Fiscal Year:	FY 2022	**Task Last Updated:**	FY 06/29/2023
PI Name:	Zhang, Ye M.D., Ph.D.		
Project Title:	Effect of Simulated Solar Particle Events and Galactic Cosmic Rays on Crop Growth and Development		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	Ye.Zhang-1@nasa.gov	**Fax:**	FY
PI Organization Type:	NASA CENTER	**Phone:**	321-861-3253
Organization Name:	NASA Kennedy Space Center		
PI Address 1:	Utilization and Life Science Office		
PI Address 2:	Mail Code UB-A		
PI Web Page:			
City:	Kennedy Space Center	**State:**	FL
Zip Code:	32955	**Congressional District:**	8
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2017 HERO 80JSC017N0001-Crew Health and Performance (FLAGSHIP1, OMNIBUS). Appendix A-Flagship1, Appendix B-Omnibus
Start Date:	10/01/2018	**End Date:**	09/30/2022
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
Contact Monitor:	Norsk, Peter	**Contact Phone:**	
Contact Email:	Peter.norsk@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: End date changed to 9/30/2022 per PI (Ed., 5/5/23) NOTE: End date changed to 9/30/2021 per PI (Ed., 8/29/20) NOTE: Change to period of performance of 10/1/2018-9/30/2020 per PI (previously 6/12/2018-9/30/2019 per HRP)--Ed., 3/14/2019		
Key Personnel Changes/Previous PI:	N/A		
COI Name (Institution):	Douglas, Grace Ph.D. (NASA Johnson Space Center) Feiveson, Alan Ph.D. (NASA Johnson Space Center) Massa, Gioia Ph.D. (NASA Kennedy Space Center) Plante, Ianik Ph.D. (KBR/NASA Johnson Space Cent)		
Grant/Contract No.:	Internal Project		
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Performance Goal Text:			

Task Description:	With the renewed goal of crewed Mars exploration, continuous fresh food production during long-term deep space missions may be a critical addition to the processed food system to meet astronauts' nutritional requirements and to provide a psychological countermeasure for crew in the isolation and confinement of deep space. However, critical knowledge gaps, such as the impact of deep space radiation on plant crops, must be addressed prior to dependence on crop systems for any portion of a deep space food system. Although the biological impact of simulated space radiation on mammalian cells and rodents has been investigated extensively, the effects of long-term exposure to deep space radiation on crop seeds and plant growth has yet to be characterized. We must investigate the impact of deep space radiation on crop foods to either confirm that nutritious and high quality produce can be reliably grown in deep space or to provide a baseline to guide future radiation countermeasure development for crop foods. We propose to investigate the effect of simulated Solar Particle Events (SPEs) and Galactic Cosmic Rays (GCRs) on several model crops at different growing stages using the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Lab (BNL). The selected crops have been grown successfully under spaceflight-like conditions (temperature, air, humidity, lighting, etc.) in ground analogs, and either have been or will be grown on International Space Station (ISS) for crew consumption. The additional knowledge of the response of each crop to deep space radiation will help identify candidate traits for successful growth on deep space vehicles. The effect of the simulated space radiation environment on crop seeds viability, seedling development, and the impact on nutritional value of fresh produce will be determined.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This project is to address the HRP Risk and Gaps: Risk of Performance Decrements and Crew Illness due to an Inadequate Food and Nutrition. Gap Food-01: We need to determine how processing and storage affect the nutritional content of the food system. (Previous Title: AFT1) Gap Food-03: We need to identify the methods, technologies, and requirements that will deliver a food system that provides adequate safety, nutrition, and acceptability for proposed long-duration Design Reference Mission operations. (Previous title: AFT4) Gap Food-04: We need to identify tools or methods that can be used or developed to help mission planners and vehicle developers determine the most effective combination of methods, technologies, and requirements to balance crew food system needs with vehicle resources. The goal of this study is to provide baseline evidence to guide future research and radiation countermeasure development for crop foods. The results will also benefit life on Earth to provide insights to improve agriculture and crop production.
	SUMMARY OF TASK PROGRESS Food crops grown in-mission have the potential to supplement crew nutritional requirements and to act as a psychological countermeasure for the crew by providing a familiar aspect of Earth in the isolation and confinement of deep space. Seeds and plant growth supplies may also be sent ahead of the crew and would need to remain viable for the length of the mission. Deep space radiation is one of the major factors that could impact viability of food crops. During a long-duration deep space mission, seeds and plants growing in space will be exposed to 1-2 mSv/day space radiation during the transit and approximately half this value on planetary surfaces. The estimated average quality factor of space radiation on the Martian surface is 3.05, compared with 3.82 estimated during transit, primarily due to the shielding variance. The total estimated mission dose equivalent is about 1.01 Sv for a round trip Mars surface mission consisting of 180 days (each way) and 500 days on the Martian surface. In this study, seeds from model plants, Arabidopsis thaliana and three crops (mizuna, lettuce, and tomato), were exposed to simulated GCR and SPE radiation scenarios to determine the impact of the space radiation environment on seeds and their ability to germinate and develop. 1. Methodology - Radiation Scenarios, Plant Model Organisms, and Post-Irradiation Analyses: Arabidopsis seeds (Col-0) were prepared from our in-house cultures, while the crop seeds were purchased from commercial vendors. Seeds were exposed to different SPE and GCR scenarios at acute or lower dose rate (LDR) in the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL). The irradiated seeds were then shipped back to NASA Kennedy Space Center (KSC) for post-irradiation grow-outs and analyses. 2. Milestones and Timelines: Due to the pandemic, we experienced significant delay (more than one year) on NSRL experiments, and RNA/DNA isolation from RNAlater-preserved samples, as well as RNA sequencing (RNAseq) analyses. RNA sequencing was finally completed in May 2022, and the downstream analyses were completed by the end of FY22. The project was complete by the end of 2022. 3. Brief Experimental Protocols: A. Preparation of seeds: Sanitized seeds were used for all the experiments. A germination test was conducted after each sanitization procedure to ensure that the seeds were in good and comparable quality across all the experiments. Dry seeds were used for GCR experiments, whereas imbibed (hydrated) seeds were used for SPE experiments. Seeds (as well as non-irradiated control seeds) were exposed to radiation (or comparable control environment) in dark. Seeds were always stored and transported in dark at 4°C before and after the exposure. B. Early seedling development on petri dishes: Two days after irradiation, control and irradiated seeds were planted onto petri dishes (6-12 seeds per dish depending on the species) containing 0.5x Murishige and Skoog (MS)-based agar media. Growth conditions were set at 22°C, 40-45% RH, 450 ppm CO_2, and 150-300 µmol·m2·s-1 light (depending on the species) with 16hr/8hr photoperiod. Seedlings were imaged at 6-7 days after planting (DAP) for Arabidopsis, and 5-10 DAP for mizuna, lettuce, and tomato (depending on the species) for morphological analyses to evaluate signs of stress and deformation. Root length was measured using Rootnav v1.8.1 software. Plant tissue samples were then preserved in RNAlater for transcriptomic and gene expression analyses. RNA extracts were isolated from whole plant tissue samples from each individual petri dish following the recommended procedure provided by the manufacturer. Arabidopsis, mizuna, and tomato seedlings grown from seeds exposed to LDR, GCR, or SPE exposure were subjected to RNAseq transcriptomic analysis using the Case Western Reserve University Genomics Core. C. Long-term grow-outs: For SPE exposure, seeds embedded in 0.5% MS-based agar plates were exposed to LDR SPE. Dry seeds were used for simulated GCR exposure. Control and irradiated seeds were then planted in peat plugs for 10 to 16 days (depending on the species). Seedling size and weight were measured. Young plants were then randomly selected and transferred to pots for a total of 28 days (mizuna and lettuce, 18 plants per treatment group) or 91-110 days (tomato, 9 plants per treatment group) grow-out. All plants were maintained under space-vehicle relevant, controlled environment conditions at 3000 ppm CO_2, 50% RH, and 23°C, and a 16/8 h photoperiod with 300 µmol·m-2·s-1 light.

Task Progress:

At the harvest, plant/fruit morphometrics and edible fresh mass were measured. In addition, nutritional analyses were performed for mizuna, lettuce, and ripe tomato fruits. Leave samples and tomato seeds were also collected for potential future molecular and multi-generation analyses.

D. Transcriptomic analysis: Libraries were prepared from purified RNA (0.3µg) using the TruSeq Stranded Total RNA Library Prep with Ribo-Zero Globin (Illumina, 20002613) according to the manufacturer's protocol. Quality control of purified RNA and RNA libraries was carried out using the Fragment Analyzer System (Agilent). Raw DE multiplexed fastq paired end read files were trimmed of adapters and filtered using the program skewer to throw out any reads with an average phred quality score of less than 30 or a length of less than 36. Trimmed reads were then aligned using the HISAT2 aligner to the Homo sapiens NCBI reference genome assembly version GRCh38 and sorted using SAM tools. Aligned reads were counted and assigned to gene meta-features using the program featureCounts as part of the Subread package.

RESULT SUMMARY

1. Exposure to simulated GCR or SPE has significant impact on seed quality and plant development from the irradiated seeds, causing reduced viability, cotyledon deformation, discoloration, shortened root length, and reduced produce quality and yield. The severity of impact is species or cultivar dependent.

2. Based on our GCR and SPE data, the level of radiation impact depends on radiation quality, quantity, and the plant type. For both GCR and SPE, 40 cGy shows some effects, but to a much lesser extent compared with 80 cGy, which may be considered as the "maximum permissible exposure" for these seed types. However, 40 cGy SPE exposure on imbibed tomato seeds still resulted in a 40% reduction of fruit production.

3. Transcriptomic analysis of Arabidopsis seedlings revealed dose-dependent alterations in gene expression profiles and pathways showing upregulated DNA repair and stress responses, and downregulated metabolic signaling pathways.

4. SPE may cause synergic detrimental effects on seeds, which are constantly exposed to GCR during a long-term deep space mission, as well as the plants grown from these seeds. Radiation shielding is highly recommended for deep space long-term seed storage, and for protecting plants from SPE caused damages.

5. These radiation impacts potentially affect the ability of crops to respond to other space environmental stressors (e.g., altered gravity, constraint environment with higher CO2 and volatile organic compounds/VOCs, water stress, long-term seed storage under unfavorable conditions, unique microbiome, etc.).

Sample Archive Tissue samples, 2nd generation tomato seeds, and RNA and DNA samples (collected from the experimental plants) will be archived for potential future analyses (e.g., mutation, grow-outs of the 2nd generation tomato seeds, or more complete transcriptomic analyses).

PUBLICATIONS

Peer-reviewed Publication: Y. Zhang, J.T. Richards, A.H. Feiveson, S.E. Richards, S. Neelam, T.W. Dreschel, I. Plante, M. Hada, H. Wu, G.D. Massa, G.L. Douglas, and H.G. Levine. Response of Arabidopsis thaliana and Mizuna Mustard Seeds to Simulated Space Radiation Exposures. Life, 2022, 12, 144. https://

Publications in Preparation: 1. *A. Dixit, *B. Richardson, A. Meyers*, J.T. Richards, S. Richards, S. Neelam, M. Hada, H.G. Levine, M. Cameron, and Y. Zhang. Early Transcriptomic Response in Seedlings Exposed to Simulated Galactic Cosmic Ray Irradiation. Frontiers in Plant Science, 2023 (invited). *Contributing equally 2. J.T. Richards, L.E. Spencer, J.J. Torres, J.A. Fischer, G.D. Massa, G.L. Douglas, and Y. Zhang. Survival, Morphology, and Nutritional Value in Plants Developed from Seeds Exposed to Simulated Space Radiation. Life, 2023 (invited). 3. *A. Dixit, *B. Richardson, *A.D. Meyers, J.T. Richards, B. Tamilselvan, G.D. Massa, G.L. Douglas, C.M. Cameron, M.J. Cameron, and Ye Zhang. Cross-Species Transcriptomic Profile Changes in Seedlings from Seeds Exposed to Simulated Space Irradiation. Plant, 2023 (invited). Contributing equally.

Oral Presentations in Conferences: Oral presentations in the American Society for Gravitational and Space Research (ASGSR) Annual Conference (2018, 2019, and 2020), the 2021 NASA Human Research Program (HRP) Investigators' Workshop (IWS), and the Committee on Space Research (COSPAR) meeting (2018 and 2021). Poster presentations in the 2022 ASGSR and the 2022 HRP IWS.

Ed. Note: See Cumulative Bibliography for more information.

Bibliography Type:	Description: (Last Updated: 01/08/2024)
Abstracts for Journals and Proceedings	Zhang Y. "Changes in plants developed from imbibed seeds irradiated by simulated solar particle events." 36th Annual Meeting of the American Society for Gravitational and Space Research, Virtual Meeting, November 5-6, 2020. Abstracts. 36th Annual Meeting of the American Society for Gravitational and Space Research, Virtual Meeting, November 5-6, 2020. , Nov-2020
Abstracts for Journals and Proceedings	Zhang Y. "Changes in plants feveloped from dry seeds irradiated by simulated galactic cosmic radiation." Committee on Space Research (COSPAR) 2021-Hybrid, 43rd Scientific Assembly, Sydney, Australia, January 28-February 4, 2021. Abstracts. Committee on Space Research (COSPAR) 2021-Hybrid, 43rd Scientific Assembly, Sydney, Australia, January 28-February 4, 2021. Abstract F2.1-0015-21. https://www.cospar-assembly.org/abstractcd/COSPAR-21/ , Jan-2021
Articles in Peer-reviewed Journals	Dixit AR, Meyers AD, Richardson B, Richards JT, Richards SE, Neelam S, Levine HG, Cameron MJ, Zhang Y. "Simulated galactic cosmic ray exposure activates dose-dependent DNA repair response and down regulates glucosinolate pathways in Arabidopsis seedlings." Front Plant Sci. 2023 Dec14;14:1284529. https://doi.org/10.3389/fpls.2023.1284529 , Dec-2023

Articles in Peer-reviewed Journals	Zhang Y, Richards JT, Feiveson AH, Richards SE, Neelam S, Dreschel TW, Plante I, Hada M, Wu H, Massa GD, Douglas GL, Levine HG. "Response of Arabidopsis thaliana and Mizuna mustard seeds to simulated space radiation exposures." Life (Basel). 2022 Jan 19;12(2):144. https://doi.org/10.3390/life12020144 ; PMID: 35207432; PMCID: PMC8879990 , Jan-2022