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FY 99 NASA Life Sciences Task Book Form

(This report compiled and submitted by Christopher Brown, October 26, 1999)

Title:	NSCORT. Calcium, Signaling and Gravity: An Integrated Molecular, Cellular and Physiological Approach to Plant Gravitational Biology.
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Project Web Address:	www.cals.ncsu.edu/nscort
Monitoring Center:	HQ
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NASA NRA Solicitation:	NSCORT
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Joint Agency:	

Co-Investigators:	**Affiliation**:
Allen, Nina S.	North Carolina State University
Boss, Wendy F.	North Carolina State University
Davies, Eric	North Carolina State University
Huber, Steven C.	North Carolina State University
Muday, Gloria K.	Wake Forest University
Robertson, Dominique	North Carolina State University
Sederoff, Ronald R.	North Carolina State University
Thompson, William F.	North Carolina State University
Tucker, Edward B.	Baruch College, City University of NY
Whetten, Ross W.	North Carolina State University

NASA Facilities / Equipment used for this project:

Number of Funded Students:

Pre-college:	3

Undergraduate:	26
Graduate:	10
Post-doctoral:	9

Was a final report for this project submitted to NASA during FY99?
No

Abstract:
The NSCORT is a consortium of 11 project leaders from 4 institutions including North Carolina State University (College of Agriculture and Life Sciences and School of Forestry), Wake Forest University, Baruch College (City University of New York), and Dynamac Corporation (which manages the Life Science support contract at NASA's Kennedy Space Center). Faculty, staff and students from all of these institutions participate in various aspects of the program. In addition, participation by non-NASA supported personnel has added to the value of the grant. This report summarizes the progress of the NSCORT in FY99. The overall mission of the NSCORT is spelled out in the two sub-headings: Research and Education/Outreach.

Research:
To advance the basic understanding of the role of calcium in gravity perception, induction and response and; To alter gravity-induced growth by altering calcium homeostasis. The group uses an integrated molecular, cellular, and physiological approach to plant gravitational biology.

Education/Outreach:
To create an innovative learning environment for postdoctoral fellows, graduate and undergraduate students through active integration of teaching, research and outreach opportunities; To communicate knowledge and enthusiasm for gravitational plant biology to K-12 teachers and students and; To enable the teachers to share what they learn.

Task Progress

Research

The approach, as stated in the Original Proposal Abstract, is as follows: *"The precise modulation of calcium homeostasis will be achieved using transgenic plants and monitored using sophisticated imaging techniques to verify the specificity and extent of transgene expression. These efforts, in combination with our expertise in local and long-distance signaling, will make a major contribution to understanding the fundamental role of calcium in orchestrating the transduction of the gravity stimulus into an autopoietic (self-regulated) response."* The research synergy is such that "Principal Investigator" and "Project" are NOT synonymous and thus it was inappropriate to make this report one of 11 independent PI/projects. This approach has resulted in 5 highly integrated research thrusts, listed below as Projects I - V.

Project I. Changing calcium homeostasis in transgenic plants: A reverse genetics approach (Robertson, Thompson, Boss).

Project II. Isolation of a novel class of mutants with impaired transduction of the gravity signal (Robertson, Muday),
Project III. Changes in gene expression during gravistimulation (Davies, Whetten, Sederoff),
Project IV. Signal transduction pathways and early responses to gravistimulation (Allen, Tucker, Boss, Muday, Davies, Whetten,
Project V. Effects of gravity on metabolism (Huber, Brown, Muday).

Our thesis is that if we genetically altered calcium homeostasis, we would affect the plant's ability to respond to gravity. The research progress of this reverse genetic approach is described in **Project I**. This has resulted in a number of constructs we now have available for the group. These include calreticulin, calmodulin, calmodulin-binding peptides, PIP 5-kinases, the ER Ca^{2+}-ATPase and tonoplast Ca^{2+}-ATPase under the control of inducible and targeted promoters. This has furnished the first evidence to support our hypothesis insofar as transgenic *Arabidopsis* plants over-expressing calreticulin in the endoplasmic reticulum, when grown on low calcium medium, have an enhanced response to gravity compared to controls. This has also enabled us to produce the first stable transformants with a reliable, inducible Top10 promoter in *Arabidopsis* and to optimize conditions for its use. We also optimized conditions for using onion epidermal peels for transient expression. The onion system greatly facilitates imaging GFP-fusion proteins for localization studies and for testing our constructs. Another focus has been the optimization of calcium- and pH- sensitive reporter proteins for expression in *Arabidopsis* to facilitate analysis of ion fluxes in response to gravity.

Project II is a mutant screen of T-DNA tagged *Arabidopsis* that takes advantage of the fact that inflorescence stems can perceive but not respond to gravity at 4^{o}C.**.** The mutants are proposed to have lesions in their **G**ravit**y P**ersistent **Si**gnaling mechanism and, therefore, they have been called GyPSi mutants. We have recovered 8 mutants that have been screened through the F2 generation and we are in the process of characterizing them with respect to phenotype, genetics, and auxin transport in mutant and wild-type backgrounds. We anticipate that the mutant screen will lead to new insights concerning control points for gravi-sensing in plants.

Project III is another approach to identifying novel genes involved in gravity-induced signal transduction and response. Changes in transcription are indicated by the fact that specific transcripts become more abundant in gravi-stimulated pine xylem and maize pulvinus tissue, while changes in translation are indicated by differences in polysome profiles in gravi-stimulated maize pulvini. Our data suggest that transient changes in gene expression occur over time in the upper and lower halves of gravi-stimulated maize pulvini and are consistent with biochemical differences reported in Project IV and V. We also are using microarrays to identify genes expressed in gravi-stimulated pine and will eventually expand these studies to maize.

Project IV addresses cellular and biochemical studies of signal transduction pathways. We have evidence for a role for the phosphoinositide pathway in the maize pulvini and MAP kinase-regulated signaling events in both gravi-stimulated pine and maize. Rapid (10s) changes in IP_3 evident in the lower side of the maize pulvinus indicate an early sensing of a change in the gravity vector. In addition, we have repeatedly detected an oscillation of the metabolic changes alternating between the lower and upper sides of the pulvinus as though the commitment to

elongate does not occur until after about 2-4h of gravi-stimulation. More complete analysis of the signaling events over time will be needed to sort out this intriguing signaling pattern. Although the maize pulvinus has been ideal for biochemical studies it is not tractable for imaging. To this end, we developed a method for isolating files of amyloplast-enriched bundle sheath cells for imaging changes in cytosolic calcium and pH during gravi-stimulation.

Although others were not able to detect changes in Ca^{2+} in gravi-stimulated root columella cells, we have shown a rapid (<1min) increase in pH of the Tier 2 columella cells on the lower side of *Arabidopsis* roots. To provide insight in the relationship between auxin transport and gravity response in the roots of *Arabidopsis,* an assay has been developed to measure auxin movements. Results from the assay of wild-type and mutant plants altered in transport, combined with inhibitor studies indicate that basipetal auxin transport is sufficient to control gravity response in *Arabidopsis* roots. In addition, proteins that bind and potentially regulate auxin transport proteins were found to bind F-actin affinity columns suggesting actin localization of the transport proteins is a potential means for regulating auxin transport.

In order to measure calcium influx in response to gravity, we used a self-referencing Ca^{2+}-selective ion probe and monitored Ca^{2+}-influx into the tip cell of a filament of *Physcomitrella.* This cell has proven to be useful for microinjecting dyes and recombinant proteins, and studies of Ca^{2+} revealed a dramatic and rapid effect of light on cytosolic Ca^{2+} which may override gravity-induced changes.

Project V focuses on the role of carbon metabolism in the gravity response and studies of starch metabolism in space flight. Rapid (10 min) changes in malate concentrations are evident in the maize pulvinus following gravi-stimulation. Changes in the intracellular distribution of the sucrose metabolizing enzyme, sucrose synthase, were also noted suggesting a mechanism regulated by protein phosphorylation that affects the membrane and cytoskeletal localization of the enzyme. The effect of gravity on the regulation of invertase activity as well as transcription of its gene has become a major focus of this work. Recent work indicates that invertase gene expression in controlled by auxin levels and that redistribution of auxin across a gravity stimulated maize pulvini precedes differential invertase expression. The potential for changes in cytosolic pH to regulate invertase activity and a possible connection to the cytoskeletal structure make this a very exciting system for regulating a growth response. Space flight grown plants have been studied with regard to their starch metabolism and were found to produce less starch and have smaller starch granules. Indications of altered starch grain structure and morphology have been noted and are under investigation.

Education/Outreach

The “Space Biology” course was made an official part of the NC State University curriculum (Botany 277) and is taken primarily by undergraduates, either science majors wanting to expand their horizons, or by non-science majors as a science elective. This has increased the awareness of space biology among the campus community. Furthermore, since the course was transmitted from 5 locations around the country, including 3 NASA centers, and was transmitted to 2 other institutions (UNC-Chapel Hill and the NC School of Science and Math), it has broadcast awareness of space biology to a much wider audience than just NCSU. In 1998, 23 students and

1 teacher took Space Biology. Ten guest lecturers participated last year, many of them external to the NSCORT. We are in the process of developing it as a model course for long distance education and have established a course website (http://www.cals.ncsu.edu/course/bo277).

The 1999 Annual Teachers Workshop "Plants, Gravity and Space" had 9 participants from 5 states and the District of Columbia. The workshop helped to forge ties with teachers both within and outside the state of North Carolina and has been the basis for other outreach activities such as "Expanding Your Horizons". These workshops also provided the impetus for our NSCORT to become a major player in the NC Science Olympiad held at NCSU in 1998. In total, the NSCORT has interacted with over 250 students and 100 teachers through our Outreach Program in FY99.

Both the 1999 and 2000 Annual Symposia *"Calcium and Gravitational Biology"* top speakers, and drew over 80 attendees each year from across the country. In addition to the main speakers, poster presentations have been given by teachers, undergraduate, graduate and post-doctoral students.

Earth Benefits:

This research will determine the mechanisms by which plants perceive and respond to several environmental stimuli, especially gravity. It will provide a fundamental understanding of basic plant processes, especially at the cellular, molecular, and developmental levels. A deeper understanding of how plants respond to gravity and other environmental conditions will improve our understanding of how they grow in various space conditions (e.g. Earth orbit, Mars) and how their growth can be modified to maximize yields on Earth. More applied work on specific plants should yield valuable by-products such as enhanced paper quality (e.g., pine seedling system and its formation of compression wood), yield of seed grains (e.g., reorientation of corn plants blown over in strong winds), improved quality of cut flowers (maintenance of straight stems during long distance shipping), and nutraceutical plants (e.g., plants with enhanced capability of storing essential nutrients such as calcium).

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Zsuppan, G., Collings, D.A., Allen, N.S. (1999) Actin in the columella cells of maize. J. Elisha Mitchell Scientific Society 115: 171. Presented at North Carolina Academy of Sciences, Elon College, Elon NC March 27th 1999 *(First place, Undergraduate student competition).*

Patents

Awards

Geary, R. Ligation of DNA in Microgravity: a Student Space Flight Opportunity. This space shuttle flight opportunity was awarded to NCSU undergraduate student and NSCORT advisors, Sarah Wyatt and Niki Robertson in response to a national competition. The project was sponsored by Instrumentation Technology Associates (ITA) and the American Society for Gravitational and Space Biology (ASGSB). It flew on STS-95 from October 29 – November 9, 1998.

Gupton, S. 3rd place, Botany Section - undergraduate research presentations, North Carolina Academy of Sciences, Elon College, Elon NC, March 27th 1999.

Yeung, N. 1999 NCSU Undergraduate Research Symposium Award Winner (Biological Sciences).

Zsuppan, G. 1st prize, Botany Section - undergraduate research presentations, North Carolina Academy of Sciences, Elon College, Elon NC, March 27th 1999.

Impact on America

Who Uses This Research

This research will determine the mechanisms by which plants perceive and respond to several environmental stimuli, especially gravity. It will provide a fundamental understanding of basic plant processes, especially at the cellular, molecular, and developmental levels. A deeper understanding of how plants respond to gravity and other environmental conditions will improve our understanding of how they grow in various space conditions (e.g. Earth orbit, Mars) and how their growth can be modified to maximize yields on Earth. More applied work on specific plants should yield valuable by-products such as enhanced paper quality (e.g., pine seedling system and its formation of compression wood), yield of seed grains (e.g., reorientation of corn plants blown over in strong winds), improved quality of cut flowers (maintenance of straight stems during long distance shipping), and nutraceutical plants (e.g., plants with enhanced capability of storing essential nutrients such as calcium).

Industrial Affiliates

Dynamac Corporation

New Technologies

Current Employers of Former Graduate Students

Magazine Covers

Scott, A.C., Wyatt, S., Tsou, P-L., Robertson, N. and Allen, N.S. 1999. A model system for plant cell biology: GFP imaging in living onion epidermal cells. BioTechniques 26: 1125-1132. "A 4 day-old Arabidopsis seedling expressing green fluorescent protein localized to the endoplasmic reticulum. The red in the leaves is chlorophyll autofluorescence."

Popular Press Coverage

Newspapers and Magazines

10/6/98 "Student's work heads to space" *Technician,* NCSU, Raleigh, vol 70 (29).

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11/4/98 "Potential Nurtured at A-B Tech" *Asheville Citizen Times*, Asheville, NC.

Fall 1998 "A new way of seeing: Images from a microscopic world" *Perspectives, The Magazine of the College of Agriculture and Life Sciences*, NCSU, Raleigh.

Nov/Dec 1998 "Far-Out Project" *The Community College Connection*, vol 1(3), Raleigh.

Winter 1998 "Out of this world" *Perspectives, The Magazine of the College of Agriculture and Life Sciences,*
NCSU, Raleigh.

2/5/99, *"Grant Program Explores New Teaching Methods"* Bulletin, NCSU, Raleigh.

8/9/99, "Summer School part of many teachers' activities", *News & Observer***,** Raleigh.

7/30/99, "Teachers Introduced to Space Biology", *Bulletin*, NCSU, Raleigh.

Television and Radio

10/21/98, WNCN (NBC,Raleigh) 3 stories (related to the “DNA Ligation in Microgravity” Space Shuttle experiment performed by undergraduate student Reathal Geary).

10/28/98, WRAL (CBS, Raleigh) 1 story (related to the “DNA Ligation in Microgravity” Space Shuttle experiment performed by undergraduate student Reathal Geary).

10/28/98, WTVD (ABC, Raleigh) 3 stories (related to the “DNA Ligation in Microgravity” Space Shuttle experiment performed by undergraduate student Reathal Geary).

10/29/98, The Today Show, NBC Network (related to the “DNA Ligation in Microgravity” Space Shuttle experiment performed by undergraduate student Reathal Geary).

Fall 1998, North Carolina Radio News Network, ran multiple times (related to the “DNA Ligation in Microgravity” Space Shuttle experiment performed by undergraduate student Reathal Geary).

7/20/99, Radio feature on "Plants, Gravity & Space" teacher workshop, WPTF-AM, Raleigh.

7/19/99, Television feature on "Plants, Gravity and Space" teacher workshop, WTVD ABC affiliate), Durham, NC, Channel 11.

7/20/99, Television feature on "Plants, Gravity and Space" teacher workshop, WRAL CBS affiliate), Raleigh, NC, Channel 5.

8/99, Feature on the College of Agriculture and Life Sciences Video Newsmagazine for State Extension Agents.