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Response of *Phaseolus vulgaris* L. plants to low-let ionizing radiation: Growth and oxidative stress ☆

C. Arena [a,*], V. De Micco [b], G. Aronne [b], M. Pugliese [c], A. Virzo De Santo [a], A. De Maio [a]

[a] Department of Biology, University of Naples Federico II, Via Cinthia 4, 80126 Naples, Italy
[b] Department of Agriculture, University of Naples Federico II, Via Università, Portici, 100-80055 Naples, Italy
[c] Department of Physics, University of Naples Federico II, Via Cinthia 4, 80126 Naples, Italy

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ABSTRACT

The scenarios for the long-term habitation of space platforms and planetary stations involve plants as fundamental part of Bioregenerative Life Support Systems (BLSS) to support the crew needs. Several constraints may limit plant growth in space: among them ionizing radiation is recognized to severely affect plant cell at morphological, physiological and biochemical level. In this work, plants of *Phaseolus vulgaris* L. were subjected to four different doses of X-rays (0.3, 10, 50 and 100 Gy) in order to assess the effects of ionizing radiation on this species and to analyze possible mechanisms carried out to overcome the radiation injuries. The effects of X-rays on plant growth were assessed by measuring stem elongation, number of internodes and leaf dry weight. The integrity of photosynthetic apparatus was evaluated by photosynthetic pigment composition and ribulose 1,5-bisphosphate carboxylase (Rubisco) activity, whereas changes in total antioxidant pool and glutathione S transferase activity (GST) were utilized as markers of oxidative stress. The distribution of phenolic compounds in leaf tissues as natural shielding against radiation was also determined.

Irradiation of plants at 0.3 and 10 Gy did not determine differences in all considered parameters as compared to control. On the contrary, at 50 and 100 Gy a reduction of plant growth and a decrease in photosynthetic pigment content, as well as an increase in phenolic compounds and a decrease in total antioxidant content and GST activity were found. Only a slight reduction of Rubisco activity in leaves irradiated at 50 and 100 Gy was found. The overall results indicate *P. vulgaris* as a species with a good potential to face ionizing radiation and suggest its suitability for utilization in BLSSs.

1. Introduction

In space, the health of organisms is strongly threatened by the exposure to protons and high-Z-energy (HZE) ions. The radiation exposure in the space environment may increase cancer morbidity or mortality risk in astronauts [1,2]. These risks may be also influenced by other space flight factors including microgravity. Actually the radiation protection of humans in space represents a challenge for extraterrestrial environment exploration and many studies have been addresses to this issue [3,4]. Beside the problem

Abbreviations: (BLSSs), Bioregenerative Life Support Systems; (GST), glutathione S transferase; (HZE), high-Z high-energy ions; (Rubisco), ribulose 1,5-bisphosphate carboxylase.

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* Corresponding author. Tel.: +39 081 679 173; fax: +39 081 679233.
E-mail addresses: c.arena@unina.it, carmenarena@libero.it (C. Arena), demicco@unina.it (V. De Micco), aronne@unina.it (G. Aronne), pugliese@na.infn.it (M. Pugliese), virzo@unina.it (A. Virzo De Santo), andemaio@unina.it (A. De Maio).

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related to human permanence in space, in order to reduce the need for supplying life support materials for long-term manned missions, it could be interesting to evaluate the potentiality of plant survival and reproduction in extra-terrestrial environments.

Plants are key organisms in Bioregenerative Life Support Systems (BLSSs) in space because they have a fundamental role in the regeneration of resources and in the psychological support of the crew. In space, plant growth is controlled by environmental factors also acting on the Earth, and by new environmental factors such as microgravity and ionizing radiation [5,6].

Ionizing radiations induce genetic, morphological, physiological and biochemical changes, that vary with plant species, irradiation dose and type [6]. Due to technical and financial constraints, the performance of ground-based experiments under simulated space conditions is a requirement for studying the effects of space radiation on plants. Even though space radiation is composed by charged particles, a large fraction of the dose is delivered by low-energy protons, whose effectiveness is not different from photons. In general, any space radiation study must be validated with X-rays or gamma-rays that may be considered the reference radiation, to explore the dose range where sensitivity is expected.

The behavior of a large number of plant species following exposure to gamma radiation has been extensively studied [7–10], on the contrary fewer studies about the effects of X-rays have been carried out [11,12]. It has been reported that X-ray exposure induces biological modifications that may affect plant cellular mechanisms as well as metabolic functions [13,14].

It is well known that low doses of X-rays may have no effect on plant growth, while high doses may reduce seed viability, germination and development. In particular, plants exposed to X-rays may be subjected to cytological changes, mutations and reduced synthesis of DNA, RNA and proteins [13,14].

Among different physiological processes, ionizing radiation has a direct impact on tissue architecture as well as on the functioning of photosynthetic apparatus. The latter may be affected in all major components, including light harvesting complexes, thylakoid electron transport and the carbon reduction cycle [6].

It has been demonstrated that irradiation of plants with heavy ions, gamma- and X-rays causes the occurrence of defective chloroplasts with alteration in the synthesis of chlorophylls [15,16].

Generally, the exposure to ionizing radiation qualities, including X-rays, leads to the overproduction of reactive oxygen species (ROS), which determines oxidative stress. The excess of ROS damages proteins, lipids, carbohydrates and DNA [17,18].

During the evolution, plants have developed a wide range of efficient strategies to counteract the oxidative stress and its harmful effects.

A first step in plant defense occurs at structural level. Generally, irradiation with gamma rays and UV leads to the accumulation of phenolic compounds that act as natural screen against radiation, limiting the absorption [7,15,19].

At biochemical level, both the enzymatic and non-enzymatic systems are effective in plant defense against oxidative stress. The enzymatic system operates with the sequential and simultaneous actions of a number of scavengers enzymes including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione-S-transferase (GST) [20,21].

The non-enzymatic defense strategy is based on the action of various plant pigments, such as anthocyanins, carotenoids and flavonoids as well as ascorbic acid, glutathione and phenolic compounds, which are effective in the removal of free radicals [22].

Usually, after the exposure to gamma radiation, the scavengers enzyme pool increases depending on radiation dose [23–25]. The capability of cells to contrast the oxidative stress and to screen ionizing radiation represents a valuable feature needed in an ideal candidate for cultivation in extraterrestrial environments.

At present, the knowledge concerning the role of the antioxidant systems in protecting plants under ionizing radiation stress is controversial and limited at very few plant species.

The aim of present work is to evaluate the effect of different X-rays doses on some growth and structural traits, photosynthetic pigments, Rubisco and antioxidant defense of dwarf bean plants. To this purpose, plants of *Phaseolus vulgaris* L. were irradiated at the stage of trifoliate leaf, in order to assess *if* and *how* this species is able to counteract the effects of X-rays. The assessment of plant potential to overcome both low and high doses of ionizing radiation will be useful in sight of its cultivation in BLSSs.

2. Material and methods

2.1. Plant cultivation

Seeds of dwarf *Phaseolus vulgaris* L. were germinated in the dark and placed 1–2 cm deep in pots of 10 cm diameter filled with field soil. All pots were located in a growth chamber under controlled conditions of temperature, relative humidity and light. More specifically day temperature, relative humidity and Photosynthetic Photon Flux Density (PPFD) were 25–26 °C, 60–65% and 90–100 μmol photons m^{-2} s^{-1}, respectively. During the whole growth period, plants were regularly watered to reintegrate water lost by evapotranspiration. Plants were cultivated between April and June 2011.

2.2. Radiation type and irradiation procedures

Four total doses of X-rays (0.3 Gy, 10 Gy, 50 Gy and 100 Gy), 200 kVp, dose rate 1 Gy/min, were supplied to dwarf bean plants. At the time of irradiation, the first trifoliate leaf was 6–7 days old and fully expanded, whereas the second leaf was just sprouted. The four doses of X-rays were applied by using a X-rays tube with a maximum voltage of 300 kV/p (Siemens, Forchheim, Germany) as one dose for each plant in order to avoid