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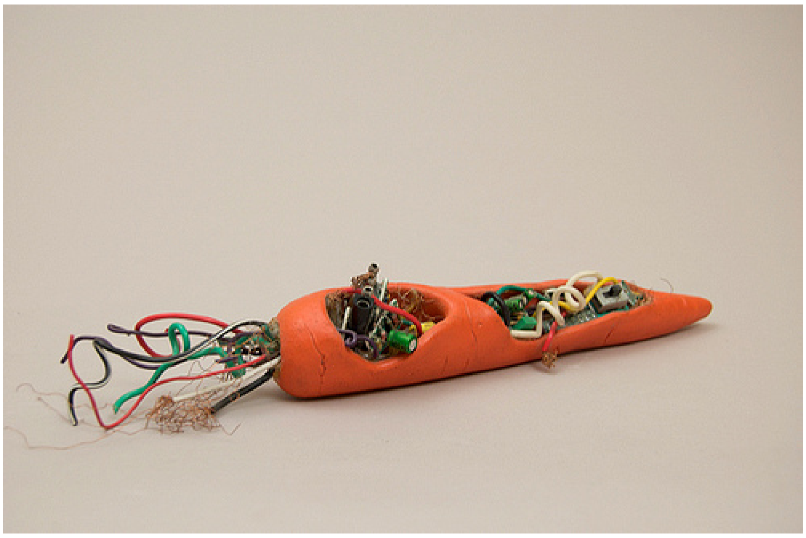
Roadmap for Research Activities on Higher Plants in Space



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1. Introduction

Future space exploration and long term missions to the Moon and ultimately Mars rely on a life support system capable of regenerating all the essentials for survival. Plants are important components in bioregenerative life support systems for long-term missions. Plants provide a regenerative food source, aid in air purification and oxygen production, contribute to water purification (Wheeler *et al.*, 2001, Brown *et al.*, 2008) and may also have a positive psychological impact on the crew (Marquit *et al.*, 2008). To ensure successful plant production and a reliable food source for human crew, it is fundamental to understand in which way the space factors and an altered physical environment affect the basic processes of a plant's physiology.

The Micro Ecological Life Support System Alternative project (MELiSSA) is a model system for an advanced life support based on different microbial species and higher plants (Binot *et al.*, 1994; Poughon *et al.*, 2009). The system is inspired by terrestrial ecosystems, and uses the combined activity of different living organisms: microbial cultures in bioreactors, a higher plant compartment, and a human crew (Hendrickx, De Wever et al. 2006). The MELiSSA loop concept is described in Figure 1. The objectives of the MELiSSA Higher Plant Chamber (HPC) are production of food and oxygen, CO_2 consumption and water purification.

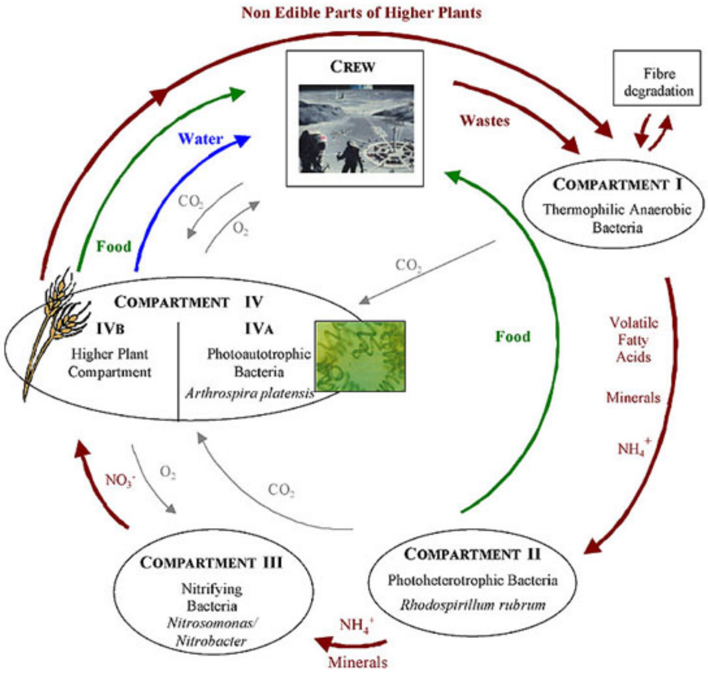

Figure 1. MELiSSA loop concept (from Gòdia *et al.*, 2002)

The Literature Review of Higher Plants in Space for MELiSSA (LiRHiPliSME) project was initiated to establish an understanding of the present state of knowledge concerning the impact of the space environment on higher plants.

The project has been focusing on the existing research which describes the effects on higher plants exposed to three physical factors on the Moon and Mars that are different from conditions on Earth:

- Gravity
- Radiation
- Magnetic field

The main objective of LiRHiPliSME has been to provide input to the MELiSSA Phase 2 – preliminary flight experiments, and to establish an understanding of the current knowledge within space plant biology. In addition, LiRHiPliSME has aimed to identify the need for future scientific research activities required before higher plants can be included in regenerative life support systems.

The scheme of the literature review process is shown on Figure 2. The study started assessing the plant sub-levels, which then lay the groundwork for evaluation of the whole plant perspective.

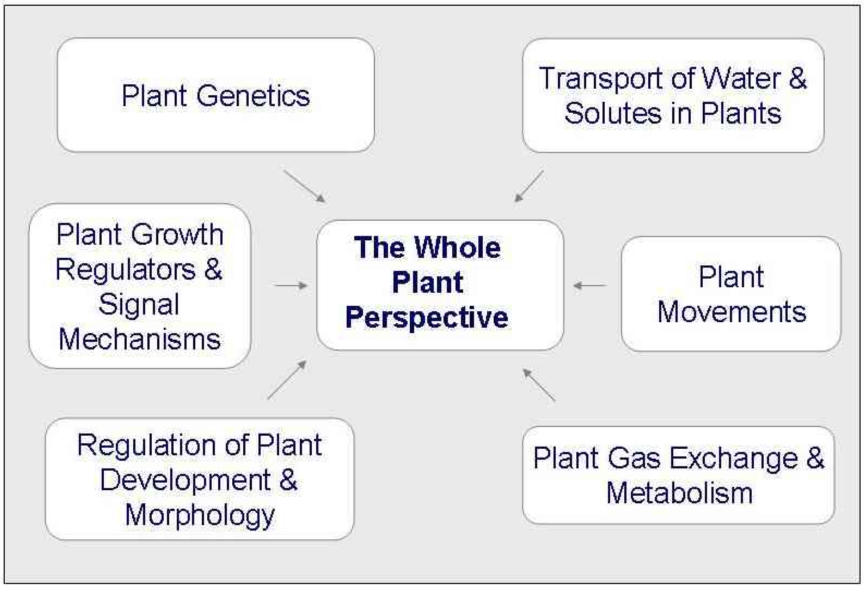

Figure 2. LiRHiPliSME Project Scheme.

1. Scope of the Technical Note

Based on the LiRHiPliSME literature review process and Synthesis of Requirements for Future Research activities on Higher Plants in Space (TN 97.10) the scope of this Technical Note is:

- To define the building blocks for a roadmap for future research activities
- To place these building blocks in a timeline
- To elaborate a roadmap for future research activities on higher plants in space

3. Main conclusions from literature review

In general, the available information regarding effects of the space environment on plant growth and metabolism is inconclusive and suffers from limitations in available flight hardware, sensor technology and research facilities that simulate space conditions. However, based on the available literature, two main conclusions can be drawn:

- Plants have demonstrated their ability to grow and reproduce in space
  → A number of long term experiments with plants have been successfully performed in space (Merkys *et al.* 1984, Ivanova *et al.* 1993, Sychev *et al.* 2001).

- Hardware is of great importance
  → Satisfactory environmental control is essential to grow healthy plants in space and under reduced gravity conditions, especially atmosphere control (*e.g.* ethylene scrub,) and air circulation both at canopy level and in the root zone (Musgrave *et al.* 1998, Kitaya *et al.* 2003, Liao *et al.* 2004).

Through the literature review process, interview with scientists and discussions in the LiRHiPliSME working group there was a decision made that future investigation should prioritise the fundamental processes required to ensure sustainable plant growth and food production in space, i.e. *effects of the space environment on plant nutritive value and the processes of photosynthesis, gas exchange and transport of water and solutes.* Space experiments should be designed to address the whole plant perspective and give a holistic view of the plants health status, before proceeding to carefully selected plant sublevels. Experiments should also, whenever feasible, include assessment of a plants complete growth cycle.

For a more detailed description of the literature reviewed and results from the literature review process see plant sublevel Technical Notes (TNs 97.03 – 97.08).

4. Requirements for future research

4.1 Primary and secondary requirements

Overall, the primary requirement for future research activities on higher plants in space is *to understand and control the entire food chain within the life support system, including the effects of the space environment on the whole-plant physiology and plant nutritive value.*

This ambitious goal can only be achieved through extensive research and technology development activities. Thus, the primary requirement can be achieved through a set of secondary requirements:

-study of selected food crops and definition of their nominal conditions (selected MELiSSA species: tomato, soya bean, potato, wheat, lettuce, rice, spinach and onion).

-standardisation of experimental design for ground and space experiments with selected species

-low chronic radiation experiments

-graded gravity experiments

-elucidation of the impact of magnetic field on plants

-development of mathematical models

These activities can be grouped together in a series of activities called *plant characterisation studies*; with the common objective of increasing the knowledge and intelligibility of the MELiSSA food crops and all the processes related to them.

During the LiRHiPliSME workshop process the LiRHiPliSME Team and project Partners defined the main groups of requirements for future research activities on higher plants in space. These main groups of requirements, forming the grounding for future scientific activities within space plant biology, are presented in figure 3.

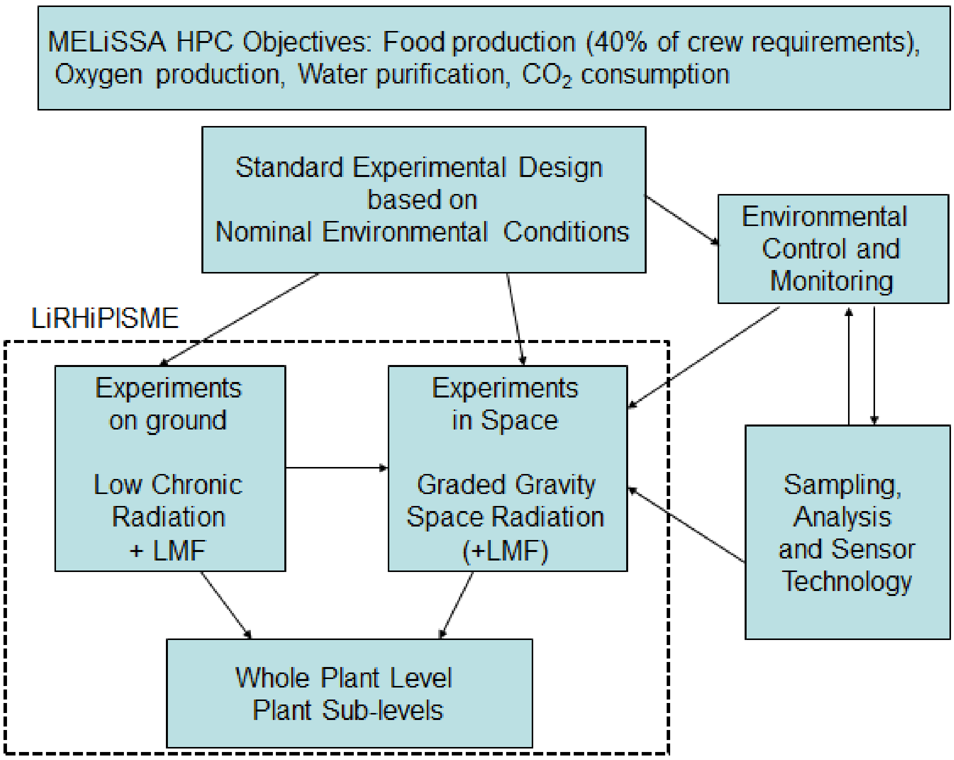

Figure 3. Requirements for future research activities on higher plants in space. HPC – higher plant compartment, LMF – low magnetic field (on ground: shielding from geomagnetic field, in space: lower than geomagnetic field)

4.2 Technological issues

Water and nutrient management is considered to be one of the most challenging aspects of plant cultivation in space. Surely, water management and plant dehydration has been a frequent reason for failure in space experiments. In addition, the most frequently reported effects of a reduced gravity environment on plant physiology are linked to changes in the plants physical environment (for a review see Porterfield 2002). Consequently, and even though optimal environmental conditions were one of the assumptions made before start-up of the literature review, these topics will be briefly considered in the following sections.

In addition, and in accordance with the ultimate goal of high yield food production systems, space plant experiments need to be performed with higher light intensity and/or selected wavelengths of narrow bandwidth light, as well as larger growing areas to measure effects on the plant canopy level. This is particularly important when considering effects on photosynthesis and plant metabolism. On the whole, a prerequisite for increased output of future plant experiments and achievement of the scientific milestones within space plant biology is the development of a new generation of higher plant chamber test-beds.

4.2.1 Water management in closed hydroponics

Water and nutrient management includes water and nutrient supply, water and nutrient availability to the plant, active and passive uptake of nutrients, water taken up and transpired by the plants and recirculation of water and nutrients within the actual system.

In future Closed Ecological Life Support Systems (CELSS), with extreme limitations on resource availability and re-supply, a closed cycle recirculating hydroponic system is considered to be the preferred solution. However, the availability and application of closed system technologies are very limited (Stanghellini et al. 2005, Massa et al. 2010). Open culture systems, or semi closed systems with circulation and discharge of water when electrical conductivity (EC) or ion concentration has reached some threshold value, are more commonly used (Pardossi et al. 2006, Massa et al. 2010). In addition, when implemented, closed-cycle hydroponic systems have led to a number of reported problems as accumulation of non-nutrient salts from irrigation water (Stanghellini et al. 2005, Massa et al. 2010), pathogens (Postma 2010) and accumulation of phytotoxic organic acids (Schwarz et al. 2005, Lee et al. 2006). With regard to salt accumulation, one solution to the problem could be reverse osmosis desalinization (Stanghellini et al 2005). To avoid plant diseases, various methods have been developed for disinfestation of the recirculated nutrient solution. Disinfestation technologies can be grouped by the following approaches; filtration, heat treatment, oxidation, electromagnetic radiation (e.g. UV), active carbon adsorption and copper ionization (Postma 2010, Othani et al. 2000). Sand filters have received special attention since they are relatively cheap and robust, and depend largely upon the establishment of an active microflora effective in removing pathogens such as *pythium* and *Phytophtora* species, which are a serious threat in soil less systems (Postma 2010). Several methods using activated charcoal have been shown effective in eliminating phytotoxic substances in hydroponics (Lee et al. 2004). However, the technology transfer value and usefulness for space applications is limited and extensive technology and sensor development is necessary before a satisfactory solution for closed hydroponics is available for CELSS. The optimal solution would be a system with a high recycling capacity and real time surveillance of plant nutrient- and water status. Automated replenishment from nutrient refill solution should be based on the collected real time data, and adapted to the different stages of the plant life cycle.

4.2.2 Environmental control and monitoring

For increased success and relevance in future space experiments it is crucial to implement full environmental control in the hardware. This includes both monitoring and control of light, temperature and atmospheric composition. To obtain such a fully controlled environment, and gain an overview picture of plant processes in space, technology development is essential in both growth chamber components and sensor technologies.

Moreover, with the foreseen restrictions on sample return, remote sensing diagnosis of plant health is needed. Such remote sensing diagnosis could include various imaging techniques and sensors for surveillance of for instance nutrient availability and plant water status, allowing for immediate detection of stress situations before the occurrence of adverse effects on plant health.

5. Roadmap elaboration

5.1 Space related plant experiments

The non-conclusive results from previous plant space experiments probably can be ascribed to the use of different plant species, differences in experimental design and also limitations in the plant growth hardware. In future space related research activities it is crucial to work with a standardised experimental design, and mandatory procedures for sampling and analysis. Hardware providing comprehensive environmental control and monitoring of environmental conditions also is a prerequisite for future work within space plant biology. Moreover, there is a general need for reference data before space conditions can be compared to conditions on Earth, and all possible scientific knowledge from pre-flight experiments must be uncovered before proceeding to space experiments.

5.1.1 Pre-flight research experiments

In future experiments with higher plants, the first logic step would be to describe the nominal conditions and cultivar characteristics of the selected MELiSSA cultivars. Secondly, a standard experimental design, describing in detail the environmental conditions for each of the selected species, as well as protocols for sowing, plant handling and sampling should be formulated. Thirdly, mathematical models describing the growth, development and metabolism of the chosen cultivars ought to be developed.

After an evaluation of the available technical solutions for simulation of space conditions, low chronic radiation exposure seems to be the most realistic variable to assess in pre-flight experiments. Because of the necessity of shielding to protect humans from high radiation doses, low chronic radiation is considered more relevant than high acute radiation. On this basis, the primary focus of ground studies for space applications should be on the effect of low chronic radiation on fundamental plant processes. Measures of priority are effects of radiation on biomass production, photosynthesis and gas exchange, gene expression profile, along with all processes affecting the plants nutrient value. Based on reported results from space experiments, several issues related to food quality- and safety need to be revealed. These include changes in the cell wall components (Hoson *et al.* 2000, 2003 and 2004; Soga *et al.* 2002) and changes in secondary metabolite production (Musgrave 2005, Allen *et al.* 2009, Tuominen *et al.* 2009). For additional references and more detailed information on the above mentioned studies, see TN 97.09. Subsequently, it is important to consider the effects of chronic radiation on morphological changes, chromosome aberrations and mutation frequency, as these are good measures of plant development and genome stability. The prioritized plant sublevels to be included in the objectives of future research related to space plant biology, are described in figure 4.

The radiation exposure should mimic space radiation as much as possible, and include at least gamma-rays, proton and neutron particles. Existing facilities for radiation experiments are the Radioactive Isotope Beam Factory RIKEN (Nishina Center for Accelerator-Based Science (RIBF)), the HIMAC (Heavy-Ion Medical Accelerator in Chiba), both located in Japan, as well as the Alternating Gradient Synchrotron in Brookhaven, USA. The available facilities for radiation experiments and simulation of space conditions are insufficient in simulating the whole radiation load in space; however a large number of rays and particles with high energies can be obtained.

The 2-dimensional (2D) clinostats and random positioning machine (RPM) are widely applied methods for simulation of microgravity. However, the spatial limitations strongly restricting sample size and method of cultivation, limit the value of experiments with 2D-clinostats and RPMs.

With regard to magnetic fields, there is a technology for shielding of Earth's natural magnetic field that could in principle be combined with radiation exposure. However, even though some studies have indicated that magnetic fields can have a direct effect on plant growth and development (LiRHiPliSME TNs 97.03, 97.04, 97.05 and 97.08), more studies are required to determine the effects of reduced magnetic fields in space on higher plants.

As mentioned in section 4.2, both water and nutrient management in closed hydroponics and environmental monitoring and control are prerequisites for increased success in future space plant experiments. While the improved control of environmental factors is considered to be in principal technological issues, there is a need for both scientific activities and technology development to solve the issues related to water and nutrient management. As pointed out by Porterfield (2002), the effects of gravity on basic physical phenomena of all matter, (indirect effects of gravity) and how these effects in turn influence on the biological system, need to be elucidated before the direct effects of gravity on the cell, tissue, organ or whole organism can be revealed. Only when the biophysical limitations of transport and exchange in a reduced gravity environment are understood, technologies can be constructed to overcome these problems (Porterfield 2002). Parabolic flights have been proven useful to gain insight to physiological transport in microgravity. On the topic of recirculation of nutrient solutions in closed hydroponics, extensive work is required on both basic plant nutrition processes and development of sensor technology for surveillance of nutrients in the solution and plant nutrient status.

5.1.2 Space experiments

With sustainable plant production as the ultimate objective, space experiments should start assessing the whole plant and focus on fundamental processes as biomass production, photosynthesis and gas exchange, and transport of water and solutes. In addition, all factors shown in the pre-flight experiments to have an influence on plant nutrient value should be prioritised. Analysis of gene expression profile should be included, but only when linked to specific plant functions.

Secondary, and based on the initial work, a set of sublevels will be defined for further studies. At this point, sublevels identified to be of potential interest are secondary metabolite production, the photosynthetic apparatus, chromosome aberrations and mutation frequency, hormonal interactions and morphological- and anatomical changes in the plant. The priority of sublevels may change as the work process moves forward. Plant experiments in space with several consecutive generations are required to study genome stability, pollination success and potential adaptation responses.

Natural low chronic exposure to space radiation, along with graded gravity generated by a centrifuge, will contribute to the understanding of the combined effects of radiation and microgravity on plants.

Future space plant experiments, assessing the whole plant and a complete growth cycle, require long duration exposure to space conditions to provide maximum output. At present, the best option for performing space experiments with higher plants is considered to be on the International Space Station (ISS). Satellites are also considered to be a good alternative to analyse short term effect on photosynthesis and gas exchange and to some degree the water management (transpiration). This scenario presupposes remote monitoring and control by use of sensors and image recording. At present, the ISS is the only platform where long duration (full or multiple plant life cycles) experiments with graded gravity by use of centrifuges can be performed. The ISS crew and the logistics established for ISS allow for the crew to interact with the plant chambers during and after the plant cultivation. The low chronic radiation on ISS also enables an assessment of combined effects of radiation and reduced gravity. Analyses of biomass production and the transport of water and solutes are feasible as long as optimal growth conditions are provided and cultivation systems are developed on the ISS platform. ISS also open up for establishing gene expression profiles of higher plants e.g. to reveal the effect on the metabolism. Furthermore, effect of graded gravity and chronic low radiation on food quality/safety, hormone interaction and chromosomal aberration and mutation frequency can be analysed by use of the ISS.

To assess the effects of total space radiation load and the lack of magnetic field, experiments need to be performed outside Low Earth Orbit (LEO). At this point and in the foreseeable future, the most realistic scenario for plant experiments outside LEO is as part of robotic mission to Moon or Mars surfaces.

Figure 4. Research objectives for future research in space plant biology. The listed plant sublevels are identified based on the reviewed literature in the LiRHiPLiSME project. The final objectives for future space experiments may differ from the ones listed above.

5.1.3 Mathematical models

To achieve the primary requirement of understanding and controlling the plant compartment processes, as well as the effects of space factors on the whole-plant physiology, mathematical models should be developed that describe the fundamental plant physiological processes; plant growth and development, water and nutrient uptake dynamics, photosynthesis, transpiration, gas exchange, microbial communities etc. A first step would be to define the parameters to be included in a model describing the higher plant compartment. A proper model for plant physiological processes should include the complex interplay between environmental, physiological, biophysical and bio mathematical factors (Gielis 2004). A model developed especially for higher plants in bio regenerative life support applications is the higher plant model. This model separates the different plant organs (leaf, stem, root and fruit, seed or storage organ) in order to study the different sub-processes to find existing laws and models (Hezard et al 2011). All these submodels fit into a general structured model. The goal of the general model is to be able to predict the CO_2 and nutrient solution consumption, as well as the O_2, clean water and food production from the higher plant compartment, at different environmental conditions (Hezard et al. 2010).

Another approach to predict and model the plant related processes in the MELiSSA higher plant compartment, is the development of a preliminary model aiming to describe the mass flux at the surface of the plant leaf (Holmberg 2011). This includes transport phenomena such as the vaporisation of water, carbon dioxide uptake, oxygen release and respiration, processes all controlled mainly by the stoma of the leaf. The flux through the stoma is induced by diffusion. The preliminary model is based on a series of existing physical laws (mainly Fick`s law: a quantitative description of the diffusion process). Also the impact of the external environment surrounding the stomata, which is parameters as humidity, temperature, airflow ad pressure, is included in the model (Holmberg 2011). It should be stressed, however, that the described preliminary model contains many constraints and should be further adapted and validated through computer simulations and computations, as well as crop specific experiments (Holmberg 2011).

To increase the relevance of the mathematical models, as well as for the preparation of future space plant experiments, more work is required to increase the understanding of physiological transport and exchange in higher plants, as well as basic fluid physics applicable in higher plant physiology.

5.2 Building blocks for roadmap

Based on the literature review process and workshop discussions, and the proposed requirements for future research activities described above (and in more detailed in TN. 97.10), the activities required to obtain the scientific readiness for further development of enclosed regenerative life support systems including higher plants, are grouped into a set of building blocks. The building blocks, forming the basis for the roadmap are presented in figure 5.

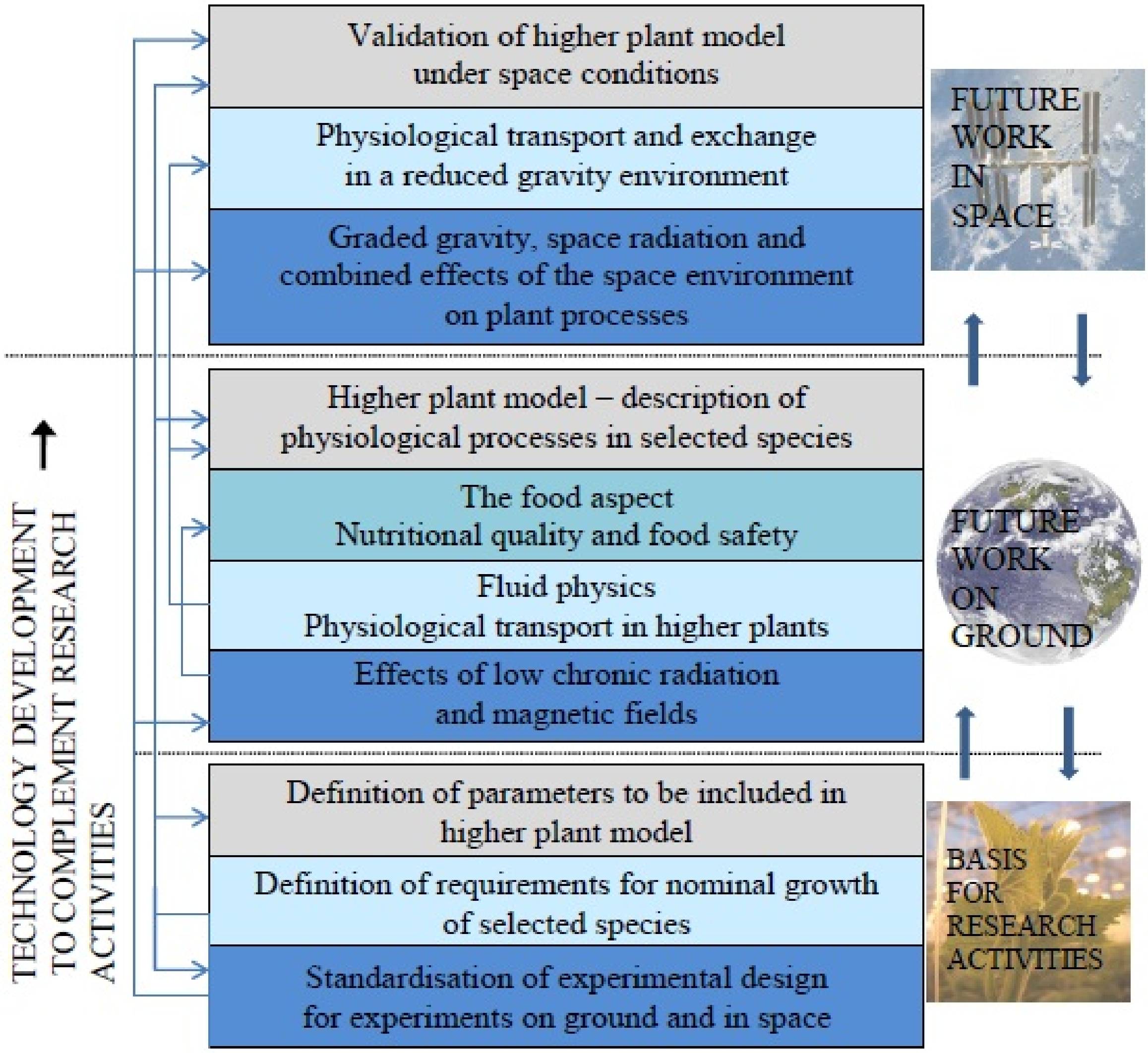

Figure 5. Building blocks (main groups of requirements) for future research activities on higher plants in space. The lower section of the figure describes activities to be performed before proceeding to ground based scientific activities (described in the middle section of the figure). The upper section builds on the lower and middle sections, and describes space experiments required to reach the scientific readiness to develop regenerative life support systems including higher plants.

5.3 Roadmap

The roadmap for future research activities on higher plants in space is presented in figure 6. The roadmap aims to describe the scientific activities leading to milestone achievements on the way towards sustainable plant growth and food production in space. Pre-flight activities and space activities are given separately. Pre-flight activities include the development of mathematical models and food characterisation studies aiming to fully describe and understand the chosen MELiSSA plants and all the processes related to them. All the parameters determined in the plant characterisation studies (describing the plants growth, development and metabolism) as well as the mathematical models developed on ground must be validated under space conditions. Moreover, future space experiments should build on the pre-flight experiments and address the whole plant perspective together with carefully selected sublevels. The pre-flight and space research activities presented in the roadmap are described in more detail in section 5.1. Figure 6 describes the proposed series of scientific activities and milestones within plant biology that need to be achieved for the realization of a regenerative life support system including higher plants.

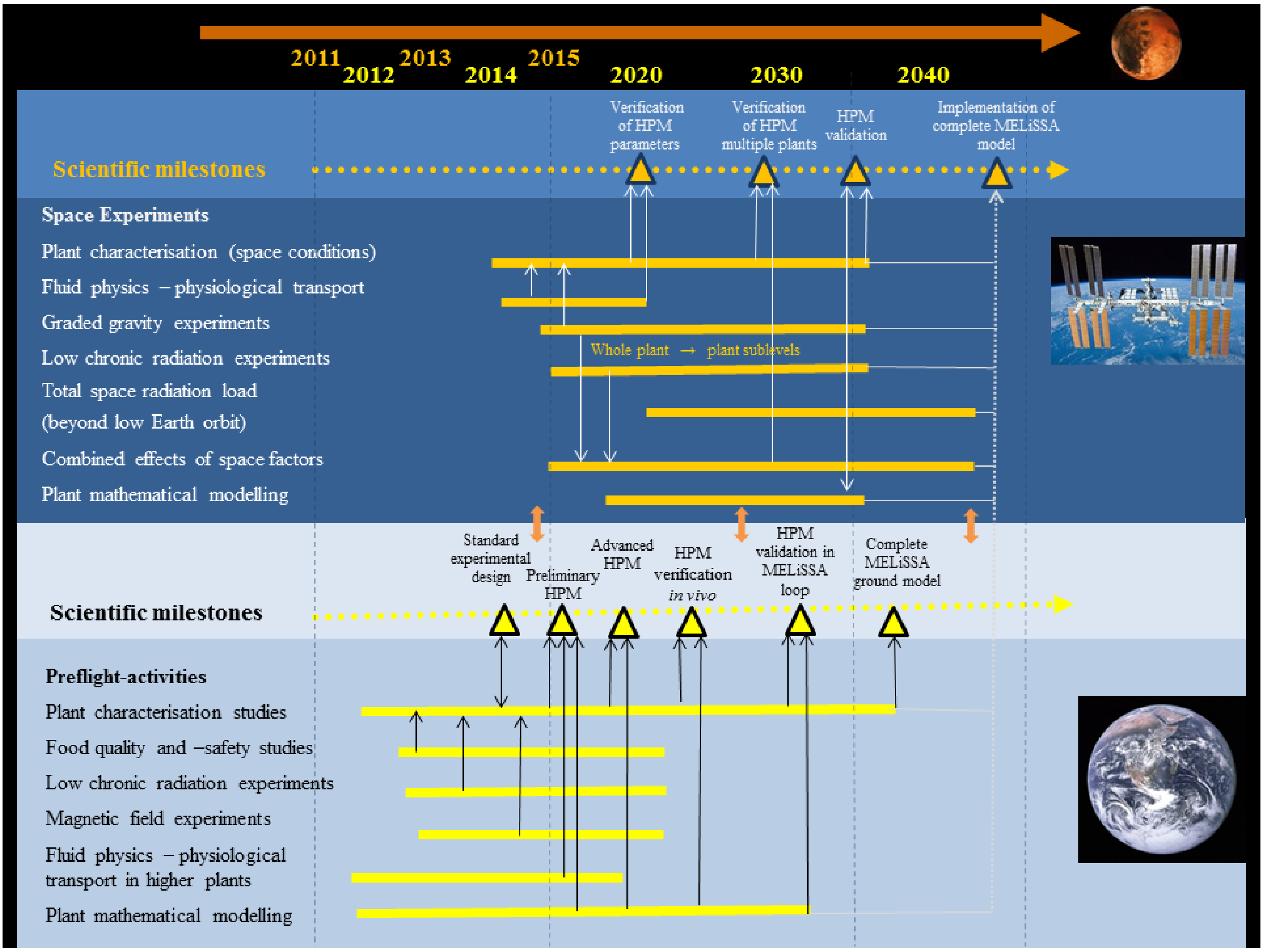

Figure 6. Roadmap for future research activities, aiming to describe the scientific activities and milestones within plant biology, that is required for the realization of a regenerative life support system including higher plants. The lower section (light blue) describes preflight activities, while the upper section (darker blue) describes future research activities in space. The food characterization studies are carried out to describe and define the chosen MELiSSA plant species and all the related processes (i.e. growth, development and metabolism), and will provide input to the development of the higher plant mathematical model (HPM). The preliminary HPM will form the basis for initial testing of HPM parameters under space conditions.

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32. TECHNICAL NOTE 97.03- Plant genetics
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36. TECHNICAL NOTE 97.07. Plant movements
37. TECHNICAL NOTE 97.08. Plant gas exchange and metabolism
38. TECHNICAL NOTE 97.09. The global plant perspective
39. TECHNICAL NOTE 97.10. Requirements for future research activities on higher plants in space.