NTNU
Samfunnsforskning AS

TECHNICAL NOTE 97.10-I1

Requirements for Future Research Activities on Higher Plants in Space

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Reference/Réference	C 90329
Issue/Edition	I1
Revision/Révision	2
Date of issue/Date d'édition	09.07.11

APPROVAL

Title *Titre*	Requirements for Future Research Activities on Higher Plants in Space	Issue *Edition*	I1	Revision *Révision*	2

Author *Auteur*	Silje Aase Wolff	Date *Date*	09.07.11

Approved by *Approuvé par*		Date *Date*	

CHANGE LOG

Issue/*Edition*	Revision/*Révision*	Status/*Statut*	Date/*Date*
D1	0	Released	09.06.2010
D2	1	Released	20.12.2010
I1	2	Released	08.07.2011

Distribution List

Name/*Nom*	Company/*Société*	Quantity/ *Quantité*
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Contents

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 how 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 MELiSSA objectives are complete recycling of gas, liquid and solid wastes during long distance space exploration. The system uses the combined activity of different living organisms: microbial cultures in bioreactors, a plant compartment and a human crew (Hendrickx *et al.*, 2006). The MELiSSA loop concept is described in Figure 1.

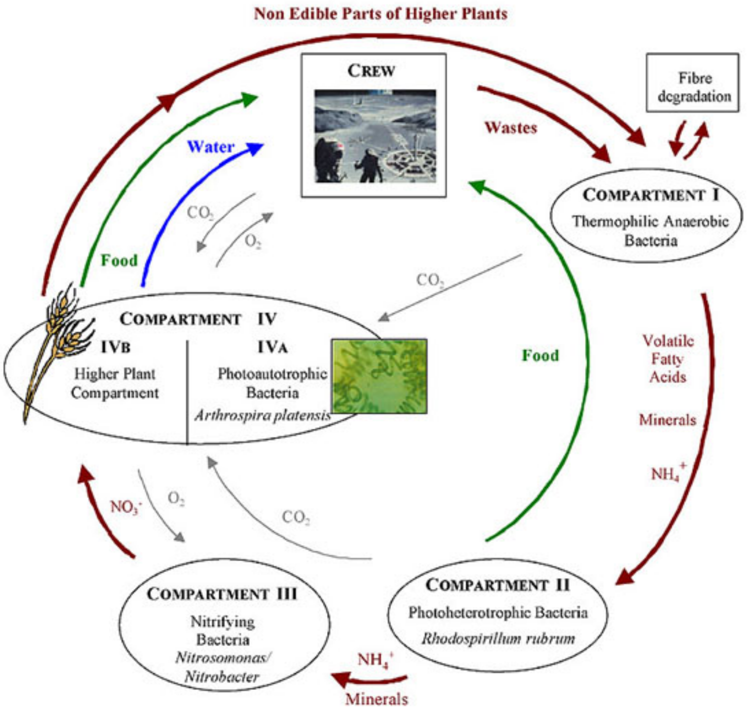

Figure 1. MELiSSA loop concept (from Gòdia *et al.*, 2002)

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 describing the effects on higher plants exposed to three physical factors on the Moon and Mars that are different from conditions on the Earth:

- Gravity
- Radiation
- Magnetic field

The aim of the LiRHiPliSME project have was to collect knowledge from the literature and the scientific community concerning how these physical factors on Moon/Mars will affect higher plants through one life cycle

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. The

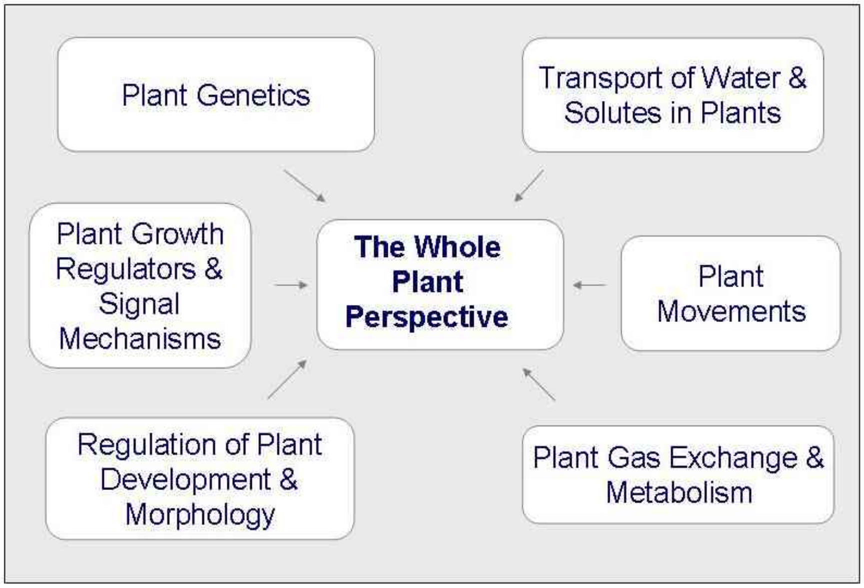

Figure 2. LiRHiPliSME Project Scheme.

2. The Scope of the Technical Note

The purposes of this Technical Note were:

- To describe the principal conclusions from the literature review process
- Based on these conclusions: to make an assessment of where new or extended scientific knowledge is needed to be able to grow plants as a stable food source and component of bio-regenerative life support systems
- To identify requirements for future research activities on higher plants in space

The requirements were drawn with the aim of reaching the scientific readiness to allow for further development of enclosed regenerative life support systems including higher plants.

3. 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 and ventilation is essential to grow healthy plants in space and under reduced gravity conditions

During the literature review discussion it was decided by the LiRHiPliSME team that the primary questions to be solved should be linked to the primary processes required to ensure sustainable plant production in space, i.e. *effects of the space environment on the processes of photosynthesis, gas exchange, transport of water and solutes and stability of the plant genome*. Experiments should also, whenever feasible, include assessment of a plants complete growth cycle. These aspects will therefore be emphasised in the following synthesis of requirements for future research activities on higher plants in space.

3.1 Gravity

In general, well-controlled studies regarding the effect of gravity on plants are very scarce. The most frequently reported effects of a reduced gravity environment on plant physiology are linked to changes in the plants physical environment. These types of effects are called indirect effects of gravity because they are not caused by gravity interacting with the mass of the plant body itself (Porterfield, 2002). As an example, the lack of buoyancy driven thermal convection (BDTC) in microgravity and consequent increase in boundary layer thickness causes biophysical limitations on the processes of gas exchange and physiological transport in higher plants (Porterfield, 2002). In the aerial plant parts this effect can be diminished by proper ventilation and forced air movement (Musgrave *et al.*, 1998; Kitaya *et al.*, 2003). In the root zone the problem is more complex, and root zone hypoxia induced by gravity dependent changes in fluid and gas distribution remains a persistent challenge in space plant experiments (Liao, 2004). However, a research based understanding of the influence of gravity on physiological transport and exchange will make possible hardware technology development and technological solutions that will overcome these difficulties.

3.2 Radiation

Due to the shielding of the experiment facility, which is a prerequisite for humans in manned space exploration, long term exposure to low chronic radiation is considered to be more relevant than high acute radiation doses. The studies performed with chronic radiation exposures are relatively few (Reall *et al.*, 2004). However, exposure to chronic radiation and ionizing radiation seems to affect the genetic structure of populations in the long term, and a reduction of genetic variability may be an adaptive process associated with chronic stress (Esnault *et al.*, 2010). Moreover, chronic exposure to very low doses of ionizing radiation has been shown to have a comparatively stronger influence on plant genetics than an acute dose (Kovalchuk *et al.*, 2000). Kovalchuk and co workers (2007) suggest that different mechanisms are involved in the response to chronic or acute exposure to radiation. While the most well represented group of genes affected by acute radiation exposure is the group of oxidative stress-related genes, chronic stress leads to a totally different response that reflects in adaptive responses by regulating genes belonging to general stress and nucleic acid metabolism. Chronic stress also induces several genes involved in photosynthesis and carbohydrate metabolism (Kovalchuk *et al.*, 2007). Different species show varying resistance to radiation damage (Bhaskaran, 1961).

Consequently, the experiments with radiation *on ground* should focus on low chronic radiation exposure and different species including food plants. This kind of experiments, assessing the impact of heavy ion radiation (i.e neutrons, protons), gamma radiation as well as secondary radiation, all which originates from space, can be achieved in facilities such as; 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 in the Alternating Gradient Synchrotron in Brookhaven, USA.

3.3 Magnetic fields

Indications of effects from weak magnetic fields on plant gas exchange and metabolism have been reported (Kursevich, 1973, Belyavskaya, 2004). However, more studies are required to understand the effects of magnetic fields on plants. At the moment only a few papers are available, but a comprehensive study is currently being finished by Professor Massimo Maffei (University of Turin, Italy). The significance of the effects from magnetic fields on plants should be reconsidered after publication of the work from the group in Turin.

3.4 Combined effects

Plant gas exchange, metabolism and photosynthesis seem to work properly in microgravity when provided with satisfactory environmental control (Stutte *et al*., 2005). A reduction in the activity of the PSII and PSI photosystem activity has been reported (Tripathy *et al*. 1996, Stutte *et al*., 2005), however, more studies are required to draw a final conclusion about the potential effects of reduced gravity on photosynthesis.

Diffusion limited gas exchange (due to microgravity effects on the plants physical environment) and root zone hypoxia can result in a reduced uptake and transport of nutrients in plants (Porterfield, 2000). Some studies indicate that the sometimes observed stunted growth in microgravity can be linked to nutritional issues (Nechitailo and Gordeev, 2001).Other studies show no differences in nutrient uptake rates between ground and flight exposed plant material (Heyenga *et al*., 2000) Thus, studies on the effects of microgravity and the space environment on plant nutrition are inconclusive and very limited. Root growth, water and nutrient uptake are strongly interdependent and should ideally be studied together (Adiku, 2008).

The Space environment induces little or no impact on the morphology of higher plants in either short or long term flights (one generation period). Some influence, however, has been observed on the ultrastructure of cell organelles (e.g. amyloplasts and chloroplasts) and of the structure of cell walls (Nedhuka, 1996; Hoson *el al.,* 2001; Soga *et al.,* 2002*).*

Gravity, radiation and weak magnetic fields seem to alter auxin levels and auxin transport in plants (Ueda *et al.*, 2000). However, many open points have to be addressed in order to understand the interaction between auxin, plant growth and the space environment.

Space factors alter gene expression levels and affect the genome through DNA damage and chromosome mutations (Nevzgodina *et al.,* 1989). At this point, however, the effects do not seem to be detrimental for plant growth and survival (for references and more information see TN 97.03). Still, and despite the fact that plants have been grown in space even during several consecutive generations (Sychev *et al.*, 2007), it is not yet known if the plant genome will manage to remain stable.

3.5 Impact on the whole plant

Spaceflight experiments with higher plants have typically focused on individual processes, functions or structures (Stankovic, 2001; Paul *et al.*, 2001). Experiments assessing the whole plant and its physiology are limited (Monje *et al.*, 2005), and the responses of plants and seeds to the space-flight environment, especially outside low Earth orbit where the protective geomagnetic shield is absent, need to be characterised (Ferl *et al.*, 2006). Experiments on plants and seeds should be preferred before cell culture studies, as cell cultures might react in a different manner than a whole plant system. Based on this, and to maximize the output from each experiment, future studies should be performed (when possible) on whole plants and seeds, and with focus on the physiology of the whole plant. This is also important because the major processes in plant physiology are strongly interconnected and hard to separate from each other.

3.6 Environmental conditions

Optimal environmental conditions were one of the assumptions made before start-up of the literature review. However, since the influence of hardware, environmental control and gravity effects on the physiological environment turned out to be such a significant part of the project discussion, these factors need to be included in this synthesis of requirements. For increased success and relevance of future space experiments it is necessary to:

- Determine nominal conditions for all environmental variables
- Monitor all conditions
- Establish priorities for technology development to obtain environmental control and monitoring of environmental conditions in future hardware

As the technology readiness for biological life support advances, the following 12 environmental variables were listed by the project team (at the LiRHiPliSME workshop) and considered to be important for the successful growth of plants in space:

- Gravity (graded gravity, Mars and Lunar fractional-g)
- Radiation (dose and duration)

- Water availability in the root zone (e.g. hydroponic system or substrate with specific range of H_2O content)
- Nutrient supply (macro/micronutrients)
- Temperature
- Photosynthetically Active Radiation (PAR) (photoperiod, spectral quality and intensity for each species)
- Oxygen concentration (in atmosphere and rootzone)
- Carbon dioxide concentration
- Atmospheric pressure (and composition in terms of main constituents O_2, CO_2, H_2O vapour, N_2)
- Humidity (water vapour pressure deficit ,WPD)
- Volatile organic compounds and trace hydrocarbons (e.g. ethylene).
- Magnetic field (on or off)

3.7 The food aspect

Another aspect that is considered important within the scope of the LiRHiPliSME project is the so called *food aspect*: How do space factors affect the ratio between edible and non-edible plant mass and also the quantity and quality of edible plant mass? Such changes may be of consequence for the plants’ role as a food supply, and for the mass balance in a closed life support system. As mentioned in Section 3.4, components of the cell wall, such as cellulose and lignin, have been shown to decrease in plants grown under microgravity conditions (Nedhuka, 1996). Furthermore, microgravity seems to cause an irreversible increase in cell wall extensibility and a decrease in the molecular masses of cell wall polysaccharides (Hoson *el al.*, 2001; Soga *et al.*, 2002*)*. These changes in cell wall properties could influence the ratio between edible and non-edible plant mass.

3.7.1 Nutrient content

There are indications that space conditions can cause changes in carbohydrate, protein and lipid content in plants (Kuang *et al.*, 2000, 2005; Musgrave *et al.*, 2005). In addition, the so called secondary effects of gravity, inducing diffusion limited gas exchange and possibly inhibition of the uptake and transport of nutrients, could reduce the mineral content of space grown plants.

3.7.2 Secondary metabolites

Spaceflight conditions seem to influence the secondary metabolite production in plants. For example, the concentration of glucosinolates and isoflavonoids has been higher in spaceflight material than in ground control samples of *Brassica* (Musgrave *et al.*, 2005) and soybean (Levine *et al.*, 2001). An increased accumulation of small secondary metabolites has also been detected in plants grown under microgravity conditions. As one example, the 3-butenyl glucosinolate concentration in space-grown *Brassica* stems was 75% higher than in stems developed on Earth (Tuominen *et al.*, 2009). Increased gravity (hypergravity) seems to have the opposite effect: glucosinolates in *Brassica* decreased by 140% over the range from micro-*g* to 4-*g* (Allen *et al.*, 2009). For a review of plant secondary metabolism in altered gravity, see Tuominen *et al.* (2009).

An increase in secondary metabolite production in food plants could in fact be a positive trait in more than one way: a diet high in antioxidants or anti-carcinogenic compounds would be advantageous; crops high in secondary metabolites often have fresh flavours and fragrances; and plant secondary metabolites are important for maintaining plant health (Tuominen *et al.*, 2009). Glucosinolates, formerly known as mustard oil glucosides, are a group of secondary plant metabolites present in the order Capparales that contains the Brassicaceae family; including the model plant *Araidopsis thaliana* as well as crop plants such as oilseed rape, white mustard, broccoli and cabbage (Kissen *et al.*, 2009). Hydrolysis of glucosinolates upon tissue damage results in a range of products (isothiocyanates, epithionitriles, nitriles, thiocyanates). Whereas glucosinolates are considered biologically inactive, their degradation products have toxic effects on fungi and bacteria (Brader *et al.*, 2006). The biological relevance of the glucosinolate hydrolysis products needs further consideration (Kissen *et al.*, 2009).

4. Elaboration of Requirements

Throughout the Literature Review process it became clear that the work performed on plants in space has given diverse and sometimes contradictory results. The varying results can be explained by the use of different plants species, differences in experimental design and also plant growth hardware. In future research activities it is crucial to work with a standardised experimental design, including standardised environmental conditions, satisfactory environmental control and monitoring of environmental conditions. This necessitates development of both sensor technology and analytical tools. In addition, mandatory procedures for sampling and analysis should be prepared. In future space experiments, sample return is expected to be very limited, and technology for remote sensing of the plants environment, health status and metabolism is required. 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.

Thus the execution of relevant space related plant experiments is required both on ground and in space, using food crops as well as model plants. Overall focus must be on the whole plant and primary plant processes. First of all, a selection of food plants for life support purposes need to be selected. Then, a standard experimental design applicable to higher plant experiments, based on the nominal conditions of the selected crops, must be determined. Subsequently, pre-flight experiments on ground can be performed to reach the scientific readiness level to proceed to space experiments.

Main groups of requirements for future research activities on higher plants in space, prepared by the LiRHiPliSME Team and Project Partners during the LiRHiPliSME workshop is presented in figure 3.

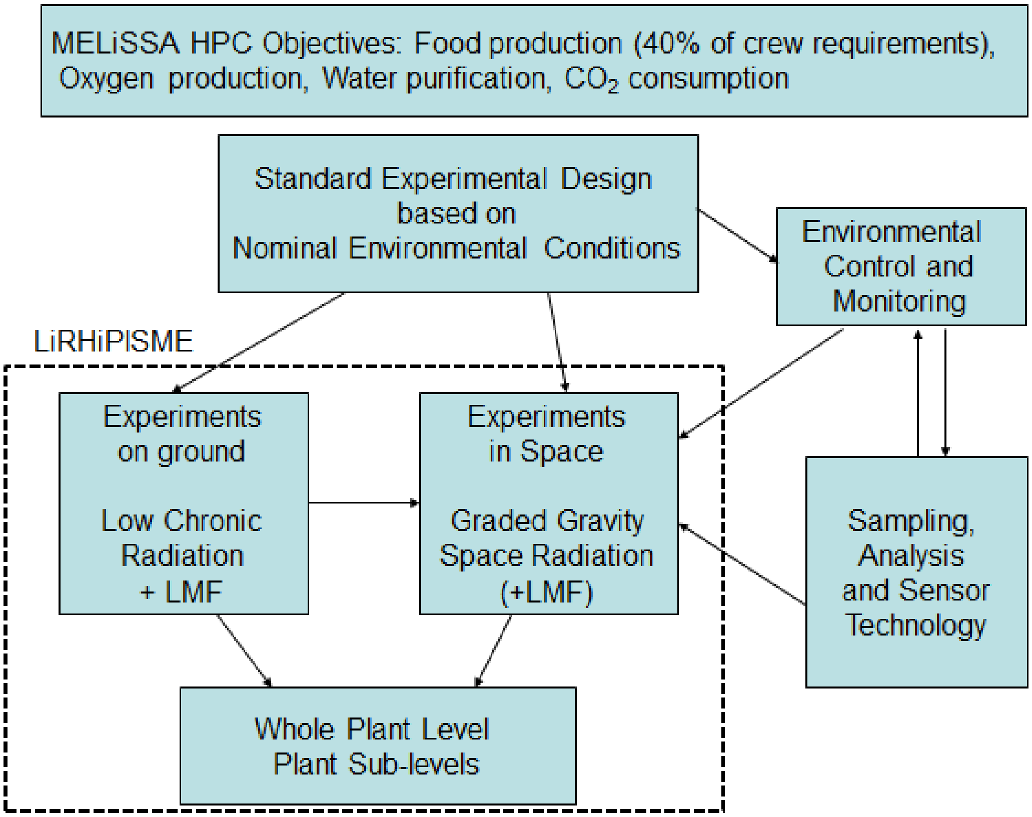

Figure 3. Main groups of requirements for future research activities on higher plants in space. HPC – higher plant compartment, LMF – low magnetic field (on ground: shielding from geomagnetic field, in space: lower than geomagnetic field).

4.1 Preliminary conclusions

In general, the knowledge about effects of the space environment on plant physiological processes is limited. To increase our knowledge and to succeed in sustainable plant production as part of life support systems, future experiments should include food plants as well as model plants, both dicotyledonous and monocotyledonous species and also the microbial associations. Experiments should be performed on whole plants and seeds, and should focus on the physiology of the whole plant and primary processes related to plant growth and metabolism.

4.2 Available space research facilities

The 2-dimensional (2D) clinostats are widely applied as a practical method to simulate microgravity (van Loon, 2007). However, already over 30 years ago it was shown that the compensation of the gravity vector by the 2D-clinostat was incomplete (Brown *et al.*, 1976). The Random Positioning Machine (RPM) is generally believed to be a suitable micro-g simulation instrument in order to reproduce the data obtained under real space microgravity conditions (Schwarzenberg *et al.*, 1999). When using the RPM, however, it is important to accommodate the sample exactly in the centre, as the rotation of the sample induces also random acceleration forces outside the centre. When using larger sample volumes or multiple samples, especially in combination with high rotation speeds, these accelerations may influence the results (Borst and van Loon, 2009). Consequently, clinostats and RPM machines have significant spatial limitations: in 2D-clinostats plants up to 3 mm can be cultivated, while RPM machines provide functional microgravity for a 3 mm^3 volume (Hauslage *et al.*, 2010). In addition, compensation of the gravity vector, even if completely effective, cannot remove chronic gravitational stimulation (Brown *et al.*, 1976).

Drop towers, parabolic flights and even sounding rockets give only a short time exposure to reduced gravity, and therefore have limited relevance for plant experiments. Satellites, on the other hand, may be used for experiments with higher plants, especially when their useful life expectancy increases. Foton retrievable capsules (unmanned) provide presently12-18 days exposure to low gravity (10^{-5}g) (European User Guide to Low Gravity Platforms, 2005).

Robotic missions to planetary surfaces could include plant science payloads. Such scientific payloads, however, have to be constructed in such a way as to guarantee no interference with other aspects of the mission. No sample return and remote sensing is to be expected within this scenario.

The best option for performing experiments with higher plants is on a space station like the ISS. However, with the retirement of the Space Shuttle programme, sample return will be unfeasible. This means that development of analytical tools and sensor technology, especially remote sensing, will become crucial for plant research in space.

Different gravitational biology methods and their suitability for plant experiments are summarised in Table 1.

Table 1. Methods in gravitational biology (microgravity)

Method	Time in microgravity	Quality of microgravity	Possibility for graded gravity	Experiments with higher plants
Clinostat	Unlimited	simulated	-	(+)
Drop tower (Bremen)	4.8-9.0 sec	10^{-6}g	-	-
Parabolic flight	20-30 parabolas 15-20 sec each	10^{-2}g	+	(?)
Sounding rockets	Up to 13 min	10^{-4}g	(+)*	?
Satellites	7-20 days (up to 3 months)	10^{-5}g	+*	+
(Space Shuttle)	(2 weeks)	10^{-4}g	+*	no longer available ☹
Robotic missions	3-4 months	0.17 g	(+)*	+
ISS	Unlimited	10^{-4}g	+*	+ No sample return!

*with on-board centrifuge

4.3 Future work

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.* The primary requirement can be achieved through a set of secondary requirements:

-selection of species and definition of their nominal conditions
-standardisation of experimental design
-low chronic radiation experiments

-graded gravity and space radiation experiments
-development of mathematical models

Future space related plant experiments should be designed to address the whole plant perspective and give a holistic view of the plants growth and development from seed to fully matured plant, before proceeding to carefully selected plant sublevels. Due to the limitations in available technology for simulating space conditions, and the elements of uncertainty associated with space experiments, propositions for future activities on ground and in space are given separately. Overall, technology status and state of the art within the actual field of research must always be well examined and documented before planning of space experiments. Surely, there is a need for baseline data before space conditions can be compared to conditions at Earth, and all possible scientific knowledge should be uncovered before proceeding to space experiments.

4.3.1 Future work on ground

Based on the conclusions from the literature review and described technology limitations, on-ground experiments should focus on *low chronic radiation exposure.* Radiation should mimic space radiation as much as possible, and should include at least gamma-rays, proton and neutron particles. 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. Threshold values for acceptable radiation doses should be determined for the selected life support food crops.

Clinorotation and RPMs could be implemented to simulate a reduced gravity environment when working with small samples on clinostats (< 1 mm) and RPM where the sample size is less critical (van Loon, 2007), and could be combined with radiation exposure experiments if practically feasible. However, because of the hard restrictions on sample size and cultivation method, clinostat and RPM unfortunately is of limited suitability for plant experiments.

The impact of magnetic fields on plant physiology needs further examination. The importance of such studies is debated, and should be reconsidered after publication of the work from the group of Professor Massimo Maffei (University of Turin, Italy).

To elucidate effects of low chronic radiation on whole plant physiology and plant metabolism, the following parameters should be prioritized in future research activities:

- Photosynthesis and gas exchange

- Biomass production
- Gene expression profile

Experiments should also, whenever feasible, include assessment of a plants complete growth cycle. Gene expression studies should be linked to well documented genes and specific plant functions. Research groups worldwide are working with collection of data through transcriptomic and metabolomic analyses of plants, and also the interpretation of such data to give a more complete picture of plant function and health status. It is estimated that mechanistic models for interpretation of descriptive data from the plants transcriptome and metabolome will improve considerably within five years (Atle Bones, NTNU Cell & Molecular Biology Group, pers. comm.)

Secondary, and based on the literature review, the following plant sublevels should be in focus:

- Nutrient content
- Chromosome aberrations and mutation frequency
- Morphological changes

Without the need for simulation of the space environment and with regard to plants as a food source the following aspects should be considered:

- Potential effects of changes in secondary metabolite production on food quality and food safety.
- Potential effects of changes in the shoot/root ratio and changes in the cell wall on the edible/non-edible plant mass ratio
- Effects of altered secondary metabolite production and cell wall components on plant susceptibility to disease.

Based on previous experiments the following hardware related issues need further consideration:

- Water management in closed systems
- Problems linked to volatile organic compounds (VOCs)

Water management includes chemical, physical, microbiological as well as plant physiological aspects and should be assessed by several cooperating groups.

In a closed environment, volatile organic compounds VOCs will be produced from man-made materials, plant and microbial communities as well as human activities. Such volatiles play a vital role in the normal growth and development of plants, but except for ethylene, threshold levels of phytotoxicity have not been established for most biogenic compounds (Stutte and Wheeler, 1997).

4.3.2 Future work in space

The two main factors affecting plant growth and development in space are reduced gravity and space radiation. Future space plant experiments, assessing the whole plant and a complete growth cycle, require extensive 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, especially when their useful life expectancy increases. Reference centrifuges in orbit are required to perform graded gravity experiments. To assess the effects of total space radiation load, 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. For an assessment of available methods in gravitational biology and their suitability for plant experiments, see table 1.

Future space plant experiments should focus on effects of the space environment on the whole plant and fundamental plant processes as:

- Photosynthesis and gas exchange
- Biomass production
- Transport of water and solutes

-provided optimal conditions

- Gene expression

-linked to specific plant functions

Furthermore, the following plant sublevels have been selected to be of particular interest for space research:

- Factors affecting mass balance and nutritional quality

-changes in carbohydrate, protein and lipid content
-shoot/root ratio and cell wall components
-production of secondary metabolites

- Morphological changes
- Hormone interactions (e.g. auxin and ethylene)

-analysis of plant hormones will help to predict morphological changes and to assess plant health

- Chromosome aberrations and mutation frequency

The prioritised sublevels may change as the work process moves forward.

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 gravity on higher plants. Plant experiments in space with several consecutive generations are required to study genome stability, pollination success and potential adaptation responses.

5. Conclusions

- Well controlled studies about effects of the space environment on plant physiological processes are scarce, and the available information is inconsistent and of varying quality
- Considerable work is required on food crops as well as on model plants
- A well-established field of research, satisfactory baseline data and knowledge for comparison of Earth and space conditions are prerequisites before proceeding to space experiments
- For better success and comparability on future research activities it is crucial to work with a standardised experimental design
- Proper ventilation and environmental control, along with monitoring of environmental conditions, are essential in plant growth facilities for space experiments
- With limited or no sample return in future missions to ISS, the Moon or Mars, remote sensing diagnosis of plant health will be required
- Development of hardware and sensor technology, for both ground-based activities and space applications, should be prioritized to complement research activities

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