

**PLANT ADAPTATION TO LOW ATMOSPHERIC PRESSURES:
POTENTIAL MOLECULAR RESPONSES**

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(Abstract)

There is an increasing realization that it may be impossible to attain earth normal atmospheric pressures in orbital, lunar or Martian greenhouses; simply because the construction materials do not exist to meet the extraordinary constraints imposed by balancing high engineering requirements against high lift costs. This equation essentially dictates that NASA have in place the capability to grow plants at reduced atmospheric pressure. Yet current understanding of plant growth at low pressures is limited to just a few experiments and relatively rudimentary assessments of plant vigor and growth.

The tools now exist, however, to make rapid progress toward understanding the fundamental nature of plant responses and adaptations to low pressures, and to develop strategies for mitigating detrimental effects by engineering the growth conditions or by engineering the plants themselves. The genomes of rice and the model plant *Arabidopsis thaliana* have recently been sequenced in their entirety, and public sector and commercial DNA chips are coming available such that thousands of genes can be assayed at once. A fundamental understanding of plant responses and adaptation to low pressures can now be approached and translated into procedures and engineering considerations to enhance plant growth at low atmospheric pressures. In anticipation of such studies, we present here the background arguments supporting these contentions, as well as informed speculation about the kinds of molecular physiological responses that might be expected of plants in low-pressure environments.

(Key Words)

Low Pressure	Molecular Responses	Mars	Plant Growth
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While the molecular responses of plants to low atmospheric pressures are currently unknown, information on responses to other environmental extremes allows reasonable inferences about what those responses might be, and suggests potential impacts on the design and implementation of low pressure extraterrestrial growing systems.

Introduction

The growth of plants in space remains a priority concern for the development of NASA strategic plans, especially for long term habitation of space and other contained environments such as would be found in early Martian bases or colonies. Plants would be an integral part of long-term bioregenerative life support systems and would impact any future planetary ecosynthesis efforts. However, the effective use of plants depends on the intrinsic ability of the plants to prosper within their environment, and this environment can be especially limiting in space flight and Martian applications.

The basis of low pressure constraints

Mass is a critical consideration for all space flight efforts. This consideration is especially relevant for human missions to Mars since mass is a dominant driver in designing propulsion systems and launch vehicles (25). The term “equivalent system mass (ESM)” has been used by NASA as a key metric by which various life-support systems can be compared (17). Conversions of ESM based on spacecraft and mission architectures are used to calculate equivalent masses for commodities such as propulsion, electrical power, thermal rejection, spacecraft volume, and human life-support components. For example, in a bioregenerative advanced life-support (ALS) strategy, if 25% of food is produced on board, then condensing the water transpired through the plant growth system can regenerate all of the required mission water (17), thereby significantly reducing the ESM. Any reductions in ESM by changing the design of a subsystem will effectively increase the permissible mass of other spacecraft or payload components. Therefore, the use of low-pressure plant growth modules is one potential way in which the ESM of the bioregenerative ALS systems can be reduced for surface missions to Mars.

Historically, low-pressure environments have been utilized throughout the USA human space exploration programs. Lower atmospheric pressures impose lower forces on structural components, thereby allowing reductions in the mass of structural and consumable components of space vehicles. Such reductions have resulted in increased mission lengths and/or increased masses of launched payloads. For example, the Mercury, Gemini, and Apollo environments were designed to operate at 34 kPa with a pure oxygen environment in order to simplify the

engineering challenges of supporting humans in space and to decrease the launched masses of these spacecraft (7,41). In contrast, the Shuttle and Space Station Alpha are generally operated at Earth-normal pressures near 101 kPa, but are lowered to 70 kPa for EVAs (62,63). Although the precise internal pressures for surface missions to Mars have not been established, it seems reasonable to expect that reduced-pressure atmospheres will likely be utilized in order to decrease structural components and atmospheric consumables. It is also reasonable to expect that human-rated modules will utilize pressures and gas compositions that are different from plant growth ALS modules. Since humans cannot effectively function below pressures of 30 to 40 kPa even in pure oxygen environments (1,2,54), while plants have been shown to be able to live at pressures as low as 5 to 10 kPa (7,41), it might well be desirable to grow plants on orbit, on the moon, or on Mars under much lower pressures than are possible for human activities.

Reduced pressure environments may also permit the use of ALS systems that otherwise would not be possible under higher atmospheric pressures. For example, estimates indicate that a transparent greenhouse structure using currently available materials on Mars could be constructed if the internal pressure of the greenhouse could be maintained below 7.5 kPa (9) (See Figure 1). If the pressures in the inflatable greenhouse structure on Mars were any higher, the structure would have to be reinforced or constructed of opaque materials. If heavily reinforced or opaque materials were used for construction, then, the ESM of the system would go up due to higher mass of the structure, higher gas consumables for more concentrated atmospheres, and higher launch and landing costs. In addition, supplemental lighting systems for plant growth would have to be added to the ALS modules because the greenhouse walls would no longer be transparent to solar irradiation. In short, the ESM of the system rises to the point of impracticality.

Plants in low pressure

Several early studies indicated that plants tolerate low atmospheric pressures quite well, depending upon the experimental set up (40), and enhancements in growth, productivity and fruit storage have been reported (3,12,13,19,20,38,45,49). However, current views of plant productivity in stressful terrestrial environments suggests that plants likely must undergo a dramatic response and adaptation process in order to survive in low atmospheric pressures. For example, low atmospheric pressures would certainly reduce oxygen availability, and plants can

survive hypoxia only if they adapt and respond. In addition to presenting incipient hypoxia, low-pressure environments place extreme demands on the transpiration of water (22,27,47), which suggests that responses similar to dehydration could occur. And with the reduction of conductive and convective cooling at low atmospheric pressures, it is possible that heat stress could occur due to lack of typical temperature control mechanisms. So even though there is no fundamental understanding of the stress responses induced by low-pressure environments per se, there exists a great deal of understanding relative to plant growth under stressful terrestrial environments. An examination of those stress responses provides key insights and expectations for plant response and adaptation to low pressures.

Plant responses to environmental stress

Plants respond to abiotic stress by both avoidance and adaptive strategies. Avoidance refers to long-term evolutionary modifications in the architecture or physiology of the organism, such as the development of sunken stomates, leaf hairs, thick cuticles and long taproots in plants growing in arid environments. Adaptive mechanisms are, however, fundamental processes that are induced by a particular environmental stress and usually involve the transcriptional activation of a subset of specialized, stress response genes. These genes encode protective proteins, enzymes that synthesize protective compounds, molecular chaperones that refold denatured proteins, or enzymes for pathways that allow alternative metabolic processes. Plants have evolved multiple adaptive stress response pathways to cope with extremes in environments such as excessive heat or cold, flooding, drought and desiccation. Although there is, as yet, no known pathway(s) specialized for low atmospheric pressures, it is expected that multiple existing stress responses may be called upon to adapt to hypobaria. For example, the low partial pressures of oxygen and reduced water vapor pressures of hypobaric atmospheres may simultaneously activate pathways originally evolved to protect against flooding and drought. It is also conceivable that plants severely stressed by low-pressure environments may accumulate denatured proteins or suffer heating of leaf surfaces by light, since rates of heat dissipation would be greatly reduced by the low pressures, and thereby activate the heat shock response.

The following examples outline well characterized stress responses that may provide metabolic pathways from which a hypobaric response might draw for adaptive strategies. In all of these examples, several relevant themes recur. The major adaptation to environmental stress is the

induction of the genes that encode specific adaptive proteins. The roles of the adaptive proteins are to mitigate or circumvent the difficulties inherent in the environmental stress. The adaptive genes are co-regulated by hierarchical transcription factor families. Progress toward engineering increased tolerance to environmental conditions has been made through understanding and manipulation of these adaptive response themes.

Hypoxia

The hypoxic stress response is a fundamental example of an adaptive plant response to a hostile environment, and the induction of plant genes by hypoxia has served as one of the major paradigms for understanding gene expression in response to environmental stresses. Higher plants typically circumvent flood-induced hypoxia by altering structural morphology to maximize the use of any available oxygen, but the initial adaptations to hypoxia include the activation of alternate metabolic pathways that can function when oxygen is limiting.

The morphological changes that develop in response to hypoxia predominate in the roots and include outgrowths of new, adventitious roots and the formation of aerenchyma tissue, which function to increase the available oxygen to the hypoxic regions of the plant. These processes are mediated through plant growth regulators such as ethylene, and ethylene itself can induce certain genes typically associated with the hypoxic stress response.

The initial adaptive response to hypoxia is the cessation of the expression of most of the genes expressed under normoxic conditions, followed by a programmed induction of genes that allow fermentative metabolism and the development of the avoidance morphology. For many plants, this means enhanced expression of genes involved in glycolysis to maintain carbon flow and energy production as the products are diverted through pyruvate decarboxylase and alcohol dehydrogenase (ADH). For some plants there is a period during the initial adaptation to hypoxia in which lactate dehydrogenase (LDH) is activated, offering yet another additional metabolic pathway for glycolytic products (4,16,51,60).

Dissecting the regulation of genes induced by hypoxia, such as Adh, has been of fundamental importance in understanding the process by which environmental signals are converted into specific adaptive gene response mechanisms (6,11,26,39,42-44,51,53,64). Intense characterization of the Adh gene has led to the discovery that Adh, like many of the other genes

involved in environmental stress responses, responds to other stress signals in addition to hypoxia. The Adh gene also responds to cold, salt, glucose, and abscisic acid (ABA) (18,23,24,32,35). This illustrates that many environmental stress-response-signaling pathways can intersect one another and that the process of mounting an adaptive response is both an orchestrated activation of specific pathways and the collateral activation of related pathways.

Heat Shock

The heat shock (HS) response is a well-established phenomenon that occurs in all organisms that are subjected to a sudden elevation of temperature or certain other forms of physiological stress (8,46,52). HS manifests itself by changes in the normal pattern of protein and mRNA synthesis; heat shock proteins (HSPs) are rapidly induced in order to protect cells from the deleterious effects of stress, in many cases by acting as molecular chaperones (61). The induction of HS mRNAs occurs very rapidly, as seen in soybean where normally silent HS genes are transcribed within three to seven minutes after application of the heat stress (34). After about two hours, the HSPs can represent over 50% of the total cellular protein synthesis. Thus, the HS response, like the hypoxia response, is a fundamental example of the activation of specific stress response genes as a means for overcoming environmental stress.

The HS genes provide a simple model for transcriptional activation under environmental stress conditions. Their rapid and robust induction is mediated by heat shock transcription factors (HSFs) that preexist in uninduced cells. In general, heat-inducible transcriptional activation is a stepwise process and is envisioned as a sequential unfolding or unmasking of functional domains within the HSF protein itself. It seems fitting that the regulation of the heat shock response is mediated by an unfolding of HSF, since a sudden elevation in temperature results in increased unfolding of cellular proteins in general. HSPs counter this process by binding to partially denatured proteins to prevent irreversible aggregation, acting as molecular chaperones to promote proper folding of denatured proteins and nascent peptides. As the free pool of HSPs is depleted by their interaction with nonnative proteins, fewer HSPs are available for interaction with HSF to maintain its folded and inactive state, thus releasing HSF to induce HS genes.

It is known that offering plants an elevated but sub-shock temperature leads to the acquisition of thermotolerance. By extension, engineering plants for over-expression of HSPs can also result in greatly enhanced survival of seedlings to severe heat stress (48). In addition, since the HSF

transcription factors control the expression of the HSPs, engineering the expression of the HSFs offers a means to coordinately regulate and manipulate the entire heat shock response, and in many cases simply increasing the expression of HSFs leads to thermotolerance.

Cold and Drought Stress

The acclimation to freezing injury is another response that involves changes in gene expression, and was first documented in spinach (31). The response can be seen within 12 to 24 hr of exposure to cold temperatures, with some genes transiently expressed and others expressed throughout the entire period of low temperature acclimation (14,36,37,58). Low temperature-induction differs, however, from the heat shock and hypoxia responses in that there is little attenuation of normal gene expression (29,30,37).

Another difference from heat shock is the lack of strict conservation of structure or apparent biochemical function among the genes induced by low temperature in different plants. However, many of the Cold Responsive (COR) proteins contain lysine rich motifs that are repeated in many of the ABA-responsive (RAB) proteins, Late Embryogenesis Associated (LEA) proteins, and desiccation induced (DHN) proteins. The COR and the RAB/LEA/DHN proteins are all soluble to boiling in aqueous solutions and are very hydrophilic (57). It has been proposed that some of these gene products function by stabilizing the inner membrane of the chloroplast by increasing the intrinsic curvature of the membrane, thereby decreasing the formation of hexagonal II phase lipid layers (5,55). Many of the COR proteins are also induced by ABA or drought conditions (as in the DHN family), or are abundant during late embryogenesis (as in the LEA proteins) (21).

In addition to the accumulation of specific COR proteins, cold acclimation is accompanied by the regulation of metabolic pathways regulating proline and sugar synthesis. Genetic evidence suggests that the *eskimo1* gene may encode a negative regulator of both proline and soluble sugar synthesis (65), while the *HOS1* gene seems to possess both negative and positive regulatory potential (32,66). More is known regarding the so-called CBF/DREB1 pathway, which is regulated by the CBF/DREB1 family of transcriptional activators. Over expression of CBF1, for example, results in the expression of the arabidopsis COR proteins and provides transgenic plants some degree of freezing protection (33). Over-expression of CBF3, another member of the CBF family, better mimics cold acclimation by inducing synthesis of the COR

proteins in addition to raising levels of proline and total soluble sugars (28). Although CBF3 over-expressing arabidopsis plants showed a marked increase in freezing tolerance, the plants exhibited a dwarf phenotype and were late in flowering.

The response of plants to dehydration shows considerable overlap and similarity that evoked by cold acclimation (10). This is not surprising since much freezing injury is due to the dehydration effects of ice on the cytosol. The types of genes induced by dehydration stress can be grouped into three general classes. The LEA-related proteins, which are expressed in vegetative tissues, compose the first class. The enzymes involved in osmolyte accumulation, protection, or signal transduction, together with non-enzymes such as ubiquitin, heat shock proteins, Kunitz trypsin inhibitors form the second class. Finally, there are the intrinsic proteins that form transmembrane channels (10). Although dehydration induced enzymes are largely independent of ABA induction, approximately 20% of the LEA-related proteins and 80% of the non-enzyme proteins are induced by ABA. Thus, a complicated but interrelated set of responses is involved in cold and drought adaptation.

Conclusions; Metabolic engineering to meet hypobaric challenges

It is most likely that plants adapt to low-pressure environments through the use stress responses that have evolved for terrestrial stresses, and reasonable inferences can be made as to which stress responses would be activated. In light of these inferences, what are the possibilities for enhancing plant growth at reduced atmospheric pressures?

Recent strides forward in understanding plant responses to environmental stresses demonstrate that metabolic engineering can directly address stress response capabilities (50,56,59,67). The historical foundation for the metabolic engineering of enhanced stress responses is based in the observations that a pre-acclimation to stressful environments can impart a degree of tolerance for that stress (15,31,34,46). Hypoxia, heat shock, cold and desiccation, all show evidence of this effect. Thus a major strategy for creating plants that are “pre-acclimated” for a particular stress is to engineer them to constitutively express specific genes that play a role in the adaptive metabolism that will be initiated by exposure to that environment (5,28,33). The engineering can take the form of introducing a gene whose product participates in the adaptive response, or it can encompass the introduction of a gene that encodes one or more transcription factors that play a role in promoting a suite of genes involved in an adaptive pathway. Both approaches

require that the genes in the metabolic processes be identified and characterized. This argues that the first step in engineering plants that are enhanced for hypobaric growth is the identification of key genes involved in the hypobaric adaptive response mechanisms.

In addition to guiding the engineering of plants for enhanced growth at low pressures, data on hypobaric gene expression patterns would also be directly useful in the design of Martian ALS systems. If the hypoxia response is activated by low pressure in a simple and direct fashion, then the lower limits of total pressure might have to be constrained, or supplemental oxygen could be used to mitigate hypoxia while maintaining low pressures. If the drought response is activated due to low actual or perceived humidity, innovative water supply and recovery systems may be necessary. However, efficacy of each of these ALS engineering alterations can and should be measured by directly monitoring the effects of the change on gene expression patterns.

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Biographical Sketches

Robert J. Ferl has a BA in biology from Hiram College and a PhD in biology from Indiana University. He is a Professor in the Horticultural Sciences Department at the University of Florida and is also and Assistant Director of the Biotechnology Program at the University of Florida. His long-standing research interests involve the signal events that regulate plant gene expression, particularly in response to adverse environments. He has used the Adh/GUS reporter gene technology in spaceflight experiments designed to understand plant responses to low earth orbit.

Andrew C. Schuerger received his BS and MS degrees from the University of Arizona and his Ph.D. from the University of Florida studying plant pathology. His dissertation included research on the effects of temperature and pH on spore attachment of the fungal pathogen, *Fusarium solani* f. sp. *phaseoli*, to roots of mung bean plants grown in hydroponic systems. Dr. Schuerger worked for 16 years at The Land (a hydroponic research and education facility) at

Epcot Center, Florida developing disease management programs for viral, bacterial, fungal, and nematode disease of vegetable and agronomic crops. His research interests have closely paralleled NASA's ALS (Advanced Life Support) program in which he has published many articles on plant-pathogen interactions in semi-closed plant growing systems. More recently, Dr. Schuerger has joined the Dynamac Corporation (a NASA contractor at KSC specializing in environmental and life sciences) to pursue research into the remote sensing of plant stress using fluorescence technologies. Furthermore, he has received two NASA research (NRA) grants for FY2000 to study the survival of terrestrial microorganisms and the growth of plants under simulated Martian conditions. Dr. Schuerger is currently located at the Kennedy Space Center, FL.

Anna-Lisa Paul has BA and MS degrees in botany and plant physiology from the department of Biology, University of South Florida, and a PhD in molecular genetics from the department of Botany at the University of Florida. She is currently an Assistant Scientist in the Horticultural Sciences Department at the University of Florida. Her continuing research focus has been in the investigation of chromatin structure and gene regulation, with a recent emphasis on higher-order chromatin and genome organization. Current research includes analyses of spaceflight associated stress responses in arabidopsis using the Adh/GUS transgenes.

William B. Gurley has a BS in biology and an MS in botany from North Carolina State University at Raleigh. He received his PhD in botany from the University of Georgia in the area of DNA reassociation kinetics. Postdoctoral studies were conducted in the Plant Pathology Department at the University of Wisconsin. He is currently a professor in the Department of Microbiology and Cell Science at the University of Florida and is studying basic mechanisms of activated transcription in plants by manipulating general transcription factors and those that regulate the heat stress response.

Kenneth Corey Ken Corey, former University of Mass/Amherst professor, received his MS and PhD at North Carolina State University in plant physiology with minors in statistics and soil science. His research has involved the study of physiological processes and responses of a wide range of agronomic and vegetable crops. As a teacher, he has developed and taught numerous courses in plant, soil, and environmental sciences, including a special topics course in advanced life support systems. For the past 11 years, Corey has been involved with advanced life support

systems research for NASA with an emphasis on the use of plants for bioregenerative purposes. Recently, his work has focused on plant responses to rarified atmospheres with applications to the design of atmospheres for extraterrestrial plant growth systems and structures.

Ray Bucklin has BS, MS and PhD degrees in Agricultural Engineering from the University of Southwestern Louisiana, Louisiana State University and the University of Kentucky, respectively. He is a Professor in the Agricultural and Biological Engineering Department at the University of Florida. His research and extension education programs during his 18 years at UF have dealt with agricultural structures and have focused on structural analysis and design of light framed structures and thin walled structural components and on ventilation and cooling systems of greenhouses, shade structures and livestock housing.

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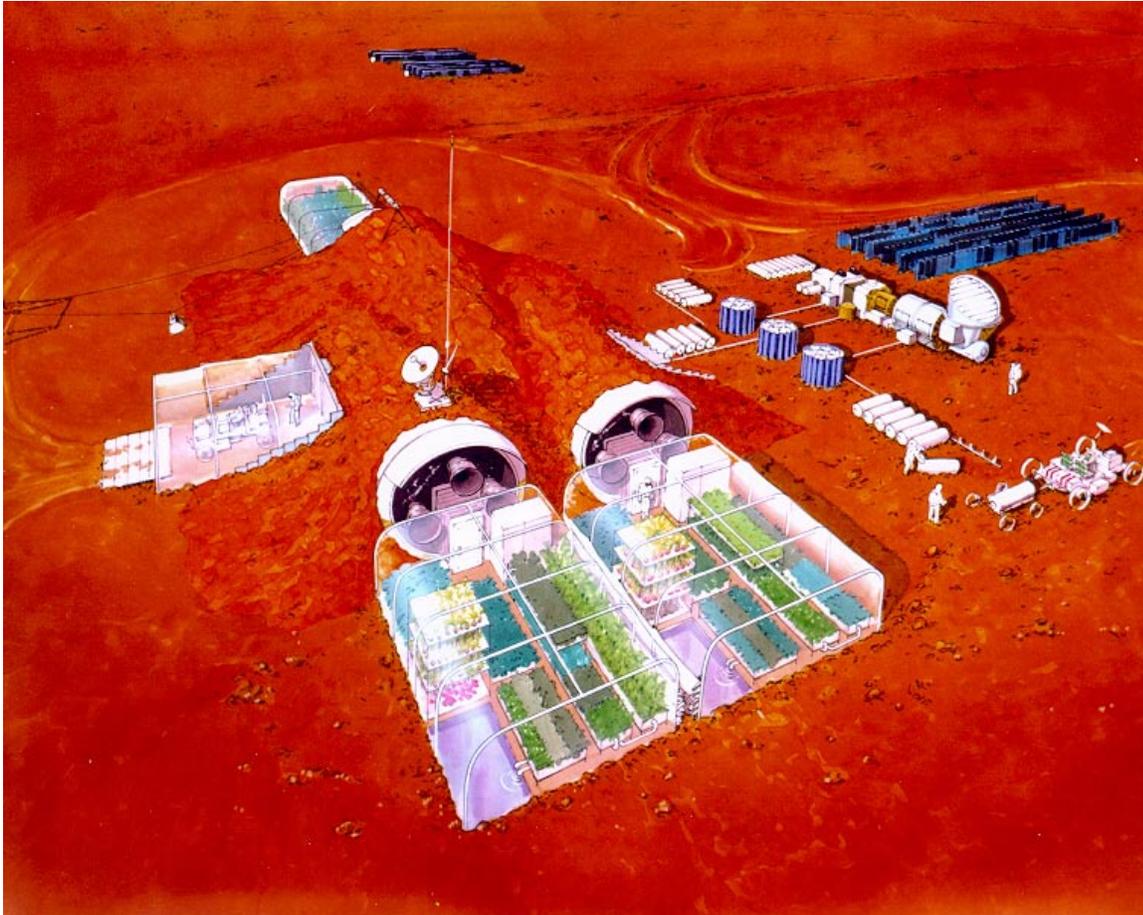


Figure 1. Artist's rendition of a potential Mars base design incorporating transparent, low pressure plant growth areas. The lightweight nature of the transparent materials in designs such as this essentially dictate that Martian plants be adapted to growth in low atmospheric pressures. (Photo courtesy of NASA.)