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Project Title:	Zhang, Ye M.D., Ph.D. Effect of Simulated Solar Particle Events and Galactic Cosmic Rays on Crop Growth and Development		
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Organization Name:	NASA Kennedy Space Center		
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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)		
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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. **Task Description:** 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:** Introduction : A long-duration mission to Mars will require humans to live in space for up to 2.5 years. The distance from Earth will prevent resupply, thus food may have to be sent years ahead of the crew. Under expected ambient storage conditions, nominally, shelf-stable food systems may be five years old by the end of the mission, and critical

storage conditions, nominally, shelf-stable food systems may be five years old by the end of the mission, and critical nutrients and quality factors may degrade over this period of time (Cooper et al., 2017; Catauro et al., 2012). This possibility creates a critical risk to the provision of adequate food and nutrition to support crew health and performance through such missions. 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 affect 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 deep space radiation and approximately half this value on planetary surfaces (Huff et al., 2016; Cucinotta et al., 2006; Zeitlin et al., 2013). The average quality factor on the Martian surface is 3.05, compared with 3.82 measured during transit primarily due to the shielding variance (Zeitlin et al., 2013; Hassler et al., 2014). There is a total estimated mission dose equivalent of ~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 galactic cosmic rays (GCR) and solar particle event (SPE) radiation scenarios to determine the impact of the space radiation environment on seeds and their ability to germinate and develop.

## Progress

1. 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. The seed conditions, radiation scenarios, and grow-out conditions were designed to be relevant to deep space missions. Seeds were exposed to different radiation scenarios in the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Lab (BNL). Dry seeds were exposed to simulated GCRs (40 or 80 cGy), whereas, imbibed seeds were exposed to SPE like radiation (40, 80, or 200 cGy). The exposures were delivered either acutely or at lower-dose rate. The irradiated seeds were then shipped back to Kennedy Space Center (KSC) for post-irradiation grow-outs and analyses. Grow-out endpoints were 1. Up to 10 days for analyzing germination, viability, and early seedling development on plates, depending on the species; and 2. 28-100 day grow-outs for mizuna, lettuce, and tomato from low-dose rate (LDR) irradiated seeds. Post-irradiation analyses were conducted to evaluate morphometric, transcriptomic, and nutritional changes.

2. Milestones and Timelines: We have successfully completed most planned experiments except for the 2020 NSRL summer run experiment. Transcriptomic and gene expression analyses are ongoing.

3. Brief Experimental Protocols:

A. Preparation of seeds: Sanitized seeds were used for all the experiments. Germination test was conducted after each sanitization procedure to ensure that the seeds were in good and comparable quality across all the 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 0.5% Murishige and Skoog (MS)- based agar media. Growth conditions were set at 22°C, 40-45% RH, 450 ppm CO2, and 150-300 µmol·m-2·s-1 light (depending on the species) with 16 hr/8 hr 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 RNA later for transcriptomic and gene expression analyses. RNA extracts were isolated from whole plant tissue samples from each individual petri dish using the Plant RNeasy Mini Kit (Qiagen) and a homogenizer following the recommended procedure provided by the manufacturer. Selected groups were subjected to transcriptomic analysis using the Case Western Reserve University Genomics Core.

C. Long-term grow-outs: Control and irradiated seeds were planted in peat plugs for 10 to 14 days (depending on the species). Seedling size and weight were measured. Viable seedlings were then randomly selected and transferred to pots for a total of 28 day (mizuna and lettuce) or 91-110 day (tomato) grow-out. All plants were maintained under space-vehicle relevant controlled environment conditions at 3000 ppm CO2, 50% RH, and 23°C, and a 16/8 h

Task Progress:

	photoperiod with 300 µmol·m-2·s-1 light. At the harvest, plant/fruit morphometric 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.
	Results Summary
	Exposure to simulated GCR or SPE has significant impact on seed quality and plant development from the irradiated seeds at 80 cGy. The severity of impact is species or cultivar dependent. Seedlings from irradiated seeds showed reduced viability, cotyledon deformation, discoloration, and shortened root length. The extent of impact also depends on radiation quality, quantity, and fluence. 40 cGy shows some effects, but to a much lesser extent, 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.
	Publications: Oral presentations in the American Society for Gravitational and Space Research Annual Conference (2018 and 2019) and the COSPAR (Committee on Space Research) meeting in 2018.
	References:
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Bibliography Type:	Description: (Last Updated: 01/08/2024)
Abstracts for Journals and Proceedings	<ul> <li>Richards JT, Spencer LE, Torres JJ, Fischer JA, Hada M, Plante I, Feiveson AH, Wu H, Massa G, Douglas GL, Zhang Y. "Changes in Plants Developed from Dry Seeds Irradiated by Simulated Galactic Cosmic Radiation." Presented at the 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019.</li> <li>Abstracts. 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019.</li> </ul>
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