Molecular Approaches for Enhancing Crop Productivity – Sustainable Crops for Advanced Life Support in Space Exploration

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Synopsis: Plants are essential components of human space exploration. They provide oxygen, remove carbon dioxide, purify water, supply fresh food, pharmaceuticals and are also beneficial for psychological health. Our research is focused on understanding mechanisms of stress signal transduction towards enhancing tolerance to environmental stresses in plants for Space Agriculture by the genetic approach.

We have two long-term goals in our current research: 1) to better understand the stress induced signaling network in plants, and 2) to create valuable biotechnological products (stress tolerant tomato and rice plants) for use in the Advanced Life Support (ALS) of NASA mission. Understanding the molecular basis of adaptation and integration of abiotic stress responses in plants should allow us to generate crop plants that will be able to tolerate conditions beyond their adaptive range on earth. Recently, we identified new *Arabidopsis* protein (lipid-binding protein with C2 domain (AtCLB)) that may play a role of negative regulator of plant stress responses. Confirmation of functions of AtCLB will open new perspectives for increase of plant stress tolerance.

To achieve our goals **we propose three specific objectives:**

1. To confirm the role of conserved C2 domain in stress signaling by characterization of functions of *Arabidopsis* genes containing C2 domains that are close homologues to C2 domain of lipid-binding protein with C2 domain (AtCLB);
2. To investigate the subcellular localization of negative regulator of stress response Ca^{2+}-dependent lipid-binding protein with C2 domain (AtCLB) in *Arabidopsis* cells using advanced methods of subcellular and subnuclear fractionation;
3. To increase stress tolerance in crops through the silencing of AtCLB crop homologues. While *Arabidopsis* is a useful model for basic research, it has a very limited value for Advanced Life Support. We are expecting to apply knowledge generated in our CRP grant (PI: Khodakovskaya; Co-PI: Srivastava) for genetic improvement of crops (tomato, rice) that were characterized as the suitable plants for ALS (NASA/TM-2003-211184). We will specifically design experiments to address the function of AtCLB crop homologues by overexpressing or suppressing (silencing) the gene activity in the respective host plant. While overexpression of AtCLB homologues is expected to confer susceptibility to stress, suppression of AtCLB homologues is expected to confer abiotic stress tolerance. These experiments are important for the validation of gene function in rice and tomato. Dr. Srivastava has developed transgenic approaches for very efficient gene over-expression and suppression (Srivastava et al., 2004; Nicholson and Srivastava, 2009). We are going to apply her technologies to the proposed project.

Relevance of the Project to NASA: The growth of plants in space remains a priority for the development of strategic plans, especially for those plans concerning the long-term habitation in space and on planetary surfaces. Interrelationships between plants and humans are complementary: plants recycle human wastes and provide human nutrients, while humans recycle plant wastes and provide plant nutrients. Therefore, plants must be an integral part of long-term bioregenerative life support systems and essential components of efforts to establish larger-scale controlled ecosystems on extraterrestrial surfaces (Ferl et al., 2002). Plants are Earth-based organisms and thus are able to successfully grow and maintain best nutritional characteristics only in limited environmental conditions. However most of environmental factors such as temperature, water availability, light, atmospheric pressure and radiation will be dramatically different in space and on other planets. Establishment of crop plants or cell cultures with novel genetic traits including tolerance to different types of environmental stress is one of the most promising approaches. Genetic improvement of crop plants for use in Advanced Life Support has been prioritized as the #1 mission critical to NASA's Bioastronautic Roadmap under Risk #42. Provision of fresh vegetables or fruit to diversify the diet and positively impact the psychological well being of people living in the confined environments of space habitats.

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Dr. Raymond M. Wheeler,
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Dr. Raymond M. Wheeler is a Plant Physiologist and Member of Advanced Life Support/Space Biology Group at KSC. He has kindly agreed to accommodate us for this outreach activity program. Dr. Wheeler's interest is development of bioregenerative life support systems including vegetable production system (VEGGIE) for producing salad type crops.

Letter of support from Dr. Raymond M. Wheeler will attached to actual proposal

Duties of participating members:

Role of PI (UALR):

- Dr. Khodakovskaya will supervise and coordinate overall research activities related to this project. She will supervise postdoctoral scientist (to be hired) and train undergraduate student (to be hired) to perform analysis of the studies related to subcellular/subnuclear fractionation and stress experiments with transgenic crops. She (together with postdoctoral scientist, graduate students and undergraduate students) will carry out number morphological, biochemical and stress experiments. She will analyze raw data, interpret data and produce written output, including presentations and manuscripts.

Role of Postdoctoral Scientist (to be hired at UALR):

- Postdoctoral Scientist will work under supervision of Dr. Khodakovskaya performing all biochemical experiments (subcellular fractionation), transformation, phenotypical studies and stress experiments with *Arabidopsis* mutants and transgenic crops.
- Role of Undergraduate Student (to be hired at UALR):
- Undergraduate Student will be trained to carry out experiments related to work with loss-of-function mutants for two genes (gene of *Arabidopsis* CLB1-like protein and gene of *Arabidopsis* NTMC2Type2.2 protein). He/she will do backcrossing, establishment of homozygous mutant

lines, analysis of gene expression for mutant lines. Undergraduate student will be actively involved in outreach activity.

Role of Co-PI (UA, Fayetteville):

- Dr. Srivastava will focus on Objective 3. The genes identified by Dr. Khodakovskaya in Arabidopsis, tomato and rice (homologues of AtCLB gene) will be used for rice transformation in Dr. Srivastava's laboratory. She will develop strategy, experimental plan, and supervise students (to be hired) for all transformation work and transgenic plant analysis.

Role of Graduate Student (to be hired at UA, Fayetteville):

- The graduate student (to be hired) will generate transformation constructs and generate transgenic rice and tomato lines overexpressing or silencing the AtCLB homologues

Role of Undergraduate student (to be hired at UA, Fayetteville):

- The undergraduate student will assist the graduate student in DNA manipulations and plant tissue culture techniques.

Project Description:

Background: Plants are essential components of human space exploration. They provide oxygen, remove carbon dioxide, purify water, supply fresh food, pharmaceuticals, and are beneficial for psychological health. During human space travel or habitation on other planets, plants will have to be grown in chambers that provide a suitable environment. However, generating an earthlike environment will be costly with respect to material transport and energy required. To minimize costs and the risks of total crop loss during system failures, it is necessary to generate plants that can withstand and adapt to the drastic changes (water deficit, atmospheric pressure, temperature stress) in the physical environment. At the same time, the plants' value for Advanced Life Support should be maximized.

Plant signal transduction pathways in response to drought, salt and osmotic stress, as well as gravitropic and phototropic stimulations, have been shown to involve inositol phosphate metabolism and changes in subcellular calcium (Ca^{2+}) concentrations. While plants have several unique Ca^{2+}-sensing proteins, the downstream components of Ca^{2+} signaling in plants remains poorly understood (Reddy, 2001). Several proteins have been reported to be activated or translocated in the presence of Ca^{2+} including cytosolic phospholipase A2 (cPLA2), phopholipase C (PLC), protein kinase C (PKC), calmodulin, and calcium-dependent calmodulin-independent protein kinase (CDPK) (Reddy et al., 2010). These proteins exhibit Ca^{2+}-binding domains that include Ca^{2+}-dependent lipid-binding (CaLB) domains or C2 domains. The C2 domain (about 130 residues) was first described as Ca^{2+} -binding site in protein kinases (Xu et al., 1997). Now, about 600 C2 domains have been identified in >400 different proteins. There are a few reports describing involvement of C2 domain proteins in plant responses to abiotic and biotic stresses. For example, it has been reported that phospholipase D and copine are involved in plant stress signaling (Young et al., 1996; Laxalt and Munnik, 2002). In tomato, C2 domain of phospholipase D (PLD) exhibited binding activity to membrane phospholipids (PA and phosphatidylinositol) during ripening and senescence (Tiwari and Paliyath, 2011).

Despite the fact that Ca^{2+} binding proteins are being identified at a rapid pace, progress in elucidating the functions of many of them is very limited (Yang and Poovaiah, 2003). Furthermore, only a few C2-domain proteins have been reported in plants compared to a wide variety of animal C2-domain proteins most of which are involved in eukaryotic signal transduction and membrane trafficking processes (Nalefski and Falke, 1996). The majority of known C2 domain-containing proteins in plants have not been functionally characterized yet. At the same time, it is well indicated that many C2 domain Ca^{2+}-sensing proteins are involved in plant stress signal transduction as positive or negative regulators of stress-signaling cascades. The discovery and characterization of such proteins will shed light on complicated Ca^{2+} -dependent stress signaling *in planta* and could potentially lead to new biotechnological strategies for improving stress tolerance in valuable crops.

Preliminary data: Previously we described new *Arabidopsis* C2 domain Ca^{2+}-dependent lipid-binding protein (AtCLB) that is able to negatively regulate stress response in *Arabidopsis* (de Silva et al. 2011). Expression of the *AtCLB* gene was documented in all analyzed tissues of *Arabidopsis* (leaf, root, stem, flower, and silique) by real-time PCR analysis. Immunofluorescence analysis revealed that AtCLB protein is localized in the nucleus of cells in *Arabidopsis* root tips (de Silva et al., 2011). We demonstrated that the AtCLB protein was capable of binding to the membrane lipid ceramide. The role of the *AtCLB* gene in negatively regulating responses to abiotic stress in *Arabidopsis thaliana* was identified. The loss of the *AtCLB* gene function in mutants confers an enhanced drought and salt tolerance (Fig. 1) and a modified gravitropic response (not shown) in T-DNA insertion knockout mutant lines.

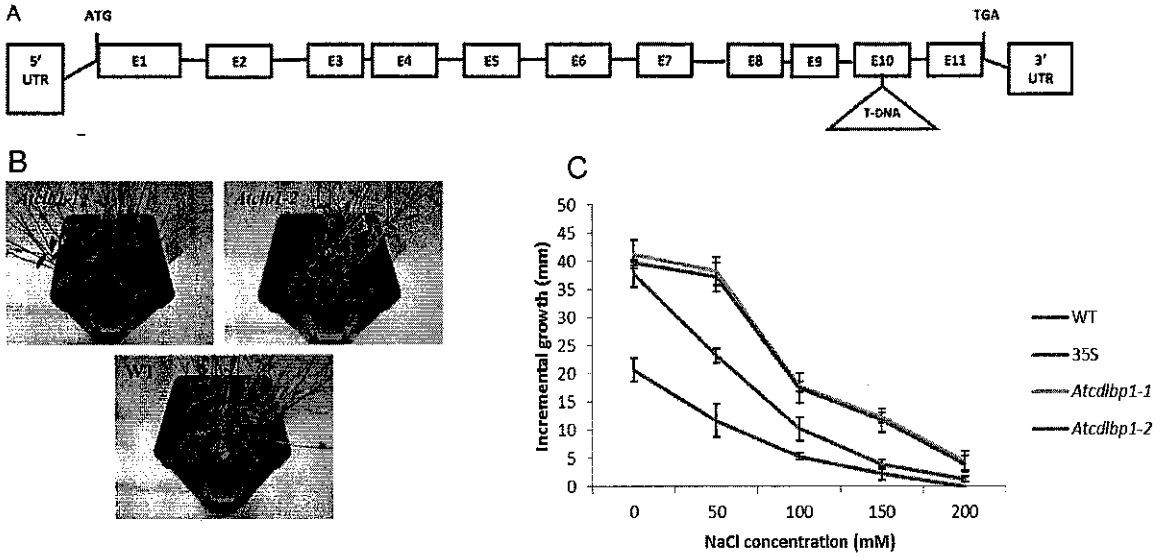

Figure 1. T-DNA insertion knockout lines *Atclb1-1* and *Atclb1-2* (A) exhibited enhanced drought (B) and salt (C) tolerance. (A) Schematic diagram of the *Arabidopsis thaliana* calcium dependent lipid binding protein (*AtCLB*) genomic structure and T-DNA insertion site in the mutant allele *Atclb1*. (B) Phenotype of wild type (*WT*) and knockout mutant lines after three weeks of withholding water. (C) Salt-tolerant phenotype of T-DNA insertion knockout lines *Atclb1-1* and *Atclb1-2*.

Our long-term goals focused on increasing tolerance to environmental stresses (water deficit, cold, salt, gravity). To extend future genetic engineering strategies in the three-year project period, we will investigate involvement of AtCLB in downstream of Ca^{+2} signaling and determine if homologues of *AtCLB* in *Arabidopsis* and valuable crops (tomato, rice) can also negatively regulate abiotic stress responses. By silencing of genes that are close homologous to *AtCLB* in tomato and rice we expect to increase tolerance to abiotic stress in both tested species.

Our team: A team of scientists with different but complementary background from two Arkansas state university campuses will participate in this study. The team consists of Dr. Mariya Khodakovskaya (UALR) and Dr. Vibha Srivastava (UA) and postdoctoral scientists and students from both campuses. This project integrates undergraduate and graduate students throughout the study providing them with educational opportunities and career development.

Each investigator will bring unique expertise in the project. Dr. Khodakovskaya (PI, UALR) is an expert in Plant Genomics and Stress Signal Transduction. She discovered and described functions of AtCLB protein. Dr. Vibha Srivastava's expertise is in Plant Transformation and Transgenic Plant Evaluations. She created two plant transformation technologies that are relevant to this proposal: (1) precise site-specific gene integration technology to allow stable over-expression of foreign gene in plant genome; (2) targeted gene silencing approach based on the expression of truncated transgene fragment.

These technologies have been validated in rice and *Arabidopsis* model plant. Their implementation in tomato will be simple and straight-forward.

Research plan and experimental design:

1. ***Confirmation of the role of conserved C2 domain in stress signaling by characterization of functions of Arabidopsis genes containing C2 domains that are close homologues to C2 domain of AtCLB.***

Justification for experiments: We expect to confirm the role of conserved C2 domain in stress signaling by characterization of functions of *Arabidopsis* genes containing C2 domains that are close homologues to C2 domain of AtCLB. We choose two *Arabidopsis* genes (gene of *Arabidopsis* CLB1-like protein and gene of *Arabidopsis* NTMC2Type2.2 protein) with sequence similarity to C2 domain of AtCLB protein for functional characterization using reverse genetics approach. We expect that T-DNA insertion knockout of both genes will lead to increase of tolerance to abiotic stress in corresponding mutants as it was observed in *Atclb* loss-of-function mutants (de Silva et al., 2011). Thus, mutant analysis may provide additional evidence about involvement of conservative C2 domain in *Arabidopsis* stress signal transduction. This experiment will also help us to identify new genes-targets for genetic crop improvement.

Methods:
Work with Arabidopsis knockout mutants (Clb1-like and *Ntmc2Type2.2* lines)
Loss-of-function mutants for two selected genes (gene of *Arabidopsis* CLB1-like protein and gene of *Arabidopsis* NTMC2Type2.2 protein) will be obtained from the Salk Institute. Established lines will be backcrossed and tested for homozygosity. Confirmed homozygous lines will be tested in a series of stress experiments (salt stress, water deficit stress, ABA stress, and gravity) as described earlier (de Silva et al., 2011).

2. ***Investigation of the subcellular localization of Ca^{2+}-dependent lipid-binding protein with C2 domain (AtCLB) in Arabidopsis cells.***

Justification for experiments: It has also been experimentally demonstrated that many Ca^{2+}-dependent proteins (e.g., calcium-dependent protein kinases, calmodulin-binding protein, and C2 domain protein HyC2d1) are localized not only in the cytoplasm but also in the nucleus (Dammann et al., 2003; Chehab et al., 2004). The ability of C2 domain proteins to migrate between cell compartments allows them to transduce signals inside the cell in a Ca^{2+}-dependent manner (Teruel and Meyer, 2002). This unique ability together with the remarkable property of C2 domain proteins to bind a variety of substrates, including lipids and proteins, puts them among the key regulators of stress signaling. However, the mechanism of transduction of C2 domain-regulated signals from Ca^{2+} to downstream components of plant stress signaling is not clear.

The major aim of this objective is to analyze the intracellular localization of new negative regulator of stress response (AtCLB) in *Arabidopsis* via subcellular and subnuclear fractionation and possible examine protein migration between compartments in a response to stress signal. This task will give us a unique insight about AtCLB involvement in downstream of Ca^{+2} signaling in plant system. We will test our hypothesis about migration of AtCLB between cell compartments (cytoplasm, nucleus) in response to stress signal.

Methods: As first step we will optimize methodology of subcellular fractionation for *Arabidopsis* and confirm purity of the established cell fractions using specific cell compartment markers. Then, we will

identify location of AtCLB protein in cells of plants grown at regular conditions and in cells of plants grown under abiotic stress (salt stress, drought stress). Methodology of proposed subcellular fractionation is summarized on Figure 2.

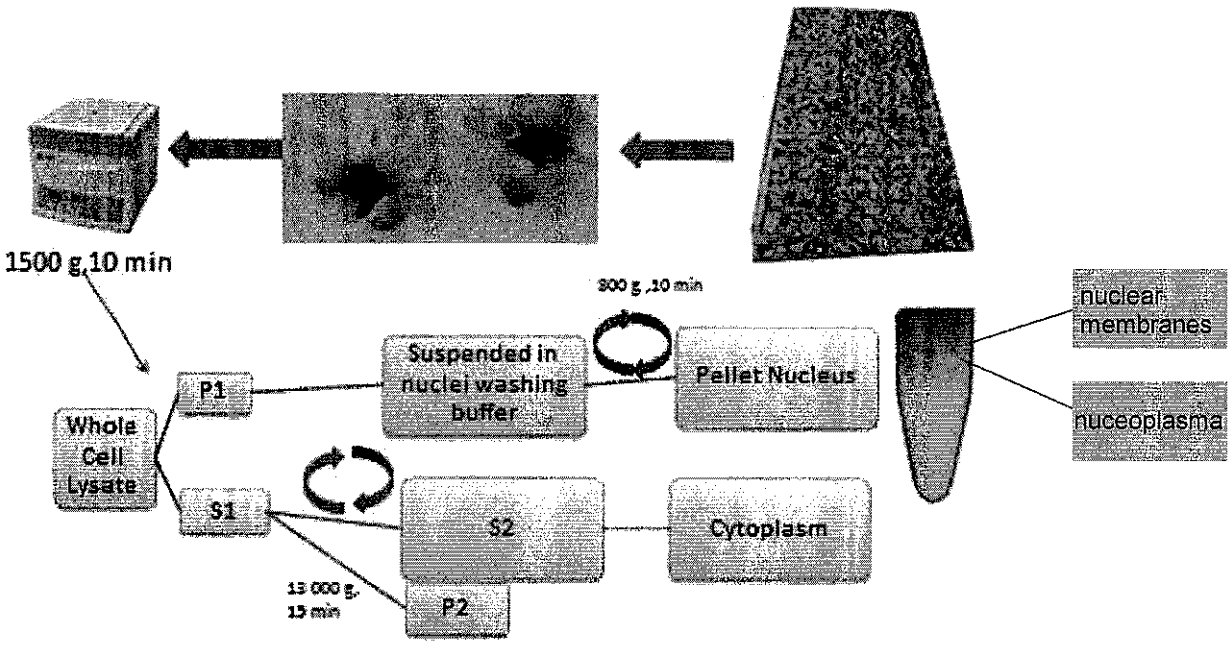

Figure 2. Proposed steps of subcellular fractionation in *Arabidopsis* system. Abbreviations: S – supernatant; P – pellet.

Particularly, we will separate cytoplasm and nucleus of young (2-weeks-old) and mature (5-weeks-old) *Arabidopsis* plants according protocol of Kay et al. (Kay et al., 1972). The purity of fractions will be confirmed using anti-Histone 3 antibody as nuclear marker and LDH enzyme assay for cytoplasmic fraction. After confirmation of the purity of separated fractions we will identify location of AtCLB in established fractions using antibody against AtCLB (antibody is available in laboratory). Standard Western blot will be performed for AtCLB identification. In case if AtCLB will be found in nucleus or in both nucleus and cytoplasm we will try to navigate location of AtCLB on nucleus. We will separate nuclear membranes and nucleoplasm according Philipp et al. (Philipp et al., 1976). Purity of established fractions will be confirmed by using AtSUN2 as nuclear membrane marker and FBL as nucleolar marker. The possible location of AtCLB inside nucleus will be investigated by Western blots using specific AtCLB antibody. After clarification of localization of AtCLB inside cell under regular conditions we will determine the possibility of migration of AtCLB between cell compartments in response to abiotic stress (water deficit stress, salt stress). Water deficit stress will be imposed and evaluated as described early (de Silva et al., 2011). The salt stress will be imposed by addition of NaCl (100 mM) into small-scale hydroponics system used for cultivation of *Arabidopsis* (available in laboratory). Several time points will be used in stress experiments. Control plants and plants exposed to stress will be harvested and used for subcellular fractionation and Western blots as described above (Figure 2). Results of AtCLB presence in different cell fractions originated from control plants and stress-treated plants will be compared and analyzed.

3. Establishment of stress-tolerant crops (rice, tomato) by silencing of AtCLB homologue genes

Justification for experiments: Bioinformatics analysis has revealed that the C2-like domain of Arabidopsis calcium-dependent, lipid-binding protein is identical to the C2 domain sequences of important crops, including tomato and rice. Expression analysis of AtCLB homologues (*SlCLB1* gene in tomato and *OsHP* gene in rice) confirmed the presence of the gene transcripts in root, stem, leaf, flower of these crops (data generated but not shown here). T-DNA knockout mutant analysis reveled that down regulation of AtCLB containing a conserved C2 domain, could confer resistance to various abiotic stress

conditions such as salinity and drought in *Arabidopsis* (de Silva et. al., 2011; Fig.1). Silencing of the AtCLB C2 domain homologues in crops will test the involvement of the conserved C2 domain in stress signaling and potentially bring resistance to abiotic stress conditions.

We will achieve over-expression and silencing of AtCLB homologues in tomato (*SlCLB1* gene) and rice (*OsHP* gene) plants by expressing over-expression or silencer DNA constructs, respectively. We will use rice (cultivar *Nipponbare*) and tomato (cultivar *Micro-Tom*) for this project. DNA constructs to allow overexpression will consist of strong promoters—maize uniquitin-1 (ubi) promoter for rice gene and cauliflower mosaic virus 35S RNA (35S) promoter for tomato genes. Full-length cDNA of *SlCLB1* and *OsHP* will be provided by Dr. Khodakovskaya. These cDNA will be cloned into existing transformation vectors in Dr. Srivastava's laboratory to allow over-expression. Similarly, truncated (~500 bp) fragments of these cDNA will be cloned into the silencing vectors available in Dr. Srivastava's laboratory to achieve silencing of *SlCLB1* and *OsHP* genes in respective host plant. In each case, strong promoters (as mentioned above) will be used to express full-length cDNA or truncated cDNA. Stress experiments with generated transgenic plants will be performed as described below. We expect that silencing of AtCLB homologues will lead to significant increase of stress tolerance in rice and tomato. On the contrary, the over-expression of *SlCLB1* and *OsHP* genes will be resulted in increase of sensitivity to abiotic stress in tomato and rice.

Methods:

Transformation: Standard rice transformation and tissue culture systems will be used as described by Srivastava et al. (2004). Briefly, rice (cv. *Nipponbare*) callus will be generated by plating mature seeds on tissue culture media, and bombarded using particle gene gun (Bio-Rad PDS 1000) to introduce DNA constructs for either gene overexpression or gene silencing. Each construct will contain a selection marker gene, hygromycin resistance or geneticin resistance to allow selection of the transformed cell lines. These lines will be regenerated into shoots using previously published regeneration protocol (Nishimura et al., 2006). The regenerated plants will be grown in greenhouse till maturity and seed collection. The transformation of tomato will be performed as described in previous paper (Khodakovskaya et al, 2010).

Stress experiments with transgenic crops:

Wild type, vector control, overexpressing and silenced plants from each species will be used to evaluate their tolerance for cold, drought and salinity stress in replicated experiments. Salt stress experiments will be carried out in a hydroponics system as described by Zhang and Blumwald (2001). Drought stress will be induced by withholding water for at least 2 weeks (or until the first plants show irreversible damage) from mature plants (wild type and transgenic lines). Changes in all lines will be monitored daily. Leaf relative water content (RWC) will be determined by measuring fresh, turgid and dry weights of leaves as described by Barr and Weatherley (1962). Cold stress in mature plants will be induced by changing the temperature in the plant growth chamber to +4°. Salt stress will be performed as described early (de Silva et al., 2011).

Appendix:

References

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Title: "Molecular Approaches for Enhancing Crop Productivity - Sustainable Crops for Advanced Life Support in Space Exploration"													
PI: Dr. Mariya Khodakovskaya (University of Arkansas, Little Rock)													
Co-PI: Dr. Vibha Srivastava (University of Arkansas)													
Budget - University of Arkansas at Little Rock													
						FIRST YEAR		**SECOND YEAR**		**THIRD YEAR**		**TOTAL UALR COSTS**	
						NASA	Match	NASA	Match	NASA	Match	NASA	MATCH
Personnel													
Salaries													
Mariya Khodakovskaya			**PI**	summer salary		15,866		15,866		15,866		47,598	
TBN			PostDoc			36,000		37,080		38,192		111,272	
TBN			Undergraduate Student			4,000		4,000		4,000		12,000	
TBN			Graduate student			-	13,000	-	13,000	-	13,000		39,000
TBN			Graduate student			-	13,000	-	13,000		13,000		39,000
Fringe benefits						12,256		12,537		12,826		37,619	
Equipment						7,500		10,500		7,000		25,000	
Materials and Supplies						17,000		17,000		17,000		51,000	
Tuition						-	11,000	-	11,000	-	11,000		33,000
Travel	domestic					4,000		4,000		4,000		12,000	
NASA Indirect Costs (21.2% salaries, wages & benefits)						14,442		14,730		15,027		44,200	
UALR Indirect Costs (	41%-21.2%		19.8				22,098.00		22,368.00		22,645.00		67,111.00
TOTAL				**50% from total**		**111,064**	**59,098**	**115,713**	**59,368**	**113,911**	**59,645**	**340,689**	178,111
				Over match									7,767

BUDGET - University of Arkansas	Date:
Proposed to (Sponsor):	
Proposed Start & End Dates:	
UA Lead Investigator:	

Notes from RSSP: (1) Do not type in grey-shaded cells.
(2) Follow instructions in cells with a red corner triangle.
(3) Delete these notes when the budget is final.

SALARIES & WAGES	Base Salary	Type Appoint.	PERSON-MONTHS CAL	AY	SMR	Yr 1 Sponsor	Yr 1 Srivastava	Yr 2 Sponsor	Yr 2 Srivastava	Yr 3 Sponsor	Yr 3 Srivastava	Cumulative Total Sponsor	Cumulative Srivastava	Cumulative Total Cost-Share
	$101,524	12 mo.	0.79				6,691		6,887		7,093		20,671	20,671
PI, summer salary		9 mo.				0		0		0		0	0	0
						0		0		0		0	0	0
Co-PI #1, summer						0		0		0		0	0	0
Co-PI #2, acad. or cal. year		9 mo.				0		0		0		0	0	0
Co-PI #2, summer						0		0		0		0	0	0
												0	0	0
Postdoctoral Associate		12 mo.				0		0		0		0	0	0
Research Associate (staff)		12 mo.				0		0		0		0	0	0
Research Assistant or Tech.		12 mo.				0		0		0		0	0	0
Graduate Assistant (Ph.D.)					mo. @	0						0	0	0
Graduate Assistant (Masters)			1	12	mo. @ 1250	15,000		15,450		15,914		46,364	0	0
Hourly, non-student(s)					hrs @	0		0		0		0	0	0
Hourly, enrolled student			1	600	hrs @ $10	6,000		6,000		6,000		18,000	0	0
Total S&W						*21,000*	*6,691*	*21,450*	*6,887*	*21,914*	*7,093*	*64,364*	*20,671*	20,671

FRINGE BENEFITS	Institutional Rate:												
Faculty/staff academic / calendar salary	27.10%		1,813		1,866		1,922		5,602		5,602		5,602
Faculty summer salary	16.00%	0		0		0		0		0		0	0
GRA(s)	3.10%	465	0	479	0	493	0	1,437	0	0		0	0
Hourly, non-student	7.30%	0		0		0		0		0		0	0
Hourly, enrolled student	0.40%	24		24		24		72		0		0	0
Total FB		*489*	*1,813*	*503*	*1,866*	*517*	*1,922*	*1,509*	*5,602*		5,602		5,602
Total Salaries + Benefits		**21,489**	**8,504**	**21,953**	**8,753**	**22,431**	**9,015**	**65,873**	**26,272**		**26,272**		**26,272**
TRAVEL - Domestic		3,000		3,000		3,000		9,000	0		0		0
TRAVEL - Foreign									0		0		0
MATERIALS & SUPPLIES (not *fees or services, which are "Other"*)		6,000		6,000		5,800		17,800	0		0		0
JOURNAL PUBLICATION FEES								0	0		0		0
OTHER DIRECT COSTS *(itemize by type; insert extra rows if needed.)*								0	0		0		0
								0	0		0		0
Subtotal Other Direct Costs		0		0		0		0	0		0		0
Modified Total Direct Costs (above subtotal costs subject to F&A Cost)		*30,489*	*8,504*	*30,953*	*8,753*	*31,231*	*9,015*	*92,673*	*26,272*		*26,272*		*26,272*
F & A COST (MTDC x RATE):	**21.2%**	**4,566**		**4,564**		**4,755**		**13,985**			**0**		**0**
F & A COST (UNRECOVERED):	**25.3%**		**9,621**		**9,739**		**9,767**		**29,128**		**28,128**		**28,128**
F & A COST (COST-SHARE):	**46.5%**		**3,964**		**4,070**		**4,182**		**12,217**		**12,217**		**12,217**
Modified Total Direct Costs (first $25K of each subaward)		*0*		*0*		*0*		0			0		0
F & A COST (MTDC x RATE)SUB(S):	0%	0		0		0		0	**67,617**		**67,617**		**67,617**

(Direct Costs not subject to F&A Cost, with the exception that the first $25K of each subaward is subject to F&A):

GRA TUITION (estimated 10% increase/Yr)	# Credit Hours: 21	Rate: $380	8,379		8,799		9,240		26,418	0		0	
EQUIPMENT @ > $2500 each									0			0	
PARTICIPANT (TRAINEE) SUPPORT									0			0	
SUBAWARD #1, total (institution):									0			0	
SUBAWARD #2, total (institution):									0			0	
SUBAWARD #3, total (institution):									0			0	
TOTAL DIRECT COST			**38,868**	**8,504**	**39,752**	**8,753**	**40,471**	**9,015**	**119,091**	**26,272**		**26,272**	
TOTAL PROJECT COST			**$43,424**	**$22,079**	**$44,408**	**$22,583**	**$45,226**	**$22,975**	**133,056**	**67,617**		**67,617**	

Note: Any slight discrepancy in calculation is due to spreadsheet rounding.

Tuition
399 8,379 419 8,799 440 9,240

TPC $ 200,673

Match Required $ 66,891

Title: Molecular Approaches for Enhancing Crop Productivity - Sustainable Crops for Advanced Life Support in Space Exploration												
CUMULATIVE BUDGET OF UALR and UA												
PI: Dr. Mariya Khodakovskaya (University of Arkansas, Little Rock)												
Co-PI: Dr. Vibha Srivastava (University of Arkansas)												
DURATION OF PROJECT - 3 YEARS												
						UALR		**UA, FAYETTEVILLE**		**TOTAL FOR TWO CAMPUSES**	
						NASA	Match	NASA	Match	NASA	Match
Salaries						170,870	78,000	64,364	20,671	235,234	98,671
Fringe benefits						37,619		1,509	5,602	39,128	7,837
Equipment						25,000				25,000	
Materials and Supplies						51,000		17,800		68,800	
Tuition							33,000	26,418		26,418	33,000
Travel	domestic					12,000		9,000		21,000	
NASA Indirect Costs (21.2% salaries, wages & benefits)						44,200		13,965		58,165	
UALR Indirect Costs (41	41%-21.2%		19.8				67,111		41,345		108,456
TOTAL						**340,689**		**133,056**		**473,745**	
		Match		**50% from total**			*178,111*		*67,617*		

JUSTIFICATION FOR BUDGET
University of Arkansas at Little Rock

"Molecular Approaches for Enhancing Crop Productivity – Sustainable Crops for Advanced Life Support in Space Exploration"

PI: Mariya Khodakovskaya

Personnel:
Dr. Khodakovskaya, the PI, will be in charge of the project at the University of Arkansas at Little Rock. She will direct all studies during the three years of the project and coordinate collaborative work with Co-PI of project (Dr. Srivastava at UA, Fayetteville). Dr. Khodakovskaya will commit 1 calendar month per year to this project. She has a 9-month appointment and is requesting summer salary of $7,933 for 1 summer month of each year ($23,799 for 3 year) from the sponsor.

A Postdoctoral Scientist (to be hired) will be involved in most of the proposed research activities for the three years of the project. His/her salary (initial salary is $36,000) for a 12-month appointment is requested. An increase in salary of 3% is requested for the second and third years. He/she will be involved in outreach activities.

A Ph.D. students (Mr. Hector Villagarcia and Mr. Mohammad Alimohammadi) will work under the supervision of Dr. Khodakovskaya and in contact with the postdoctoral scientist performing experiments related to objectives 1 and 2. The doctoral students will have funding (as UALR match) for 3 years (12-month appointment, $13,000 per year). They will be involved in all described outreach activities.

An undergraduate student (to be hired) will work together with the graduate student on the portion of the project related to the investigation of the involvement of AtCLB protein in ABA-stress signaling (work with *Arabidopsis* mutants, transformation of *Arabidopsis*, analysis of established transformants). He/she will also be involved in outreach activities. $12,000 is requested to support undergraduate student whole during project.

Fringe benefits: Fringe benefits (total $37,619 for 3 years) were calculated as 18% of summer salary for the PI, 26% of annual salary for the Postdoctoral Scientist, and 1% of the annual salaries for the graduate and undergraduate students.

Equipment: An E24R temperature-controlled benchtop shaker (Fisher, Inc.) is requested ($7,500) for incubation of bacteria used for molecular cloning (incubation of *E. coli* and *Agrobacterium* in liquid medium). An ultralow-temperature (-86^0 C) Fisher freezer ($10,500) is requested for bacteria/DNA, RNA samples storage. Eppendorf ThermoCycler (Mastercycler gradient) ($7,000) is requested for performing all PCR reactions (objectives 1 and 2).

Travel (domestic):
Funds of $12,000 ($4,000 for each year of project) are requested support the doctoral student and Postdoctoral Scientist to attend Annual Meetings of the American Society of Plant Biologists (ASPB), Southern Section of ASPB (ssASPB), and Annual Meeting of American Society for Gravitational and Space Biology (ASGSB) during the course of the project.

Materials and Supplies:

A total of $51,000 is requested for materials and supplies during the duration of this project. These include the following: Antibodies for subcellular fractionation (AtSUN, FBL, Histone-3) reagents for LDH assay, protein purification resins, chemicals for gel electrophoresis (Bio-Rad), agar, medium, solutions and units for small-scale hydroponics system, greenhouse supplies, other chemicals and plastic supplies.

Other direct costs:
Tuitions for 2 graduate students ($33,000 for whole project) as UALR match is requested. Estimated tuition fees for the one doctoral student will be 5,500 per year (2 semesters per year, 9 credits per semester, in-state rate).

Indirect Costs:
21.2% of salaries and fringes ($44,200) are requested in accordance with the policies of NASA. Equipment and tuition costs are exempt of charge.

Total amount requested for project by UALR campus: $340,689

JUSTIFICATION FOR BUDGET
University of Arkansas, Fayetteville

"Molecular Approaches for Enhancing Crop Productivity – Sustainable Crops for Advanced Life Support in Space Exploration"

Co-PI: Vibha Srivastava

Personnel:
Dr. Srivastava, the Co-PI, will be in charge of the project at the University of Arkansas, Fayetteville. She will focus on Objective 3 (transformation of rice and tomato) during the three years of the project and coordinate collaborative work with the PI of project (Dr. Khodakovskaya at UALR). Dr. Srivastava will commit 10% of her time to this project. No salary is requested for her from the sponsor.

A graduate student (to be hired) will work under the supervision of Dr. Srivastava in performing experiments related to Objective 3. A stipend of $15,000 per year is requested for the graduate student, a part of which will be matched by UAF. He/she will be involved in all described outreach activities.

An undergraduate student (to be hired) will work together with the graduate student on plant tissue culture and transformation. He/she will also be involved in outreach activities. A total of $6,000 per year is requested to support undergraduate student hourly wages.

The budget includes an annual 3% increase in GA salaries and fringe.

Fringe benefits: Fringe benefits rates for UAF are 27.10% for staff, 3.10% for graduate student, and 0.4% for undergraduate students.

Equipment: No equipment is requested for UAF.

Travel (domestic):
Funds of $9,000 ($3,000 for each year of project) are requested to support the travel of Co-PI and graduate student to attend Annual Meetings of the American Society of Plant Biologists (ASPB) and Society of In Vitro Biology during the course of the project.

Materials and Supplies:
A total of $17,800 is requested for materials and supplies for 3 years period of this project. These include the following: plant tissue culture media and plastic supplies, particle bombardment supplies, PCR enzymes and chemicals, radioisotopes and molecular biology chemicals, and greenhouse supplies.

Other direct costs:
Tuition ($26,418 for whole project) is requested. Estimated tuition fees for the graduate student will be ~$8,379 per year (21 credit hours x $380/hr with a 10% estimated annual increase).

Facilities And Administrative Costs (formerly Indirect Costs)
46.0% of MTDC in accordance with F&A Rate Agreement approved by DHHS.

Total amount requested for project: $133,056 is requested from NASA EPSCoR and $67,616 will be provided as cost share by UAF

Mariya Khodakovskaya

Education

Far Eastern Federal University (Vladivostok, Russia)	Biology	M.S. (Diploma), 1992
Institute of Biology and Soil Science, Russian Academy of Sciences (Vladivostok, Russia)	Plant Physiology	Ph.D., 1997
University of Connecticut (Storrs, CT, USA)	Biotechnology	Postdoctorate, 2004

Professional Experience

From 07/2012- Associate Professor, Dept. of Applied Science, University of Arkansas at Little Rock (AR, USA)

07/2008- 06/2012 - Assistant Professor, Dept. of Applied Science, University of Arkansas at Little Rock (AR, USA)

01/2005-06/2008 - Researcher, Dept. of Plant Biology, North Carolina State University, (NC, USA)

9/2000-12/2004 - Visiting Scientist of University of Connecticut (CT, USA)

2000 - Senior Researcher and Scientific Secretary, Institute of Biology and Soil Science, Russian Academy of Sciences (Russia)

11/1998- 11/1999 - Visiting scientist, Institute of Molecular Biology and Genetics, Chonbuk National University (S. Korea)

07/1992-11/1998 - Engineer, Junior Researcher, Scientific Researcher , Senior Researcher, Institute of Biology and Soil Science, Russian Academy of Sciences (Russia).

Publications (over 30 peer-reviewed publications; 44 conference proceedings)

Five publications most closely related to this proposal

1. Villagarcia H, Morin A-C, Shpak ED*, **Khodakovskaya M*** Modification of tomato growth by expression of truncated ERECTA protein from *Arabidopsis thaliana Journal of Experimental Botany* 2012, doi:10.1093/jxb/ers305
2. **Khodakovskaya M*,** Kim B-M, Jong Kim JN, Biris AS, Alimohammadi M, Dervishi E, Cernigla CE. Carbon nanotubes as fertilizers: effects on tomato growth, reproductive system and soil microbial community. *Small,* 2012, DOI: 10.1002/smll.201201225
3. Alimohammadi M, de Silva K, Ballu C, Ali N, **Khodakovskaya M*** Reduction of inositol (1,4,5)–trisphosphate affects overall phosphoinositol pathway and leads to modifications in light signaling and secondary metabolism in tomato plants. *Journal of Experimental Botany*, 2012, 63(2): 825–835.
4. de Silva K, Laska B, Brown C, Winter Sederoff H, **Khodakovskaya M*** Arabidopsis thaliana calcium-dependent lipid-binding protein (AtCLB) – a novel repressor of abiotic stress response. *Journal of Experimental Botany*, 2011, 62(8): 2679-2689.
5. **M. Khodakovskaya,** C. Sword, I. Perera, W. Boss, C. Brown, H. Sederoff* Expression of inositol-1,4,5-triphosphate metabolism affects drought tolerance, carbohydrate metabolism, and phosphate-sensitive biomass increases in tomato. *Plant Biotechnology Journal,* 2010, 8: 170-183.

Patents:

- R. McAvoy, M. Khodakovskaya, Yi Li "Method and composition for increasing branching and flowering response in plants" Patent 7741548 Issued on June 22, 2010.
- McAvoy, M. Khodakovskaya, Yi Li "Method and composition for increasing plant survival and viability under cold and/or dark storage conditions". Submitted
- M. Khodakovskaya, A. Biris "Method of using carbon nanotubes to affect seed germination and plant growth" Submitted

Awards of Research Grants:

Served in 9 external funded projects (as PI in 6 of them, as Co-PI in 3 of them). During 2008-2012 total dollar amount of awards is $4.426,472, Khodakovskaya's portion is ~ $767,093

Teaching experience: **Courses:** Tissue Culture and Genetic Engineering (class ASCI 7399-03); Recombinant DNA Methods and Applications (class ASCI 7386); Biochemistry of Biological Molecules (class ASCI 7375)

Total graduate student supervised: 7; **Total undergraduates supervised:** 6

Vibha Srivastava

Education

Ph.D.	1991	Life Sciences, Jawaharlal Nehru University, New Delhi, India
M.S.	1986	Biochemistry, G. B. Pant Univ. Agric & Tech., Pantnagar, India
B.S.	1983	Chemistry, Dayalbagh University, Agra, India

Professional Experience

2012-	Professor, Dept. of Crop, Soil, and Environmental Sciences, Department of Horticulture, Univ. of Arkansas, Fayetteville, AR.
2006-2012	Associate Professor, Dept. of Crop, Soil, and Environmental Sciences, Department of Horticulture, Univ. of Arkansas, Fayetteville, AR.
2001-2006	Assistant Professor, CSES, HORT, Univ. of Arkansas, Fayetteville, AR.
1996-2001	Post-Doctoral Research Associate, Univ. of California-Berkeley, CA
1993-1996	Post-Doctoral Research Associate, Univ. of Florida, Gainesville, FL

Areas of Research Specialization

My research group focuses on genetic engineering of plants using rice as a model crop. In this project, we develop technologies for both stabilizing the expression of foreign genes and suppressing endogenous genes to modulate plant phenotype. In addition, we incorporate existing techniques of the removal of antibiotic resistance genes from the transgenic plants in order to develop 'clean' transformation technologies. Using molecular and genetic approaches, we study the expression of foreign genes introduced by the newly developed methods to evaluate the efficacy of the technology. Thus, my research covers both basic and applied aspects of plant genome modifications.

Teaching Experience

Courses:	Teaching 1 graduate course (CSES 5233) and frequently teaching special topics (CSES 504V) course.
Advising:	Advisor of 4 Ph.D., 6 M.S. and 3 undergraduate students. Coordinator of undergraduate minor program, Crop Biotechnology (CPBT-M). Advisor of 4 Postdoctoral Associates and 4 Research Specialists

Grants

Federal:	6 Research grants, 1 conference grant. Total research funding: $1,927,463
State:	4 Research grants, 1 teaching grant. Total research funding: $528,815

Patent

U.S. Patent No. 6,114,600 (Ow D and Srivastava V) *"Resolution of Complex Integration Patterns to Obtain Single Copy Transgenes".*

Publications

Journal articles:	33
Invited review articles:	4
Book review:	1
Book chapters:	3
Conference proceedings:	27