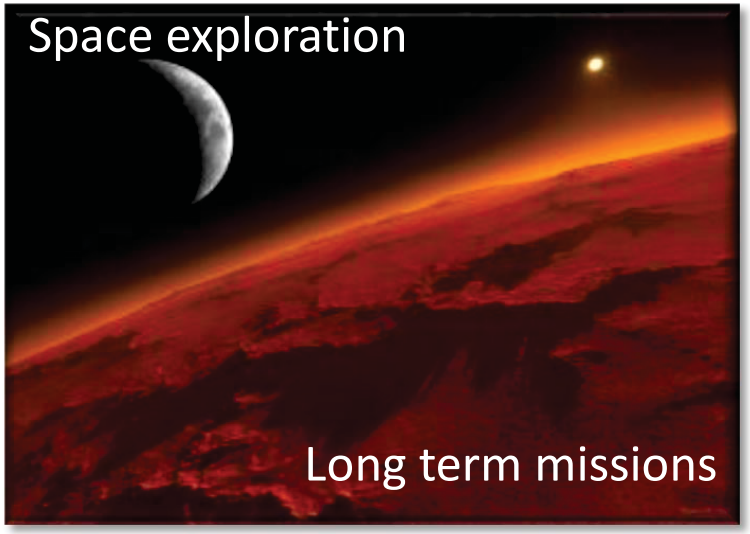
Agenzia nazionale per le nuove tecnologie,
l'energia e lo sviluppo economico sostenibile

Engineering tomato as a "space biofactory" fortified in anti-oxidants content and in free radical scavenging activity

Roma, 18/05/2018

Laboratorio Biotecnologie

SILVIA MASSA, PhD – Plant Biotechnologies
ENEA – Biotechnology Laboratory (**BIOTEC**) - Biotechnologies and Agroindustry Division (**BIOAG**); Sustainability Department (**SSPT**)

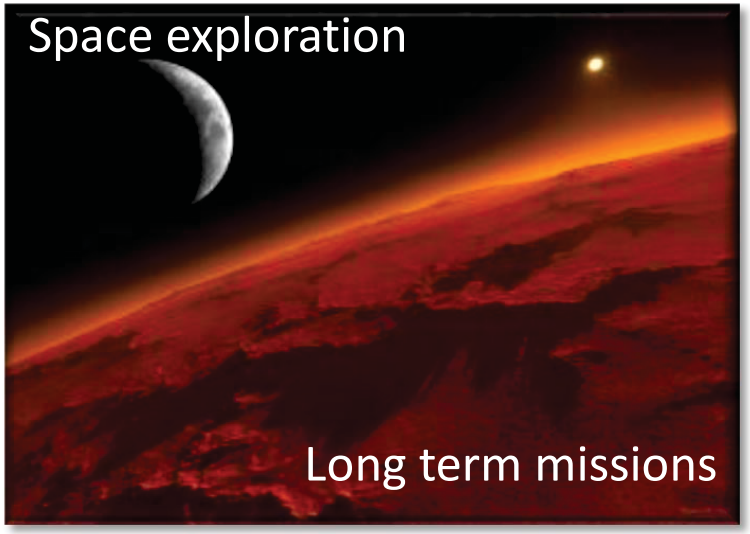


Limited availability of supplies from Hearth:

- weight and size of the load
- expiration time

Cultivation of plants as a source of:

- **fresh food**
- **bioactive molecules** possibly counteracting the effects of permanence in confined environments exposed to astro-physical stimuli



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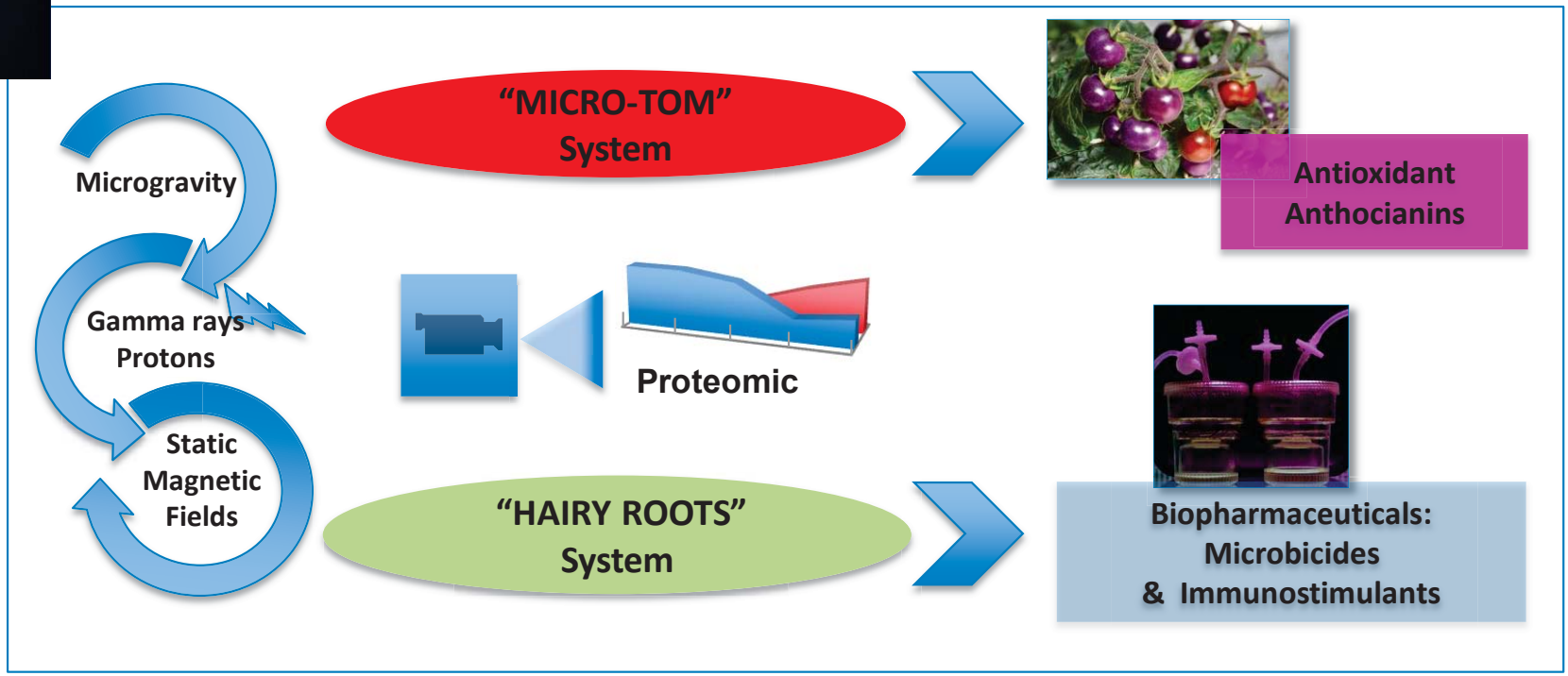
‘Plant BIOfactories for the formulation of bioactive molecules with antioxidant activity for life in exTREME conditions’

BIOxTREME project

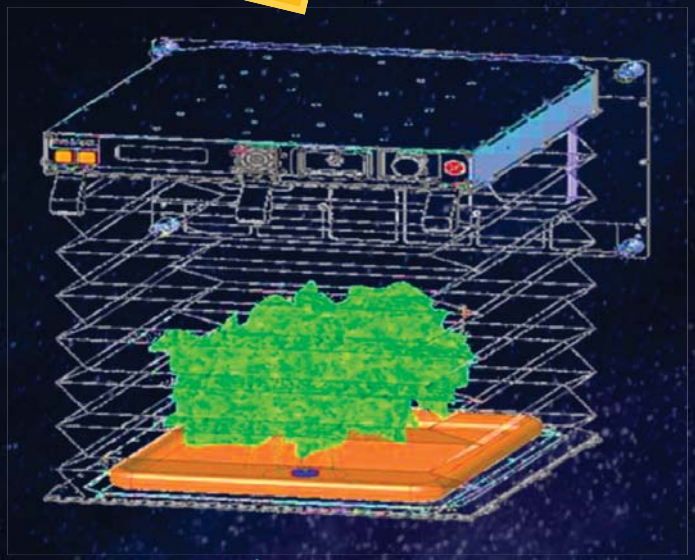
Plant **BIO**factories for the formulation of bioactive molecules with microbicidal, immunostimulatory and antioxidant activity for life under e**XTREME** conditions.



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)

PLANTS IN SPACE

Light



O_2

H_2O

Food

CO_2

CO_2

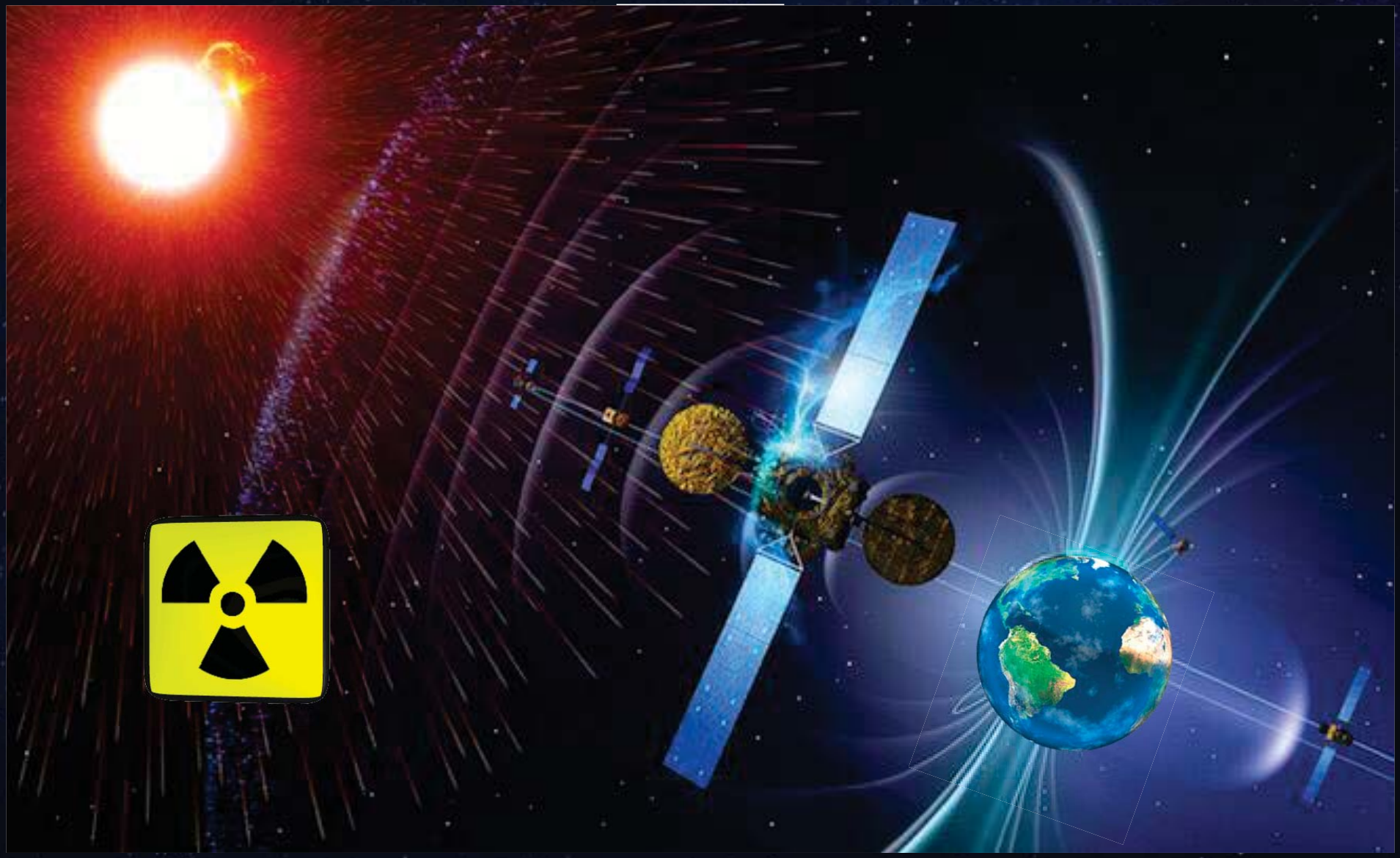
H_2O
Nutrients

Waste Processor
(e.g. Microbial
Bioreactor)

Waste H_2O &
Solid Waste

Engineering tomato as a "space biofactory " fortified in anti-oxidants content and in free radical scavenging activity

SPACE ENVIRONMENT = PRO-OXIDANT



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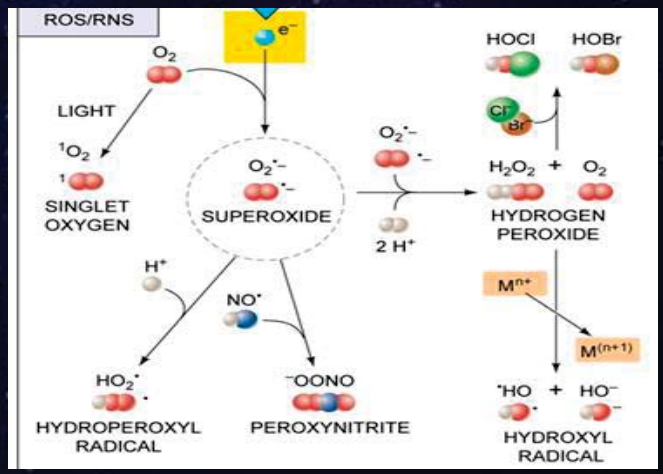
RADIATION EFFECTS ON LIVING STRUCTURES

- 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

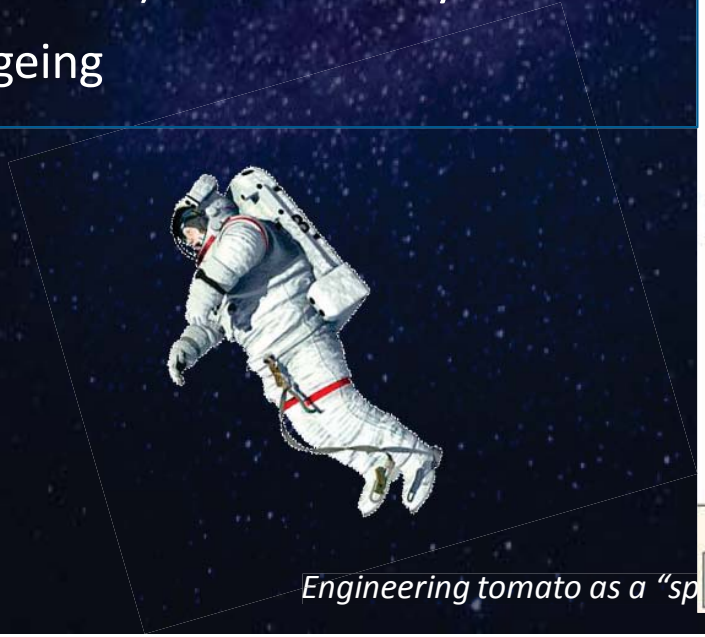


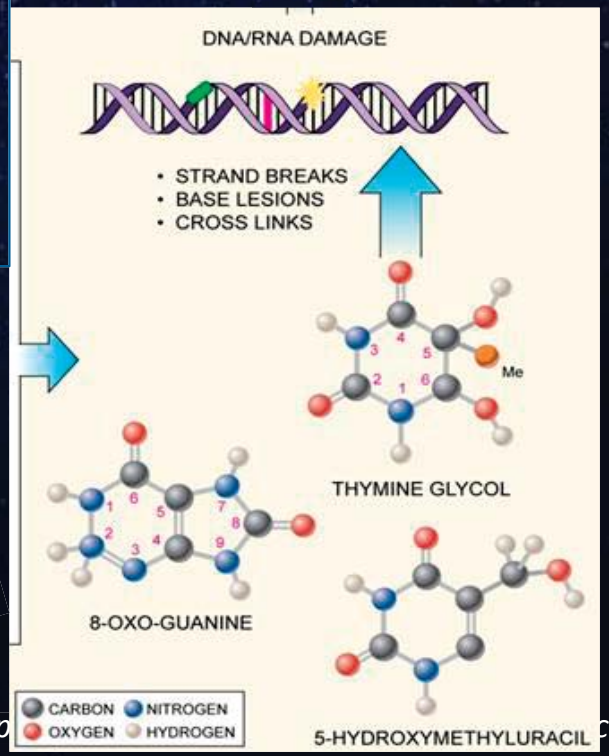
Pathological conditions:

Neurodegenerative disease

(Chronic) Inflammatory disease

Ageing





Mutations

Cytotoxicity

Cytostasis

Proliferation

Engineering tomato as a "sp… content and in free radical scavenging activity

ANTI-OXIDANTS

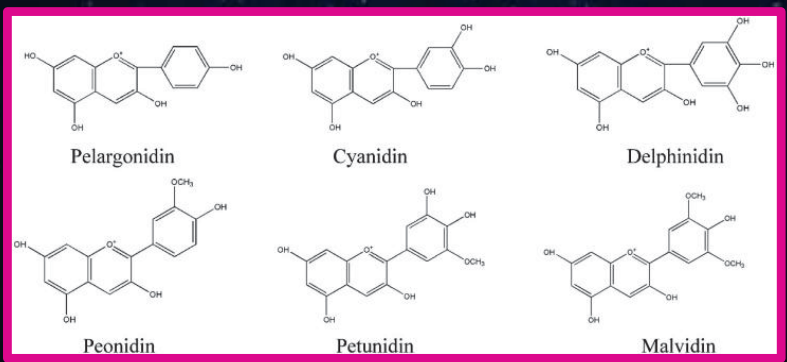
Ascorbic Acid

Vitamin E

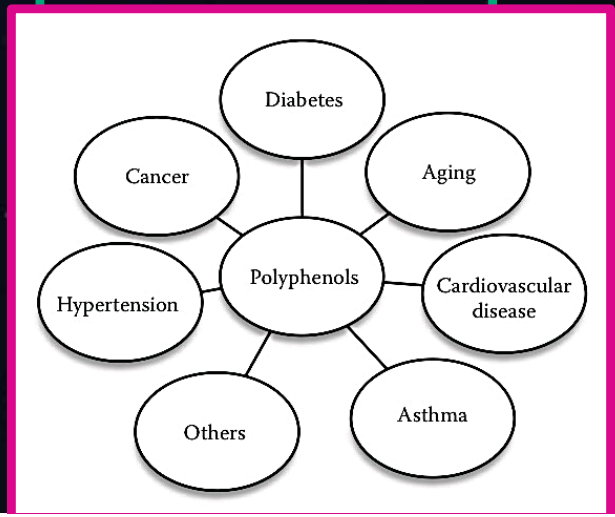
Beta Carotene

Flavonoid

Polyphenols

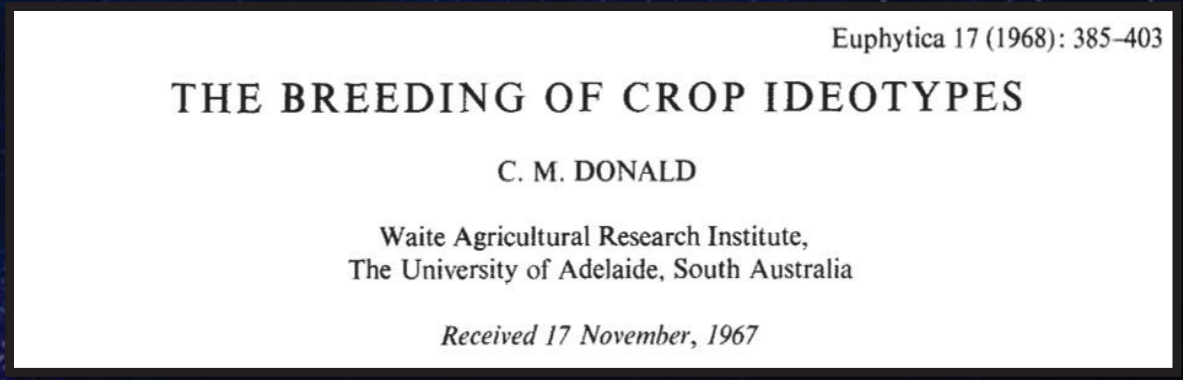


Anthocyanins



IDEOTYPE

In broad sense an Ideotype model which is expected to perform or behave in a predictable manner within a defined environment.



Euphytica 17 (1968): 385–403

THE BREEDING OF CROP IDEOTYPES

C. M. DONALD

Waite Agricultural Research Institute,
The University of Adelaide, South Australia

Received 17 November, 1967

- Development of conceptual theoretical model
- Selection of base material
- Incorporation of desirable characters into single genotype
- Selection of ideal or model plant type

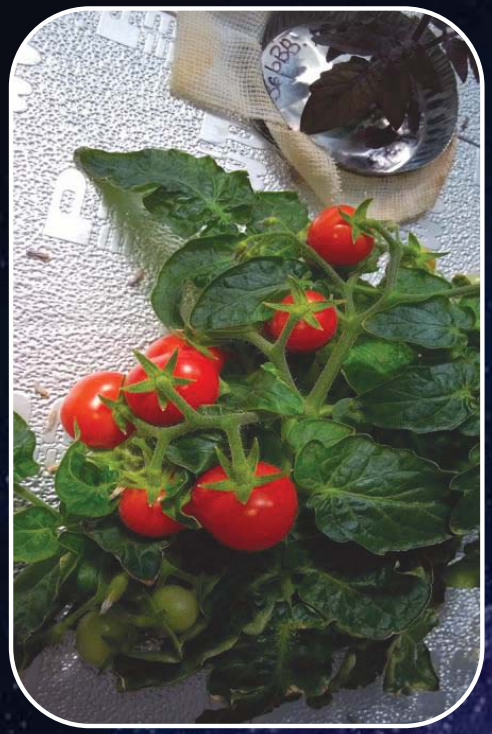
MICRO TOM

- ✓ Model cultivar for tomato research
- ✓ Small size (15-20 cm)
- ✓ Short life cycle (seed-seed 70-90 days)
- ✓ Able to grow under fluorescent light
- ✓ Easy to cultivate
- ✓ High photosynthetic efficiency
- ✓ High productivity (20-30 fruits/plant; 2-5 gr/fruit; mean diameter of fruits 15 mm)
- ✓ Continuous flowering
- ✓ Can be grown at high density (> 100 plant/m^2)
- ✓ Better performances in hydroponics

Scott JW, Harbaugh BK. 1989. Micro-Tom. A miniature dwarf tomato. *Florida Agricultural Experimental Station* Circular S-370, 1-6

Engineering tomato as a "space biofactory " fortified in anti-oxidants content and in free radical scavenging activity

Optimization of the production of natural anti-oxidants present in plants



TOMATO
Solanum lycopersicum

SOURCE OF BIOACTIVE MOLECULES WITH BENEFICIAL EFFECTS ON HEALTH

- anti-oxidant and anti-tumoural properties of:
ascorbic acid and other vitamins, lycopene, polyphenols, flavonoids.

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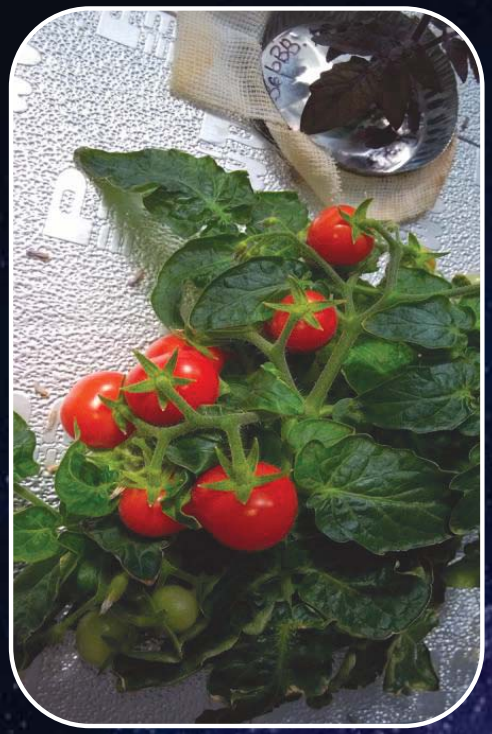

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ANTHOCYANINS:

Generally not expressed

Nutraceutical role

protect plants **against oxidative stress**

(i.e. due to ionizing radiation), softening, rotting, fungal infections

Hong MJ et al. Int J Radiat Biol. 2014; 90:1218-28

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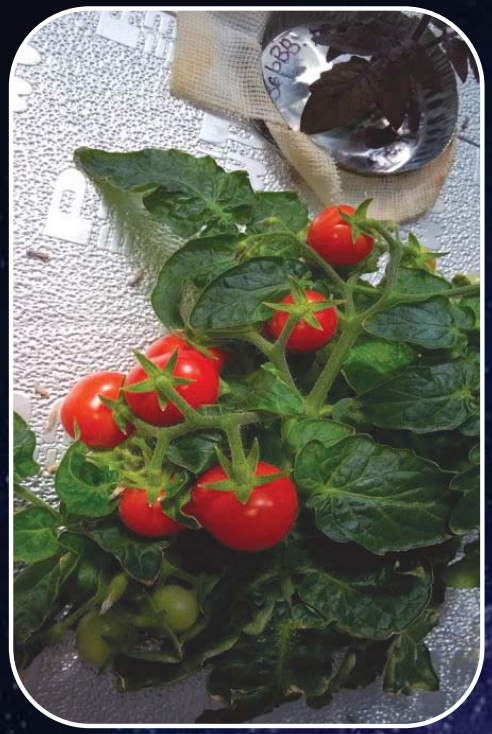

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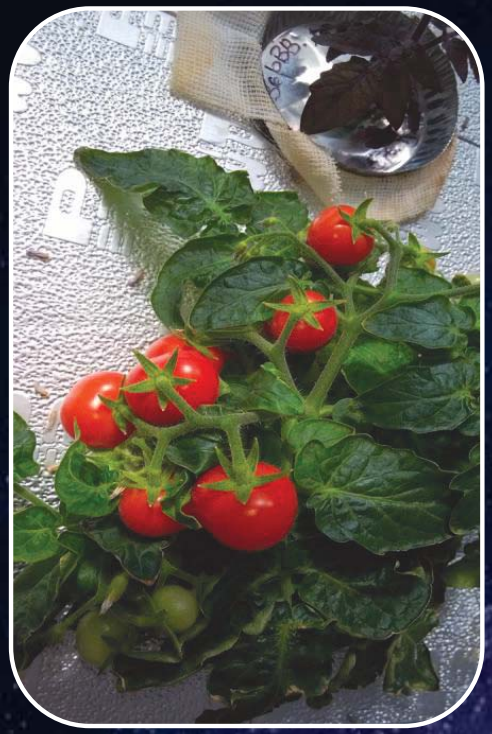
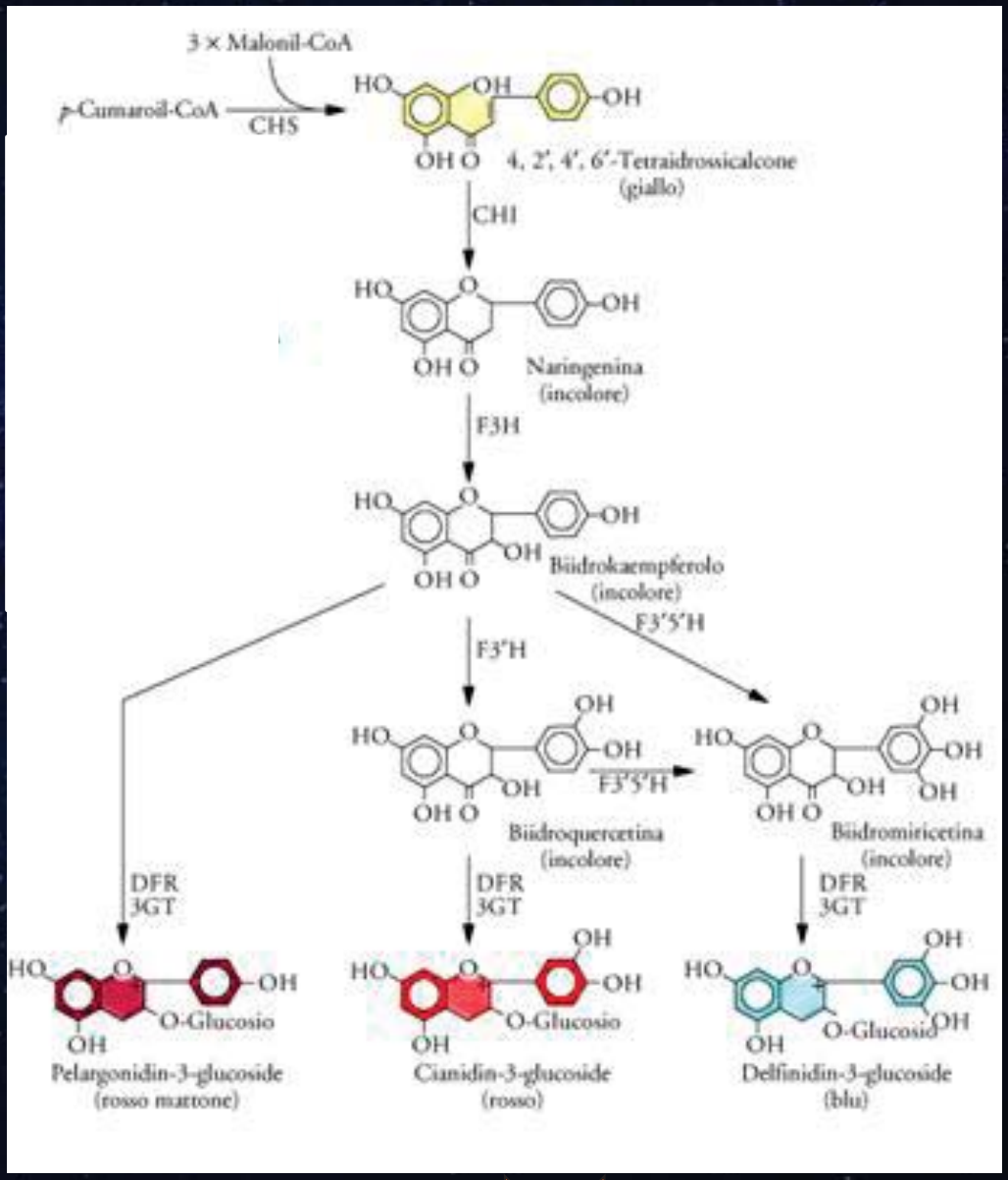
BUILD-UP A TAILOR-MADE ANTHOCYANIN-FORTIFIED TOMATO CELL SYSTEM

Optimization of the production of natural anti-oxidants present in plants



ANTHOCYANINS:

-Specific class of flavonoids responsible for petal and fruit color
-Synthesized from phenilalanine
-Lateral chains determines the final colour



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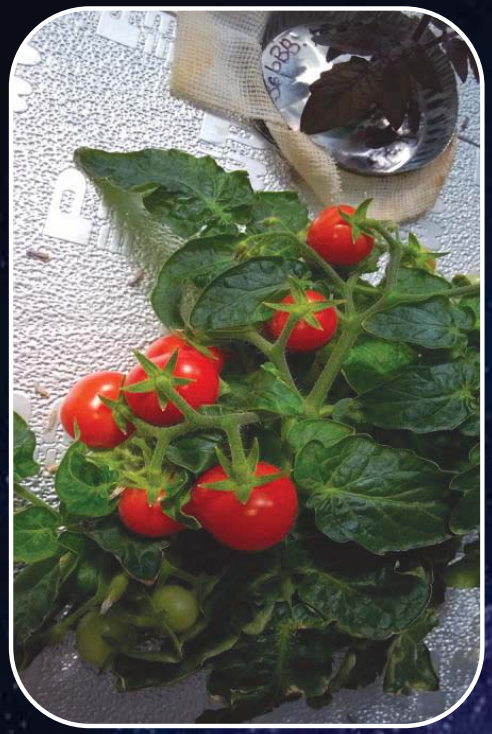
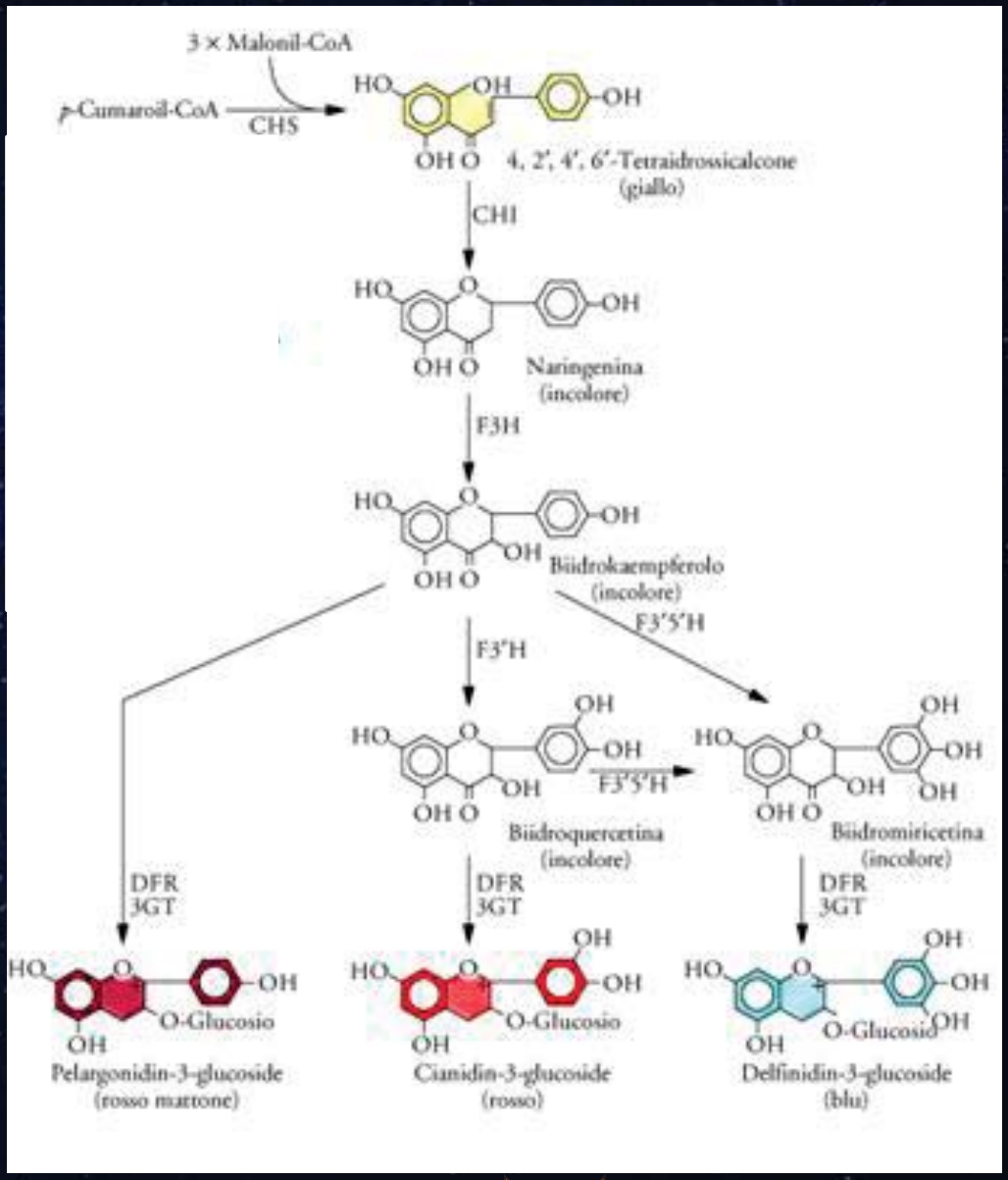


Modulating expression of pathway enzymes (e.g. by over-expression) an enhancement of bioaccumulation is possible.



BUILD-UP A TAILOR-MADE ANTHOCYANIN-FORTIFIED TOMATO CELL SYSTEM

Optimization of the production of natural anti-oxidants present in plants

In *Petunia* flower, AN4 is a transcriptional factor that mediated the activation of AN1, a protein needed for anthocyanins synthesis (flavonoid 3-O-glucosyltransferases)

↓

In tomato

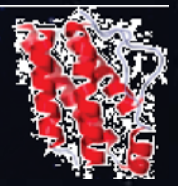
Department of Molecular Cell Biology, Graduate School of Experimental Plant Sciences, VU University, Amsterdam, The Netherlands.

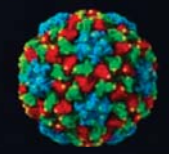
Engineering tomato as a "space biofactory " fortified in anti-oxidants content and in free radical scavenging activity

HAIRY ROOT CULTURES (HRCs)

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

- anti-tumoral antibodies (2016)
- enzymes (2008)
- hormons (2003)
- anti-HIV microbicides (2013)
- antigens (2007)





HAIRY ROOT CULTURES (HRCs)

- Mitotic, genetic and biochemical stability
- Very cost-effective (simple media, no hormones)
- No vectors of human pathogens (unlike mammalian cells bioreactors)
- Not time-consuming
- Very high biomass accumulation
- High scalability
- GMP-like (contained, sterile)

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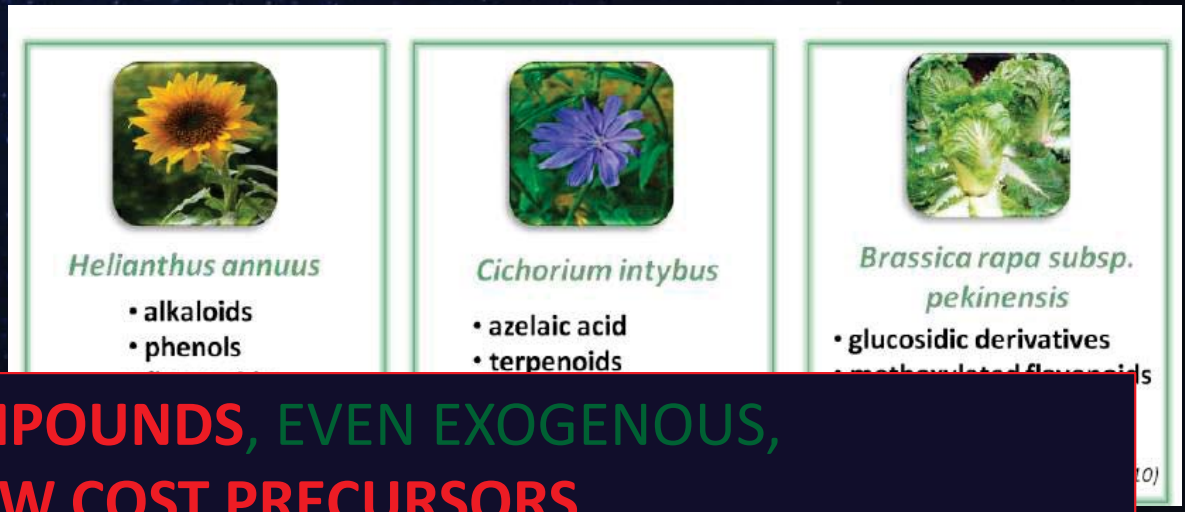
HAIRY ROOT CULTURES (HRCs)

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

* Same phytochemical pattern of the corresponding organ stimulated by A. rhizogenes *rol* genes.
* possibility of creating new phytochemical patterns



HIGH VALUE COMPOUNDS, EVEN EXOGENOUS,
FROM **LOW COST PRECURSORS**

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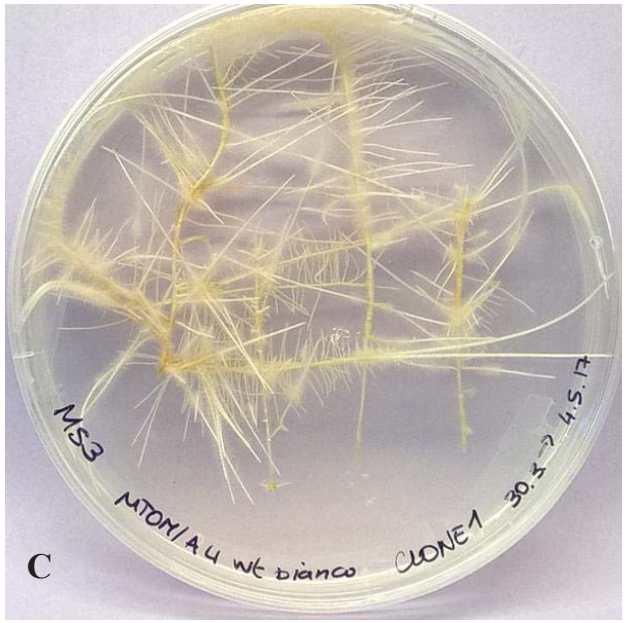
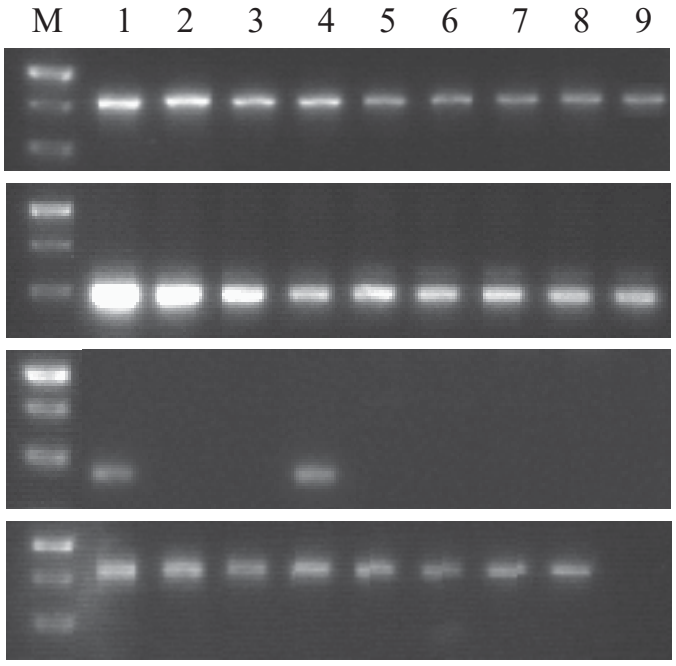
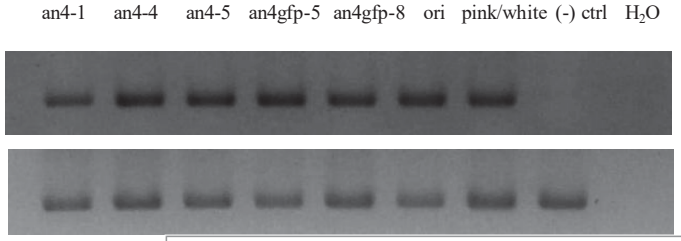
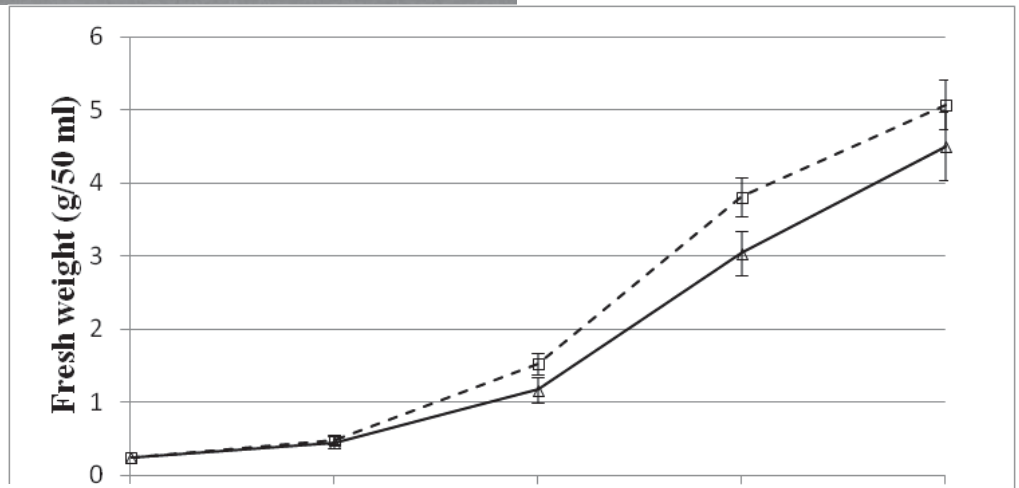




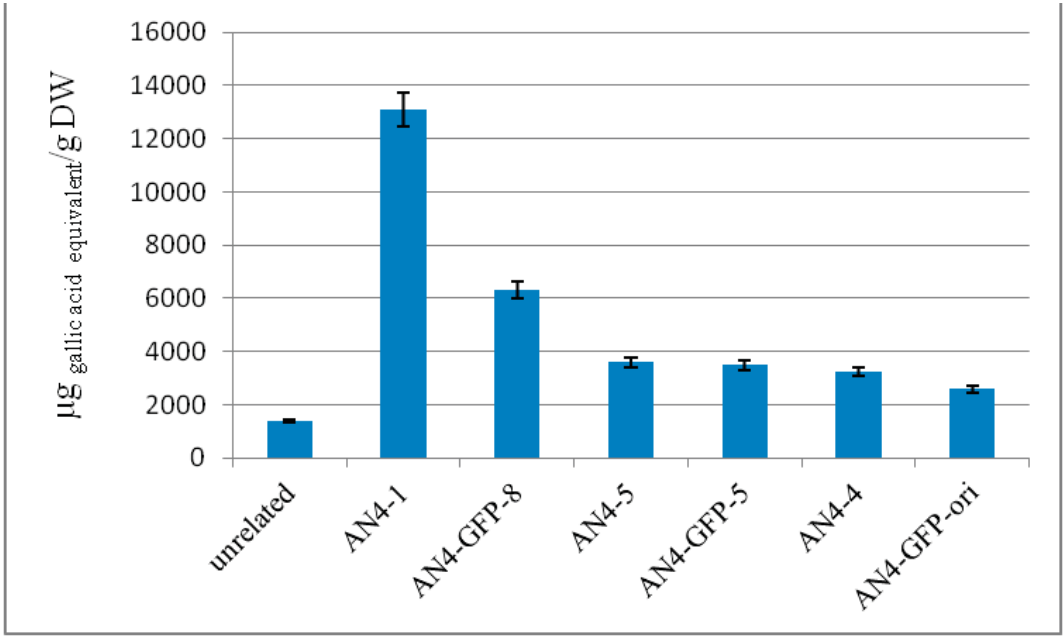


purple phenotype and normal growth

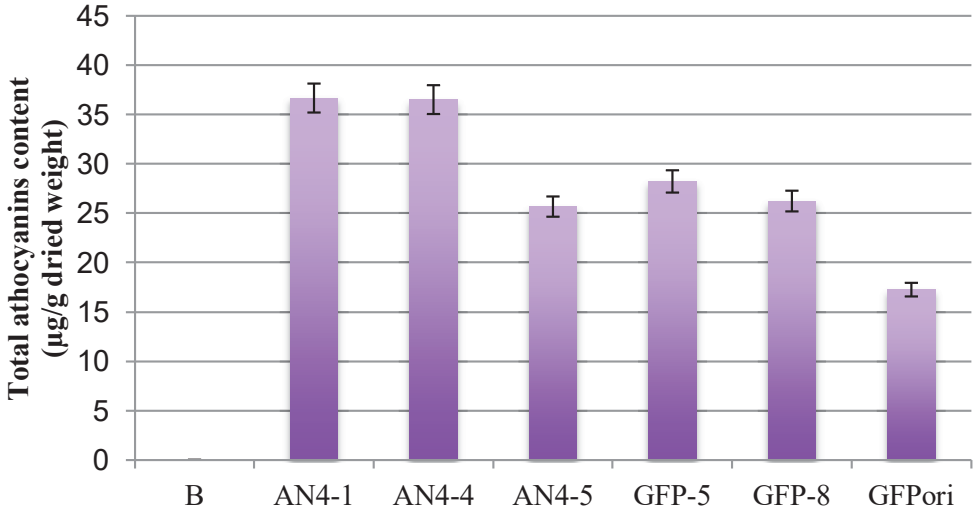


Molecular analysis demonstrated that the *PhAn4* construct was together with the typical array of genes by the Ri plasmid (i.e. *rolC* and *rolB* genes).

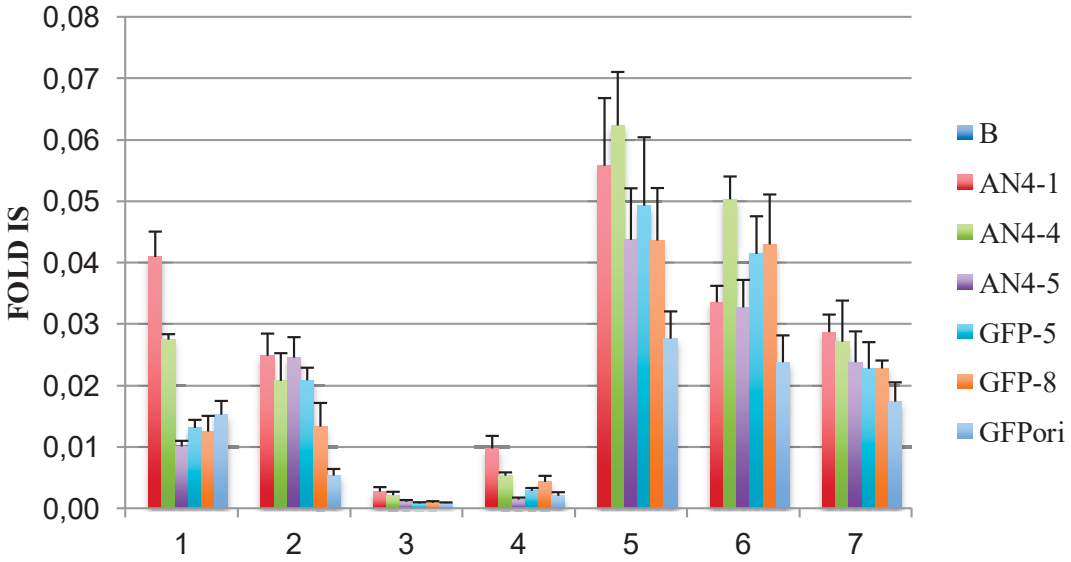
As a downstream effect of *PhAn4* transformation, a significant increase of total polyphenol content was observed



Anthocyanins content of transgenic tomato hairy roots



A



B

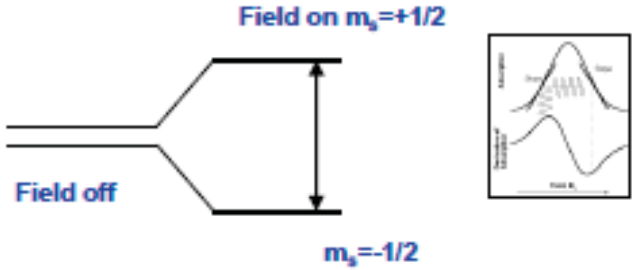
Total anthocyanins content (**A**)

1: Delphinidin 3,5-O-diglucoside;
2:Delphinidin-3-(p-coumaroyl)-rutinoside-5-glucoside;
3: Malvidin-3-O-(4‴coumaroyl)-rutinose-5-O- glucose;
4: Pelargonidin-3-glucoside;
5: Petunidin-3-(p-coumaroyl)-rutinoside-5-glucoside1;
6: Petunidin-3-(p-coumaroyl)-rutinoside-5-glucoside2;
7: Petunidin-3-feruloyl-rutinoside-5-glucoside.

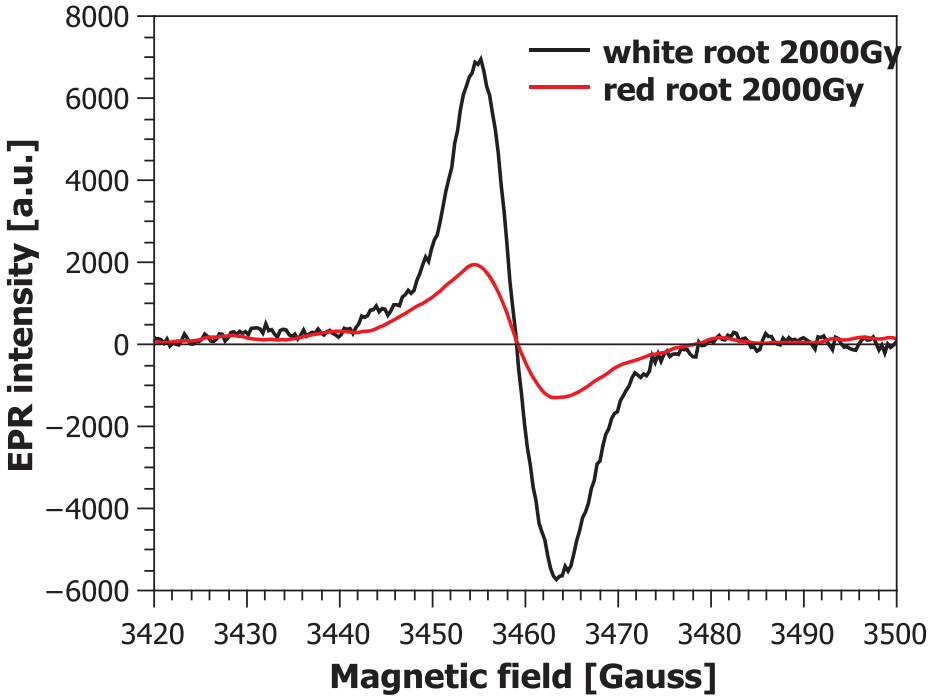
- pelargonidin-3-glucoside can be absorbed in intact form into the gastrointestinal wall,
- enters the systemic circulation as 4-hydroxybenoic acid, considered one of the main responsible for the systemic health effects of anthocyanins.
-play a major role in reducing genotoxic stress induced by environmental toxicants

-Malvidin-3-O-glucoside, significant effects on major cardiovascular risk factors (Juturu, 2014).

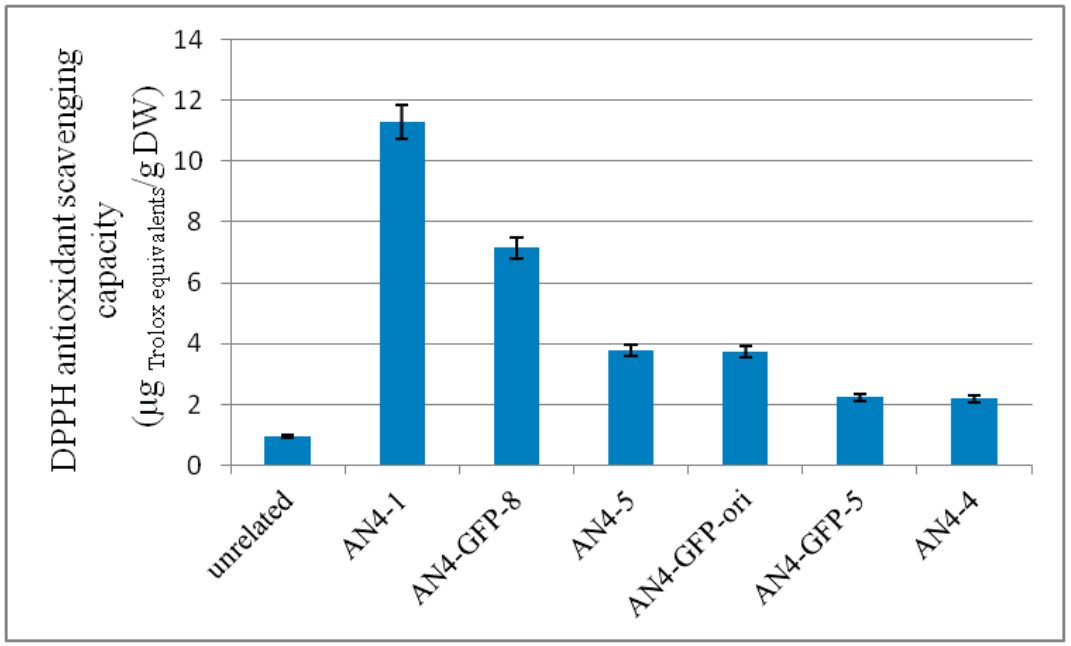
Electron Spin Resonance spectroscopy (ESR) specific for the investigation of paramagnetic species *(free radicals)* (RT, air) after gamma irradiation



ESR signal is proportional to the free radicals number



X-band ESR spectrometer, e-scan Bruker



Same absorbed dose, more free radicals generated in white than in purple HRCs

white HRCs undergo main oxidative damage

purple tissues have available ready-made free radicals scavengers resulting in a considerable decrease of the singlet intensity.

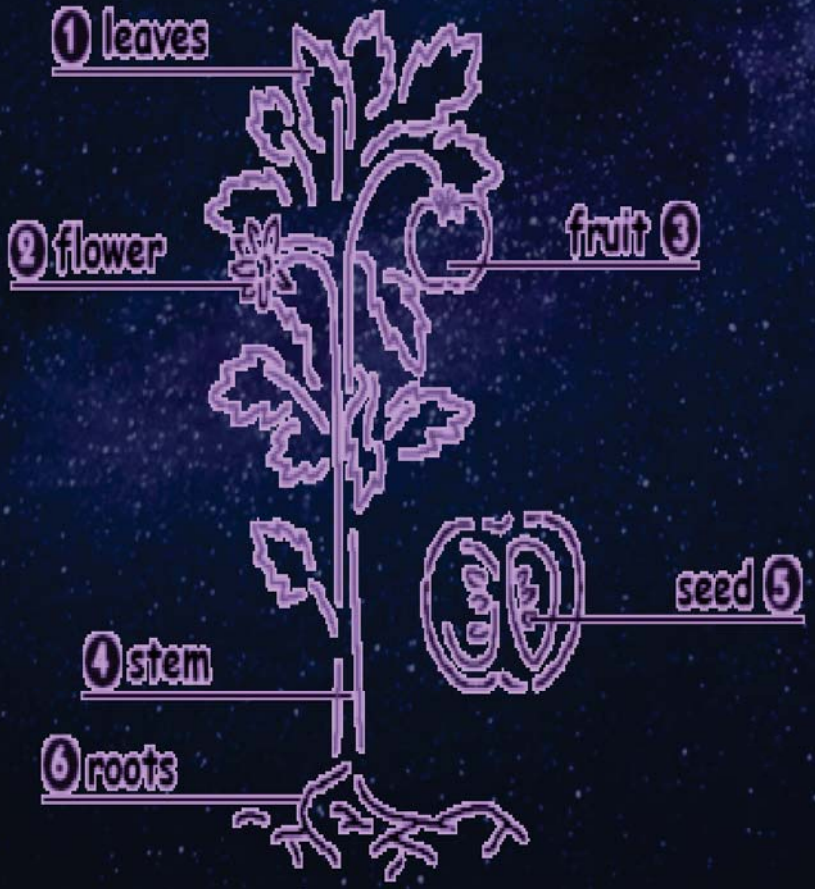
correlated with the antioxidant activity of anthocyanins and polyphenols, due to their phenolic nature.

Reduction of free radicals levels was also supported by evidence of DPPH assay results.

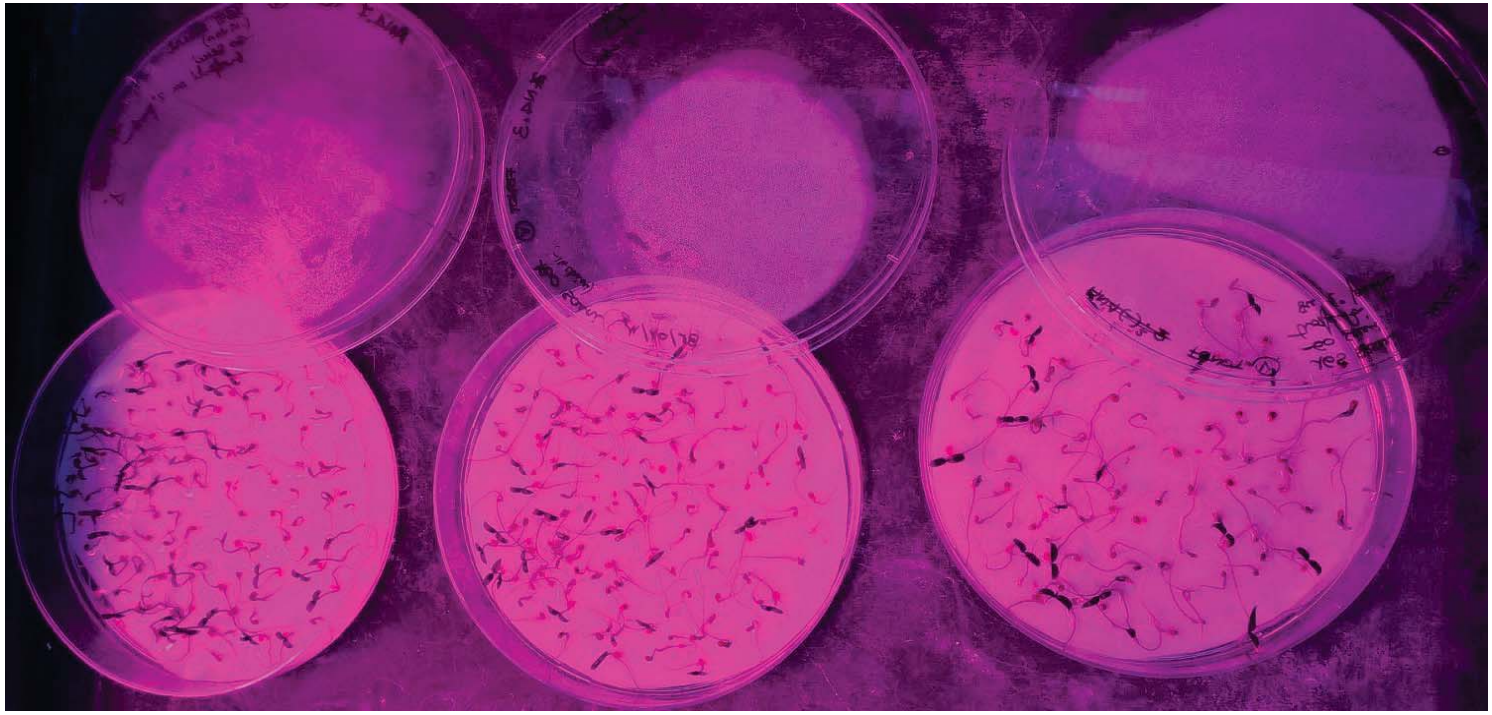
These results suggest that the activities of anthocyanins and polyphenols induced by *PhAn4* may counteract oxidative stress in normal conditions and under gamma-irradiated conditions.

Optimization of the production of natural anti-oxidants present in plants (Micro Tom whole plants)

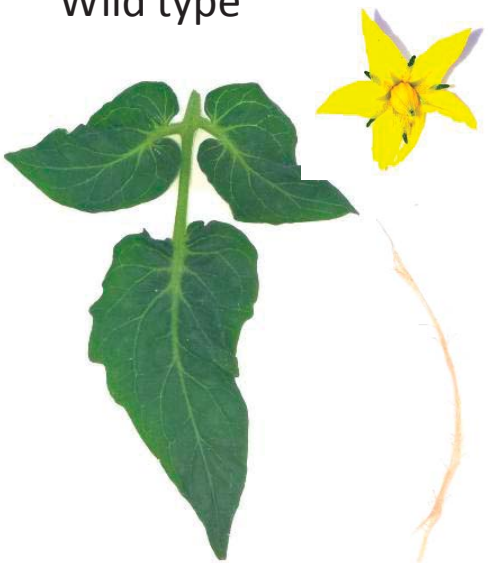
Agro-Transformation



MicroTom plants with enhanced anthocyanin level



Wild type

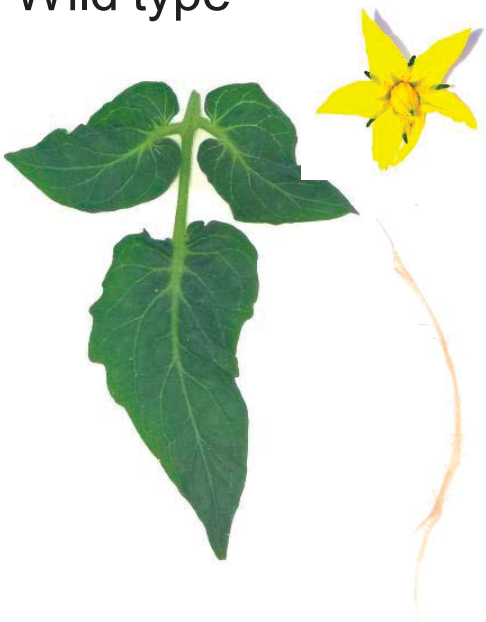


Wild type

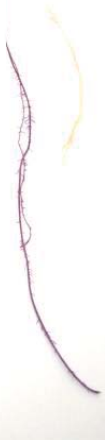
Dark red phenotype

Dark red phenotype

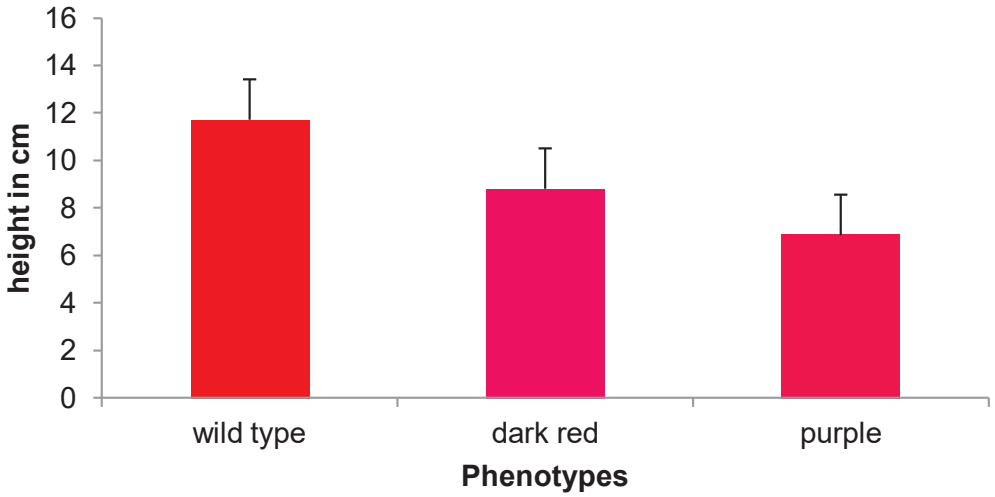
Wild type



ENEA

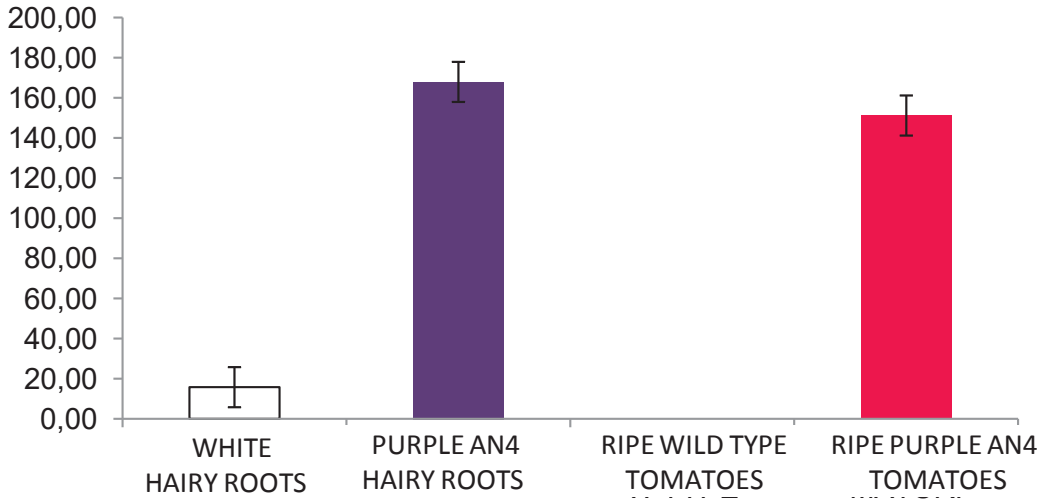


Plant height at mid life cycle



6,856371801	1,956585
8,806332837	2,538982
11,71333333	3,361927

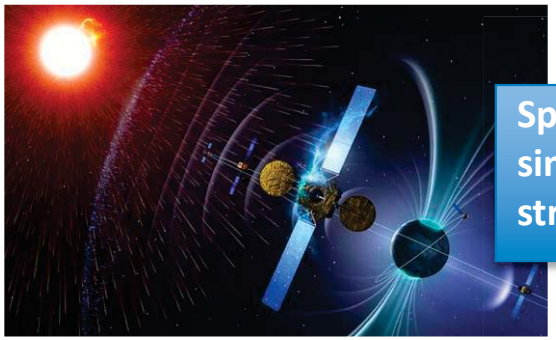
Anthocianins (µg/g)



Fare anche per numero e peso frutti?

As a downstream effect of *PhAn4* transformation, the bioaccumulation of anthocyanins was observed in ripe an4 tomatoes with respect to wild types.

Final goal: Cultivating in space …

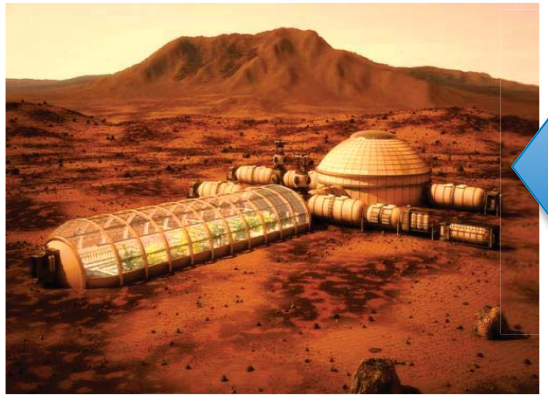


Space simulation stresses

Plant response to simulation

Cultivation in simulated missions

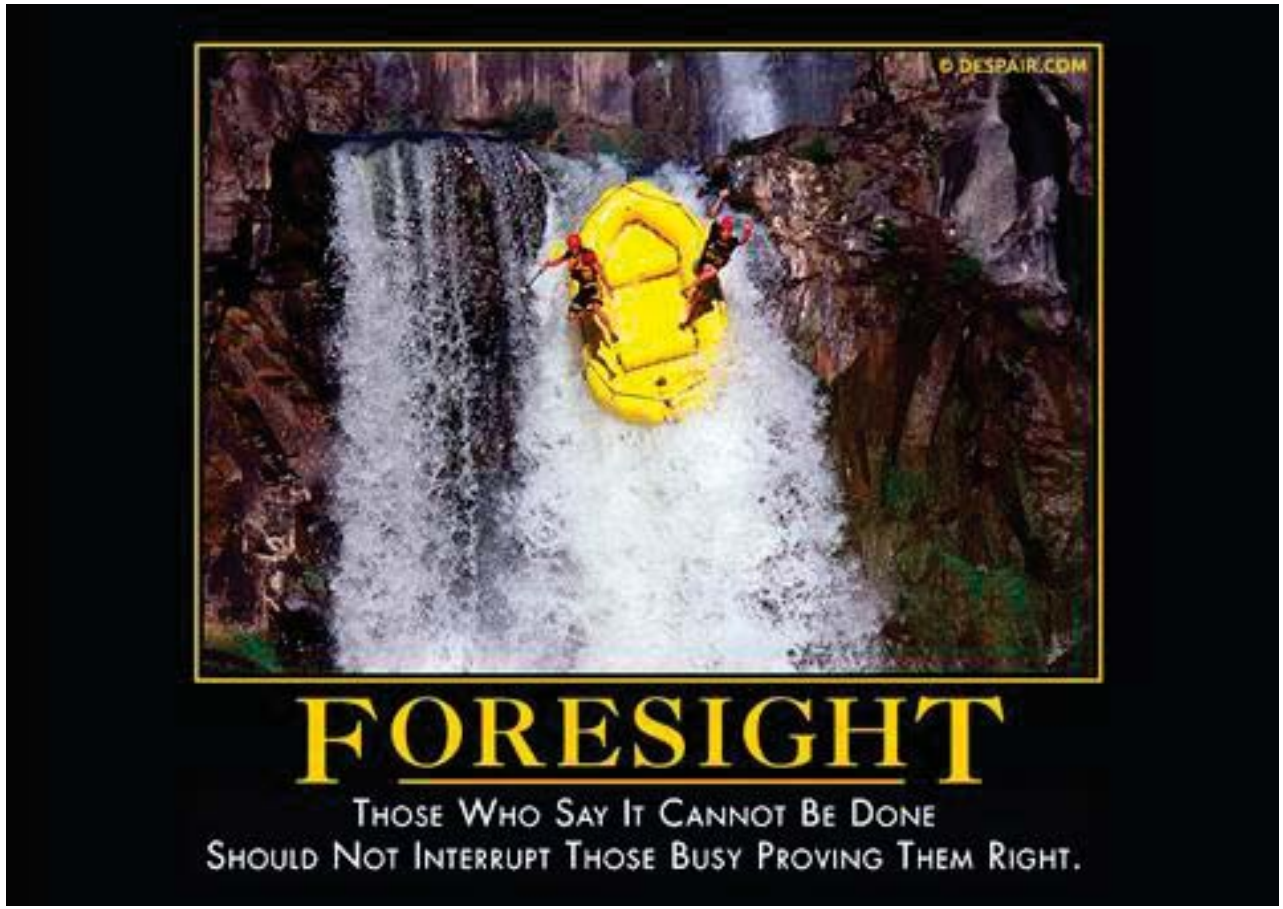
Space horticulture !



✓For the first time, Purple HRCs were obtained from MicroTom accumulating anthocyanins.

✓Tomato do synthesize anthocyanins and, with no further optimization of the culture medium/system, we were able to obtain total anthocyanin concentrations similar or superior to those yet reported for other plants

✓Purple HRCs might be a good candidate for the bio-production of this class of compounds. Further studies will reveal how this tool might become a 'portable' bioreactor for the production of ready-to-use bioactive molecules in space missions (final aim of the project).



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THANKS FOR YOUR ATTENTION

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