

## PLANTS, PLANT PATHOGENS, AND MICROGRAVITY—A DEADLY TRIO

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### ABSTRACT

Plants grown in spaceflight conditions are more susceptible to colonization by plant pathogens. The underlying causes for this enhanced susceptibility are not known. Possibly the formation of structural barriers and the activation of plant defense response components are impaired in spaceflight conditions. Either condition would result from altered gene expression of the plant. Because of the tools available, past studies focused on a few physiological responses or biochemical pathways. With recent advances in genomics research, new tools, including microarray technologies, are available to examine the global impact of growth in the spacecraft on the plant's gene expression profile. In ground-based studies, we have developed cDNA subtraction libraries of rice that are enriched for genes induced during pathogen infection and the defense response. Arrays of these genes are being used to dissect plant defense response pathways in a model system involving wild-type rice plants and lesion mimic mutants. The lesion mimic mutants are ideal experimental tools because they erratically develop defense response-like lesions in the absence of pathogens. The gene expression profiles from these ground-based studies will provide the molecular basis for understanding the biochemical and physiological impacts of spaceflight on plant growth, development and disease defense responses. This, in turn, will allow the development of strategies to manage plant disease for life in the space environment.

### INTRODUCTION

As on earth, efficient production of crops in space requires an understanding of the physical and biological requirements and parameters for optimal growth of the crop plant. This is a major focus of NASA efforts to support space-based plant growth technologies both for shuttle-based experimentation, and onboard the international space station. The efficiency of crop production worldwide testifies to the benefits of the application of this knowledge on earth. In the United States alone, the agribusiness sector contributes over \$1 trillion annually, and accounts for nearly 15% of the U.S. Gross Domestic Product. Our agricultural exports amount to approximately \$60 billion annually, with nearly a \$20 billion positive contribution to our balance of trade.

Despite the efficiency of cropping systems on earth, the exploration of the unique requirements for crop production in space is in its infancy. Several studies

indicate that microgravity has a profound impact on plant cell development, cytology, and physiology (for review, see Cowles et al., 1989; Dutcher et al., 1994; Halstead and Dutcher, 1984; Krikorian et al., 1992; Krikorian and Levine, 1991; Nedukha, 1997; Tripathy et al., 1996). For example, carbon partitioning is different after growth in spaceflight than after growth in unit gravity, that is, a shift from the storage of starch (which is under the control of enzymes in the plastid) toward carbon accumulation as lipid (under cytosolic regulatory control) occurs (Brown et al., 1995a; Brown et al., 1999; Brown and Huber, 1987; Brown and Huber, 1988). Therefore, microgravity factors are important in the regulation of plastid events, and certainly play a role in regulating the expression of genes encoding important proteins. Results from another experiment (BPAC; *Cooperative US/Ukrainian Experiment; CUE*, STS-87, 1997) document that photosynthetic processes are altered during spaceflight. Key differences were observed in thylakoid membrane stacking, photosynthetic electron transport rates and a reduction in the amount of the photosystem I complex (authors, unpublished).

These alterations may not only reduce plant yields, but may also have a significant impact on the plant's ability to appropriately perceive and respond to stresses, whether abiotic (environmental factors) or biotic (pathogens). Indeed, there is mounting evidence that plants grown in microgravity are more susceptible to colonization by both opportunistic microorganisms and pathogens (Bishop et al., 1997; Nedukha et al., 1998; Ryba-White et al., 2001). Taken together, the evidence from space flight experiments with plants clearly indicates that growth in microgravity has a significant impact on the overall physiology of plants, and therefore, by reduction, the overall gene expression of the plant. Unfortunately, these past studies were limited in that only a few physiological responses or biochemical pathways could be explored at a time, and the selection of those pathways was understandably biased by the expertise of the scientists involved. Now, with advances in genomics research, we have the tools to minimize this bias and to look at the global impact of growth in spaceflight on plant gene expression. These advances include an expanding database of genomic and cDNA sequences for a number of plants, including rice, and new technologies that allow large-scale systematic gene expression studies (DNA-array technologies for gene expression profiling).

Although a relatively new technology, gene expression profiling is widely accepted as the means to

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obtain a global perspective on the changes an organism experiences when exposed to different treatments (Desprez et al., 1998; Ramsay, 1998; Ruan et al., 1998). By comparing gene expression profiles between treatments, researchers have been able to identify and target key pathways or genes induced specifically by a particular treatment. The applications of this global information are immense (for review, see Kehoe et al., 1999; Mazur et al., 1999). In the research arena, DNA array technologies are used for detection of mutations, gene discovery, and large-scale gene expression profiling and gene mapping. In the commercial arena, the technologies are applied to pharmacogenomics and human genetic diagnostics.

The intent of this review is to introduce the development of genomic and genetic tools that will allow a more global analysis of the impact of growth in microgravity on plant/pathogen interactions. In a recent Space Shuttle experiment, we found that plants grown during spaceflight were more susceptible to pathogen attack than ground-grown plants. In the process of doing the experiment, it became clear to us that to sufficiently determine *why* those plants were more susceptible to pathogenic colonization would require the development of a model system more amenable to the unique requirements of spaceflight experimentation and a more comprehensive means of analyzing the materials that returned from spaceflight. Thus, in the second part of this review, we will discuss our progress towards developing a model plant system and adapting newly available genomic tools (cDNA subtraction and gene expression profiling) that will allow an unprecedented analysis of the molecular pathways involved in the plant's response to growth in microgravity or to plant stresses such as disease.

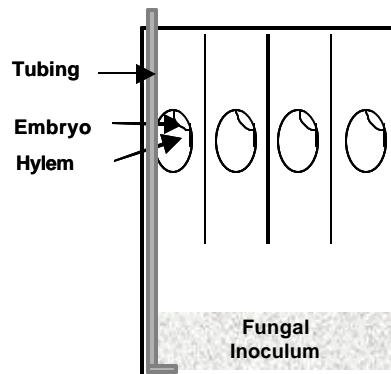
#### PLANTS GROWN IN SPACEFLIGHT ARE MORE SUSCEPTIBLE TO PATHOGEN ATTACK THAN THOSE GROWN IN UNIT GRAVITY

The potential impacts of altered growth of plants in microgravity are numerous. In addition to the major concern of reduced yield due to altered development or physiology (reduced food production), there is the concern that plants grown in microgravity are more susceptible to colonization by microorganisms (Bishop et al., 1997; Nedukha et al., 1998; Nedukha, 1997; Ryba-White et al., 2001). Microbes that can colonize plants have frequently been isolated from spaceflight hardware (Brockett et al., 1978; Nelson, 1987; Taylor et al., 1973; Taylor, 1974), and evidence of microbial contaminants colonizing and damaging plants has been documented in spaceflight experiments (Bishop et al., 1997). For example, an experiment on wheat plants was destroyed during a spaceflight experiment by a fungus that is not normally a pathogen of wheat (Bishop et al., 1997). This work indicated that growth during space flight predisposes plants for colonization by microorganisms that would not normally be able to colonize or cause disease on the plant (an opportunistic pathogen). What

would happen if a real pathogen had been introduced into the system? Interestingly, there were no studies in the literature designed to systematically address this question, i.e., what would happen if a spaceflight-grown plant were to encounter a *bona fide* pathogen?

To address this question, we developed an experimental system using a plant pathogenic fungus, *Phytophthora sojae* that causes root rot on soybean. The experiment, called SOYPAT (SOYbean PATHology), was part of the Collaborative US/Ukraine Experiment (CUE) that was flown aboard the Space Shuttle Columbia, flight STS-87, in November, 1997. The soybean (host) and *P. sojae* (pathogen) disease interaction was selected for this study because of the amenability of the interaction to spaceflight conditions and the extensive documentation of the genetic and molecular mechanisms of the interaction (Enkerli et al., 1997a; Enkerli et al., 1997b; Graham and Graham, 1999; Schmitthenner, 1989; Schmitthenner et al., 1994; Schmitthenner, 1985). In addition, the effects of gravity on soybean seedling physiology had been studied extensively (Brown and Huber, 1987; Brown and Huber, 1988; Brown and Piastuch, 1994; Brown et al., 1995b; Kuznetsov et al., 1997).

Using this experimental system, the susceptibility of soybean roots to *P. sojae* during spaceflight or in ground-based controls was quantitatively evaluated (Ryba-White et al., 2001). Soybean seeds were germinated during spaceflight in sterile pouches designed to direct the growth of the roots towards bottom of the pouch, which contained fungal inoculum (Fig. 1).

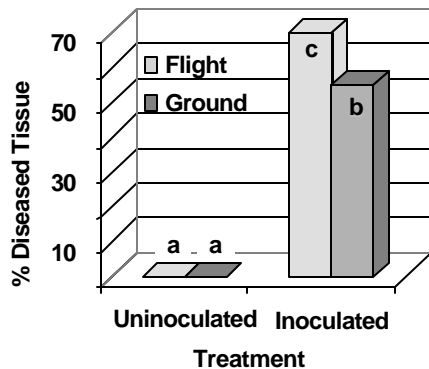


**Figure 1. Schematic drawing of autoclavable germination pouch for SOYPAT experiment.** Troughs were created in the plastic growth pouches by heat-fusing to insure directional growth during spaceflight. Surface sterilized soybean seed was oriented in the pouch as shown and held in position using Guar gum. Fungal inoculum was introduced to the bottom of the pouch through the tubing.

Control pouches did not have inoculum. Photographs were taken and the seedlings were sampled and fixed for microscopy at three timepoints during the spaceflight. Comparable samples were also collected from pouches kept at unit gravity.

To assess differences between spaceflight and ground-grown plants, parameters known to be affected by the pathogen during disease development were evaluated,

e.g., root length, number of lateral roots, disease symptom expression, fungal spore formation, and colonization of the root tissues. The most striking finding was the direct measurement of disease symptoms, i.e., more symptoms were measured on spaceflight grown, inoculated seedlings than on ground grown plants (Fig. 2). Furthermore, microscopic analysis showed that the fungal pathogen colonized the spaceflight-grown tissue more extensively than the ground-grown tissue (data not shown). Based on the amount of disease (damage to the host tissues) and the extent of fungal ramification through the tissues, we concluded that soybeans grown in spaceflight conditions were more susceptible to disease than the ground-grown controls (Ryba-White et al., 2001).



**Figure 2. Percent diseased root tissue in flight- or ground-grown soybeans.** Soybean seeds were germinated in growth pouches with or without *Phytophthora sojae* in conditions of spaceflight or in ground controls. Photographs were taken at flight day 7, and the amount of brown and macerated tissue (disease symptoms) versus healthy tissue was evaluated.

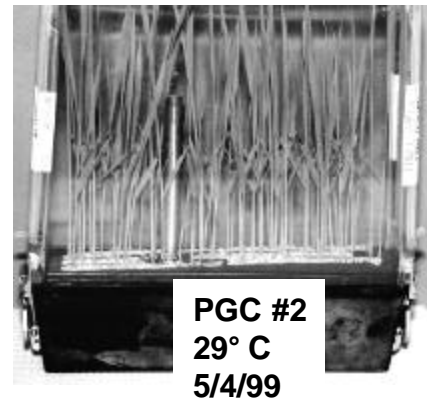
#### RICE LESION MIMIC MUTANTS: A MODEL FOR UNDERSTANDING DISEASE RESPONSE PATHWAYS

Our results from the SOYPAT experiment raised the important question of ‘Why are the spaceflight grown plants more susceptible to pathogenic colonization?’ To answer this question we need to understand precisely what differences exist in the growth, development, and responsiveness of spaceflight- vs. ground- grown plants. An elegant story is emerging for induction and manifestation of plant defense in unit gravity (for review, see Leach et al., 1996; Leach et al., 2000). The rice defense response is correlated with the induction of a number of genes collectively referred to as defense response genes, the secretion of enzymes that are the produce of some of the defense response genes such as peroxidases and chitinases, and, finally, plant cell death (for review, see Leach et al., 2000). As of yet, we do not know if these complex interactions occur in the same magnitude or time course in microgravity. A major constraint to critically addressing this question is the difficulty of manipulating plant/pathogen interactions appropriately in spaceflight. For example, the preparation of inoculum and the inoculation of plants with pathogens

during spaceflight requires the training and time of a Mission Specialist, and, depending on the host/pathogen interaction, application of the inoculum may be difficult in microgravity conditions.

To dissect out and critically analyze the defense response component of the plant/pathogen interaction under conditions of spaceflight, we have been developing a model system involving rice lesion mimic mutants. Lesion mimic mutants erratically form patches of dead cells in the absence of a pathogen (Dangl et al., 1996; Dietrich et al., 1994; Leach et al., 2001; Yin et al., 2000). The patches of dead cells formed in lesion mimic mutants resemble lesions formed during the development of disease or the rapid cell death or hypersensitive response (HR) that is characteristic of the defense response in plant/pathogen interactions (Leach, 2001). These mutants are widely used in *Arabidopsis* and maize to dissect the genetic and biochemical pathways associated with plant cell death and, by analogy, the HR (reviewed in Dangl et al., 1996; Johal et al., 1994).

In addition to their mimic of disease and defense responses, rice lesion mimic mutants are ideally suited for spaceflight experiments. Because the lesion mimics form lesions that mimic plant disease resistance in the absence of the pathogen, manipulations by Mission Specialists are minimized. Lesions on the mutants are developmentally regulated, and, depending on the mutant, are first observed at about 6-10 days after leaf expansion (9-12 days post germination), suitable for many Shuttle missions or for experiments on the Space Station. The temporal regulation of lesion development on the leaves allows us to do time course experiments for gene regulation during lesion development. Furthermore, rice is ideally suited to growth in the high humidity provided in the NASA Plant Growth Chambers (PGC, Fig. 3) that fit into the NASA Plant Growth Facility (PGF). Thus, the lesion mimic mutants are ideal models to study the effects of spaceflight on rice growth and development and activation of defense response pathways.



**Figure 3. Rice production in the Plant Growth Chamber (PGC) from the Plant Growth Facility (PGF).** Twelve-day-old rice seedlings in the PGC; up to 100 seeds can be planted per PGC. The PGF consists of six independent PGCs. Each PGC has its own temperature monitoring system. Hoagland’s solution (1X) is added to initiate seed germination, and then, every 4 days thereafter.

To obtain a collection of lesion mimic mutants for these studies, we screened more than 10,000 chemically induced rice deletion mutants (Leach et al., 2001). From this screen, 21 lesion mimic mutants that exhibit varying lesion phenotypes were identified (Fig. 4).

Based on genetic analysis of the mutants, three of the 21 lesion mimic mutations are controlled by single dominant genes, while the others were controlled by single recessive genes (authors, unpublished). All dominant lesion mimic genes showed a dosage effect, that is, more lesions are observed in homozygous plants than in heterozygous plants.

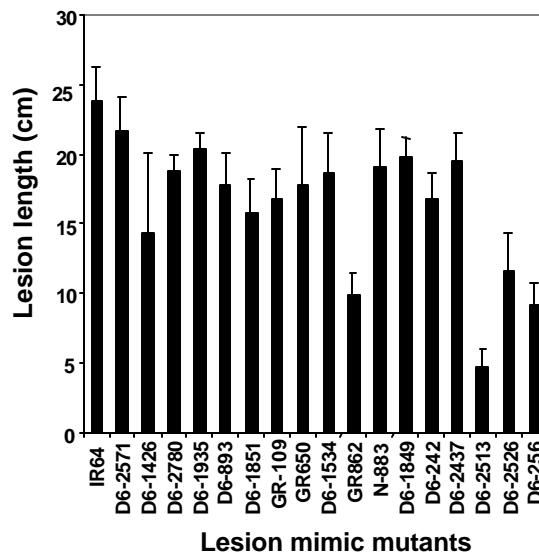
The expression of lesions by the rice lesion mimic mutants exhibits several similarities with the development of the HR. Both the HR and lesion mimic lesion formation require light. The occurrence of the HR in plants is associated with reduced pathogen multiplication and spread, or resistance (Leach, 2001). Some but not all lesion mimic mutants exhibit enhanced resistance to bacterial blight disease, and, interestingly, the amount of resistance contributed by different mutants varies (Fig. 5).



**IR64 Lesion mimic mutants**

**Figure 4. Various phenotypes of rice lesion mimic mutants.** Lesion mimic mutants were selected from a population of chemically mutagenized rice cultivar IR64.

Finally, the defense response genes associated with the HR are induced during lesion formation in some lesion mimic mutants. For example, gene expression studies show that at the onset of lesion development, as with the HR, expression of a pathogen-inducible peroxidase gene is transiently induced in developing lesion mimic leaves (Fig. 6). The general trend of the transient expression of this peroxidase gene in some lesion mimic mutants is similar to that observed for defense response genes during the HR, i.e., mRNA levels drop prior to or early in the cell death response (Chittoor et al., 1997). These findings indicate that the set of mutants now available would be valuable for identifying several genes controlling plant cell death or the HR.



**Figure 5. Rice lesion mimic mutants exhibit continuous variation in enhanced resistance to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*.** Rice leaves were inoculated with bacterial suspensions by the clipping method, and lesion lengths were measured at 14 days after inoculation. Compared to the wild type control (IR64), some lesion mimic mutants exhibited shorter lesions after inoculation, indicating enhanced resistance to *X. oryzae*.

#### GENE EXPRESSION PROFILING TO EVALUATE THE EFFECTS OF MICROGRAVITY ON PLANT GROWTH AND DEVELOPMENT

Several studies strongly suggest that microgravity has a significant impact on the physiology and growth of plants, and each study contributes incrementally to the picture of what complex changes are occurring (Cowles et al., 1989; Dutcher et al., 1994; Halstead and Dutcher, 1984; Krikorian et al., 1992; Krikorian and Levine, 1991; Nedukha, 1997; Tripathy et al., 1996). Largely due to the problem of getting suitable RNA samples from spaceflight-grown tissues, there are very few documented studies that have critically evaluated the impact of microgravity on plant gene expression, and those that exist are targeted to a limited set of genes (those regulated by particular promoters; Ferl et al., 1999; Li and Wu, 1995). All of these studies focused on one or a few plant cell processes and attempted to correlate differences in those processes with microgravity-imposed alterations. Targeted approaches such as these, while valuable, do not allow a clear perspective on the complex changes in plant growth in microgravity. Recently, with the development of DNA array technologies, the tools to analyze such complex changes became available. These technologies allow the analysis of changes in expression of large numbers of genes simultaneously (for review, see Brown and Botstein, 1999; Somerville and Somerville, 1999). Therefore, it is now possible to study the response of plants to a variety of developmental and external stimuli,

without limiting the analysis to a few known genes selected on the basis of preconceived notions as to which genes may be regulated differently. An understanding of the global expression patterns will enable accurate prediction of the biochemical processes that are modified, and will therefore provide targeted guidance to subsequent physiological studies.

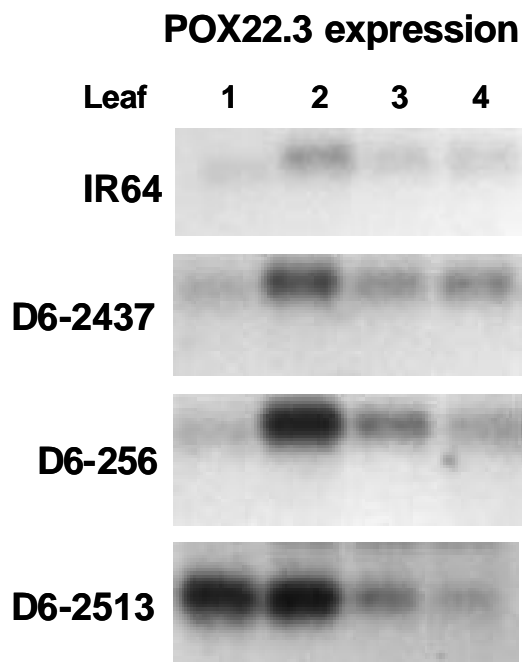
Although the literature on gene profiling studies is rapidly increasing (e.g., Cho et al., 1999; Desprez et al., 1998; Maleck et al., 2000; Somerville and Somerville, 1999; Reymond et al., 2000), there are few examples related to space biology (Lewis et al., 1999). Because of the limited number of published studies and the infancy of the technologies involved, we will spend the next few paragraphs describing gene expression profiling.

**Arraying techniques.** Several arraying techniques can be used for determining global expression profiles, including oligonucleotide DNA chip arrays (Lipschutz et al., 1995), serial analysis of gene expression (SAGE, Velculescu et al., 1995) and large-scale sequencing of primary libraries (Adams et al., 1995). The latter two methods are not readily applicable to comparisons of large numbers of experimental conditions. Although they have many excellent features, the oligonucleotide chips, synthesized by photolithography, are relatively inflexible, expensive to produce and use, and are dependent on prior knowledge of gene sequence. Due to public sequencing efforts, a publicly available rice oligonucleotide chip is feasible within the next 5 years, but currently these types of arrays are available only for *Arabidopsis*. As rice is the model here, we will focus on membrane filter and glass slide microarray technologies.

Microarrays are formed by depositing specific fragments of DNA at indexed locations on membrane filters or on glass slides. The fragments can originate from EST clones, anonymous cDNA clones, or anonymous genomic clones. Current technology allows for spotting of as many as 10,000 spots/3.24 cm<sup>2</sup> on glass slide microarrays. Given that the *Arabidopsis* genome contains about 25,000 genes, the entire genome could, in theory, be displayed on one glass microscope slide (Abeles et al., 1992).

Once produced, the arrays are hybridized with radioactive (filter arrays) or fluorescently-labeled (Fig. 6) mRNA-derived probes. The signal emitted from each spot is a reflection of the abundance of the corresponding sequence in the original probe, and thus, quantitative information about mRNA levels for a large number of genes can be collected concurrently.

For filter arrays, cDNAs are arrayed in a high density on nylon membrane filters using a replicator and template. The membrane arrays are hybridized with cDNA probes synthesized from the mRNAs of interest and the resulting expression patterns are compared by using a phosphoimager. The advantages of the membrane filter array technology are that it does not require expensive arraying or analysis equipment. Disadvantages, relative to glass slide or other higher density techniques, are that larger amounts of



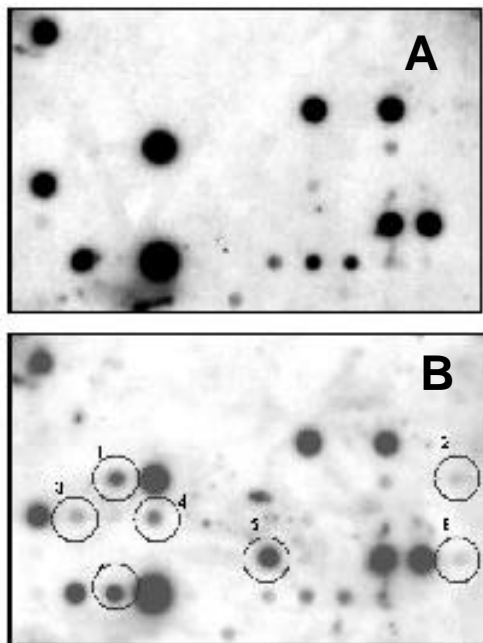
**Figure 6. Expression of a defense response peroxidase gene (POX22.3) is enhanced in some lesion mimic mutants.** mRNA from leaves of lesion mimic mutants and the wild type IR64 were collected from leaves at different developmental stages, with leaf 1 being the youngest and leaf 4 being the oldest. RNA blots were hybridized with labeled POX22.3, a pathogen-inducible peroxidase. Although the wild type plant exhibited POX22.3 transcript at leaf position 2, in general, more transcript was observed in lesion mimic mutants at all time points. The transcript accumulation was transient, and was reduced in the older leaves.

hybridization solutions are required and pair-wise comparisons cannot be made on the same membrane.

The advantage of high-density glass slide arrays is that very small hybridization volumes are needed (in comparison to the filter arrays) and by using high probe concentrations (feasible in the small volumes), rare mRNAs can be detected. The use of fluorescent probes in slide microarray is particularly useful because pair-wise comparisons can be made. Two fluorescent tags with different excitation and emission optima can be used to label two distinct probes (e.g., space vs. ground or lesion mimic vs. wild type rice). After hybridization, the ratio of fluorescence emission at the two wavelengths reflects the ratio of the abundance of that sequence in the two probes. Changes in expression pattern as low as two-fold and transcripts as rare as 1 in 500,000 have been detected using this technique (Schena et al., 1996). The disadvantage of this technique is the specialized equipment required for arraying and post-hybridization analysis.

**Preliminary results.** Although our ultimate plan is to use glass slide arrays, we have used filter arrays containing part of an existing collection of genes that are known or predicted to be involved in plant stress or

defense responses (the *KSU Defense Gene Collection*, *KSU-DGC*, <http://www.ksu.edu/ksudgc/>, ID guest, password: ksqtldataG). To monitor expression of this limited set of known defense genes, RNA was extracted from PGF-grown rice and *Brassica napus* plants to produce cDNA probes for analysis of membrane arrays containing the defense response genes (Gartner et al., 1999). Plants were grown in the PGF (containing six Plant Growth Chambers, PGCs) for 14 days. In three PGCs, the seeds were hydrated with a single treatment of Actigard™, a Syngenta product that contains BTH, a salicylic acid derivative that induces defense response gene expression in plants. Seed in the other PGC did not receive the chemical treatment. At 1 day after treatment, total RNA was extracted from the shoots and leaves and was used to synthesize cDNA probes for hybridization of arrays containing 95 defense response gene clones from the *KSU-DGC*. Interestingly, we did not detect differences in expression patterns in the rice, suggesting that rice does not respond to BTH when applied through the roots. However, we did see differential induction of seven genes in the BTH-treated *Brassica* plants (Fig. 7).



**Figure 7. Membrane filter arrays of plant defense response genes hybridized with cDNA from *Brassica napus* after treatment of roots with BTH.** After 1 wk growth in the PGC, the roots were treated with 0.15 mM benzothiadiazole (BTH) in IX Hoagland's solution. After 1 day, RNA was extracted from the seedlings and used for synthesis of cDNA radioactive probes. Seven known defense response genes were differentially expressed in BTH-treated seedlings (B) compared to untreated controls (A).

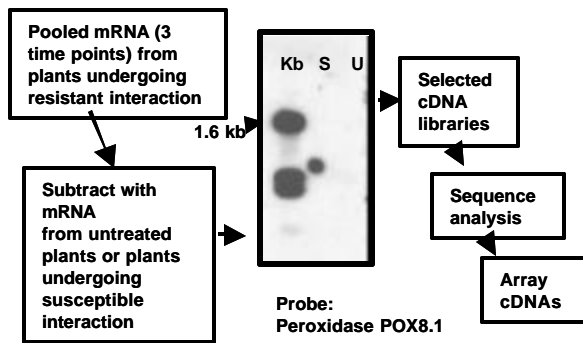
Two of these genes have been shown to be induced after application of BTH to leaves (Ryals et al., 1996); note that application of BTH through the roots had not been previously described in the literature.

#### WHAT TO ARRAY? COLLECTIONS OF STRESS AND DEFENSE RESPONSE GENES

Our interest is in detecting genes that are differentially expressed during development of defense responses in plants during growth in microgravity vs. ground. Because cDNA libraries of genes from rice grown in microgravity are not yet available, and in particular, libraries from lesion mimic plants that are developing lesions, we have focused on developing arrays containing sublibraries that are enriched for genes that are differentially induced during defense responses or during disease development in unit gravity. This enrichment was achieved through the use of suppression subtractive hybridization (SSH, Diatchenko et al., 1996). This technique combines a high subtraction efficiency with an equalized representation of differentially expressed sequences, which is achieved by a specific form of polymerase chain reaction (PCR) called suppression PCR (Diatchenko et al., 1996). Suppression PCR permits the exponential amplification of cDNAs that differ in abundance, whereas amplification of sequences of identical abundance in the two populations is suppressed.

The SSH strategy was used to construct a cDNA library that is enriched for rice defense and disease response genes (Fig. 8). To ensure that enrichment had occurred during the subtraction steps, we hybridized amplified cDNA with probes constructed from genes known to be induced during the defense response, e.g., a pathogen-inducible peroxidase POX8.1 (Chittoor et al., 1997). The hybridization of POX8.1 to only the lane containing the subtracted cDNA indicated that enrichment for the defense response genes had occurred (Fig. 8). Sequence analysis of the library, which now contains over 100 non-redundant cDNAs, confirmed that enrichment had occurred because many of the clones identified were known defense response genes. Perhaps of most interest, more than 20 cDNA clones from the library do not show any identity to known genes in the public databases.

The clones from this enriched library will be combined onto glass slide arrays with clones from the *KSU Defense Gene Collection*, which contains over 150 genes from different plant species (heavily focused towards monocots) that were cloned because of their involvement in disease defense or stress (salt or drought) responses. Although microgravity and disease defense responses will activate different sets of genes, since each is a type of stress response, there is likely to be a significant overlap in the biochemical pathways and, therefore, genes induced. Thus, these combined collections will be particularly valuable because these genes are known to play a role in stress and defense, and in some cases, much is known about the biochemical pathways in which they are involved.



**Figure 8. Suppression subtractive hybridization strategy for identification of cDNA libraries enriched for defense response genes.** Amplified cDNA that had been subtracted hybridized to probe *POX8.1*, a pathogen-inducible peroxidase (Chittoor et al., 1997), indicating that the enrichment of defense response genes had occurred.

Although our SSH library is enriched for defense response genes induced under conditions of unit gravity, an exciting prospect for the future is to develop SSH libraries enriched for rice genes induced in spaceflight conditions. Libraries could be enriched for genes involved in rice development or growth (subtract cDNA extracted from ground-grown plants from cDNA extracted from spaceflight-grown plants) or enriched for genes induced during development of hypersensitive-like lesions under conditions of spaceflight vs ground using one or more lesion mimic mutants and the wild type parent.

### SUMMARY AND FUTURE PERSPECTIVES

When grown in conditions of spaceflight, plants are more susceptible to attack by microorganisms. It is alarming that we have no plans for how to control disease in this environment. However, if we are proactive, our efforts towards understanding why the plants are more susceptible when grown during spaceflight could yield strategies to control plant diseases not only in this exotic environment, but also in unit gravity. Already, significant advances have been made in our understanding of plant responses to growth and stress in spaceflight conditions. However, because studies have focused on particular physiological processes, independently, they provide only narrow snapshots of the global changes that are occurring, and may not focus our attention on the most important pathways. The technologies and model systems are now available to determine global gene expression profiles of rice grown in spaceflight conditions compared to ground controls. These expression profiles will be useful tools to guide the directions of future physiological and biochemical studies.

### ACKNOWLEDGEMENTS

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