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## Abstract:

The space colonization Era has just begun and in the near future the first colonies will rise on the Moon with the ambition of reaching Mars. The colonization of space will depend on the ability to provide fresh and healthy foods for the crew members, with minimal re-supply from Earth. This result can be achieved through Bioregenerative Life Support Systems (BLSS) in which plants and microorganisms could be used to produce oxygen and also food, and to treat human and vegetable waste. The efficient development of plants in space is constrained by different factors (i.e., microgravity, ionizing radiation, hypobaria, etc.) that are recognized to deeply influence organism's growth at molecular, morpho-structural and physiological levels.

Studies on the effects of space environment on plants growth and development have already been carried out, but there is a lack of information about the cumulative effects of the different stresses on plant physiology particularly at the seedling level (i.e., ionizing radiation and microgravity together). It is well known that resistance to environmental factors of plant seeds is stronger due to structural and metabolic traits compared with organs at different life stages and, since the most harmful moment of plant development is represented by the seed to seedling transition, we have chosen microgreens to study the effect of different stress combinations.

Microgreens are fast-harvesting vegetables able to accumulate high concentration of phytochemicals such as antioxidants and vitamins, and we study them as a model food production system inside a space colony that would help astronauts face the problems of life in extreme environments.

The ongoing activities are conducted inside the "Calliope" <sup>60</sup>Co gamma irradiation facility (ENEA "Casaccia", Rome, Italy) with microgreens growing inside a confined controlled environment on a 2D clinostat to simulate microgravity and chronic exposure of seeds and seedlings to gamma rays. To monitor the combined effects of both stressors, morphometric and non-destructive analysis (Fluorometric, Thermal, visible light imaging) will be conducted during the cultivation process. Preliminary results of the cumulative stress effects on microgreens plants will be studied.

## Space environment simulation

### Microgravity:

Microgravity can be simulated with the use of a 2D or 3D (1 or 2 axis) clinostat that maintains the plants in continuous rotation simulating weightlessness at root level due to the absence of statoliths sedimentation.

### Ionizing radiation:

To simulate radiation effects of 15 days satellite flight on plant growth, we will expose *Lepidium sativum* from seed to seedlings stage to an equivalent total dose of 36Gy gamma rays on-ground (dose rate 2,4Gy/day). Experiments will be held inside "Calliope" <sup>60</sup>Co Gamma irradiation facility at ENEA Casaccia Research Center for chronic irradiation test.

### Hypobaria:

We will test plant growth and development in hypobaric condition on a small scale inside a transparent box maintained at 0.5atm, the pressure inside the cubesat during the flight to avoid problems in outer space.

### Morphometric & growth analysis:

Leaf area and hypocotyl length measurements will be collected via visible image analysis during growth. Fresh and dry weight will be measured with the use of a thermobalance.

## Plant growth analysis

### Fluorimetric and hyperspectral analysis:

Non-Destructive analysis will be performed using Multiplex (Portable fluorimeter, Force-A, France) for the measurement of Chlorophyll and Anthocyanins fluorescence indexes and by using Specim IQ (Hyperspectral camera, Spectral Imaging Ltd., Finland) to evaluate the effect of multiple stress on plants based on their different spectral response curves.



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