ABSTRACT

While much progress has been made in designing biospheres for optimal plant growth and long term habitation in space, plant productivity remains a major limiting factor for sustainable bioregenerative life support. To meet these challenges, we propose that plant biologists should work with engineers to redesign plants. For example, the mechanisms that plants engage to sense environmental cues can be replaced or modified to eliminate Earth-based responses no longer needed in space and to add responses that will improve growth in a biosphere. In addition, synthetic approaches can be used to fine tune plant responses to stress by expressing genes from organisms that thrive in extreme environments. Producing extremophilic proteins in crop plants can increase a plant’s tolerance to rapid changes in temperature and improve growth in environments which might otherwise be sensed as stressful. Future efforts to redesign plants provide unlimited promise for developing sustainable bioregenerative life support systems and will lead to new fundamental insights into the regulation of plant growth and development.

INTRODUCTION

Bioregenerative life support is essential for long term space exploration. NASA’s goals, as enumerated in 2007, were to have test modules on Mars by 2035 and to have life support systems that would meet 85% of food and 100% air regeneration for a crew of four by 2050. Ray Wheeler summarized the endeavors of many scientists in the Controlled Ecological Life Support Systems (CELSS) and Advanced Life Support programs (ALS) in the 2009 NASA Roadmap and Strategy for Crop Research for Bioregenerative Life Support Systems (NASA/TM-2009-214768) and in his review (Wheeler, 2010). Based on these efforts and those of the MELISSA (European) and CEEF (Japanese) projects (summarized by Lasseur et al., 2010 and Tako et al., 2010, respectively), there is no doubt that tremendous progress is being made to improve both the biology and engineering essential for closed life support systems on earth. This work is impressive and provides good evidence for the feasibility of biological life support for humans and other animals on Earth; however, the challenges imposed by the environment in space are daunting.

Although engineers can develop biospheres to ameliorate some of the environmental challenges for organisms, and breeders continue to select the best crops to withstand inhosпитable environments that will be encountered in space, more reliable, stress-tolerant plant systems are required. Furthermore, sustained plant growth must be failsafe to provide for the long term needs of remote space stations. It is time to extend our efforts beyond designing biospheres that attempt to mimic Earth-like environments and to redesign the plants so that they can survive during long term spaceflight and in the lunar and planetary habitats that are being built. Ultimately, these plants also must be capable of surviving short durations of biosphere malfunction.

SYNTHETIC APPROACHES FOR REDESIGNING PLANTS

Exactly how might one redesign plants? Space flight physiologists have already given extensive consideration to selecting plants that produce the product desired (food) while purifying air and water and using minimal space and energy. They have predicted the requirements for human life support and have tested these predictions in Earth-based biospheres (Wheeler, 2010; Lasseur et al., 2010; Tako et al., 2010). The first goal for plant scientists should be to redesign these select species to significantly improve their productivity and survival in more extreme habitats.

Technically, with the developments in molecular and synthetic biology, the only limitation for redesigning plants is one’s imagination. Examples of recent advances in synthetic biology using microorganisms to produce complex chemical factories are given by M. J. Prather and others (Prather et al., 2010; Antunes et al. 2009; Yeung et al. 2009). Importantly, these very successes reveal the complexities encountered when a metabolic pathway is altered. In reality, our understanding of the complex regulation of the biology of multi-cellular organisms limits the predictability of the outcomes of synthetic biology. Even in single cell systems, it is difficult to anticipate the results of metabolic engineering.
If controlling the metabolic flux in a single-celled organism is challenging, this complexity will be amplified many-fold in plants (Bowen, et al. 2008; Antunes et al. 2009). Therefore, modification of each crop will require an understanding of species-specific regulatory pathways controlling vegetative and reproductive growth. We are far from reaching this level of fundamental knowledge; however, rather than waiting for predictable outcomes, plant biologists need to begin the journey and empirically test current models and assumptions about plant physiology by using synthetic approaches to redesign plants. These studies will help meet the needs for future space flight and increase our understanding of plant metabolism.

We posit that one can redesign plants to optimize properties which support space flight such as their abilities to purify air and water and provide food and pharmaceuticals for the crew, and furthermore, that these attributes can be expanded to include production of building materials and other bio-based materials to meet the needs of remote communities. While this may seem rather futuristic, it is not at all. There are many organisms on Earth that have desirable attributes that have yet to be fully utilized by humankind. To make progress in this exciting endeavor, plant biologists should use the unique properties of select organisms and take a synthetic approach to generate new organisms. The newly designed organisms can then be evaluated in synergistic partnerships with biosphere engineers.

Significant advances in agriculture have been made by expressing genes from other organisms to develop plants’ resistance to viruses (producing viral coat protein to inhibit virus spread) and to decrease caterpillar predation (producing proteins from Bacillus thuringiensis to inhibit the digestion function of caterpillar larvae) and to generate herbicide resistance (producing bacterial 5-enolpyruvylshikimate-3-phosphate synthase) (Christou et al. 2006; Romeis et al. 2006; Estruch et al. 1997). In general terms, these could be considered synthetic eukaryotic biology in that one has redesigned one organism (plants) with genes from other organisms. These examples demonstrate that if there is a specific, known target, expressing foreign genes can be very effective.

**Potential Targets**

Altering a plant’s tolerance to cosmic radiation, freezing temperatures, high CO₂ and improving growth under low-light are but a few of the potential areas to target for increasing survival during space flight (Paul and Ferl 2002; Paul et al. 2004; Porterfield 2002; Wolverton and Kiss 2009). Ideally, one would like to optimize plant growth for biosphere environments that will have limited light, high CO₂ (if crews are present) or low CO₂ (if no human crew), low pressure, variable, probably low temperatures and high cosmic radiation.

With this in mind, consider some options that might be used to redesign plants for space flight. A major challenge for biosphere engineers is to efficiently generate enough light intensity in the wavelengths that will support robust plant growth and maximum biomass production. One approach to address this challenge would be to increase photosynthetic efficiency. For example, some photosynthetic aquatic organisms and microbes have adapted to efficiently capture light energy in low fluence light in ocean depths or murky ponds (Morgan-Kiss et al. 2006). Genes encoding these light harvesting complexes could, in theory, be expressed and coupled to plant photosynthetic electron transport to enhance light harvesting and photosynthetic efficiency. Specifically, one might imagine redesigning plants in this manner so that they would produce more biomass when grown under artificial light provided by light emitting diodes (LEDs). When the limits of improving light capture and photosynthesis by redesigning plants have been met, another option would be to co-cultivate crops with microorganisms that use waste products in order to generate a carbon source for the plants.

In addition to the fundamental role of light in photosynthesis, we need to consider the effects of light on plant morphology, photomorphogenesis. Plants are sessile organisms, and as such, they have evolved to respond to Earth’s environmental cues in order to optimize their survival; however, these traits may be counter-productive in non-Earth environments (Hasenstein 2009; Paul et al. 2004; Wolverton and Kiss 2009). One could alter or remove plant mechanisms that sense cues that are advantageous on Earth but of no value or are detrimental to growth in space and in so doing enhance helpful attributes and minimize those that might negatively impact plant growth in space. In this manner, plant physiologists could redesign plants to specifically function within a particular bioregenerative life support system.

For example, plants respond to light signals during seed germination, chloroplast development and greening, leaf expansion, and induction of flowering and senescence. More specifically, most plants will grow taller in the shade or light enriched in far-red wavelengths; many plants regulate the transition from vegetative to reproductive state based on the wavelength of light and day-length (the duration of dark-cycle), and of course, plants respond to light in order to maximize the light impinging on the photosynthetic tissue (phototropism and heliotropism). In space environments, the crew would need to control the plant’s photomorphogenic response to maximize biomass production and meet their needs for life support (Massa et al. 2006).

While one option would be to eliminate light sensors such as phytochrome, another option would be to redesign the photosensors so that the plant “senses” that there is light even when there is none. Additionally, one might design the plants with sensors so that the crew could regulate when the plants sensed that there was light. Using a remote
control device, the crew could regulate the timing and intensity of the plant’s response (Chatnia et al. 2009). Recent advances in our understanding of phytochrome biochemistry make these options a possibility. For example, expressing a mammalian gene to inactivate the chromophore of phytochrome, can render the plants insensitive to some of the developmental cues (Warnasooriya and Montgomery 2009). By the same token, we should be able to redesign the chromophore or protein such that it causes the plant to sense that it is receiving red light even in the dark. Thus, rather than eliminating a receptor or a transcription factor, which might have undesirable effects on overall morphology or seed production, altering one family of sensors so that the plant “thinks” there is red light could enable downstream response mechanisms to proceed unimpeded.

**Fine Tuning Responses by Altering Second Messengers**

Receptors, such as phytochrome, “transduce” an external stimulus into a biological response by producing second messengers that amplify the signal and transmit it throughout the system. Although genetically changing plant photoreceptors and other sensors is exciting and holds a lot of potential for redesigning plants for space, altering existing receptors or adding new ones could be considered course tuning of a response. Because each environmental cue initiates a multitude of downstream signaling events, course tuning has a broad impact on growth and development. Another approach is altering key regulatory factors (transcription factors); however, transcription factors have broad effects that would need to be carefully regulated. For example, increasing the transcription of a set of genes may increase tolerance to severe drought stress, but also might divert plant resources under optimal conditions such that growth is compromised. Alternatively, one might want to “fine tune” a response to change a specific metabolic pathway. For example, one might want to increase the production of a subset of pathways that contribute to cold or drought tolerance without diverting major resources from primary metabolism. One approach could be to alter a subset of signaling molecules, second messengers, within selective cells or tissues.

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**Figure 1.** Second messengers produce oscillating signals. A. Oscillations arise from the rapid synthesis and removal of a second messenger. When the receptor (R) is activated, X (the second messenger) increases. X could be a metabolite or an ion. If X is a metabolite, the concentration of X will decrease as it is converted to Y. If X is an ion, it will be transported out of the cell or into cellular compartments to lower the concentration in the cytosol. B. The frequency of the oscillation (indicated by the arrows) conveys information about the type of stimulus. The amplitude conveys information about the intensity of the stimulus. C. Multiple second messengers can be produced in response to a stimulus. The second messengers will amplify and transduce the signal through downstream effectors. The type and number of second messenger/effector interactions will determine the nature and intensity of the response. It is difficult to identify the downstream effectors of the individual second messengers. However, if one second messenger is selectively removed (dashed line), the downstream effectors should not be activated, and downstream events will be revealed. The final response may or may not be affected depending on the roles of the other second messengers.
In response to a stimulus, second messengers are rapidly generated and are rapidly removed. Their metabolism generates an oscillating signal as shown in Figure 1A. The frequency of the signal conveys information about the type of stimulus, and the amplitude reflects the intensity of the stimulus (Figure 1B). In vivo, the frequency of the oscillations may be seconds or less. These rapid signals are difficult to measure, and observed frequencies are often biased by the timing of the sampling. While the concentration of second messengers will vary, if they cannot be removed, they will usually inhibit growth and eventually kill the cell. For second messengers that are cellular metabolites, their rate of synthesis and catabolism control the level of second messenger. For second messengers that are inorganic ions such as calcium, the rate of influx and efflux (removal from the cell or sequestration into intracellular compartments) will define the signal. The rates of both synthesis and removal of the second messenger regulate the frequency and amplitude of the signal.

One approach to fine tune signaling is to constitutively increase the synthesis of a second messenger. This would increase the amplitude of the signal and could potentially decrease the frequency depending on whether the cell could efficiently remove the increased signal. All responses downstream of the selected second messenger should increase; however, not all responses downstream of the receptor would be affected. Increasing a selective second messenger would contribute to our fundamental knowledge by revealing events down stream of the second messenger that also increased. If this subset of responses contributed to a desired phenotype such as increased stress tolerance without affecting other desirable traits, increasing the second messenger could be used to fine tune plant responses. The potential problem with increasing a second messenger is that it could put a constant and increased demand on downstream metabolic pathways and, in all likelihood, would affect plant growth under optimal growth conditions. In effect the plant would “sense” a constant stress.

Alternatively, one could constitutively remove a second messenger so that its concentration never rises above background. This would prevent the initiation of any signaling event downstream of the selective second messenger but would not block the turnover of the receptor. Specifically, as shown in Figure 1C, if only one second messenger is removed, the subset of the downstream signals (effectors) that respond to the increase in the second messenger (in this instance InsP₃) would not respond. Comparing the responses of plants that normally produce an increase in the second messenger (wild type) with those in which the stimulus-induced increase is dampened (redesigned plants), would reveal downstream events normally regulated by the second messenger. Furthermore, events normally repressed by the basal or unstimulated level of second messenger signal will be revealed as they will be derepressed in the redesigned plants. Because of the complexity of intracellular signaling events, it has been difficult to study the myriad of signaling pathways in vivo. Dampening selective second messengers should aid in identifying downstream effectors and contribute new fundamental knowledge about complex signaling pathways.

How would altering second messengers affect plant growth? Specifically, if rapidly removing a second messenger selectively eliminated downstream responses mediated by that second messenger, would the plants die faster because they do not have a signal to induce adaptation to the new environment, or would the plants continue to grow even when confronted with environmental cues that would normally modify growth patterns? In the absence of a second messenger, would compensatory pathways be induced, and would these pathways enhance plant survival during times of stress? Another possibility is that the increased rate of removal of the second messenger would affect the frequency of the signal, and the plant would respond to the increase in flux even though the signal is below a threshold level (Hasenstein 2009; Paul et al. 2004). The outcomes of “fine-tuning” signaling pathways are difficult to predict because of the complexity of the signaling networks.

**Selectively Removing The Inositol(1,4,5)trisphosphate Signal**

Recent experiments using two model systems, Arabidopsis and tomatoes, have shed some light on the potential impacts of dampening signals. The inositol signaling pathway was dampened by rapidly hydrolyzing the second messenger inositol(1,4,5)trisphosphate (InsP₃). For these experiments, InsP₃ was constitutively removed by expressing a human gene encoding InsP 5-ptide. The human InsP 5-ptide enzyme was used because it was more efficient and effective at selectively hydrolyzing InsP₃ than any of the plant InsP ptases (Perera et al. 2002; Perera et al. 2008; Torabinejad and Gillaspy 2006) and because using a human gene reduced the potential for transcriptional regulation.

Arabidopsis plants expressing the human InsP 5-ptide grow normally under optimal growth conditions even though the basal InsP₃ levels are reduced to about 2-5% of wild type (Perera et al. 2006). Importantly, not only was the unstimulated (basal signal) low but also in response to all stimuli tested (gravity, osmotic stress, and salt stress), InsP₃ never rose above the basal, wild type levels (Perera et al. 2006, Perera et al. 2008). The transgenic InSP 5-ptide expressing Arabidopsis plants, were slower to respond (30% decrease) to a reorientation at room temperature. The delay was more pronounced in the cold (60% less bending) (Perera et al. 2006). The delayed response to gravity was evident in both shoots and roots indicating that InsP₃ was a universal signal that contributed to gravitropic bending. The data also indicated InsP₃ had a more dominant function in gravity signaling at cooler temperatures.
Furthermore, fundamental insights into plant signaling were revealed by comparing the response of the transgenic plants to wild type plants. For example, about 30% of the total calcium signal induced by osmotic stress and cold shock was mediated by InsP$_3$ (Perera et al. 2008). While these results might have been predicted based on our previous understanding of InsP$_3$ and calcium signaling (Gilroy et al. 1990), others were not.

The transgenic InsP 5-ptime plants were drought tolerant. One would have anticipated that a decrease in InsP$_3$ and calcium signaling would enhance drought sensitivity. Indeed, the production of abscisic acid (ABA), a universal stress hormone, was delayed when the InsP 5-ptime plants were not watered (Perera et al. 2008). What was discovered from these studies was that unpredicted, compensatory pathways increased when InsP$_3$ was constitutively lowered, and these pathways appeared to enhance drought tolerance in spite of the lower ABA and calcium signal. Specifically, a subset of DREB2A-regulated transcripts were up-regulated in the plants with a dampened Ins(1,4,5)P$_3$ signal. A total of 7 transcripts associated with enhanced drought tolerance were constitutively increased in the Arabidopsis plants expressing the human InsP 5-ptime. The major impact of these findings was that they revealed an unknown InsP$_3$-regulated transcript which, if induced, could render the plants drought tolerant while not affecting growth under normal growth conditions.

Using a different model system, tomatoes (Solanum lycopersicum cv Micro-Tom), (Khodakovskaya et al. 2010) et al. showed that the phenotype was similar; however, the underlying mechanisms controlling the response appeared to be quite different. When the human InsP 5-ptime gene was constitutively expressed in tomatoes, like Arabidopsis, the plants had lowered InsP$_3$ and increased drought tolerance. The tomato plants, however, had increased glucose and fructose (potential osmolytes) and when grown in phosphate (0.25 mM) had increased leaf and root dry wt (2-4 fold and 4-7 fold, respectively). Both increased osmolytes and increased root growth would contribute to the enhanced drought tolerance.

The tomato transgenics revealed that altering the flux through a signaling pathway could have a primary effect on basal metabolism and biomass production that were not reported for Arabidopsis. In tomatoes, the low levels of InsP$_3$ appeared to have affected phosphate sensing in that the InsP 5-ptime plants had increased uptake and utilization of phosphate. An increase in cytosolic phosphate would favor the export of triose phosphates out of the chloroplast, a decrease in starch biosynthesis and an increased net sucrose synthesis and source/sink partitioning. Transcript profiling showed an increase in the apoplastic invertase inhibitor which would favor increased sucrose transport to sink tissues. As a consequence of the lowered InsP$_3$ and InsP$_3$-mediated events, the transgenic tomato plants seemed to sense they were phosphate starved even though they were not, and as a result the uptake and utilization of Pi increased.

Was the increase in stress tolerance and biomass production in the InsP 5-ptime tomato plants the result of delayed InsP$_3$-mediated signaling, an increased flux of the downstream metabolites, an increase in responses normally repressed by basal InsP$_3$ (including inhibiting phosphate uptake), other compensatory pathways, or all of the above? Clearly more research is needed to understand the underlying mechanisms involved; however, there is no doubt that in both tomato and Arabidopsis drought tolerance was increased without sacrificing normal growth under optimal conditions. Furthermore, the fundamental insights gained from selectively altering the metabolism of a second messenger revealed new approaches for regulating plant growth.

### Synthetic Approaches For Lowering Reactive Oxygen Species

A major problem for living organisms during space flight is the induction of reactive oxygen species (ROS). ROS are generally defined as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. These ROS are produced as a part of normal metabolism; however, their intracellular generation is increased by many stimuli including temperature, anoxia, light and cosmic radiation. An initial burst of ROS can initiate a signaling cascade, but continued ROS will lead to cell death. For these reasons, cells have developed extensive mechanisms to effectively remove ROS. Our work has focused on superoxide which is metabolized by superoxide dismutase (SOD). Superoxide dismutase is an essential enzyme found in all aerobic organisms (Davies 1995).

Many studies have shown that increasing the expression of endogenous plant SODs enhances stress tolerance in several different plants (McKersie et al. 1996; McKersie et al. 1999; McKersie et al. 1993; Samis et al. 2002). Although increasing endogenous SOD should increase the need for inducing mechanisms to reduce downstream secondary oxygen species such as peroxide (Grene 2002; Foyer and Noctor 2005a; Foyer and Noctor 2005b), overexpression of SOD targeted to chloroplast enhanced resistance to paraquat (Slooten et al. 1995) and increased oxidative stress tolerance in general (Van Camp et al. 1996; McKersie et al. 2000; Van Breusegem et al. 1999; Gupta et al. 1993c; Gupta et al. 1993d) indicating that adequate ROS-metabolizing enzymes downstream of SOD were present. The importance of organellar SOD for plant growth was shown indirectly in studies where decreased expression of mitochondrial Mn-SOD resulted in reduction of root growth in young seedlings and a change in the redox balance (Morgan et al. 2008). Increasing cytosolic SOD also should affect stress tolerance; however, both the cytosolic and chloroplast Cu/Zn SOD are negatively regulated by miRNA (Sunkar et al. 2006; Dugas and Bartel 2008; Abdel-Ghani and Pilon 2008). Although mutating or suppressing miR$_{398}$ increased the production of both
Cu/Zn SODs, the effect on tolerance to high light, heavy metals, and other oxidative stresses seems to vary with the growth conditions of the seedlings (Sunkar et al. 2006; Dugas and Bartel 2008) suggesting that we need a greater understanding of the regulation of SOD expression and biosynthesis in plants in order to design functional strategies to improve plant stress tolerance.

Our thesis was that if we could produce a more effective means of removing superoxide in plants, the plants would survive longer when grown under stressful conditions such as high heat or light. To avoid endogenous regulatory mechanisms, our approach was to constitutively express a gene from a hyperthermophilic archaeon, *Pyrococcus furiosus* that lives in deep sea hydrothermal vents (Fiala and Stetter 1986). These hydrothermal vents are anaerobic; however, from time to time, *P. furiosus* is expelled into the cold, oxygenated sea water. To avoid ROS damage, *P. furiosus* uses the extremely efficient enzyme, superoxide reductase (SOR) to reduce $O_2^-$ (Jenney et al. 1999; Grunden et al. 2005). SOR is the key component of the *P. furiosus* ROS detoxification system shown in Figure 2. The *P. furiosus* oxygen detoxification enzymes, include SOR, rubredoxin (Rd), and NAD(P)H:rubredoxin oxidoreductase (NROR) (Jenney et al. 1999). The hydrogen peroxide produced by the reduction of superoxide is removed through reduction by enzymes such as rubrerythrin reductase (Rr) (Jenney et al. 1999; Weinberg et al. 2004).

There are several advantages in using this heterologous system to constitutively dampen cytosolic $O_2^-$ signaling and reduce ROS toxicity. First, in contrast to plant SODs, *P. furiosus* SOR reduces $O_2^-$ without producing $O_2$ thereby lowering the potential for further ROS generation (Jenney et al. 1999; Weinberg et al. 2004; Jenney and Adams 2001). Second, *P. furiosus* SOR has a functional temperature range of 4-100°C and is an extremely stable enzyme (Jenney et al. 1999; Grunden et al. 2005). Third, SOR has a higher affinity for $O_2^-$ and a higher $K_{cat}$ than *Escherichia coli* Fe-SOD and bovine Cu/Zn SOD (Jenney et al. 1999; Emerson et al. 2003). Fourth, when the gene is expressed in heterologous systems, the active site ferrous ions of SOR can complex with exogenously added ferrocyanide to reduce $O_2^-$ to $H_2O$ without forming detectable $H_2O_2$ (Molina-Heredia et al. 2006; Kovacs and Brines 2007). Fifth, because SOR is not a plant enzyme, it should not be regulated either transcriptionally or post-transcriptionally in the same manner as SOD.

The first challenge was to produce *P. furiosus* SOR as a functional protein *in planta*. This was done using a green fluorescent protein fusion construct so that protein production could be easily monitored in tobacco cells grown in suspension culture (Im et al. 2005). The next step was to generate transgenic Arabidopsis plants (Im et al. 2009). Like the tobacco cells, the transgenic plants had increased ability to reduce superoxide as determined by an *in vitro* biochemical assay that measured both SOD and SOR activity. To confirm that the recombinant SOR protein was functional, it was immunoprecipitated from Arabidopsis leaf extracts using antibodies raised against *P. furiosus* SOR. The immunoprecipitate from the transgenic lines reduced $O_2^-$, and there was no activity in the control extracts.

To test the impact of expressing the SOR gene, Arabidopsis plants were exposed to heat and light stress, both of which lead to the production of ROS and oxidative damage (Mittler 2006; Suzuki and Mittler 2006; Volkov et al. 2006). In all instances, the SOR transgenic plants were more stress tolerant. The SOR seeds and seedlings were more heat tolerant based on both basal and acquired thermal tolerance assays. The SOR seeds and seedlings were more heat tolerant based on both basal and acquired thermal tolerance assays. The SOR seedlings also were more tolerant of combined heat and light stresses, and the seeds could germinate and grow at relatively high concentrations of paraquat (1 µM). These data indicated that even though the SOR protein was expressed in the cytosol, it could enhance tolerance to ROS generated from the chloroplast and mitochondria.

![Figure 2. Model for detoxification of reactive oxygen species in *P. furiosus* (modified from Jenney et al. 1999). Abbreviations: SOR, superoxide reductase; Rd, rubredoxin; Rr, rubrerythrin reductase; NROR, NAD(P)H:rubredoxin oxidoreductase.](image-url)
Stress tolerance in the SOR plants seemed to reach beyond simply reducing superoxide in the cytosol. Stress-induced proteins such as the heat shock protein 70 (HSP70) and the ER binding and chaperone protein (BiP) were lower in the SOR plants compared to the wild type plants under normal conditions and induction of these proteins in the SOR plants was delayed under stress condition. In addition, the induction of ROS-mediated transcription factors and of transcripts for genes encoding ROS-scavenging enzymes such as ascorbic acid peroxidase was delayed in the SOR transgenic plants compared to the wild type controls. Paradoxically, the plants were more heat tolerant.

What caused the heat tolerance? The SOR plants showed no evidence of increase in anthocyanin biosynthesis or compensatory H2O2-scavenging mechanisms similar to those reported in the apx double mutants (Miller et al. 2007). The plants responded as though the rapid reduction of O2 decreased their ability to sense the heat stress. There may be other metabolic pathways that were induced when P. furiosus SOR was expressed and these pathways may have enhanced heat tolerance in a manner similar to the enhanced drought tolerance observed in the InsP 5-ptase plants. More extensive molecular and biochemical studies are necessary to understand the full impact of expressing SOR in planta.

Because of the transient nature of second messengers in general and of the difficulties of studying reactive molecules such as superoxide, the SOR plants provide an important model system to identify superoxide mediated events. As with the InsP 5-ptase plants, events normally repressed by basal ROS as well as those induced by ROS during stress signaling, will become evident. These fundamental insights will advance our basic understanding of signal transduction while expanding potential approaches for increasing stress tolerance.

**SUMMARY AND CONCLUSION**

Dogma states that the early induction of a stress response acclimates plants to the stress and enhances their survival. This theory of acquired tolerance has been substantiated over the years in both laboratory and field experiments (Iba 2002; Thomashow 1999). Paradoxically, the SOR plants and the InsP 5-ptase plants, which delayed their response to environmental cues at the molecular and biochemical levels, were more stress tolerant. The rapid removal of selected second messengers dampened selective, second messenger signaling and likely delayed the second messenger-mediated responses normally induced by the respective pathways (Figure 1C). How then could the plants become more stress tolerant? It is possible that because these second messengers are produced at low levels all the time, if levels were lowered in the plants to below the normal basal level, i.e. if a new steady state was established in the plants in response to the expression of the transgenes, compensatory pathways were induced or derepressed. These compensatory pathways enhanced survival of the plants under stress conditions but did not affect their normal growth. It is also possible that by lowering the basal second messenger signaling and reducing stress signals under normal conditions, the plants were simply more robust and could withstand stressful environments longer, much like in humans where lowering stress will enhance tolerance to subsequent stress. For example, a good night of sleep increases one’s tolerance for normal work-day stresses.

Regardless of the mechanism(s) involved, the synthetic plants are more tolerant to stress and provide new fundamental insights into downstream events normally regulated by the respective second messengers. Second messengers are inherently difficult to study because of their transient nature. Pharmacological approaches such as the addition of exogenous second messengers are non-selective and usually generate secondary species before they penetrate plant cell membranes (Gapper and Dolan 2006; Halliwell 2006). The challenges presented by the short-life and low membrane permeability of ROS (especially O2-) and InsP3 make a compelling argument for more model systems such as the SOR and InsP 5-ptase transgenics to selectively produce and/or dampen specific signals in order to dissect interacting sensing and response pathways (Laloi et al. 2007). Finally, the results in the model plants, Arabidopsis and tomato, are quite intriguing, and in addition to providing plants that can be used reliably for bioregenerative life support, the research will reveal fundamental insights into the systems being studied.
and expand our knowledge of the complex pathways that regulate plant growth and development.

We encourage young scientists not to be restricted by dogma and to design new experimental synthetic systems. Redesigning plants may be a matter of trial and error as each crop is predicted to respond differently to changes in metabolic flux and altered developmental cues (Bowen et al. 2008; Antunes et al. 2009). In spite of these challenges, we should not hesitate to move forward. As shown in figure 3, our future plants may have little resemblance to our current plants except for the edible parts. We need to work as a team (plant physiologists, molecular biologists, biological engineers and biosphere engineers) to redesign plants for optimal growth in space.

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