory represents discrete chambers designed to perform specific tasks within a multi-step protocol.

Once programmed protocols are complete, an increased spin rate of the CD forces the product into the next microstructure for the subsequent step. Following this type of design, one can envision a wet chemistry microlaboratory where the innermost ring of microchambers contains the necessary reagents for alkaline lysis of the tissue. Increased spin of the CD forces the lysate into the next concentric ring of chambers where the nucleic acid fraction is separated by a microresin column. Elution of the RNA fraction leads into the next series of microchambers where primers, nucleotides, reagents, and enzymes are present to reverse transcribe the mRNA. Each step of the procedure can be discretely contained within each series of chambers, with products from the preceding steps automatically being directed into the next experimental chamber where they will serve as template for the next reaction. The use of centrifugal force solves one of the major problems with carrying out wet chemistry in microgravity conditions and the technology for spinning CDs is, of course, already well perfected.

Spot Arrays

Significant advances are being demonstrated for spotted array technology. Smaller pin sizes, greater uniformity in spot diameter, and precision robotics are enabling the construction of greater oligonucleotide probe sets into smaller spaces on each physical support. Similar technologies will thus enable microarrays to be prefabricated onto the CD microlaboratory support. Labeled samples would flow through the microfluidic channel and hybridize against the spotted array. Signal detectors can be easily built from existing technologies already designed to read the information and save the raw data

Data Analysis and Modeling

Raw data sets have little intrinsic value until meaningful information can be extracted from them and presented in an informative way to generate knowledge. Many software packages are currently available to perform these tasks, however, no single standard has been established within this field and different algorithms and statistical methods are used to interpret microarray data. One attempt to standardize the analysis methodology has been presented by the statistical analysis company, SAS® (http://www.sas.com/industry/pharma/mas.html). The ability to consistently analyze, access and share microarray data, regardless of research methods, data types or platforms used, is integral to establishing a coherent and integrated knowledge base.

After data analysis, integration of the information as it relates to gene pathways or networks and whole plant physiology is the most crucial challenge facing post-genomic biology. Making sense of complex biological systems requires a common language, understanding of its meaning, and methods for organizing the complex datasets. Ingenuity Systems (http://www.ingenuity.com/) has developed an ontology-based approach that systematically encodes and structures information in a consistent, object-based model. Ingenuity's ontology is the primary organizing principle for structuring the biological knowledge and is presented in three layers of increasing complexity. The first layer is a controlled vocabulary where words and phrases are represented as defined objects within domains. The second layer explicitly classifies the interrelationships between objects while the third layer identifies the complex experimental biological relationships. Use of this ontology generates a standardized language and system to structure biological knowledge that integrates molecular, cellular, and organismal processes. The structured approach reduces the complexity inherent within the biological system and allows for computational analysis to demonstrate relationships between each unit.

For physiological processes that are under genetic control, the final results of these ongoing efforts will allow scientists to relate measurable gene function with changes in an organism's behavior. For example, increased expression of genes in the nitrogen metabolism pathway will be the plant's signal for stresses induced by atmospheric changes. Furthermore, once the gene pathways are well understood, altering gene expression profiles will allow for control and optimization of the organism's physiology.

SUMMARY

The general goal of our project is to advance the concepts necessary to use plants to their fullest advantage while supporting life in space. In some cases, plants will supply food for humans and other animals. In other cases, plants will recycle water, gases, and nutrients needed to sustain humans and all other life from earth. The physiological processes of plants that are needed to sustain life in space include photosynthesis, respiration, transpiration, and nutrient use. Establishing links between plant gene expression and environmental change involves new technologies in engineering and biology, and is essential for controlling plant physiology and growth in space.

Taking fullest advantage of plants in space will require monitoring plant genes collected from living plant tissues, using advancing technologies to determine which genes are being expressed, and altering environmental conditions in space to activate genes needed to induce desired changes in genetic expression and plant metabolism. Such changes in gene expression and metabolism will enable those in space to alter the physiological processes of plants to optimize the production of food and to provide other services. In addition, development of the microarray and bioinformatics technologies will have many applications in monitoring and maintaining human health in space environments.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge NIAC Phase II funding as well as the willingness of the following industry contacts to share information: Dr. Raymond Cho (Ingenuity Systems, Inc., Mountain View, CA), Dr. Sue Flood (SAS Institute, Inc., Cary, NC), Dr. Per Andersson (Gyros AB, Uppsala, Sweden), and Dr. Weijia Zhou (Wisconsin Center for Space Automation and Robotics, Madison, WI).

REFERENCES

Brazma A., Hingamp P., Quackenbush J., Sherlock G., Spellman P., *et al.*2001. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nature Genetics* 29:365–371.

Goff S.A., Ricke D., Lan T.H., Presting G., Wang R., *et al.*2002. A draft sequence of the rice genome (*Oryza sativa L*. ssp. japonica). *Science* 296:92–100.

Henninger, D. 2002. Advanced life support: Environmental sentinals 2002. (http://advtech.jsc.nasa.gov/EnviroSent2002/henninger/main.htm).

Musgrave, M. E., Kuang, A., Xiao, Y., Stout, S. C., Bingham, G.E., Briarty, L. G., Levinskikh, M. A., Sychev, V. N. and Podolski, I. G. 2000. Gravity-independence of seed-to-seed cycling in Brassica rapa. *Planta* 210(3): 400–406.

The *Arabidopsis* Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–815.

Yu J., Hu S., Wang J., Wong G.K., Li S., *et al.*2002. A draft sequence of the rice genome (*Oryza sativa L.* ssp. indica). *Science* 29:79–92.