Effects of simulated space radiations on plant roots investigated by a proteomic analysis

Angiola Desiderio
ENEA, Biotechnologies and Agroindustry Division
Rome, Italy
Simulating space conditions

ENEA: A multidisciplinary research center

Gamma radiation
Calliope

X radiation
Eldorado 6

Proton beams
Top Implant

Static magnetic fields

Clinostats 2D
BIOxTREME project

Plant BIOfactories for the formulation of bioactive molecules with microbicidal, immunostimulatory and antioxidant activity for life under eXTREME conditions.

HORTSPACE "New plant ‘ideotypes’ for a space garden" (ASI-ENEA Agreement). Design and construction of a greenhouse for experimental cultivation in space conditions (HortExtreme, Mission AMADEE-18)

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
Radiation effects on living structures

**DIRECT** energy transfer on biological macromolecules (DNA, proteins, membranes, polysaccharides,...)

**INDIRECT** energy transfer through other molecules (mainly water) forming very reactive radical species.

**STRUCTURAL DAMAGE**

Critical target: DNA

Possibility of irreversible damage, not compatible with survival

Other macromolecules

Structures more easily regenerable, thanks to a continuous turnover
How to study the space stress effects on plant?

Research goal

- Identifying the capacity of the biological system to withstand stress.
  - Experimental conditions pushed to the survival limit (at short or long term)
  - High dose radiations

- Understanding if and how the biological system is able to acclimate to space stress
  - Experimental conditions close to those actually experienced during space missions
  - Lower dose radiations

Investigation level

- **Genomics:** chromosome aberrations, mutations, unrepaired fragmentation, reproductive sterility, …
- **Transcriptomics, Proteomics, Metabolomics:** functional response
Proteome role in stress response

GENOME
perpetuates life by maintaining the levels of active proteins

PROTEOME
actively sustains life by:
- regulating metabolism,
- repairing genome,
- adapting growth and physiology,
- detoxifying cells,
- eliminating damaged macromolecules,
- …

Are plants able to acclimate to space environment stress conditions?

Can we use plant to produce food during long term space missions?

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
A plant for the space: the ideotype

Bioengineering

Activation of anthocyanins synthesis through the expression of the transcription factor AN4 (c-Myb) of petunia.

- Micro Tom a dwarf tomato cultivar
- Small size (15-20 cm)
- Short life cycle (seed-seed 70-90 days)
- High photosynthetic efficiency under fluorescent light
- High productivity (20-30 fruits/plant; mean diameter of fruits 15 mm)
- Continuous flowering
- Easy to cultivate at high density (> 100 plant/m²)
- Better performances in hydroponics

Solid platform for the production of valuable molecules, including metabolites and pharmaceutically relevant recombinant proteins.

Plant chosen system: Tomato ‘hairy roots’

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018

Antimicrobial peptides

Immunostimulatory molecules

Antifungal antibodies

Antifungal antibodies
Gamma radiation

ENEA National Institute of Ionizing Radiation Metrology (INMRI-ENEA)
Dr. Maria Pimpinella

- Decay of Co-60
- Average energy released: 1250 KeV
- Dose rate: from $1.3 \times 10^{-2}$ to $3.9 \times 10^{-3}$ mGy/min
- Exposure doses: 0.5 Gy, 5 Gy, 10 Gy
- Samples: 3 biological replicates for each experimental condition
Radiations in space

100 mSv: Level above which cancer is more likely

Earth

\[ 2.4 \text{ mSv} \]

Nuclear power station

\[ 20 \text{ mSv} \]

International Space Station

\[ 200 \text{ mSv} \]

Interplanetary space

\[ 600 \text{ mSv} \]

Galactic and extragalactic cosmic rays

Jovian electrons

Solar X-rays

Solar flare neutrons and \( \gamma \)-rays

Trapped particles

Solar flare electrons, protons and heavy ions

mSv = millisievert (a measure of the biological effects of radiation)

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
X radiation

CHF 320G X-ray generator
ENEA - Physical Technologies for Security and Health Division
Dr. Claudio Pioli

• Operating conditions: 250 kV, 15 mA
• Filters: 2.0 mm Al and 0.5 mm Cu
• Exposure doses: 0.5 Gy, 5 Gy, 10 Gy
• Samples: 3 biological replicates for each experimental condition
Differential proteomics approach

2D-DIGE Technology (GE Healthcare)

Reference sample

Treatment sample

Protein separation on the same 2D gel

Bioinformatic analysis and identification of differential proteins

Mass spectrometry for protein identification

Automated spot picking

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
Dose related response of root proteome

Principal components analysis

Hierarchical clustering analysis

Multivariate statistical analysis showed that doses of gamma and X radiation up to 0.5 Gy do not significantly influence plant proteome.

A functional response is evident at 5 Gy and does not vary with increasing exposure up to 10 Gy.
Predicted interactions among differential proteins

STRING analysis (functional protein association networks)

**Gamma**

**X rays**

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
Proteins involved in stress response

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
Proteins involved in oxidation-reduction processes

**Gamma**

- Malate dehydrogenase
- Insulinase
- Dihydrolipoyl dehydrogenases
- Formate dehydrogenase, mitochondrial
- Glutathione S-transferase
- Histone H4
- Peroxidase

**X rays**

- Malate dehydrogenase
- Insulinase
- Aldehyde dehydrogenase
- Ascorbate peroxidase, cytosolic
- Dehydroquinate dehydratase
- Elicitor-activated gene 3-2
- Glutamate dehydrogenase
- Glutathione-disulfide reductase
- Isocitrate dehydrogenase, cytosolic
- Monodehydroascorbate reductase
- NADH dehydrogenase, mitochondrial
- Transketolase

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
Proteins involved in protein folding and refolding

Gamma

<table>
<thead>
<tr>
<th>Protein</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaperonin 60</td>
<td>↑</td>
</tr>
<tr>
<td>Heat shock protein 60, mitocondrial</td>
<td>↓</td>
</tr>
<tr>
<td>Heat shock protein 70</td>
<td>↑</td>
</tr>
<tr>
<td>Protein disulfide isomerase</td>
<td>↑</td>
</tr>
</tbody>
</table>

X rays

<table>
<thead>
<tr>
<th>Protein</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaperonin 60</td>
<td>↑</td>
</tr>
<tr>
<td>Heat shock protein 60, mitocondrial</td>
<td>↓</td>
</tr>
<tr>
<td>Heat shock protein 70</td>
<td>↑</td>
</tr>
<tr>
<td>Protein disulfide isomerase</td>
<td>↑</td>
</tr>
</tbody>
</table>
Proton beams

Pulsed linear accelerator TOP-IMPLART
ENEA - Physical Technologies for Safety and Health Division
Dr. Monia Vadrucci

- Beam power: 27 MeV at the accelerator output
- Dose rate: 0.2 Gy/s with $10^9$ protons/cm$^2$
- Exposure doses: 0.5 Gy, 5 Gy, 10 Gy
- Samples: 3 biological replicates for each experimental condition

Experiments in progress …
Effects of static magnetic fields

Active systems of magnetic shielding from cosmic radiation

[European Space Radiation Superconducting Shield - CERN Project SR2S]

Residual magnetic field in the habitat: $\sim 10^{-1}$ T

[P. Spillantini et al, 2010]

Biological effects?
Static magnetic fields

Magnetic devices specially designed and assembled to mimic magnetically shielded space habitats

ENEAA - Physical Technologies for Security and Health Division
Dr. Vanni Lopresto

Samples: 4 biological replicates for each experimental condition

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
Roots response to static magnetic fields

Proteomic results

No statistically significant variation of proteome, after exposure at different SMF intensities (250 and 500 mT), for different periods (1 and 10 days).

Morphometric results

No significant growth modifications at 250 and 500 mT, in terms of both total root length and number of lateral roots.
Final goal: Cultivating in space ...

Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018
Effects of simulated space radiations on plant roots investigated by a proteomic analysis, May 2018