Agenzia nazionale per le nuove tecnologie,
l'energia e lo sviluppo economico sostenibile

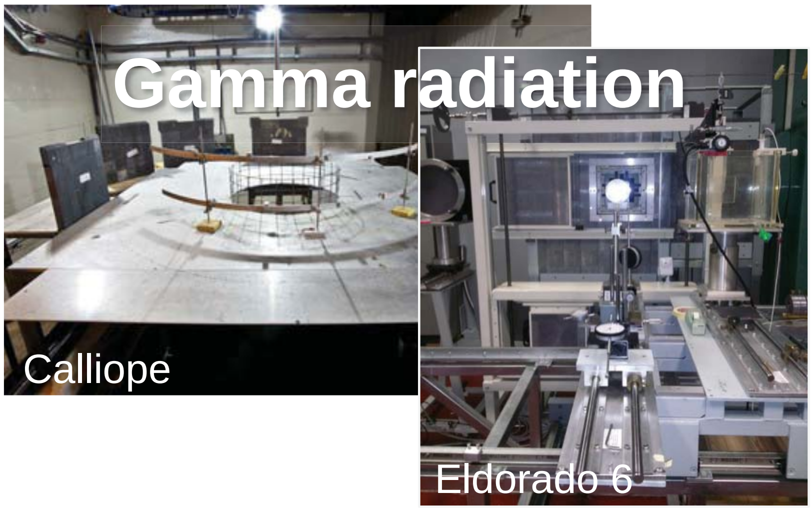
Laboratorio Biotecnologie

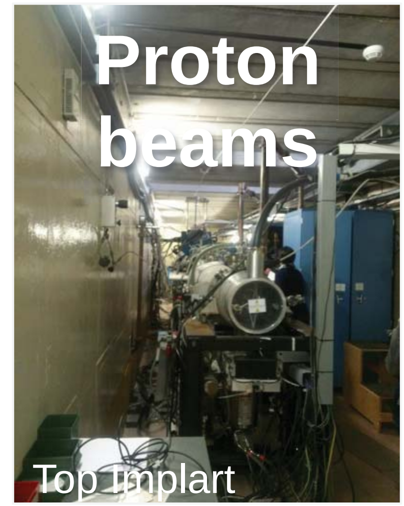
Effects of simulated space radiations on plant roots investigated by a proteomic analysis

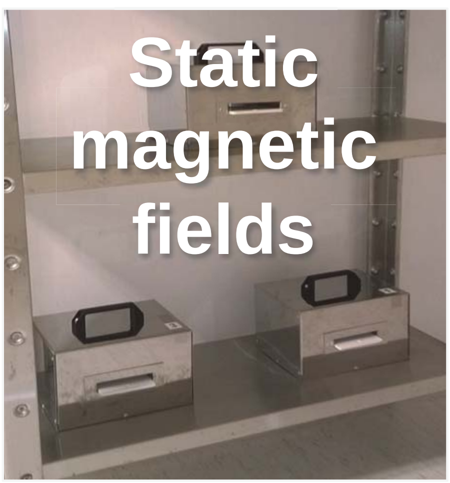
Angiola Desiderio
ENEA, Biotechnologies and Agroindustry Division
Rome, Italy

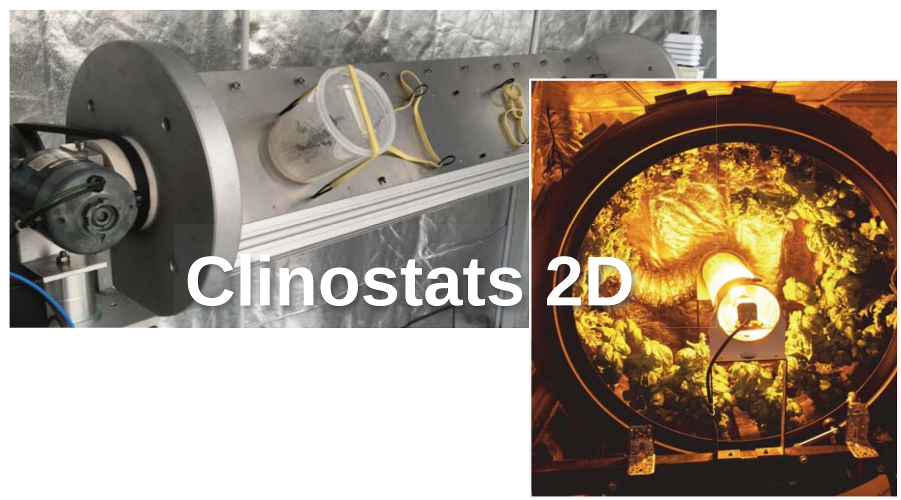
Simulating space conditions

ENEA: A multidisciplinar research center



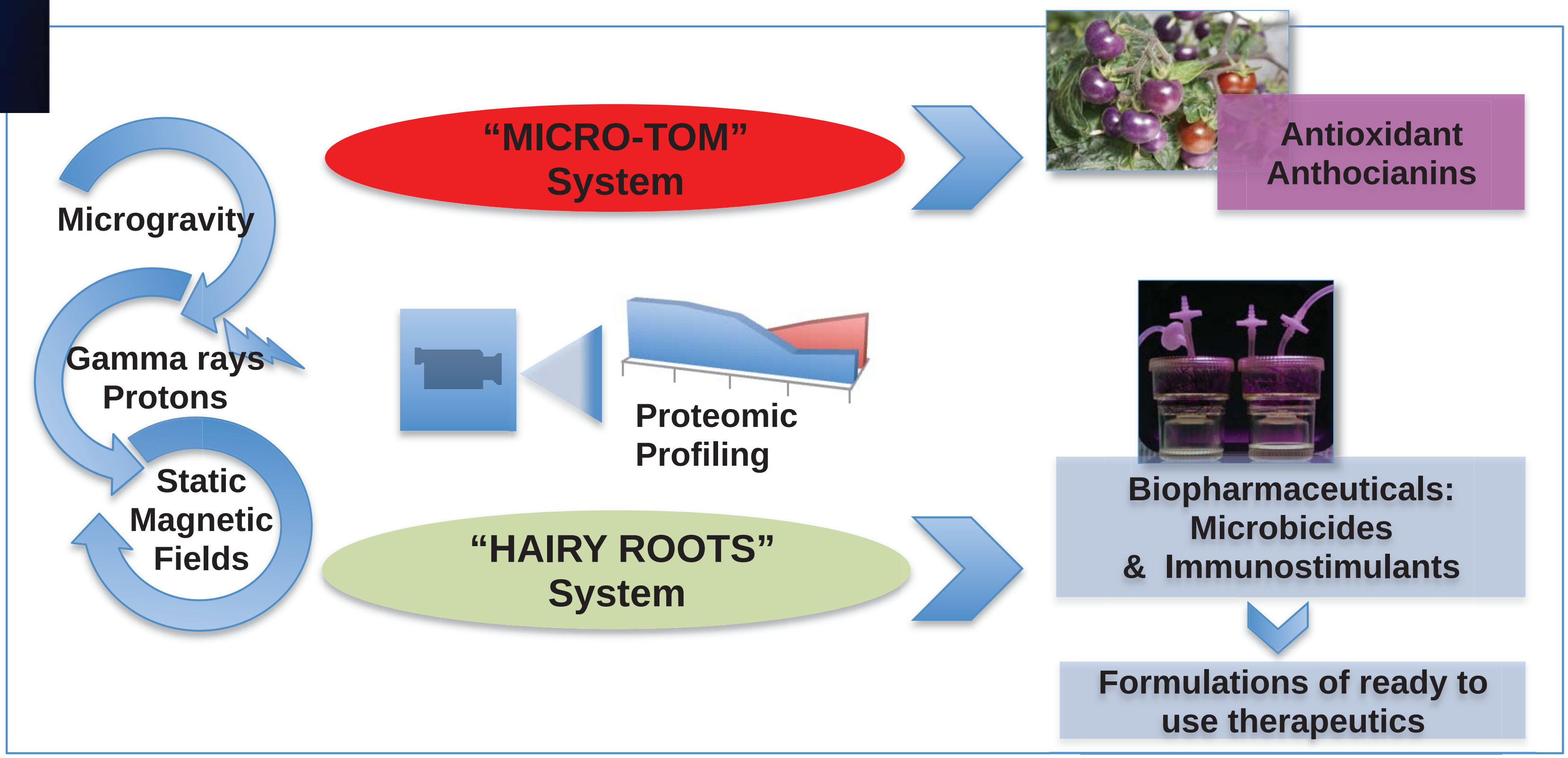






BIOxTREME project

Plant **BIO**factories for the formulation of bioactive molecules with microbicidal, immunostimulatory and antioxidant activity for life under e**XTREME** conditions.



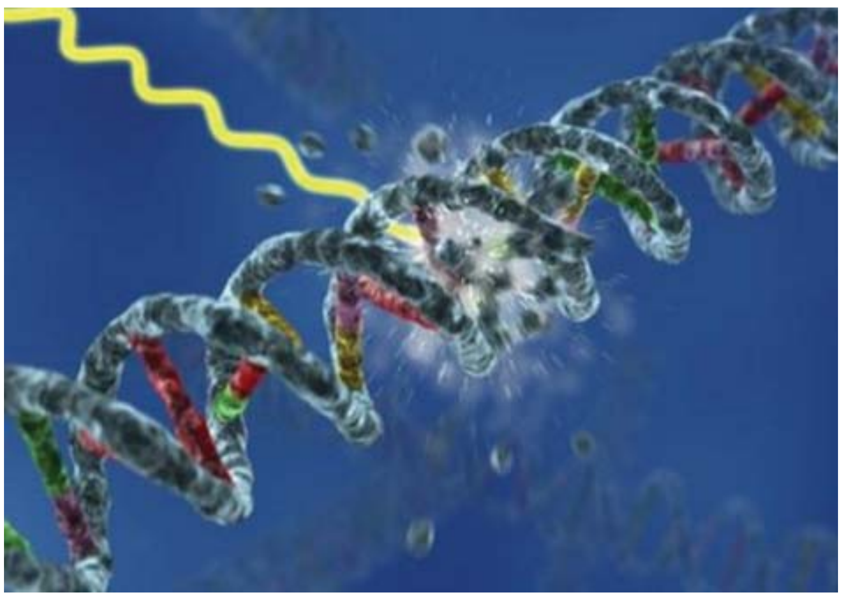
HORTSPACE "New plant 'ideotypes' for a space garden" (ASI-ENEA Agreement). Design and construction of a greenhouse for experimental cultivation in space conditions (HortExtreme, Mission AMADEE-18)

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.

Understanding if and how the biological system is able to acclimate to space stress

Experimental conditions pushed to the survival limit (at short or long term)

Experimental conditions close to those actually experienced during space missions

High dose radiations

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?

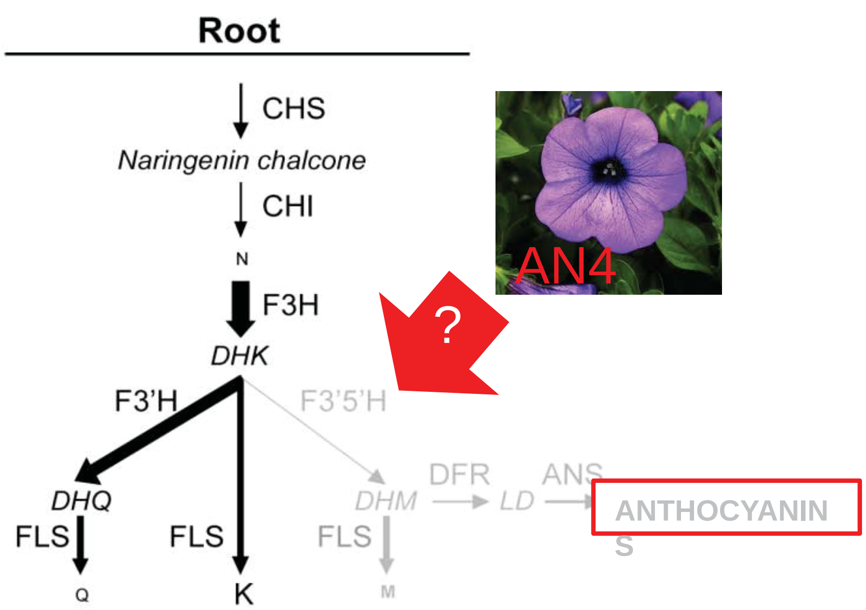
A plant for the space: the *ideotype*

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/m^2)
- Better performances in hydroponics

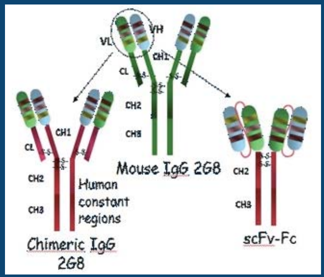
[Scott & Harbaugh 1989. Florida Agr. Exit. Sta. Circ S-370]



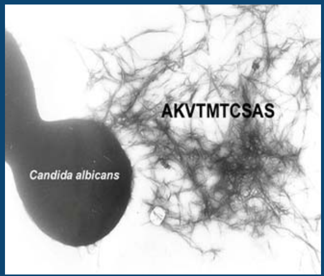
Plant chosen system: Tomato ‘hairy roots’

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

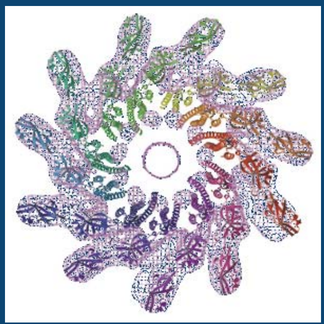
Antifungal antibodies



Antimicrobial peptides



Immunostimulatory molecules



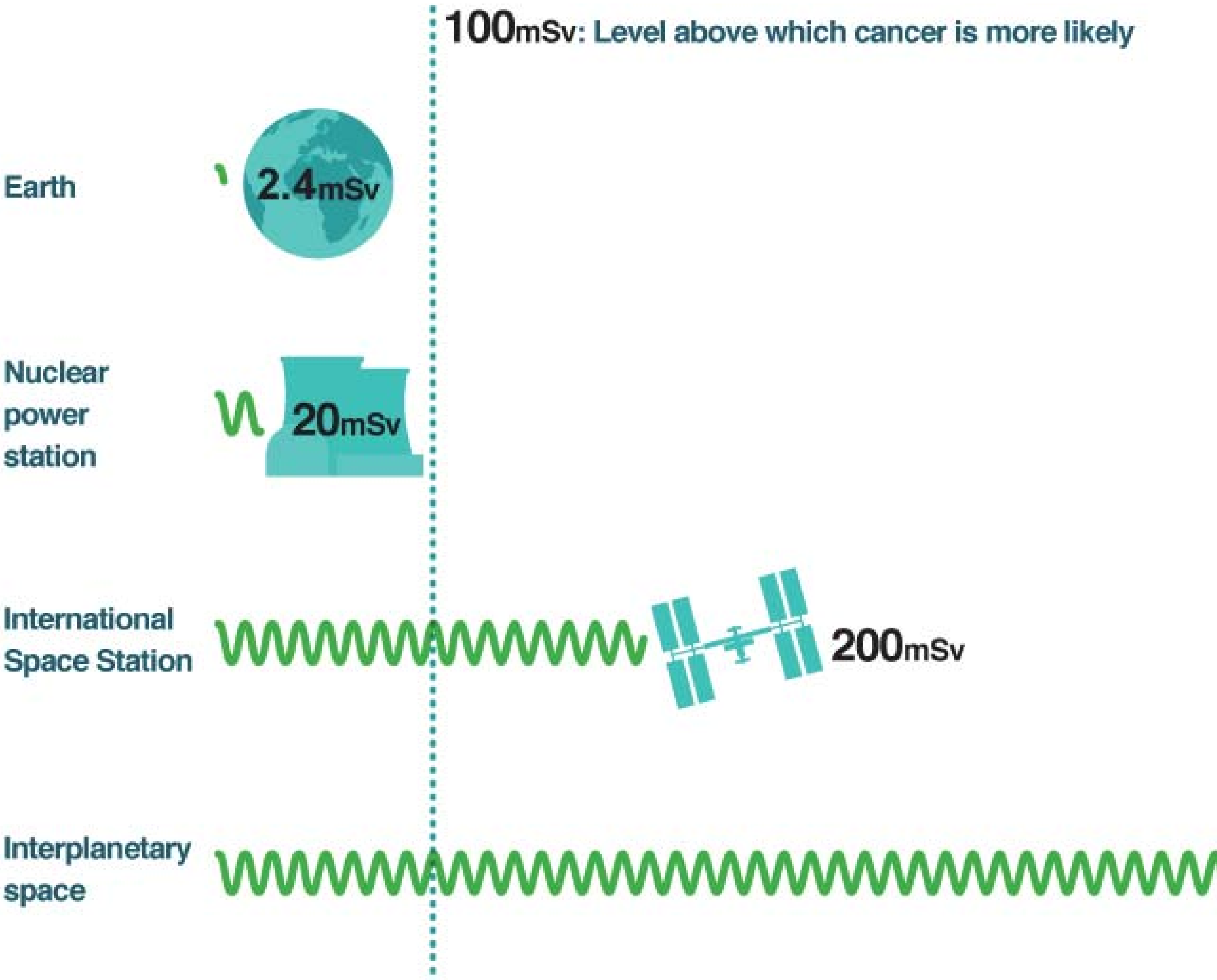
Gamma radiation

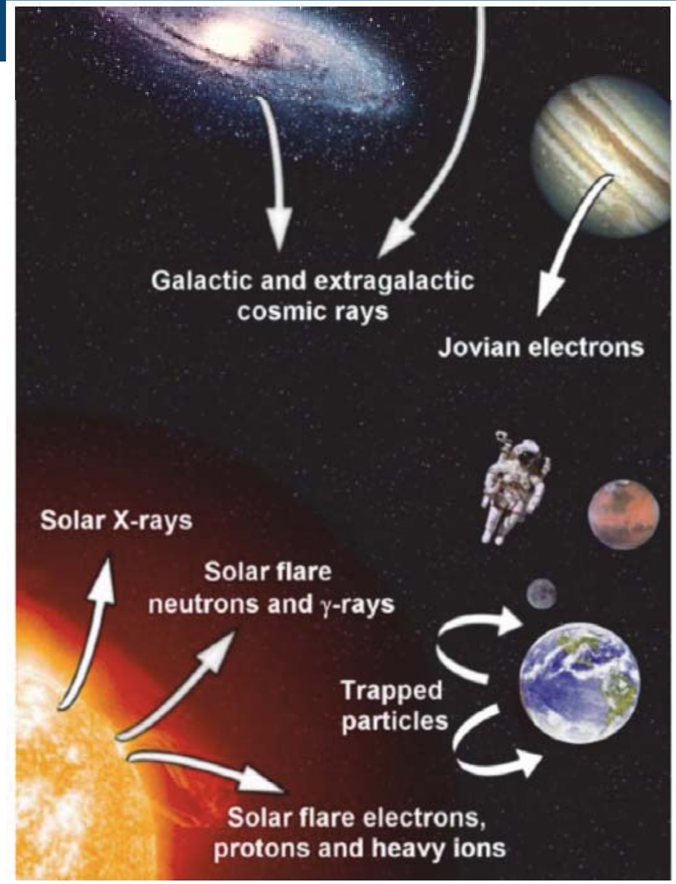
ENEA National Institute of Ionizing Radiation Metrology (INMRI-ENEA)

Dr. Maria Pimpinella

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

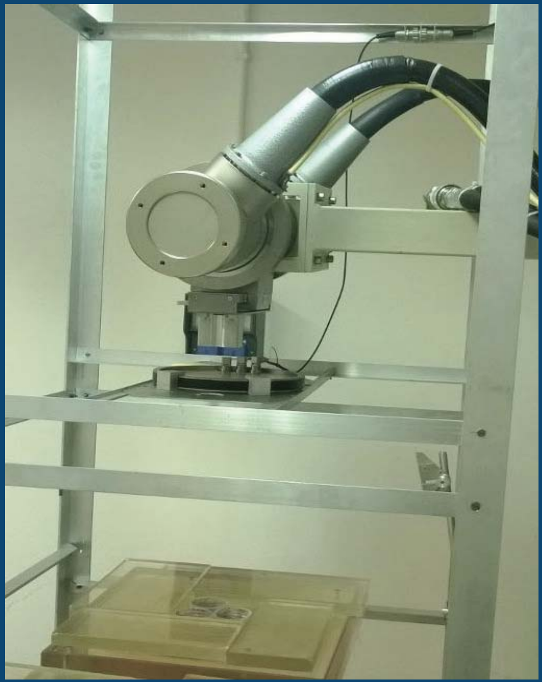
Radiations in space

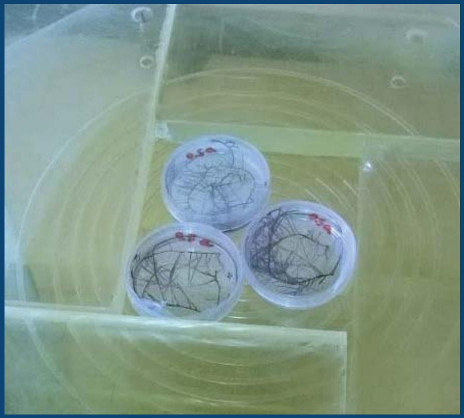




mSv = millisievert (a measure of the biological effects of radiation)

X radiation





CHF 320G X-ray generator

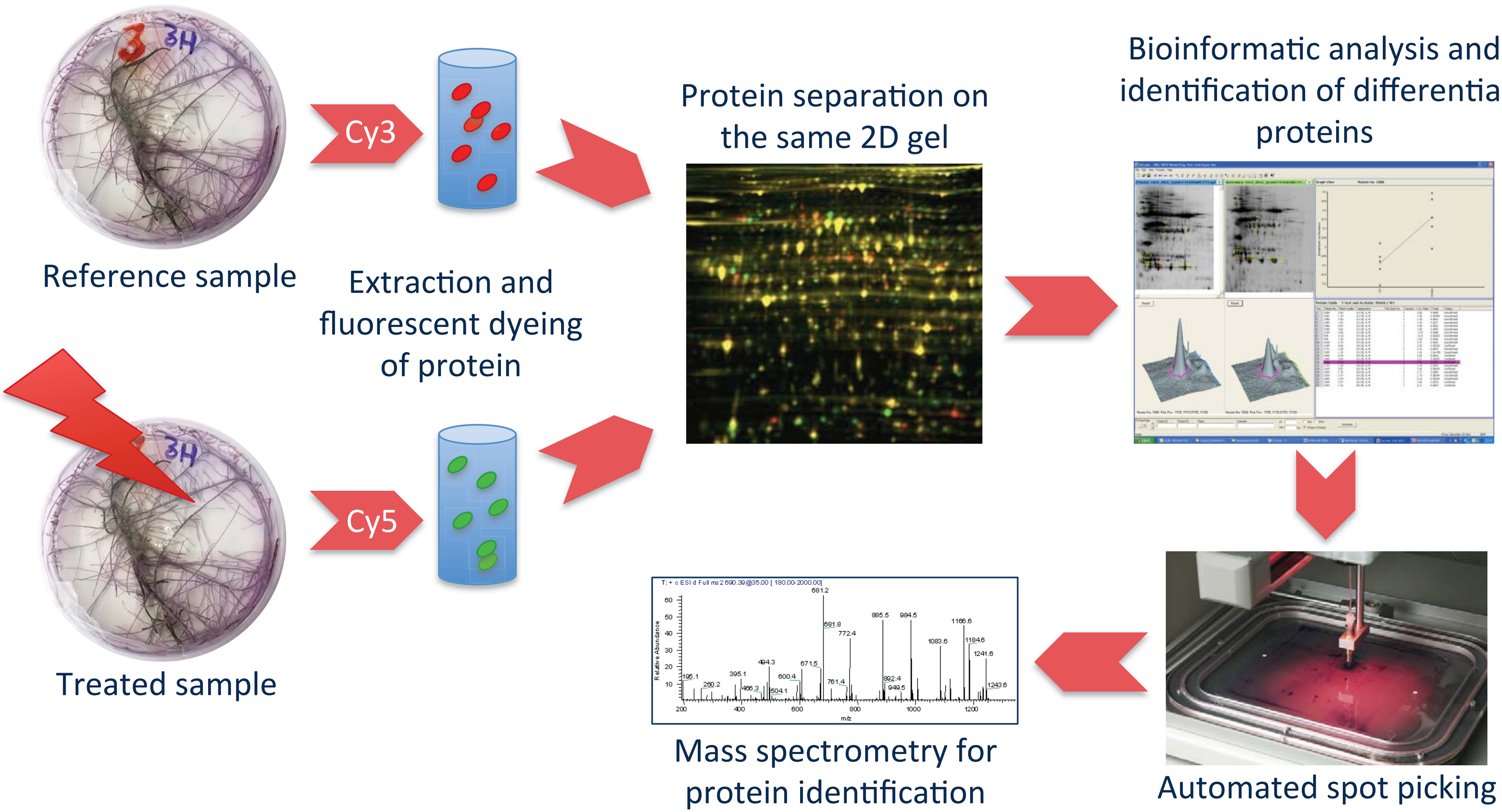
ENEA - Physical Technologies for Security and Health Division

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

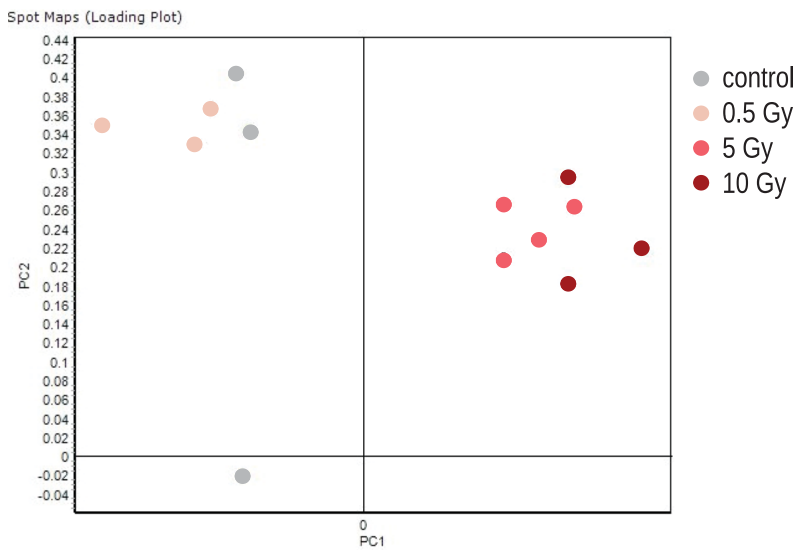
Differential proteomics approach

2D-DIGE Technology (GE Healthcare)

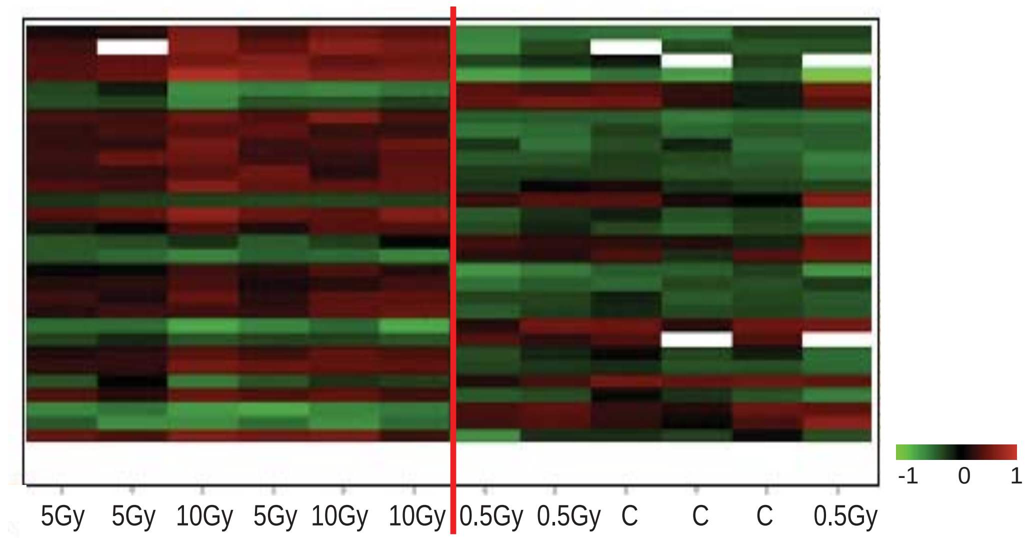


Dose related response of root proteome

Principal components analysis



Hierarchical clustering analysis



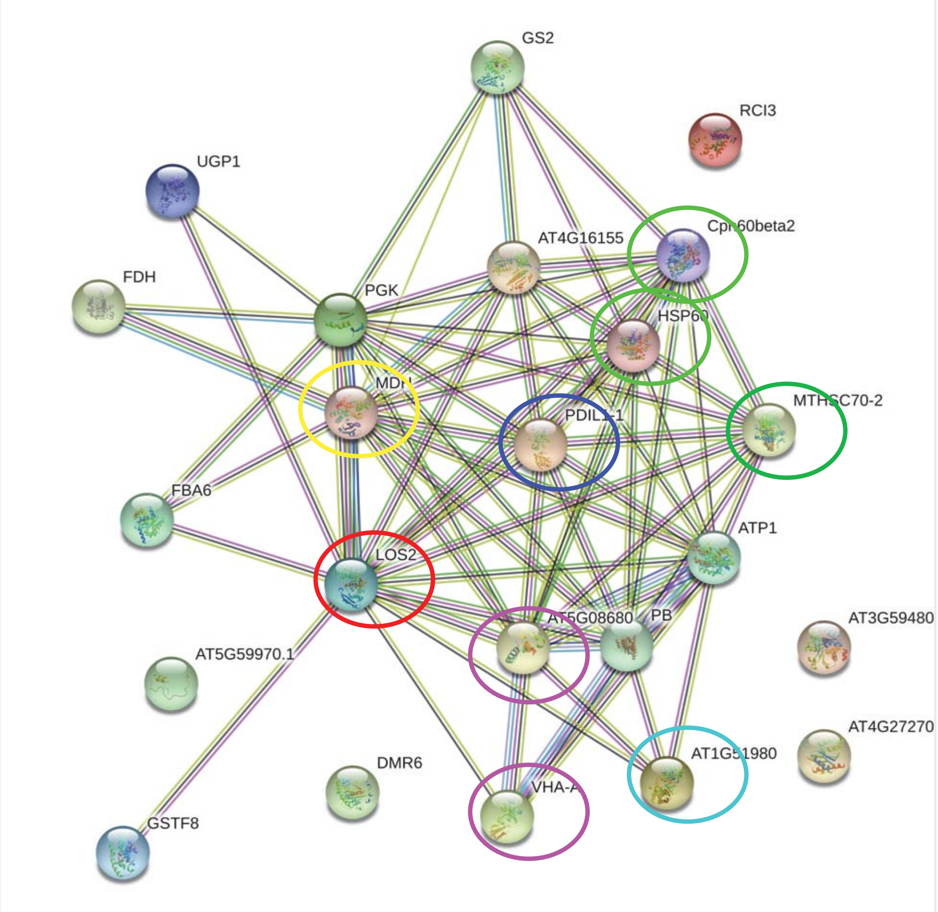
Multivariate statistical analysis showed that doses of **gamma** and **X radiation** up to **0.5 Gy** do not significantly influence plant proteome.

A functional response is evident at **5 Gy** and does not vary with increasing exposure up to **10 Gy**.

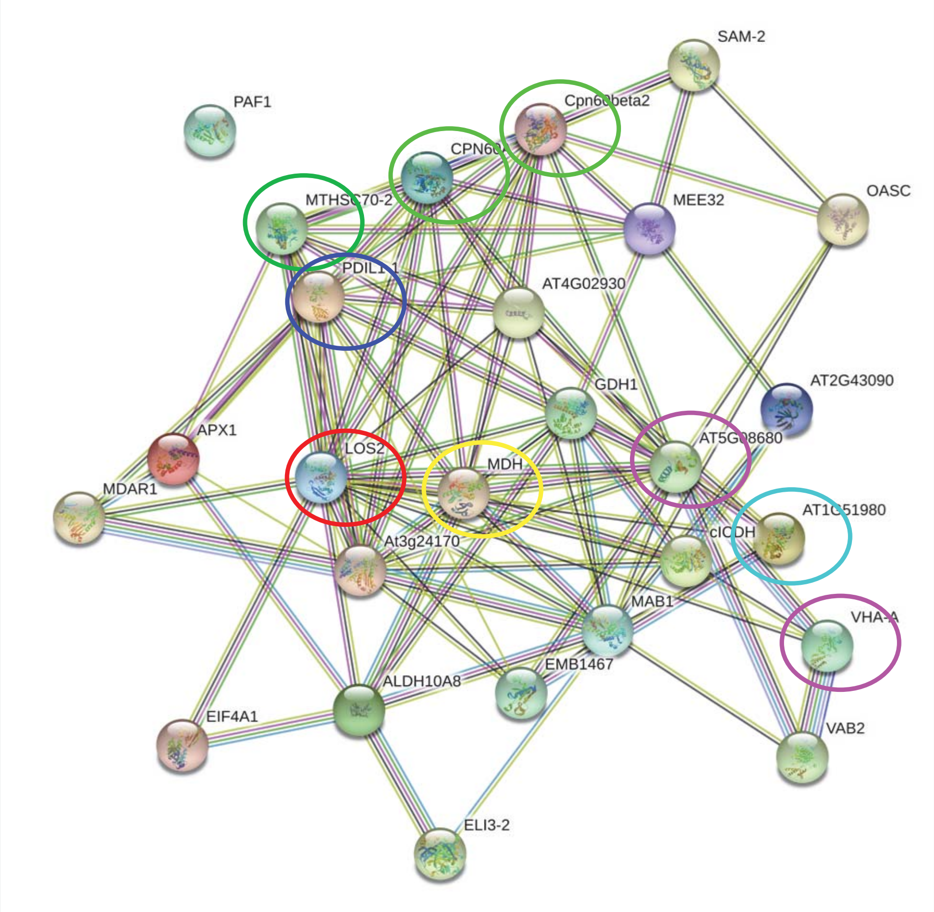
Predicted interactions among differential proteins

STRING analysis (functional protein association networks)

Gamma

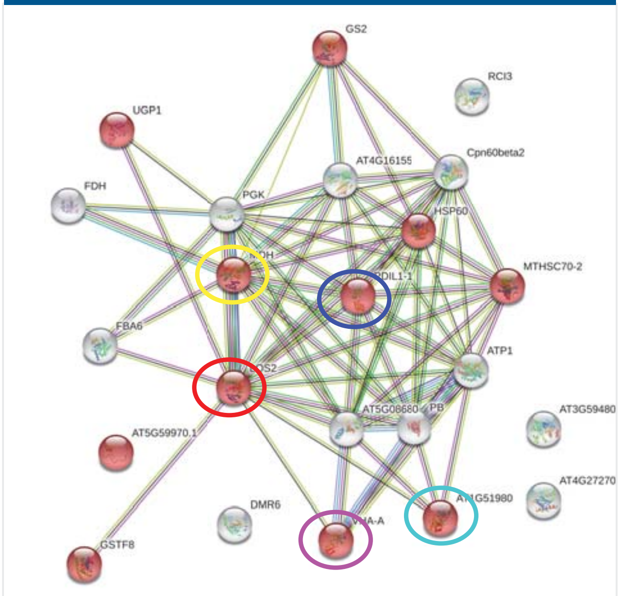


X rays

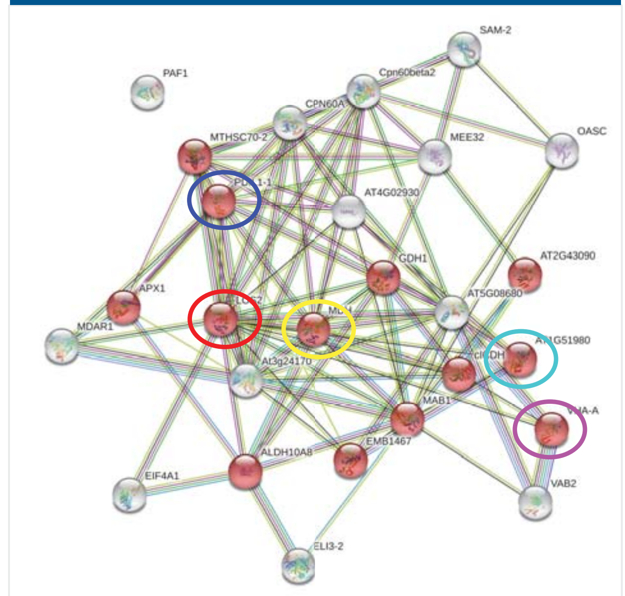


Proteins involved in stress response

Gamma



X rays

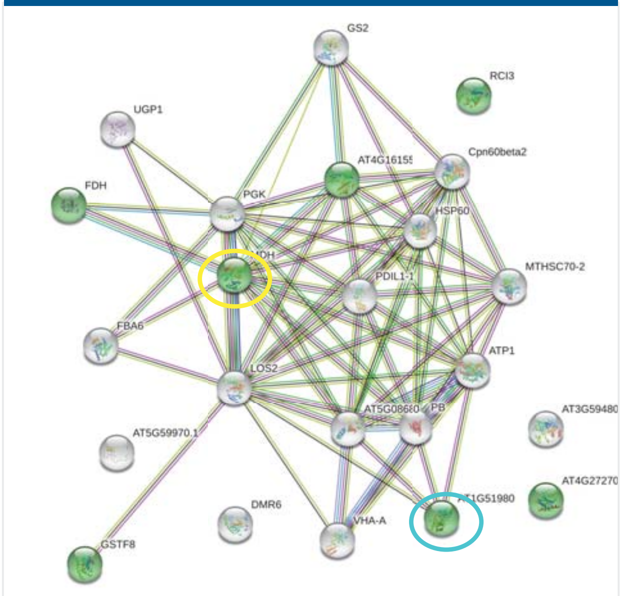


ATP synthase, vacuolar		↓
Enolase	↑	
Heat shock protein 70, mitochondrial	↑	
Insulinase	↑	
Malate dehydrogenase, NAD-dependent	↑	
Protein disulfide isomerase	↑	
Fructokinase-2	↑	
Glutamine synthetase	↑	
Glutathione S- transferase		↓
Heat shock protein 60, mitochondrial		↓
UDP-glucose pyrophosphorylase	↑	

ATP synthase, vacuolar		↓
Enolase	↑	
Heat shock protein 70	↑	
Insulinase	↑	
Malate dehydrogenase	↑	
Protein disulfide isomerase	↑	
Aconitase/3-isopropylmalate dehydrogenase	↑	
Aldehyde dehydrogenase		↓
Ascorbate peroxidase 2, cytosolic	↑	
Glutamate dehydrogenase	↑	
Isocitrate dehydrogenase, cytosolic NADP-dependent	↑	
NADH dehydrogenase, mitochondrial		↓
Transketolase	↑	

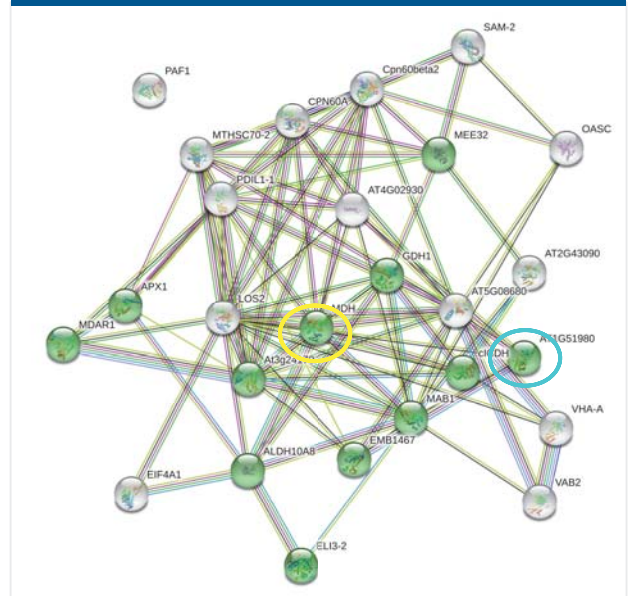
Proteins involved in oxidation-reduction processes

Gamma



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

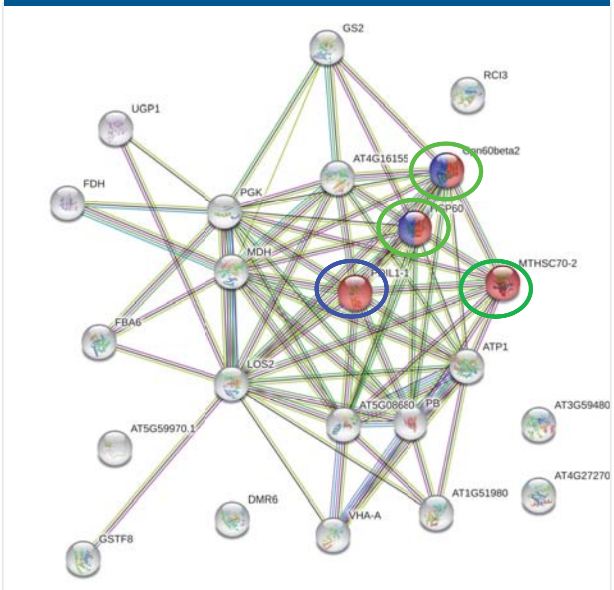
X rays



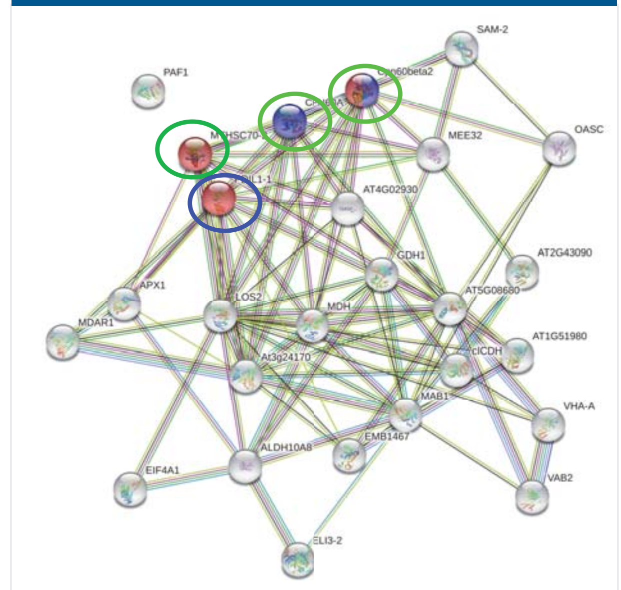
Malate dhydrogenase	↑	
Insulinase	↑	
Aldehyde dehydrogenase		↓
Ascorbate peroxidase, cytosolic	↑	
Dehydroquinate dehydratase	↑	
Elicitor-activated gene 3-2	↑	
Glutamate dehydrogenase	↑	
Glutathione-disulfide reductase		↓
Isocitrate dehydrogenase, cytosolic	↑	
Monodehydroascorbate reductase		↓
NADH dehydrogenase,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

Proteins involved in protein folding and refolding

Gamma



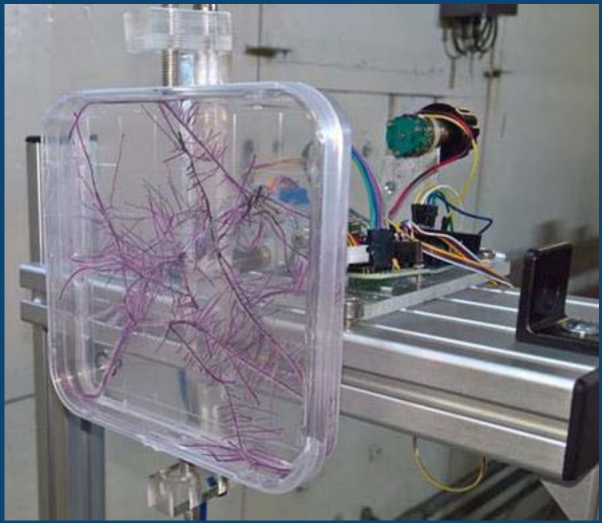
X rays



Chaperonin 60	↑	
Heat shock protein 60, mitochondrial		↓
Heat shock protein 70	↑	
Protein disulfide isomerase	↑	

Chaperonin 60	↑	
Heat shock protein 60, mitochondrial		↓
Heat shock protein 70	↑	
Protein disulfide isomerase	↑	

Proton beams



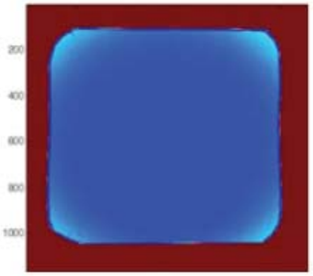
Pulsed linear accelerator TOP-IMPLART

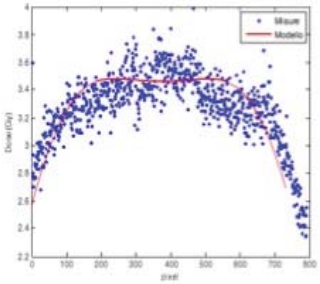
ENEA - Physical Technologies for Safety and Health Division

Dr. Monia Vadrucci

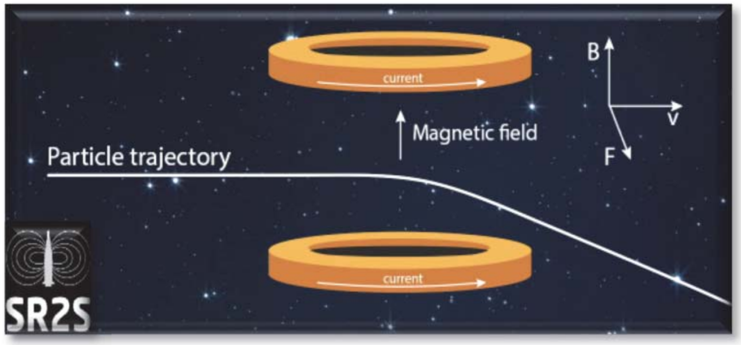
- Beam power: 27 MeV at the accelerator output
- Dose rate: 0.2 Gy/s with 10^9 protons/cm^2
- Exposure doses: **0.5 Gy**, **5 Gy**, **10 Gy**
- Samples: 3 biological replicates for each experimental condition

Experiments in progress …





Effects of static magnetic fields

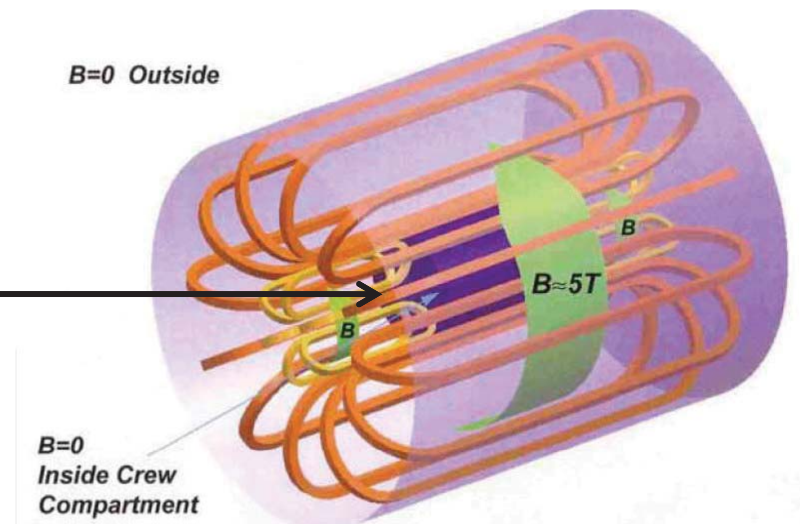


Active systems of magnetic shielding from cosmic radiation

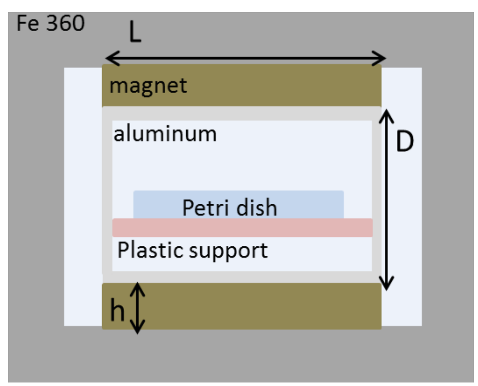
[*European Space Radiation Superconducting Shield* - CERN Project SR2S]

Residual magnetic field in the habitat: ~ 10^{-1} T

[P. Spillantini et al, 2010]

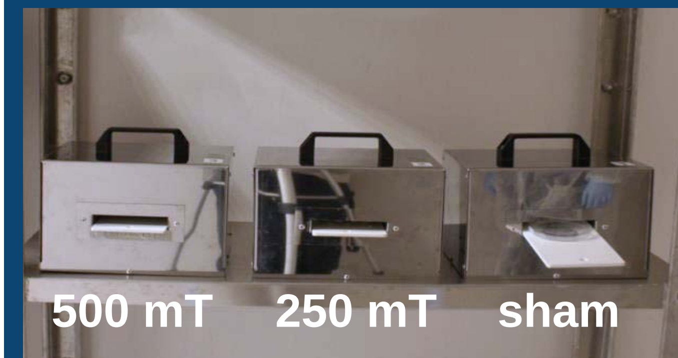


Biological effects ?



[V. Lopresto et al, 2015]

Static magnetic fields

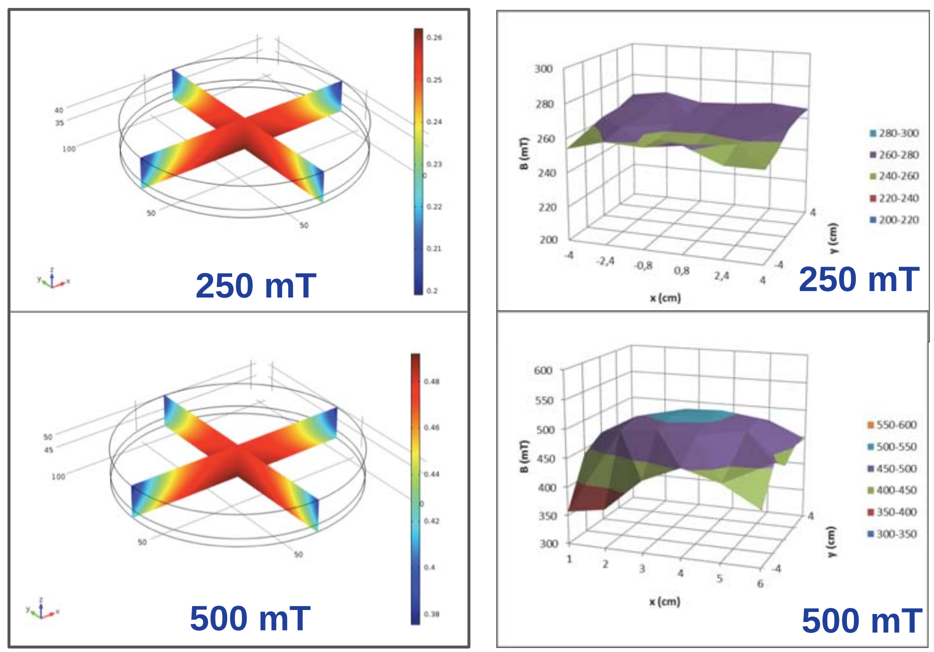


Magnetic devices specially designed and assembled to mimic magnetically shielded space habitats

ENEA - Physical Technologies for Security and Health Division

Dr. Vanni Lopresto

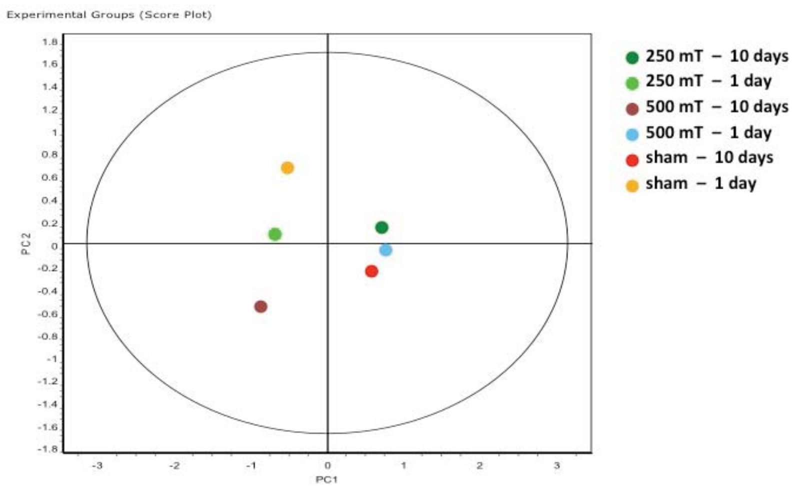
Samples: 4 biological replicates for each experimental condition

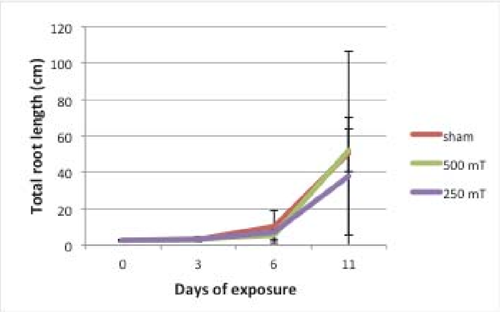


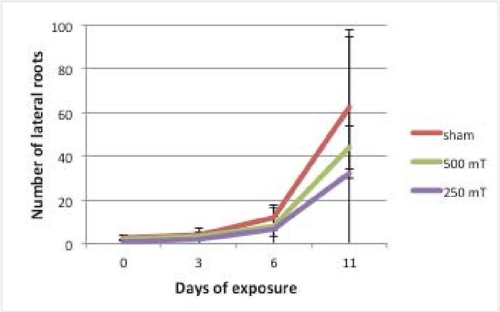
Roots response to static magnetic fields

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)





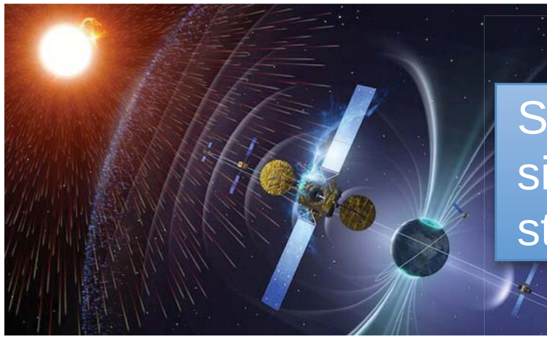


EZ Rhizo software analysis

Morphometric results

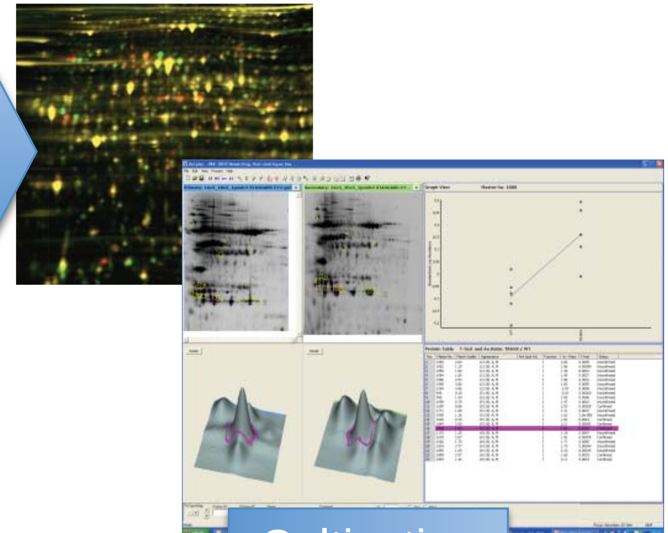
No significant growth modifications at 250 and 500 mT, in terms of both total root lenght and number of lateral roots.

Final goal: Cultivating in space …



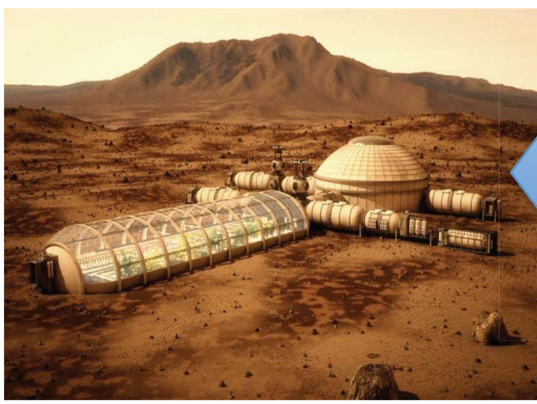
Space simulation stresses

Plant response analysis



Cultivation in simulated missions

Space horticulture !



ENEA Team

Head of Biotechnology Laboratory:
Dr. Eugenio BENVENUTO

Researchers:
Luca NARDI
Silvia MASSA
Maria Elena VILLANI
Elisabetta BENNICI
Ombretta PRESENTI
Maria PIMPINELLA
Vanessa DE COSTE
Claudio PIOLI
Vanni LOPRESTO
Stefania BACCARO
Alessia CEMMI
Monia VADRUCCI

External collaborations

Prof. Francesca QUATTROCCHIO
University of Amsterdam (NL)

Dr. Andrea SCALONI
Dr. Anna Maria SALZANO
CNR-ISPAAM Naple (IT)

Prof. Flavia GUZZO
University of Verona (IT)

From: *Il Tempo*

biotecnologie@enea.it

Thank you

biotecnologie@enea.it

Angiola Desiderio

Laboratorio Biotecnologie

ENEA