Italian National Agency for New Technologies, Energy and Sustainable Economic Development

"Biomedicina Spaziale per le Future Missioni di Esplorazione Umana dello Spazio: a Call to Action"

Nutrizione e tecnologie per la produzione e conservazione di alimenti nello spazio (NUT)

ProduzIoNe di alimenti VEgetali *iN viTro e* mediante biotecnologie e 3D (bio)printing per Applicazioni spaziali (INVENTA)

PARTNERSHIP:

EltHub S.r.l., Oricola (L'Aquila); Italy.
Ing. Giorgia Pontetti
CREA-AN

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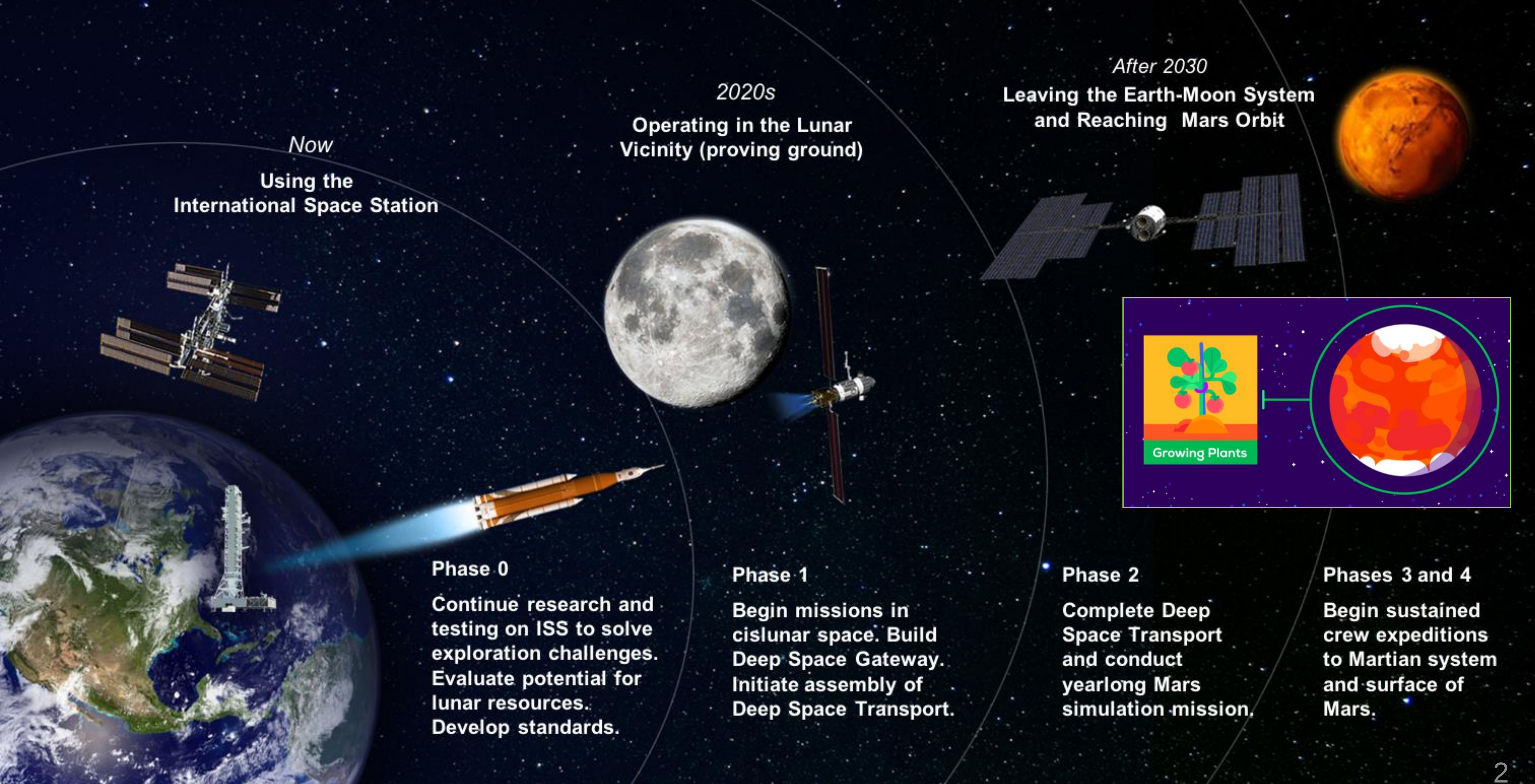
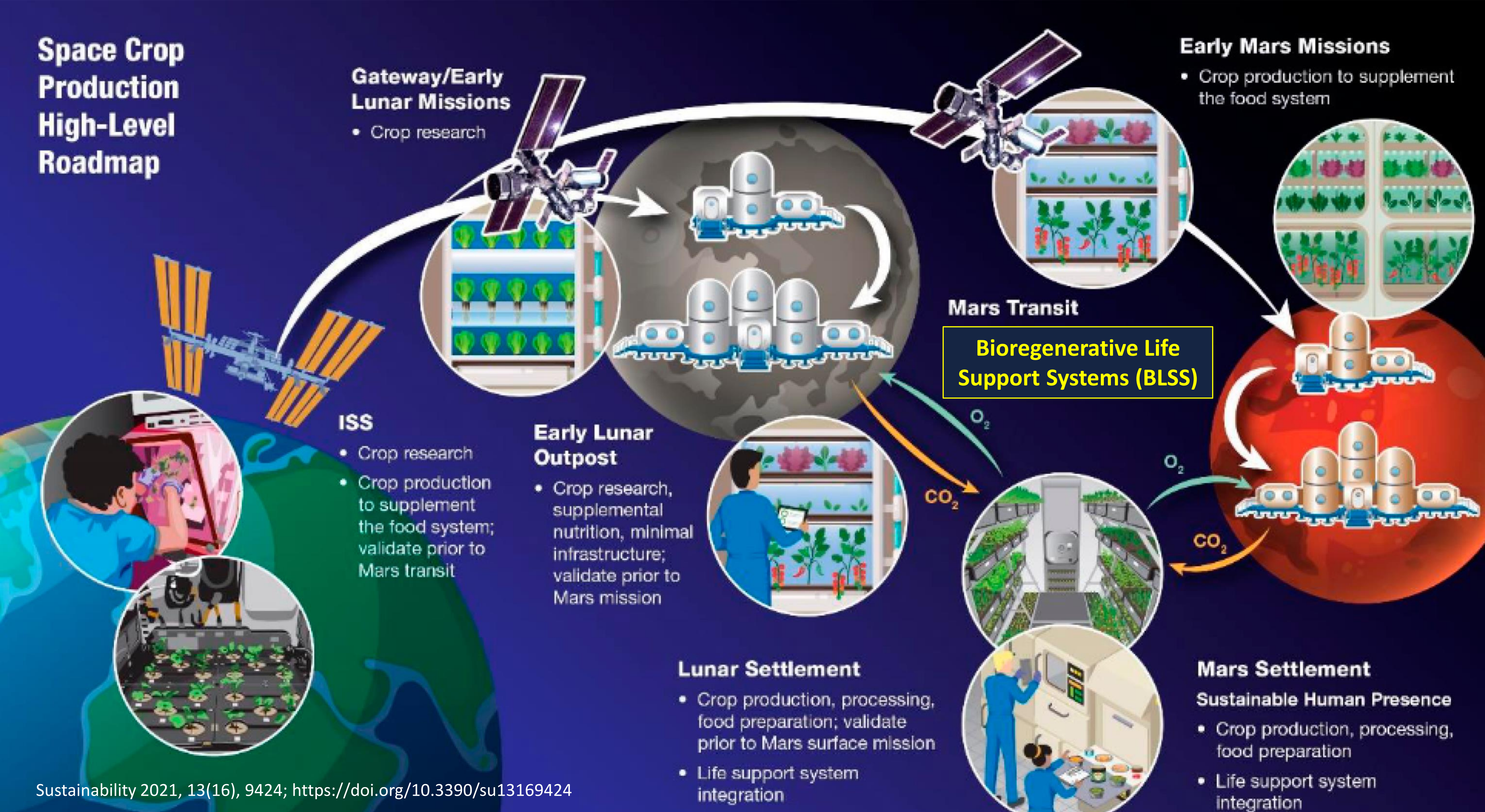
NASA ROADMAP OF HUMAN EXPLORATION OF DEEP SPACE





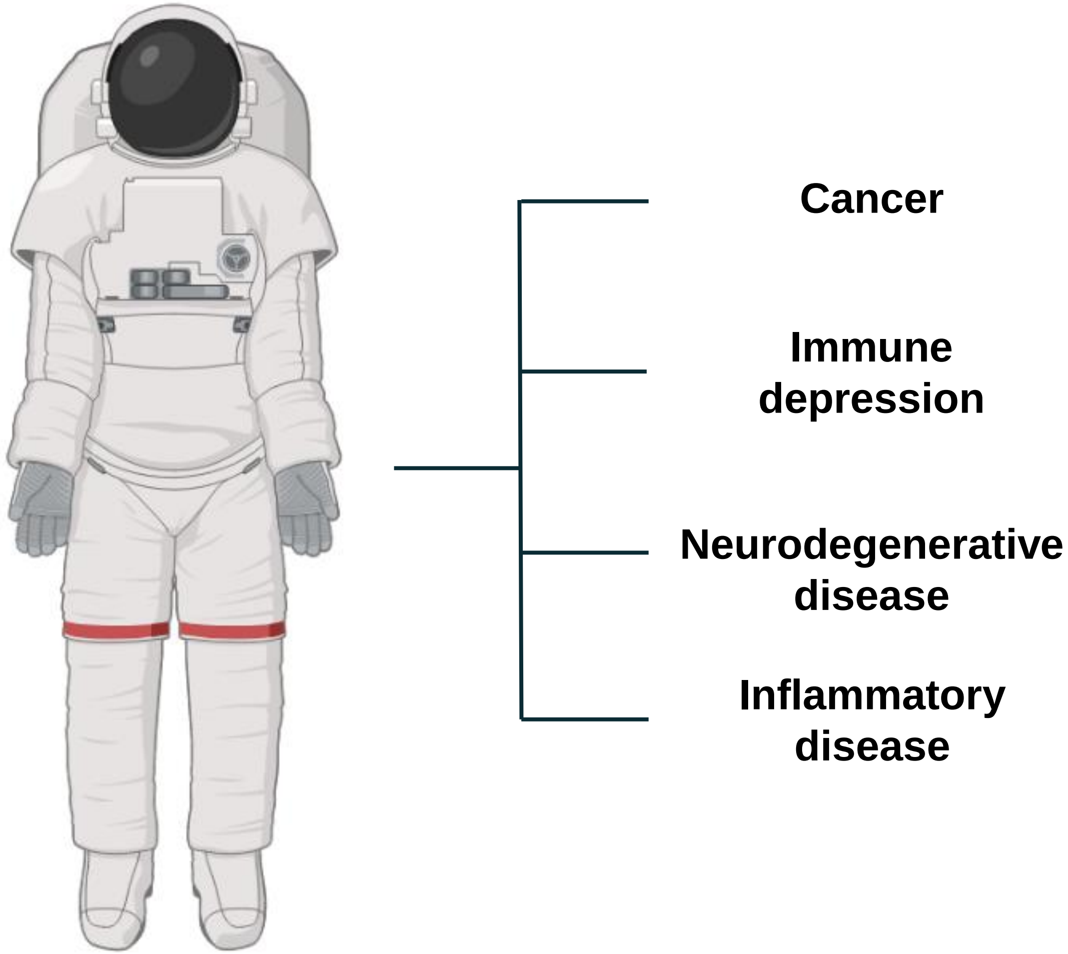
Sustainability 2021, 13(16), 9424; https://doi.org/10.3390/su13169424

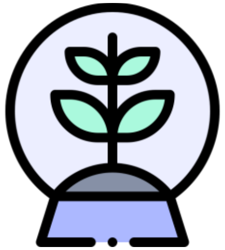
HOW PLANTS ARE EXPECTED TO ASSIST HUMAN LIFE IN SPACE

- oxygen production, carbon dioxide uptake and water purification in BLSS

Growing crops in space:

- Earth-independent, healthy, fresh food;
- Source of bioactive molecules to counteract the effects of permanence in confined environments exposed to astro-physical stimuli;
- Physical and psychological support for crewmembers forced to live in a confined and harsh environment





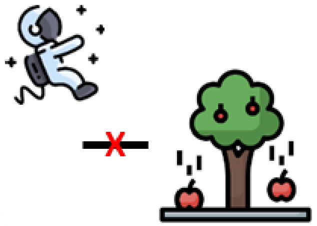
in situ cultivation of plants in various formats, resistant and capable of producing molecules with nutraceutical/pharmaceutical value, will assume a crucial role for the supply of fresh food and molecules with high added value (e.g. antioxidants)

Factors affecting plants in space

Distance

Confined environment

Gravity



Radiation
(e.g., alpha particles, neutrons, protons, **gamma rays**, X rays)

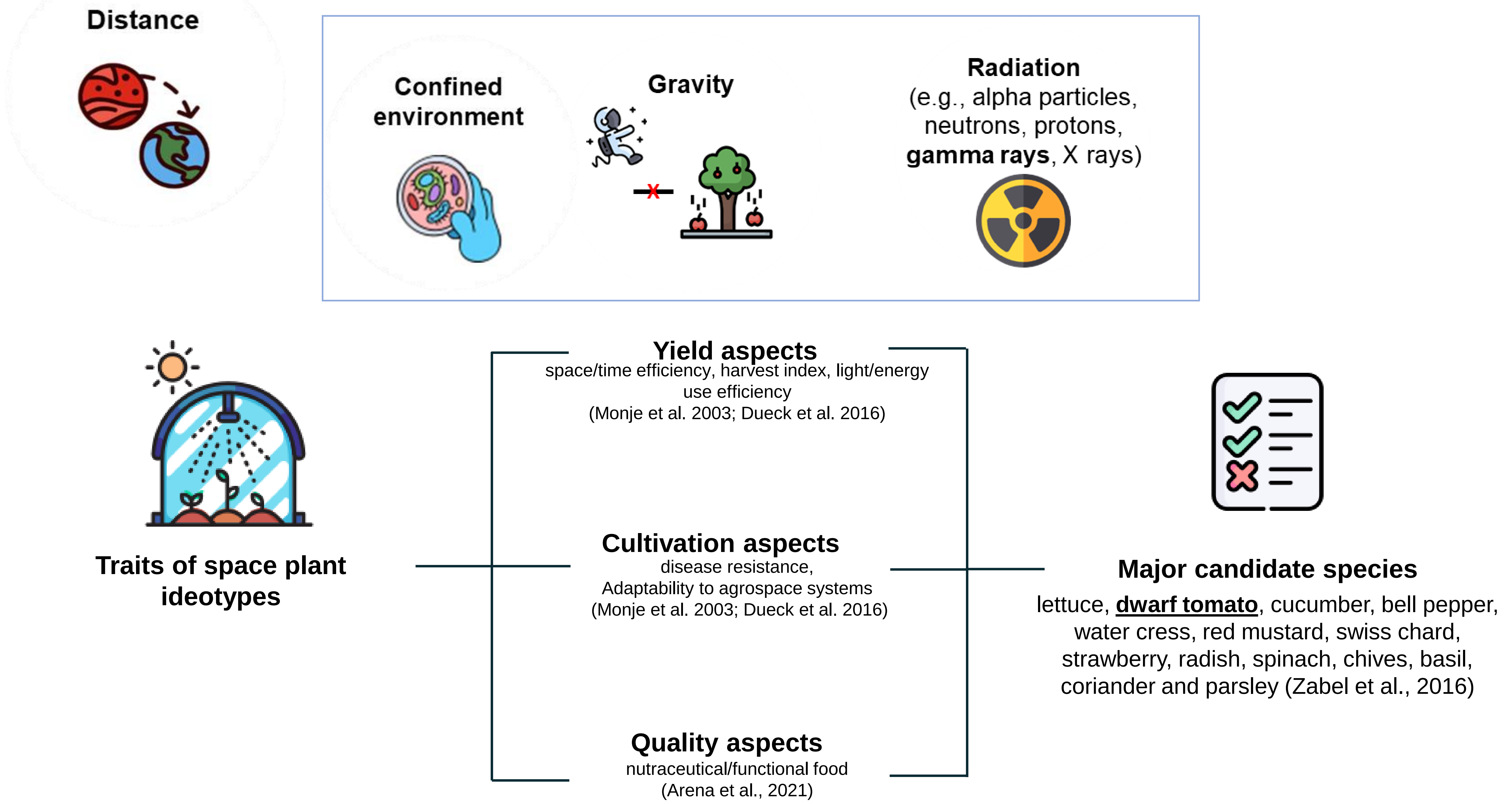
Effects on genome and proteins

Effects on photosynthesis and growth

Effects on morpho-anatomical traits

Effects on food quality

Plant systems and ideotypes for 'agrospace purposes'



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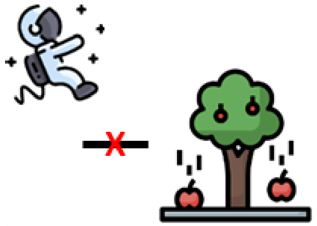

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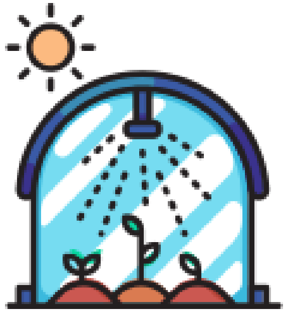


Traits of space plant ideotypes

Yield aspects
space/time efficiency, harvest index, light/energy use efficiency
(Monje et al. 2003; Dueck et al. 2016)

Cultivation aspects
disease resistance,
Adaptability to agrospace systems
(Monje et al. 2003; Dueck et al. 2016)

Quality aspects
nutraceutical/functional food
(Arena et al., 2021)

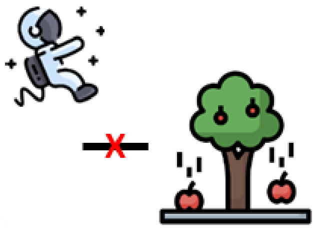
- lycopene, ascorbic acid, alpha-lipoic acid, choline, folic acid and lutein;
- small size (height: 15-20 cm at maturity);
- short life cycle (seed-seed: 70-100 days);
- high productivity (20-30 fruits/plant; 2-5 gr/fruit);
- LED light tolerance;
- high density growth (≥ 100 plant/m^2);
- good performances in soilless conditions
- Autogamous (no need of impollination under certain conditions)

Plant systems and ideotypes for 'agrospace purposes'

Distance

Confined environment

Gravity



Radiation
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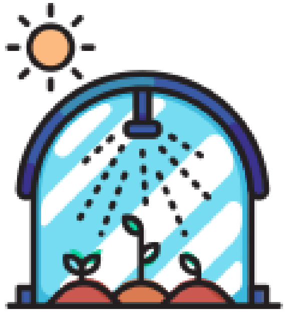

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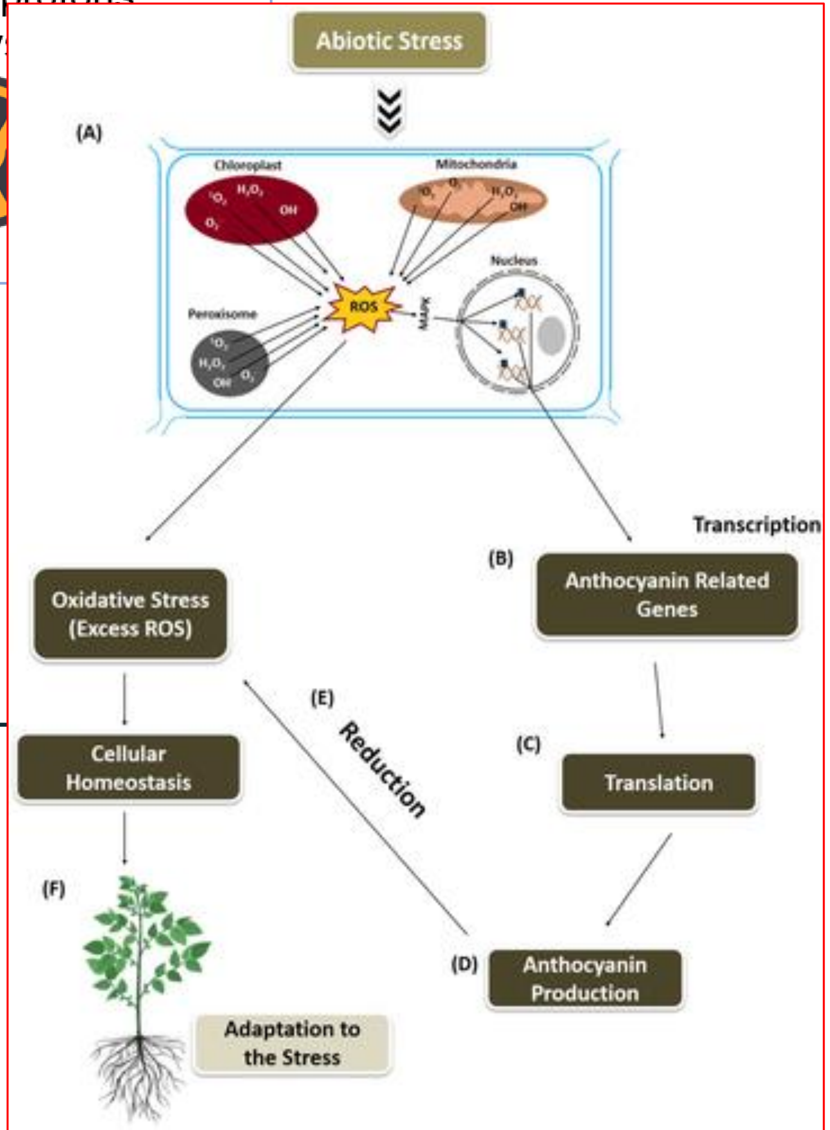

Our space ideotypes: a biofortified MicroTom accumulating anthocyanins

frontiers | Frontiers in Astronomy and Space Sciences

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Designing a novel tomato ideotype for future cultivation in space manned missions

Riccardo Pagliarello[1,2], Elisabetta Bennici[1], Alessia Cemmi[3], Ilaria Di Sarcina[3], Cornelis Spelt[4], Luca Nardi[1], Antonella Del Fiore[5], Patrizia De Rossi[6], Francesca Paolini[7,8], Ronald Koes[4], Francesca Quattrocchio[4], Eugenio Benvenuto[1] and Silvia Massa[1]*

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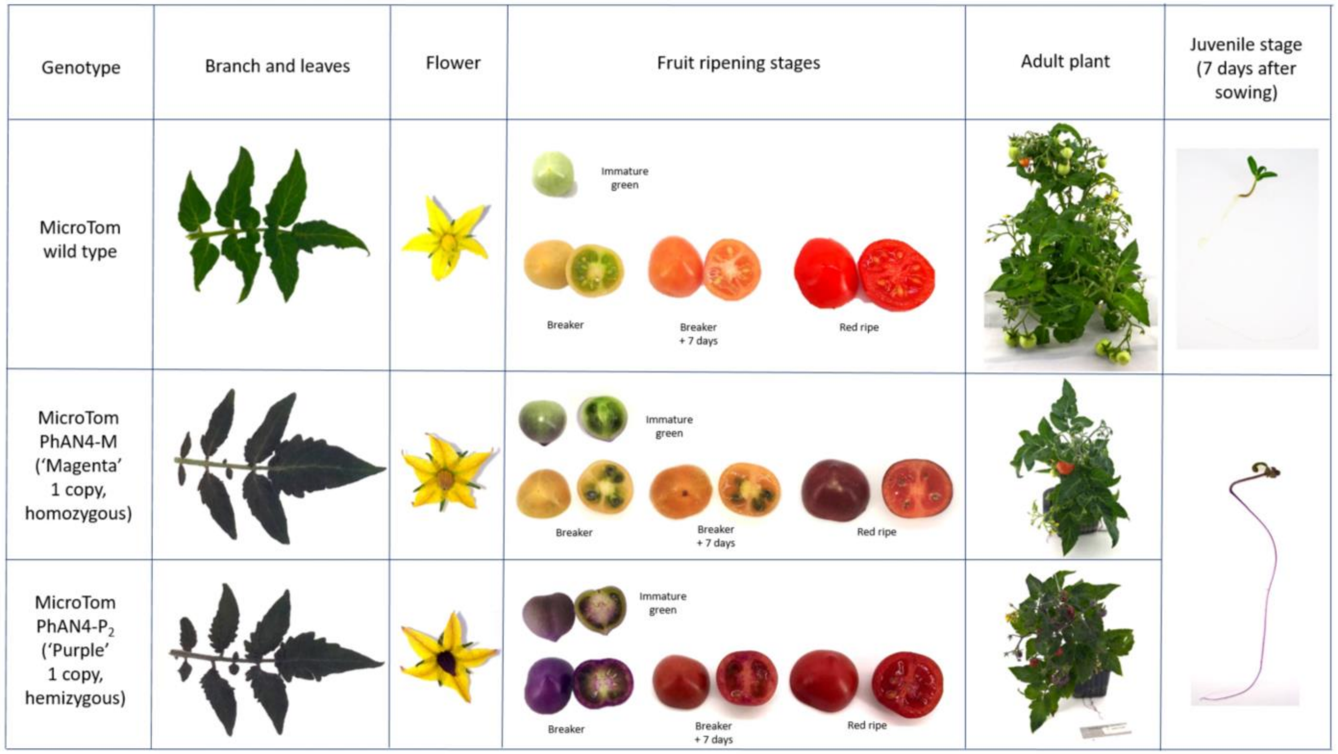
Introduction: Methods for production of fresh, health food are needed in view of long-term, deep-space manned missions. To this end, crops tailored for better performance under non-terrestrial conditions may be obtained by the exploitation of biochemical patterns related to specialized metabolites known to confer protection against environmental challenges and to be beneficial to human health.

Methods: In this work, for the first time, MicroTom plants have been engineered specifically for agrospace applications to express *PhAN4*, a MYB-like transcription factor able to regulate the biosynthesis of anthocyanins that influence tomato genes possibly involved in agrospace-relevant functions.

Results: *PhAN4* engineering underpinned the genetic background of the dwarf tomato MicroTom while maintaining yield and photosynthetic capacity. *PhAN4* expression resulted in the accumulation of anthocyanins and polyphenols, a differential carotenoid profile, increased antioxidant scavenging capacities of fruits compared to the original genotype. Improved ability to counteract ROS generation and to preserve plant protein folding after *ex-vivo* gamma irradiation was observed.

Discussion: These results highlights that the manipulation of specific metabolic pathways is a promising approach to design novel candidate varieties for agrospace applications.



MicroTom plants accumulating anthocianins

Agenzia Spaziale Italiana

(AN4 gene from *Petunia hybrida*)

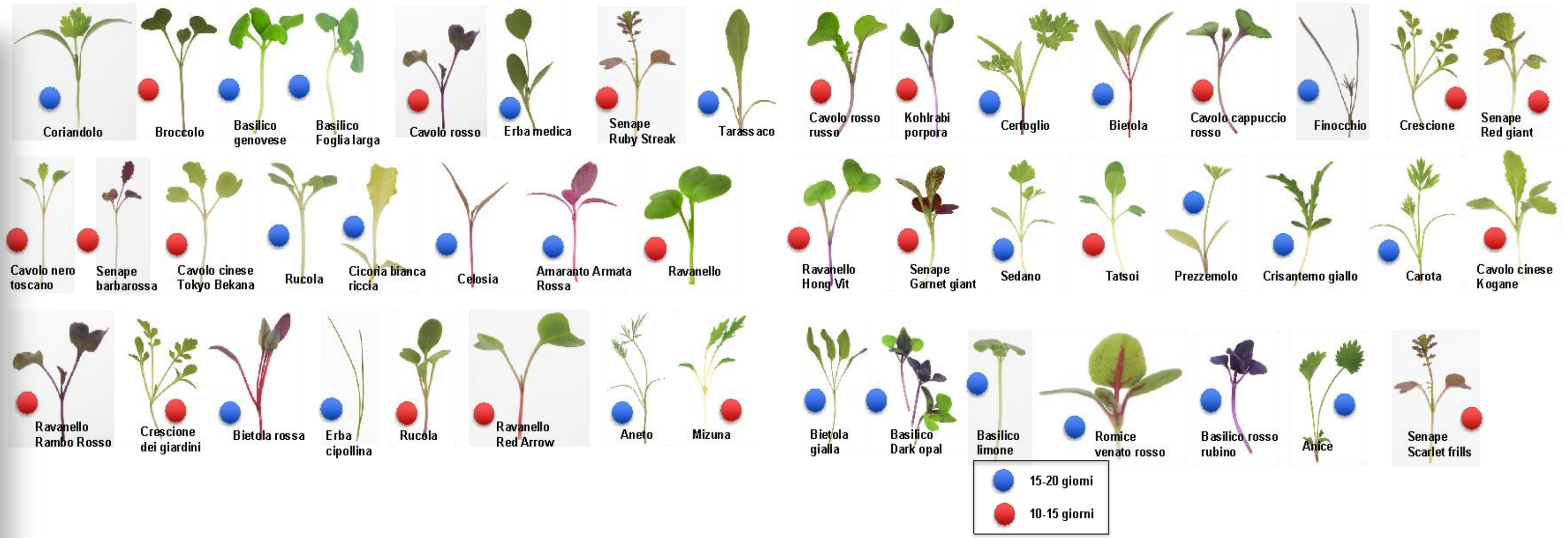
Italian National Agency for New Technologies, Energy and Sustainable Economic Development

- No effects on yield traits and photosynthetic performances;
- Ripe fruits show significantly higher ability to counteract ROS accumulation and protein oxidation upon IR-related pro-oxidant stimulus compared to wild type.
- AN4 gene: possible beneficial effects on other resistance traits

Our space ideotypes: microgreens

Microgreens (‘pick-and-eat’)

- edible *plantulae* produced from various species of vegetables, herbaceous crops, aromatic herbs, and wild plants;
- harvested as early as 8-15 days after sowing, when the cotyledons are fully developed and the first true leaves have appeared;
- show an immense variety of colors, flavors, aromas, and textures, and can serve also to reduce the so called ‘menu fatigue’;
- Several scientific studies have shown that they have higher density of vitamins, some minerals and other phytonutrients compared to the plant at the adult stage.



Agenzia Spaziale Italiana

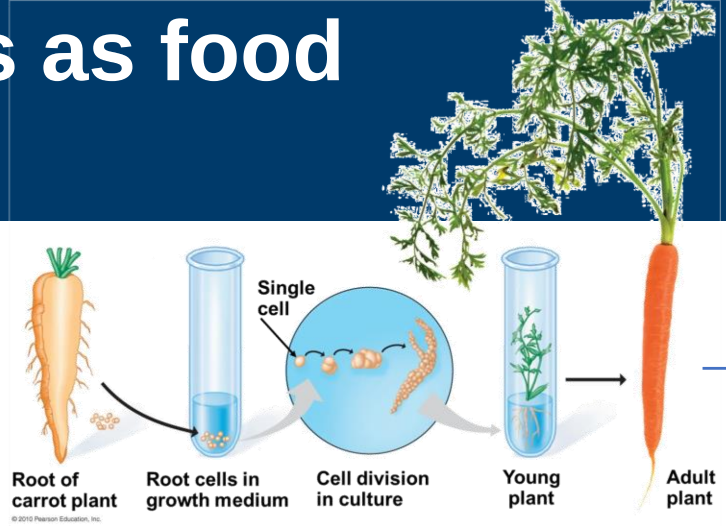
Soilless, contained cultivation of microgreens at ENEA

Italian National Agency for New Technologies, Energy and Sustainable Economic Development

Our space ideotypes: Plant cell cultures as food

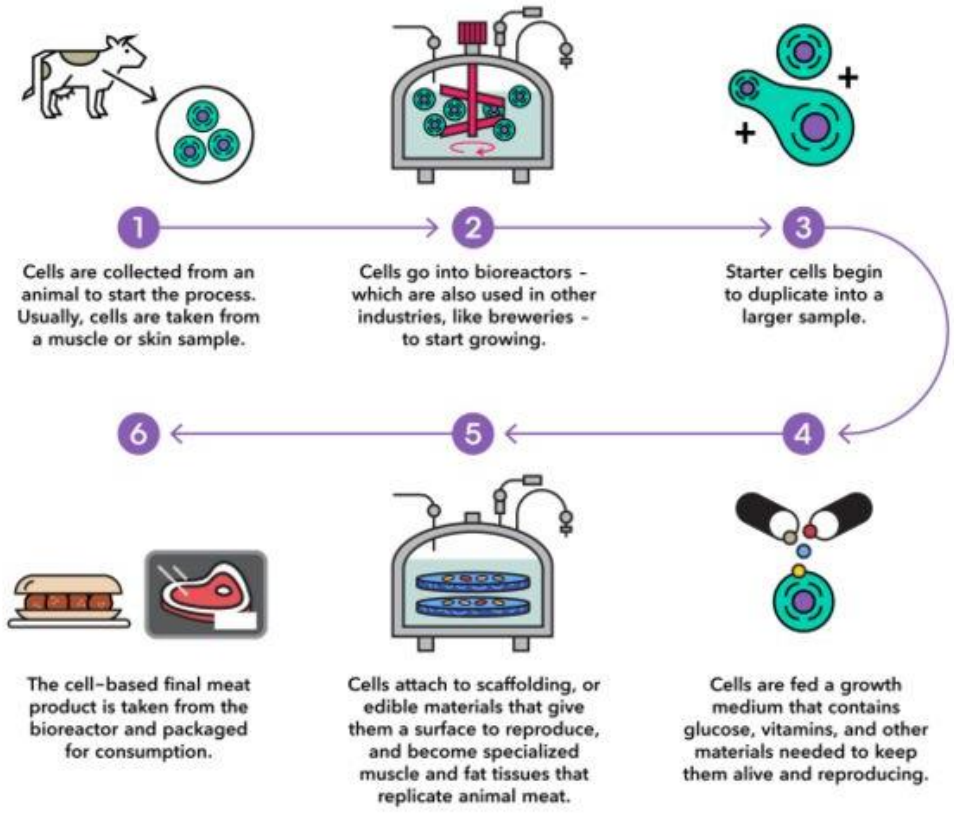
✓ PLANT CELL AGRICULTURE

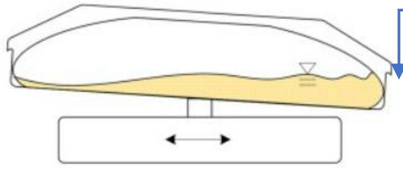
biotechnological 'in-lab' production of plant food from plant cell cultures with implications also on Earth in the next future →



Explant

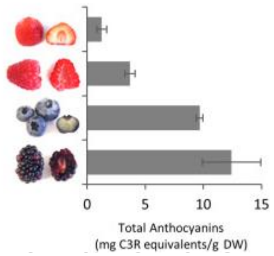
✓ ANIMAL CELL CULTURE





Bioreactors

Plant cell cultures from plant explants

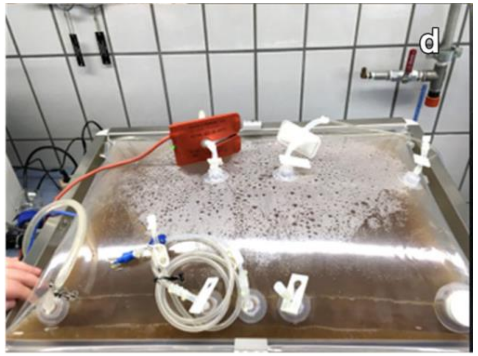


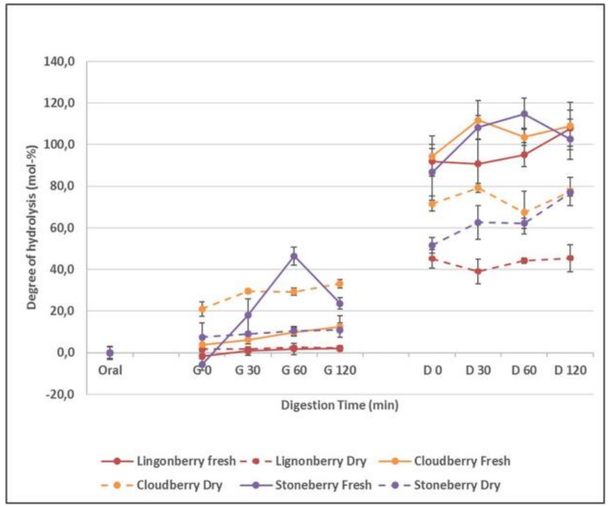
INVENTA (ProduzIoNe di alimenti VEgetali iN viTro, mediante biotecnologie e 3D (bio)printing per Applica

Our space ideotypes: Plant cell cultures as food

✓ **PLANT CELL AGRICULTURE**

biotechnological 'in-lab' production of plant food from plant cell cultures





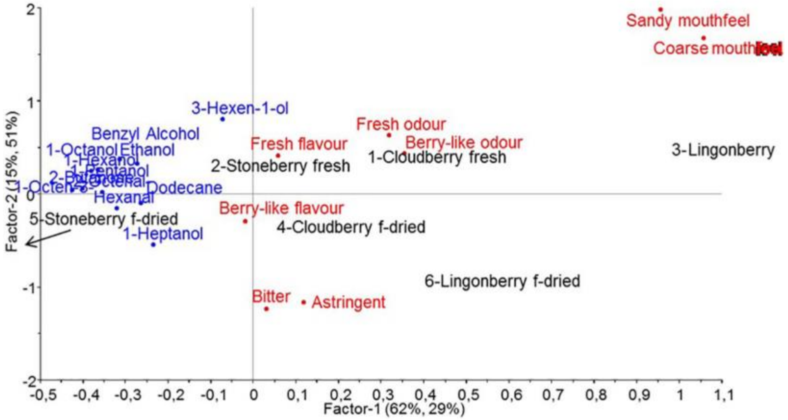


Table 3
Starch, dietary fibre (DF, including insoluble (ISDF) and soluble (SDF) dietary fibre, and acid insoluble and soluble material) and sugar composition of cloudberry, lingonberry and stoneberry cell cultures. The results are expressed as % or mg/g dw ± SD (n = 3).

	Cloudberry	Lingonberry	Stoneberry
Starch [%]	0.5 ± 0.0	0.3 ± 0.0	1.3 ± 0.1
DF total [%] (sum ISDF + SDF)	36.7	34.5	21.2
DF insoluble [%]	31.9 ± 1.3	30.0 ± 0.2	18.3 ± 0.2
DF soluble [%]	4.8 ± 1.2	4.5 ± 0.5	2.8 ± 0.6
Acid insoluble material [%]	3.5 ± 0.3	17.3 ± 1.4	2.1 ± 0.2
Acid soluble material [%]	7.6 ± 0.6	5 ± 0.4	3.8 ± 0.3
Free sugars [%] (Sum of Glu, Xyl, Ara, Gal, Man, Rha, Fru, Suc)	33.6	23.3	17.6
Free sugar composition [mg/g]			
Glucose	124.5 ± 2.8	53.9 ± 2.7	153.4 ± 1.7
Xylose	< 0.4	0.9 ± 0.0	< 0.4
Arabinose	< 0.4	< 0.4	< 0.4
Galactose	1.3 ± 0.0	0.8 ± 0.0	1.5 ± 0.1
Mannose	1.2 ± 0.0	< 0.4	0.4 ± 0.0
Rhamnose	< 0.4	< 0.4	< 0.4
Fructose	110.4 ± 3.3	100.0 ± 2.8	21.0 ± 0.2
Sucrose	72.5 ± 4.8	16.3 ± 0.4	1.65 ± 0.2
Total sugar composition [mg/g]			
Glucose[a]	142.2 ± 2.1	153.2 ± 2.3	268.3 ± 4.0
Xylose	11.9 ± 0.2	11.4 ± 0.2	6.5 ± 0.1
Arabinose	29.5 ± 0.4	19.9 ± 0.3	6.7 ± 0.1
Galactose	21.8 ± 0.3	27.6 ± 0.4	14.8 ± 0.2
Mannose	5.2 ± 0.1	3.3 ± 0.0	2.4 ± 0.0
Rhamnose	4.9 ± 0.1	3.4 ± 0.1	2.3 ± 0.0
Fructose[b]	nd	nd	nd

Table 4
Amino acid composition of PCC samples. The results are expressed as mg/g dw ± SD (n = 3). The calculated sum of the amino acids is considered as the total protein content of the sample.

Amino acid content [mg/g] dw	Cloudberry	Lingonberry	Stoneberry
His	5.0 ± 0.2	3.4 ± 0.5	4.5 ± 0.2
Ser	13.2 ± 0.2	9.9 ± 0.4	12.0 ± 0.8
Arg	13.6 ± 0.6	8.4 ± 0,6	11.3 ± 0,7
Gly	8.5 ± 0.1	6.5 ± 0,2	7.3 ± 0,4
Asp	22.5 ± 0.5	13.7 ± 0,5	17.2 ± 0,8
Glu	22.8 ± 0.6	18.4 ± 0,5	21.9 ± 1,4
Thr	8.8 ± 0.4	6.0 ± 0.7	7.0 ± 0.7
Ala	12.1 ± 0.2	7.4 ± 0.3	9.7 ± 0.5
Pro	7.9 ± 0.3	6.3 ± 0.5	6.3 ± 0.5
Cys	3.0 ± 0.1	2.8 ± 0.3	2.7 ± 0.2
Lys	15.3 ± 0.4	11.8 ± 0.6	12.2 ± 0.3
Tyr	5.4 ± 0.1	4.1 ± 0.3	4.3 ± 0.3
Met	4.9 ± 0.2	3.9 ± 0.3	3.7 ± 0.3
Val	10.4 ± 0.1	7.4 ± 0.2	8.3 ± 0.6
Ile	9.4 ± 0.4	6.8 ± 0.6	7.6 ± 0.6
Leu	15.2 ± 0.2	11.7 ± 0.3	12.1 ± 0.6
Phe	9.3 ± 0.6	6.6 ± 0.5	7.3 ± 0.6
Trp	1.4 ± 0.0	1.5 ± 0.1	1.4 ± 0.0
SUM as %	18.9	13.7	15.7

Table 5
Lipid compounds in berry cells. The results are expressed as mg/g dw ± SD (n = 3). Abbreviations: SaFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FFA, free fatty acids.

Lipid	Cloudberry	Lingonberry	Stoneberry
Class/compound	[mg/g] dw	[mg/g] dw	[mg/g] dw
SaFA	6.68 ± 0.31	4.95 ± 0.23	4.45 ± 0.21
MUFA	1.49 ± 0.04	1.01 ± 0.03	0.98 ± 0.03
PUFA	11.80 ± 0.59	7.03 ± 0.35	8.35 ± 0.42
FFA	0.63 ± 0.03	0.49 ± 0.03	0.79 ± 0.04
Campesterol	0.05 ± 0.01	0.16 ± 0.01	0.03 ± 0.00
Stigmasterol	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
β-Sitosterol	1.78 ± 0.12	0.86 ± 0.09	0.87 ± 0.04
Other Sterols	0.39 ± 0.03	1.25 ± 0.20	0.73 ± 0.14
Squalene	0.05 ± 0.01	0.28 ± 0.04	0.06 ± 0.00
Oleanolic acid	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.01
Amyrins (α + β)	nd	0.47 ± 0.11	nd

nd, not detected.

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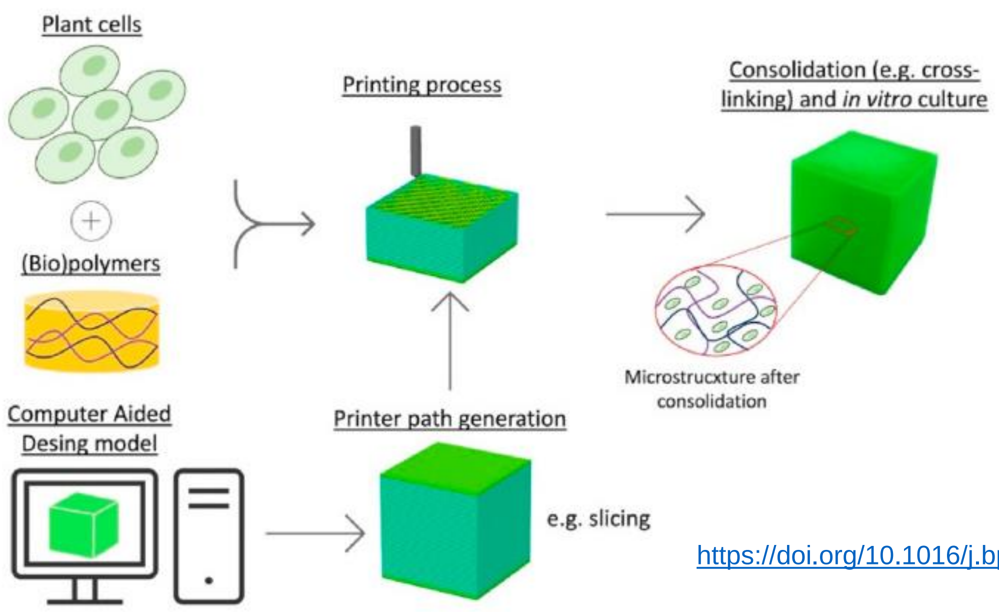
DOI: 10.1007/s00253-018-9279-8

Plant cell cultures as food matrices for 3D(bio)-printed foods

3D(bio)-printing of plant cells



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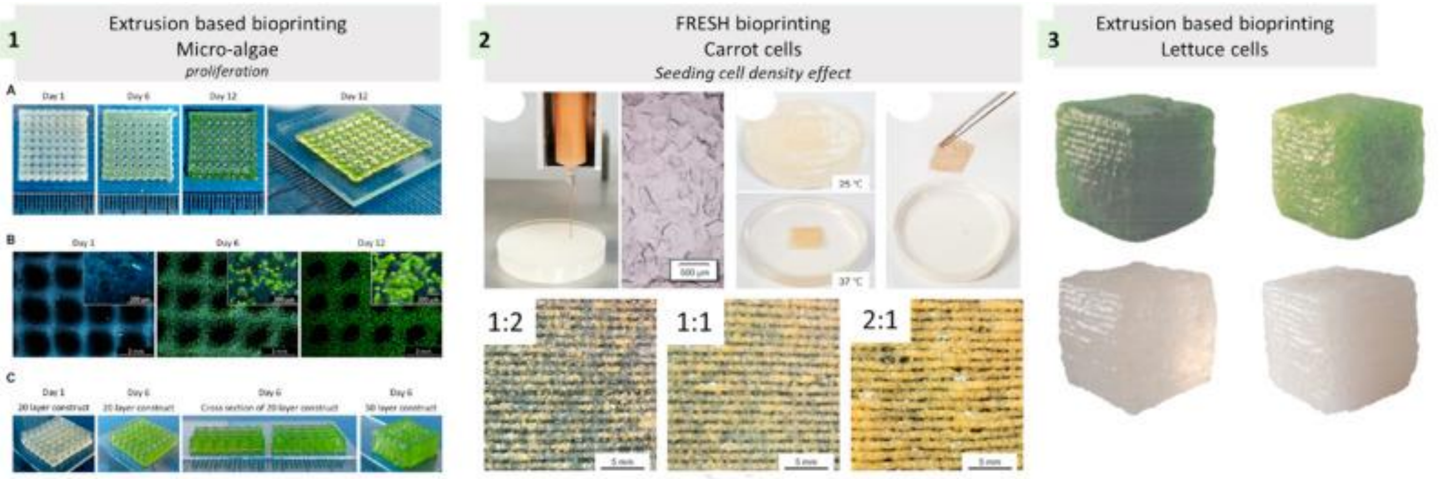
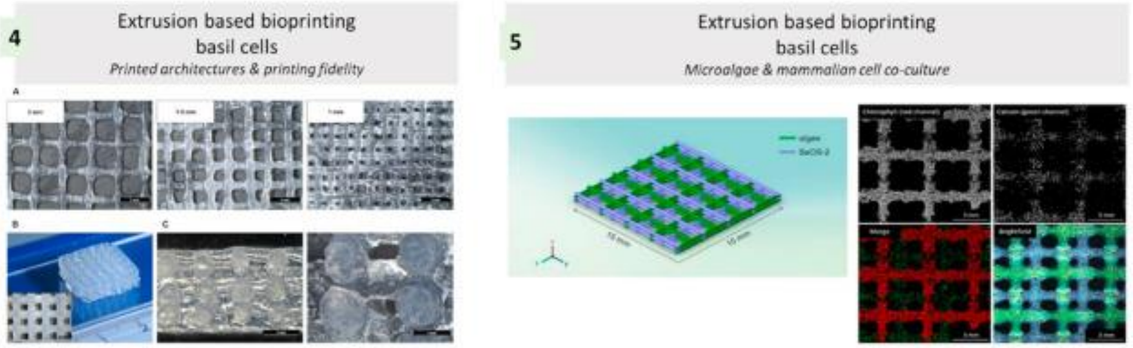




https://doi.org/10.1016/j.bprint.2022.e00216

<u>PROPOSTA TECNICO PROGRAMMATICA</u>

Bando di finanziamento per le

"SVILUPPO DI PROGETTI/ESPERIMENTI SCIENTIFICI PER LA LUNA"

ProduzIoNe di alimenti VEgetali iN viTro, mediante biotecnologie e 3D (bio)printing per lo spazio:

Food with a high nutritional profile, nutraceutical and sensory properties, profiled for the specific needs of the astronauts to contribute to their nutritional status and psychophysical well-being.

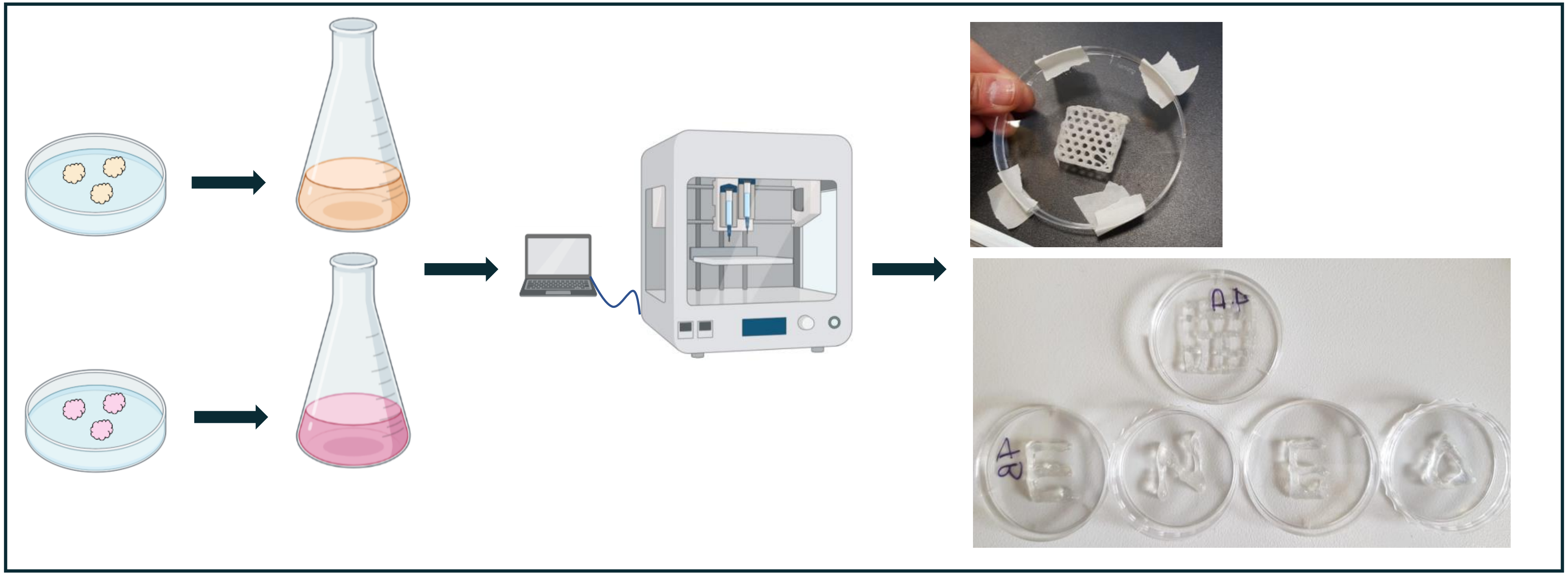
Plant cell cultures as food matrices for 3D(bio)-printed foods

✓ PLANT CELL AGRICULTURE

ENEA
Italian National Agency for New Technologies, Energy and Sustainable Economic Development

PARTNERSHIP:

EltHub S.r.l.
CREA-AN



- Use of plant material derived from *in vivo* smart farming of plant species and *in vitro* plant cells with well-known nutritional properties to create inks suitable for 3D(bio)-printing of food matrices.
- Various printing hypotheses: fundamentally extrusion-based, mold and casting to counteract the effects of microgravity on printing efficiency.

Bioinks must:

- Have the necessary chemical-physical and rheological characteristics for extrusion (speed, resolution, substrate) at room temperature and for printing based on cell cultures.
- Preserve nutritional, functional, and biological properties of plant cells.

Heritage - The space…in the laboratory

Gamma irradiation facility

L'impianto Calliope è una facility di tipo a piscina, con sorgente radioisotopica di ^{60}Co (energia 1.25 MeV) in una cella schermata ad alto volume (7,0 x 6,0 x 3,9 m^3). Ha un'intensità di dose massima di 7.3 kGy/h misurata con le seguenti tecniche dosimetriche:

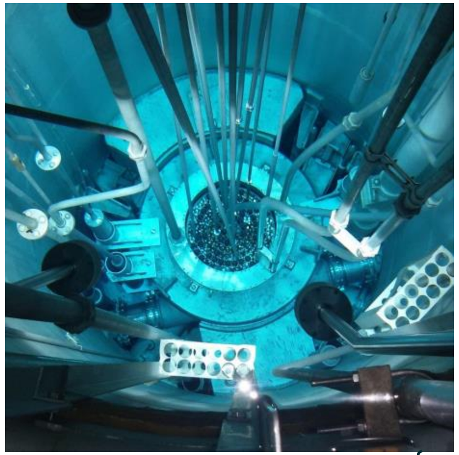
- Dosimetria assoluta Fricke (20 - 400 Gy);
- Dosimetri EPR con alanina (1 Gy – 500 kGy);
- Red-Perspex (5 - 40 kGy) e pellicole radiocromiche (1 kGy 3 MGy);
- Dosimetri a termoluminescenza (TLD) (1 mGy – 100 Gy);

RADFET elettronica (0.01 Gy – 1 kGy).

Radiazioni da neutroni

I reattori TRIGA RC1 e RSV TAPIRO sono stati qualificati come facility supportate da ASI (Accordo di Collaborazione ASIF):

- TRIGA RC1 fornisce il valore massimo per Φeq (r, 1MeV) pari a 5 x 1017 neutroni / cm2 in un irraggiamento di 5 ore e un valore corrispondente del parametro di durezza uguale a 0.35;
- RSV TAPIRO, assicura un valore massimo di Φeq (r, 1MeV) pari a 1.14 x 1016 neutroni / cm2 in un irraggiamento di 5 ore e corrispondentemente un valore di H(r) pari a 0.55



Piscina con sorgente radioisotopica ^{60}Co impianto Calliope;

Pozzo reattore impianto TRIGA;

Impianto RSV-TAPIRO

Heritage –laboratories

- Piattaforma CITOFLOW di Citometria a flusso e microscopia;

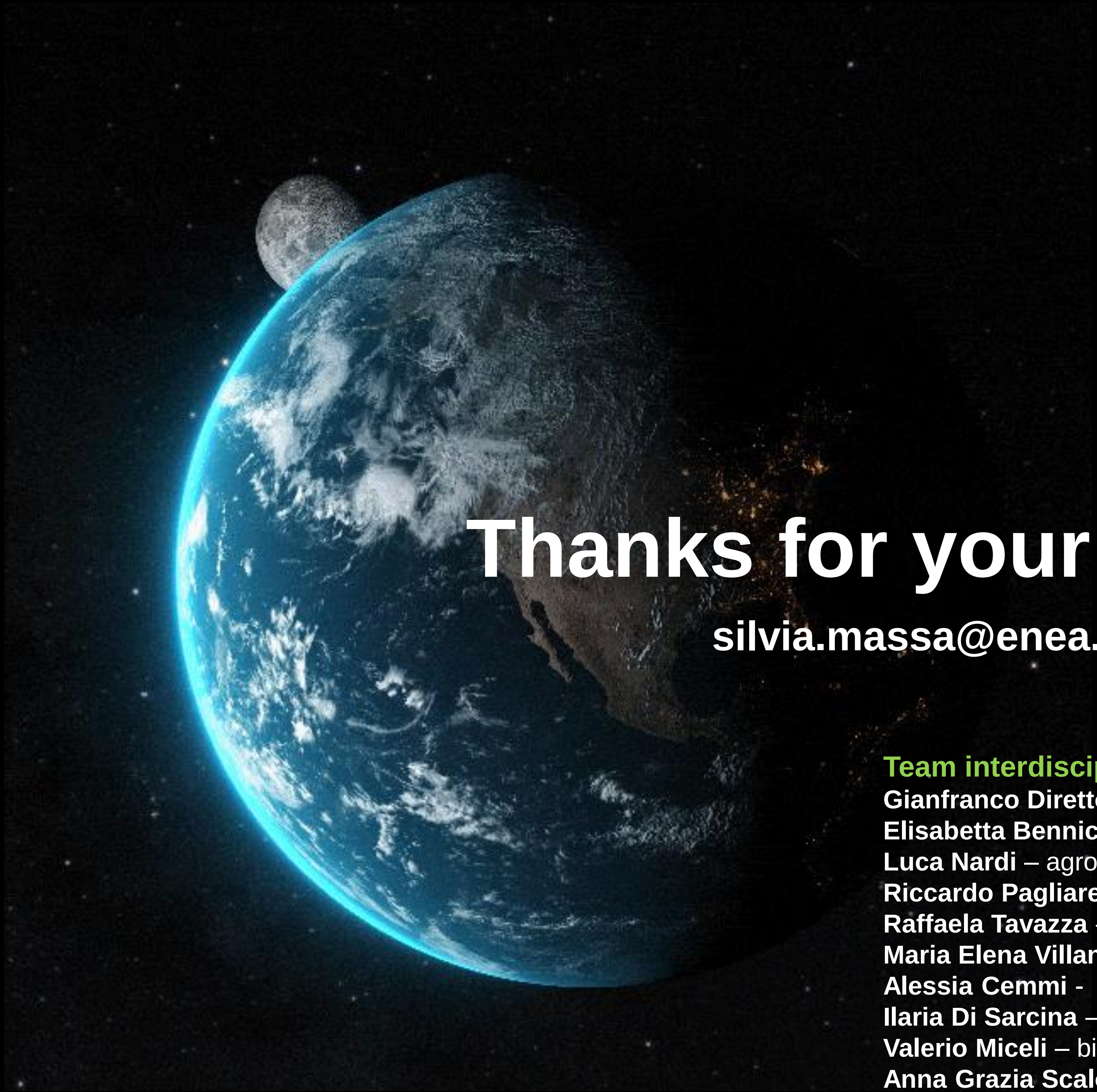
- Fitotroni, camere a contenimento, prototipi HORT^2 e HORT^3 multilivello per la coltivazione a LED e con sistemi di gestione del controllo e sensori;

- Facility di metabolomica

- Laboratori per colture cellulari vegetali, cappe a flusso laminare, incubatori;

- Bio stampante 3D Cellink BioX (TwinHelix).

- Know-how:
  - ❑ coltivazione high-tech
  - ❑ Biotecnologie vegetali
  - ❑ Colture cellulari vegetali per la produzione di metaboliti
  - ❑ Analisi metabolomiche
  - ❑ Effetti delle radiazioni ionizzanti e microgravità sui sistemi vegetali
  - ❑ Sistemi biorigenerativi



Thanks for your attention!

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