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Literature Study: The Plant Global Perspective

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The Plant Global Perspective

1. Introduction

After the realisation of the International Space Station (ISS), human missions to Moon or Mars are widely considered as the next logical step of human involvement in a peaceful, cooperative space exploration on a global scale.

The human expansion into the cosmos must coincide with the development of a life support system that is capable of regenerating all the essentials for survival. Using the primary processes of photosynthesis (air revitalisation and biomass production) and transpiration (water purification), plants provide a continuous supply of food, oxygen, and clean water for humans (Wheeler *et al.*, 2001, Ferl *et al.*, 2002). The development of a plant-based, biologically regenerative life support system could play an important role in providing the fundamental needs of a human crew (Brown *et al.,* 2008). Plants recycle human wastes and provide human nutrients, while humans recycle plant wastes and provide plant nutrients (Wheeler *et al.*, 2001, Ferl *et al.*, 2002).

Plants are critical and fundamental components of advanced life support systems.

The first plant materials were brought into space in 1960 – wheat, pea, maize and onion seeds – on board Sputnik 4. This was followed by photosynthetic measurements of Chlorella and Spirodela (Duckweed) (Ward *et al.,* 1970) and wheat seedlings and pepper plants on Biosatellite II (Johnson & Tibbitts, 1968).

Spaceflight experiments with higher plants have typically looked at individual processes, functions or structures. These include examinations of chromosomal damage and plant cell division, embryogenesis, photosynthesis, biomass production, cell wall formation, starch metabolism, amino acid composition, lipid and fatty acid content, pathogenicity, reproductive development and gene expression (Stanković, 2001, Paul *et al.,* 2005).

Information about experiments dealing with whole plant physiology in space flight is limited (e.g. Monje *et al.*, 2005) and the responses of plants and seeds to the space-flight environment, especially outside low Earth orbit, need to be characterized (Ferl *et al.*, 2006).

2. The scope of the technical note.

The purpose of this WP is to consider the plants from a global perspective. The focus will be on three major areas:

1. Assess the overall plant adaptation to the effects identified during the plant sub-level studies.
2. Based on findings from the other TNs, review effects of the space environment on the "Food" aspect: the ratio between edible plant mass and non-edible plant mass, the quantity (total amount of mass in e.g. dry weight, fresh weight or carbon) of the edible plant mass, and the edible plant mass content: macro elements (proteins, lipids and carbohydrates), nutrients (minerals and vitamins), and fibre.
3. Consider if there is a potential impact on the "Mass balance" aspect.

3. Plant adaptation to space conditions

To provide food for the human crew living in microgravity onboard a spacecraft or in planetary bases crops must be adapted to grow optimally in the new environment with new biophysical combinations. The process of adaptation to a new environment can be quite similar to what farmers have done in the past when new environments on Earth were colonized (Monje *et al.*, 2003).

Plants have demonstrated their ability to grow in space. A number of long-term experiments were successfully performed in space: a full life cycle of *Arabidopsis thaliana* has been completed on Salyut-7 (Merkys *et al.*, 1984), the first space vegetables were produced in 1993 in the Svet growth chamber on board Mir (Ivanova *et al.*, 1993), two and a half generations of Wisconsin Fast Plants (*Brassica rapa*) were grown in the Mir Greenhouse (Musgrave *et al.*, 2000), and also two generations of wheat (Sychev *et al.*, 2001).

Plants have various kinds of mechanisms for adaptation to environmental stimuli. They cope with novel environmental challenges by adapting endogenous stress management strategies. Spaceflight plants showed e.g. characteristics similar to those of plants experiencing hypoxic stress in terrestrial models (Paul *et al.*, 2001; Porterfield *et al.*, 1997, 2000; Stout *et al.*, 2001). Plants, especially those living in extreme environments, have metabolic adaptive pathways. Through the evolution plants have adapted to stress by responding with changes in metabolism in order to meet the new challenges. Even simple plants like *Arabidopsis thaliana* L. are capable of surviving short-term stress by initiating new metabolic processes to meet the challenge of the new environment. An overview of the molecular aspects of such adaptive responses to stress is presented by Paul and Ferl (2002).

Spaceflight-associated stress has been the topic of investigation since the first terrestrial organisms were exposed to this unique environment. Organisms that evolved under the selection pressure of Earth-normal environments can perceive spaceflight as a stress, either directly because gravity influences biological processes, or indirectly because of secondary effects by environmental conditions changed by a spaceflight. Different organisms and even different organs within an organism adapt to a spaceflight environment with a diversity of tactics (Paul and Ferl, 2002).

In the context of the overall plant adaptation the following topics will be dealt with:

The effects of physical factors with focus on **reduced gravity, space radiation, lower magnetic field** and **combined effects** of these factors. For information on the particular conditions found on the Moon and Mars, see CEAS paper ”Literature Study of Higher Plants in Space for MELiSSA (LiRHiPliSME)- Input to MELiSSA Phase II project” (Kittang *et al.,* 2009).

In the discussion the following experimental features were assumed: optimal control of temperature, light, pressure (1000 hPa), water and gas supply/composition; optimal root support, availability of water and nutrients.

3.1 Microgravity effects

A microgravity environment induces significant limitation to basic physiological and biochemical processes within the aerial and root zone portions of the plant. Porterfield (2002) has reviewed some of the biophysical limitations created by the microgravity environment.

In microgravity buoyancy-driven thermal convection (BDTC) is completely inhibited. Under these conditions boundary layers become large and the transport is diffusion limited. The inhibition of BDTC airflow in microgravity would potentially affect many basic physiological processes:

- Transpiration. The movement of water in xylem would be reduced with thicker boundary layers. This causes a negative effect on the whole plant physiology.

- Mineral nutrient transport. Transport of nutrients, such as Ca, which are closely tied to transpiration, could be affected.

- Photosynthesis (CO_2 uptake/O_2 release). CO_2 uptake would be more difficult with thicker boundary layers. Decrease of photosynthetic functions leads to the general decline in overall plant status.

- Respiration (O_2 uptake/CO_2 release). O_2 uptake could be reduced with thicker boundary layers.

In the root zone one can also expect problems with metabolic gas exchange and mineral nutrient transport. Limiting the bioavailability of oxygen in the root zone has a direct impact on the metabolism and physiological function of the root system. The most serious effect in terms of whole plant physiology and productivity is the inhibition of ion nutrient uptake and transport to the shoots (Porterfield, 2002).

The continuous air flow is absolutely necessary for successful plant growth in microgravity. Before the importance of ventilation was recognised, a decrease of photosynthetic functions was detected in many plant experiments in microgravity. It was observed that starch content and starch grain size were decreased in pea leaves developed during space flight (Aliyev *et al.*, 1985), and slight swelling of the chloroplast grana was found in maize seedlings following the 19 days flight of Cosmos-1129 (Tairbekov *et al.*, 1981). Chloroplasts with no starch or with smaller grains and partial destruction of thylakoids were observed in protonema cells of *Funaria hygrometrica* flown on Salyut 6 for 96 days (Kordyum *et al.*, 1981). Photosynthetic capacities of chloroplast extracts from dwarf wheat leaves that had formed in space, were slightly depressed as compared to the ground controls leading Tripathy *et al.* (1996) to conclude that microgravity partly inhibits the photosynthetic apparatus. Space-grown plants showed 25% reduction in maximum net photosynthetic rates and the shoot fresh weights of space-grown seedlings decreased by 25% (Tripathy *et al.*, 1996).

Musgrave and co-workers (1997 and 1998) demonstrated in the CHROMEX studies that the absence of proper ventilation in microgravity reduced the carbohydrate content by 40% (especially glucose and starch) and impaired reproduction when compared to ground controls. When a continuous airflow was provided to the plants, no difference in carbohydrate and chlorophyll concentration was found, and reproductive development became comparable between spaceflight and ground control plants. The results from

Table 1. An overview of the different CHROMEX experiments performed in a differently controlled environment. Focus was on the effects of cultivation conditions on plant reproduction and photosynthesis.

Experiment name	**Chamber configuration**	**Effects on reproduction**	**Effects on metabolism**
CHROMEX-03 *STS-54* Duration : 6 days	Closed plant growth chambers	Male and female gametophyte development aborted at an early stage in the flight material	Lower carbohydrate concentration (especially glucose) than in the ground control. Higher chlorophyll concentration than in the ground control.
CHROMEX-04 *STS-51* Duration : 10 days	Closed chamber with carbon dioxide enrichment	Reproductive development proceeded normally until the pollination stage, when there was an obstacle to pollen transfer in the spaceflight material	Starch concentration higher than without carbon dioxide supplementation but lower than in the ground control. Higher chlorophyll concentration than in the ground control.
CHROMEX-05 *STS-68* Duration : 11 days	Air-exchange with filtered cabin air in the growth chamber	The spaceflight plants apparently had reproductive development comparable to the ground controls, and immature seeds were produced	No difference in carbohydrate and chlorophyll concentration between the flight and the ground control.

these experiments are summarised in Table 1, demonstrating the importance of an atmospheric controlled environment for plant reproduction and photosynthesis.

The plant development is apparently not affected by microgravity. The success of the repeated seed-to-seed experiments conclusively demonstrates that gravity is not absolutely required for any step in the plant life cycle (Musgrave *et al.*, 2000). During the Ukrainian-American experiment (STS-87) the comparative cytoembryological investigation on *Brassica rapa* L. embryos showed no clear influence of microgravity on the embryogenesis of plants (Popova *et al.*, 2009).

The PESTO (Photosynthesis Experiment Systems Testing and Operations) experiment was the first long-term replicated test to obtain direct measurements of canopy photosynthesis in space under well-controlled conditions. The PESTO experiment consisted of a series of 21–24 days growth cycles of dwarf wheat (*Triticum aestivum* L. cv. USU Apogee) onboard ISS. Some of the results indicated that leaves developed in microgravity (µ*g*) get a thinner cross-sectional area than the 1*g* grown plants (Stutte *et al.,* 2006). Although the leaf area was significantly reduced in microgravity-grown plants compared to ground control plants, leaf area distribution was not affected (Monje *et al.*, 2005). At the morphological level, there was almost no difference in the development of cells of wheat under μ*g* conditions, nor was there any significant difference in chloroplast density and the chlorophyll content (Stutte *et al*., 2006).

The results of the PESTO experiments also demonstrated that there was no difference in canopy P_{net} (the rate of net canopy carbon assimilation) between μg and 1g plants grown under moderate light conditions (Monje *et al*., 2005). No differences were observed in the contents of starch, soluble sugar, or lignin in the leaves grown under μ*g* or 1*g* conditions (Stutte *et al*., 2006). Plant stand evapotranspiration, net photosynthesis and water use efficiency were not altered by microgravity (Monje *et al*., 2005). Dry mass per plant was not significantly different between the flight and ground treatments (Stutte *et al*., 2005). Furthermore, no differences in gene expression were detected in leaf samples collected at μ*g* from 24-day-old leaves of dwarf wheat, leading the authors to conclude that the spaceflight environment had minimal impact on the metabolism in wheat (Stutte *et al*., 2006).

One can conclude that plant gas exchange, metabolism and photosynthesis seem to work properly in the microgravity environment when the plants are provided adequate ventilation and grown at moderate light levels. The hardware has been shown to be of great importance on the results found under microgravity conditions. Good environmental control and ventilation are essential to grow healthy plants in space and to avoid the indirect effects of microgravity e.g. the lack of buoyancy driven forces (TN 97-08).

3.2. Radiation effects

Radiation that can damage plants and act as mutagens are (1) ionizing radiation which includes UV-A (partly), UV-B and UV-C radiation, gamma rays and X-rays; and (2) solar wind (particles) and (3) cosmic rays from outer space (galactic cosmic rays) including (a) protons, alpha (helium nuclei) and beta particles (electrons) and (b) high energy atomic nuclei (HZE particles).

The known effects on plants from the different radiation sources are presented in Table 2.

Ionizing as well as non-ionizing radiation can cause damage to the genetic apparatus, in the form of an increased level of mutations and various types of aberrations (TN 97-03).

Enhanced UV-B, UV-C, and gamma irradiation have been shown to decrease auxin levels in plants, leading to growth inhibition (TN 97-04).

Table 2. The effects of variable radiation sources on plant cells and plant metabolism

Radiation	**Effect**	**Reference**
UV-A (320-400 nm)	Both negative and positive effect on photosynthesis	TN 97-08
UV-B (290-320 nm)	Mutation and DNA damages	TN 97-03
	Decreased auxin level Growth inhibition	TN 97-04
	Damage of Photosystem II (inactivation) and Photosystem I (activity decreased by 35%).	TN 97-08
UV-C (180-290 nm)	Decreased auxin level Growth inhibition	TN 97-04
	Decrease of photosynthetic and transpiration rate	TN 97-08
Gamma rays	Mutations and chromosome aberrations	TN 97-03
	Decreased auxin level Growth inhibition	TN 97-04
	Damages on the photosynthetic system	TN 97-08
X-rays	Mutations and DNA damages	TN 97-03
Protons	No information	
Neutrons	Mutations and chromosome aberrations	TN 97-03
HZE	Mutations and chromosome aberrations.	TN 97-03
	Tumor induction in *Arabidopsis thaliana* seeds. Mutation induction in *A.thaliana* and *Zea mays* seeds. Anomalies in *Nicotiana tabacum* seeds. Multiple chromosome aberrations in *Lactuca sativa* seeds	Horneck, 1994*
Space radiation	Altered gene expression. Mutations, chromosome aberrations and DNA damages.	TN 97-03

*Horneck, 1994 is a review article i.e. comparable with the TNs.

The effects of enhanced UV-B on higher plants have been intensively studied. Enhanced UV-B appeared to be harmful to the photosynthetic apparatus, affecting different key proteins and enzymes (TN 97-08).

UV-C and gamma radiation were also harmful on the photosynthetic apparatus (TN 97-08), but the extent of the damage and the effects on the whole plant need to be further studied. Based on their higher energy levels, one would expect their effects to be very damaging.

Gamma radiation affects seed germination (delayed, decreased germination percentage), growth (decreased shoot and root length of seedlings) and protein contents (decreased after 100 to 800 Gy dose, increased after 900 and 1000 Gy) in chickpea (*Cicer arietinum* L.) (Hameed *et al.*, 2008).

Gamma exposure significantly reduced the root weight in *Arabidopsis*. Leaf weight and stem yield were significantly reduced at the highest irradiation level (2339 μGy h^{-1}) after 54 days of exposure (Vandenhove *et al.*, 2009).

There are some indications that neutrons can induce variation in growth and can also affect the expression of genes involved in auxin transport (TN 97-04).

We have not found any studies that focused on the effects of protons and neutrons on plant photosynthesis and metabolism. Since protons account for 90% of the space radiation it is important to understand which effect there is on the photosynthetic apparatus.

The effects of radiation on plant gas exchange, metabolism and photosynthesis, transport of water and solutes have not been studied under space conditions (low Earth orbit).

Studies of spaceflown plants, however, showed an increase in morphological deformations of the seedlings (atrophy of cotyledons, roots and stems), and the seeds hit by HZE particles showed a high percentage of abnormalities (TN 97-03).

In seeds of *Arabidopsis thaliana* hit by HZE particles the development is significantly disturbed. Both loss of germination (early lethality) or embryo death (delayed lethality) were shown. Chlorophyll deficiency occurs as a consequence of passage of a single HZE particle close to the shoot or root meristem. *Lactuca sativa* seeds hit by HZE particles demonstrated high frequency of multiple chromosomal aberrations (Horneck, 1994). For the review of HZE effects in space - see Horneck (1994).

The radiation load is perhaps the most important factor in space flights that causes a higher chromosome aberration ratio. Dormant seeds are more resistant to aberrations than actively developing systems. Moreover, there might be synergistic damaging effects between radiation and microgravity, but it is difficult to separate the effects of these two factors unless in-flight control centrifuges are used (TN 97-03).

3.3. Low magnetic field effects

Though early studies on the influence of electromagnetism on living systems dated back to the late nineteenth century and specific studies on plants which were initiated more than 70 years ago, comparatively little is known about magnetoperception in plants.

The central questions in this context

1. whether or not plants can perceive the Earth's magnetic field and
2. whether or not the geomagnetic field has any bearing on their survival,

have remained largely unanswered (Galland and Pazur, 2005).

The geomagnetic field is static, homogeneous (for most practical purposes), and relatively weak – 35 µT near the Equator, 70 µT near the magnetic poles of the Earth (Zhadin, 2001).

Plants that are raised in a weakened geomagnetic field show characteristic anatomical alterations in the root meristem. Specifically, mitochondria in the meristems of *Pisum sativum*, *Linum usitatissimum* L. and *Lens culinaris* roots display abnormal morphology (Belyavskaya *et al.,* 1992; Zhadin, 2001), and RNA and protein synthesis is somewhat suppressed (Fomicheva *et al.,* 1992a,b ; Zhadin, 2001). In meristematic root cells of *Pisum sativum*, very low magnetic fields (magnetically shielded boxes) induce ultrastructural changes such as accumulation of lipid bodies and altered Ca^{2+} homeostasis (Belyavskaya, 2001). Branching of plants is also affected in such shielding experiments (Govorun *et al.*, 1992; Zhadin, 2001). Germination, seedling growth, root and stem length, and growth in tissue cultures were shown to be unaffected. Respiration was reduced by 40 % in lettuce (Walkinshaw and Galliano, 1990). However, the significance of this is expected to be minimal in a CO_2-enriched environment. Low magnetic field strengths are not expected to have a detrimental impact on crop growth (Hender, 2007).

Weak magnetic fields can cause growth inhibitions, and there are indications that auxin plays a substantial role in this process. However, no direct evidence has been found yet (TN 97-04).

Plant gas exchange and metabolism also seem to be affected by a weak magnetic field (shielding from the Earth's MF): respiration and photosynthetic pigments were reduced (TN 97-08).

3.4. Combined effects

Exposure of *Arabidopsis thaliana* plants to spaceflight environments results in differential gene expression. Expression of most of the 21 000 *Arabidopsis* genes represented in an array was essentially unaffected by spaceflight, but 182 genes were altered in their expression by more than 4-fold (Paul *et al.,* 2005). The gene sets most notably induced in the spaceflight samples were involved in responses to heat shock i.e. Heat Shock Proteins (HSPs). These genes are known to respond to different kinds of stress such as heat, inflammation, hypoxia, and toxins (Paul *et al.,* 2005).

One can conclude that there are factors in space that stress the *Arabidopsis* plant and result in an induction in the stress genes after 5 days in flight. Whether it is microgravity or radiation alone or a combination of them and other factors responsible for this changed expression level, is unknown up to now (TN 97-03).

However, as mentioned in Section 3.1, there was no difference found in gene expression in leaf samples collected at μ*g* from dwarf wheat leaves (Stutte *et al.*, 2006). The different results can possibly be explained by the difference in plant species, experimental design, or plant growth hardware.

One of the most important questions in space biology is whether long-term spaceflight exposure could cause changes in subsequent generations. Wheat flown on Mir for 167 days in 1991 formed viable seeds when returned to the Earth. These seeds were grown on the ground for three additional generations. Microarray analysis showed no statistically significant difference in expression between wheat that had spaceflight exposure in their lineage and plants with no spaceflight exposure. These results indicate that exposure to the spaceflight environment for one generation does not result in changes in gene expression that are heritable (Visscher *et al*., 2009).

The space effects on morphology and DNA polymorphism in pea plants were also investigated on Mir. Four consecutive generations of peas were grown in the Lada greenhouse, and neither morphological differences from ground control during four generations nor genetic polymorphism were found in the pea plants grown in flight from the 1st generation (Sychev *et al.,* 2007). However, results of genetic analysis of 2nd-4th generations have not been published yet.

4. The food aspect

Already the second man in Space, the Soviet cosmonaut German Titov, consumed food during his flight in August 1961 but came back rather hungry, in spite of his scientifically balanced menu. It became absolutely necessary to provide cosmonauts and astronauts with food ever since. For long-term missions, especially for missions exceeding one year, it may therefore be necessary to cultivate plants for food production during the flight. Based on the current knowledge of space flight nutrition and food science, joint US–Russian nutritional recommendations were developed and implemented. Table 3 shows a representative daily composition of nutrients for a crew member (for one crew member, we assume) planned for a one year stay on the ISS.

A considerable work was performed to design appropriate menus for Closed Loop Food Systems (Salisbury & Clark, 1996, Mas *et al.*, 2005, Vanrobaeys *et al.*, 2005, Masuda *et al.*, 2005 & 2006). In the study of Mas *et al.* (2005) plants were selected according to their nutritional value, feasibility of culture requirement and suitability to create acceptable menus. Nutritional content, yield (edible biomass produced per surface and volume of the greenhouse), generated waste and micronutrients, and the mineral content were considered. The process resulted in two alternative crop sets with four crops in each. MENU 1 (low waste, high volume) is anticipated to produce less inedible biomass but requires more cultivation volume than MENU 2 (high waste, low volume). These menus were designed to provide a six-person crew with 40% of its dietary needs (Mas *et al.*, 2005). The menu composition, the daily intake in grams (fresh weight) to meet a 40% recommended daily allowance (2700 kcal, 135 g proteins, 371 g carbohydrates and 71 d lipids) per crew member (Vanrobaeys *et al.*, 2005), the waste generated and the volume and surface needs are shown in Table 4.

These two combinations should be considered as two extreme solutions; an intermediate menu composition can also exist. In MENU 1 quinoa meets volume needs, it is also a good energy and mineral provider. Kale is part of MENU 1 for its minerals and vitamins. In MENU 2 wheat is included for its energy density, minerals and vitamins. French beans and garden cress are selected since they do not need substantial cultivation volumes but still provide minerals and vitamins. Finally - soybean is included for its energy potential, reflected in the protein and lipid contents (Vanrobaeys *et al.*, 2005).

However, the information about crops nutrition values considered in the selection process was obtained on Earth grown plants (Vanrobaeys *et al.*, 2005) and critical technologies for food production processes in closed loop food systems in space include “Fundamental research on plant physiology and metabolism in Space” and “Basic research on growth of plants in microgravity/hypogravity conditions” (Mas *et al.*, 2005).

Table 3. Daily nutritional requirements for the crew on the ISS missions for up to 360 days (from Lane and Feeback, 2002).

Nutrient	Units	Requirement
Energy	KJ (kcal)	WHO* equation
Protein	%Total energy consumed	12–15
Carbohydrate	%Total energy consumed	50
Fat	%Total energy consumed	30–35
Fluid	mL/MJ consumed or mL/kcal	238–357 or 1.0–1.5 or 2000 mL/d
Vitamin A	μg retinal equivalent	1000
Vitamin D	μg	10
Vitamin E	mg α-tocopherol equivalent	20
Vitamin K	μg	80
Vitamin C	mg	100
Vitamin B12	μg	2
Vitamin B6	mg	2
Thiamin	mg	1.5
Riboflavin	mg	2
Folate	μg	400
Niacin	NE or mg	20
Biotin	μg	100
Pantothenic acid	mg	5
Calcium	mg	1000–1200
Phosphorus	mg	1000–1200 <1.5 times Ca intake
Magnesium	mg	350
Sodium	mg	1500–3500
Potassium	mg	3500
Iron	mg	10
Copper	mg	1.5–3.0
Manganese	mg	2.0–5.0
Fluoride	mg	4
Zinc	mg	15
Selenium	μg	70
Iodine	μg	150
Chromium	μg	100–200

* Individual energy requirements are calculated with the WHO (World Health Organization) equation, accounting for weight, age, sex, and moderate activity levels.

The goal of this chapter is to discuss how eventual changes on a “Plant sub-level” might affect overall plant function and food production. This section is also based on findings of the other TNs, in which one might expect an impact on the ratio of edible and non-edible plant mass and the quantity and content of the edible plant mass.

The effects of microgravity, radiation and low magnetic fields on the plant anatomy and physiology, which can be of consequence for the plant food role, are summarised in Table 5.

Table 4. MENU 1 and MENU 2 characteristics. The values are per crew member and based on the fresh weight (modified from Mas *et al.*, 2005).

	MENU 1 Low waste High volume	MENU 2 High waste Low volume
Selected crops	• quinoa (284 g/d) • soybean (50 g/d) • kale (100 g/d) • sweet potato (408 g/d)	• French bean (90 g/d) • soybean (60 g/d) • wheat (180 g/d) • garden cress (100 g/d)
Produced waste	• 322 g/d	• 433 g/d
Required volume	• 20.9 m^3	• 12.4 m^3
Required surface	• 36.6 m^2	• 28 m^2

4.1. The ratio between edible and non-edible plant mass

Components of the cell wall, such as cellulose and lignin, were reduced when the plants were growing under microgravity conditions in space (Nedukha, 1996). Further research showed a decreased level of cellulose and the matrix polysaccharides in microgravity in rice and *Arabidopsis*. Microgravity caused an increase of cell wall extensibility (irreversible) and a decrease in the molecular masses of cell wall polysaccharides (Hoson *et al.*, 2000, 2003 and 2004; Soga *et al.*, 2002).

The changes in cell wall properties under microgravity conditions during spaceflight can affect the ratio between edible and non-edible plant mass. In general, one can assume that with thinner cell walls the production of non-edible plant mass will be lower. However, more experiments are necessary to clarify the influence of space conditions on the cell wall component production. The potential effect of the changed cell wall on the ratio between edible and non-edible plant biomass was also not investigated. No experiments were made under reduced gravity levels (e.g. Mars and Moon) in a spaceflight.

4.2. The quantity of the edible plant mass

Without a sufficient gas flow around the plants, the gas exchange in microgravity will be diffusion-limited, and the carbon fixation by the plants will be slow (Musgrave *et al.*, 1997). Tripathy *et al.* (1996) found that the shoot fresh weight of space-grown dwarf wheat seedlings was decreased by 25%. However, this result was obtained without ventilation of the growth chamber.

Table 5. Possible effects of space conditions on the plant food role.

Aspect		**Effect**	**Reference**
The ratio between edible and non-edible plant mass		Thinner cell wall (*Funaria hygrometrica* Hedw., moss protonema) Decreased level of cellulose and matrix polysaccharides in microgravity (rice)	Nedukha, 1996 Hoson *et al.*, 2000, Soga *et al.*, 2002, Hoson *et al.*, 2003 and 2004, TN 97-05
The quantity of the edible plant mass	Fresh weight , Dry weight, Carbon content	Fresh weight decreased by 25% (*Triticum aestivum*) No difference in dry weight per plant	Tripathy *et al.*, 1996 TN 97-05 Stutte *et al.*, 2005
The edible plant mass	Carbohydrates	Retention of starch in microgravity (*Brassica*)	Musgrave *et al.*, 2007, TN 97-08
	Proteins	Decreased in microgravity (*Brassica*) Decreased in low magnetic field (pea, flax and lentil)	Kuang *et al.*, 2000, 2005 Musgrave *et al.*, 2005 Fomicheva *et al.*, 1992a, b Zhadin, 2001
	Lipids	Increased volume of lipid bodies in meristem cells (onion)	Belyavskaya, 2004
	Minerals	Reduced uptake of nutrients	TN 97-06
	Secondary metabolites	Higher concentration of glucosinolates in microgravity (*Brassica* and soybean) Lower concentration of lignin (*Vigna radiata*)	Allen *et al.*, 2009 Levine *et al.*, 2001 Musgrave *et al.*, 2005 Tuominen *et al.*, 2009 Cowles *et al.*, 1984

Later it was found that with proper ventilation the dry mass per plant was not significantly different when the flight (0.113 g) and the ground (0.122 g) treatments were compared (Stutte *et al.*, 2005).

4.3. Carbohydrates

When the oilseed crop field mustard (*Brassica rapa* L.) was cultivated in microgravity, changes in seed storage components were detected. Cytochemical localization of storage reserves in mature cotyledons of *Brassica rapa* L. showed that starch was retained in the space flight material, whereas proteins and lipids were the primary storage reserves in ground control seeds (Kuang *et al.*, 2000). Later Musgrave *et al.* (2005) found that *Brassica rapa* (cv. Astroplants) seeds, which were produced in space (Biomass Production System on ISS), had higher concentrations of chlorophyll, starch, and soluble carbohydrates than the ground controls. Cytochemical localization of storage reserves showed differences in starch accumulation between spaceflight and ground control *Brassica* seeds. In spaceflight seeds starch was retained after cotyledon elongation stage (9 day postpollination), while the starch grains in the ground control seeds decreased in size (Kuang *et al.*, 2005).

4.4. Proteins

Kuang *et al.* (2000) found that the protein bodies detected in mature *Brassica* cotyledons produced in space were 44% smaller than those in the ground control seeds.

They also found that large and well-developed protein bodies could be observed in cotyledon cells in ground control seeds at a stage 15 days after pollination. However, this development was delayed in the seeds produced during spaceflight (Kuang *et al.*, 2005).

Musgrave *et al.* (2005) have also observed that the protein content in *Brassica rapa* (cv. Astroplants) seeds was significantly lower in spaceflight material.

4.5. Minerals and vitamins

According to Porterfield (2002) under microgravity conditions one should expect problems with metabolic gas exchange and mineral nutrient transport in the root zone. Limiting the bioavailability of oxygen in the root causes the inhibition of ion nutrient uptake and transport to the shoots (Porterfield, 2002; TN 97-06). When nutrient uptake and transport are inhibited, the mineral content in the plants might be reduced.

There are no data on how the vitamin content is influenced by space conditions. Gitelson *et al.* (1995) analysed the productivity, biochemical composition, including content of several vitamins (ascorbic acid, thiamine, riboflavin and caroten) vitamin in edible biomass of plants grown on Earth under continuous illumination and a “lunar” photoperiod. They found that frigostable cultures like barley, carrots, red beet, radish and dill can be successfully grown in the “lunar” illumination rhythm and food value of edible biomass is equal in plants grown under continuous illumination and “lunar” photoperiod. However, these data can hardly be relevant for the present study, as experiments did not include microgravity, radiation or magnetic field influence.

4.6. Secondary metabolites

The secondary metabolite production seems to be influenced by spaceflight conditions. For example, the concentration of glucosinolates and isoflavonoids was higher in spaceflight material than in 1-g samples of *Brassica* and soybean (Musgrave, 2007). 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 (at 1*g*). Interestingly enough, increased gravity (hypergravity) had the opposite effect on the glucosinolate production since Allen *et al.* (2009) observed that glucosinolates in *Brassica* decreased by 140% over the range from micro-*g* to 4-*g*. For a review of plant secondary metabolism in altered gravity - see Tuominen *et al.* (2009).

The potential effect of gravity on plant secondary metabolism would influence the food production in a BLSS (Bioregenrative Life Support System) under microgravity conditions in at least three ways. Firstly, a diet high in antioxidants or anti-carcinogenic compounds would be advantageous. Secondly, it may be desirable from a psychological point of view, to add fresh flavours and fragrances to the stowed foods provided to the crew. Thirdly, plant secondary metabolites are important for maintaining plant health (Tuominen *et al.*, 2009).

In taste trials of four leaf vegetables produced in microgravity in 2000, Russian cosmonauts indicated a preference for red mustard (*B. juncea* L., cv. Red Giant) and mizuna (*B. rapa* L. var. *nipposinica* (Bailey) S. Kitamura) over two other varieties of *B. rapa*. Glucosinolate profiling indicated that red mustard and mizuna both contain higher total levels of these compounds than the other two varieties of *B. rapa*. Therefore, it seems likely that increased glucosinolate levels produced in a microgravity environment would be beneficial (Tuominen *et al.*, 2009).

However, the food value of glucosinolate-containing plants can be affected by degradation of glucosinolates. Whereas glucosinolates are generally considered as biologically inactive, their degradation products have toxic effects on fungi, bacteria and also function as insect pests (Kissen *et al.*, 2009). Mechanical damage, infection or pest attack induces cellular breakdown in the plant and the stored glucosinolates become exposed to degradative enzymes (myrosinases). The glucosinolate degradation products, thiocyanates, of a food plant can inhibit the uptake of iodine into the thyroid and large amounts are known to have negative effects on the liver (Bones and Rossiter, 1996). The anti-nutritional properties of glucosinolate degradation are well documented with goitrin and thiocyanate as the main anti-nutritional factors. Goitrin is an inhibitor of thyroid peroxidase and prevents oxidation of iodide to iodine for subsequent iodination of tyrosine residues in the biosynthesis of the thyroxines T3 and T4. Thiocyanate anions act as a competitive inhibitor of iodide and thus prevent iodide uptake by the thyroid (Bones and Rossiter, 2006).

These compounds are also known to be potential mutagenic and carcinogenic in nature but they also may have anti-carcinogenic properties (Bones and Rossiter, 1996). Some glucosinolate hydrolysis products are believed to have anti-carcinogenic properties in humans (Kissen *et al.*, 2009). The isothiocyanate sulphoraphane is a particularly potent anti-cancer compound, which induces phase II detoxification enzymes that are thought to induce apoptosis in cancer cells (Bones and Rossiter, 2006).

Isothiocyanates can potentially generate new compounds, particularly during cooking processes, and this area of research warrants further investigation (Bones and Rossiter, 2006).

The potential negative effects of glucosinolates require further examination.

5. Mass balance

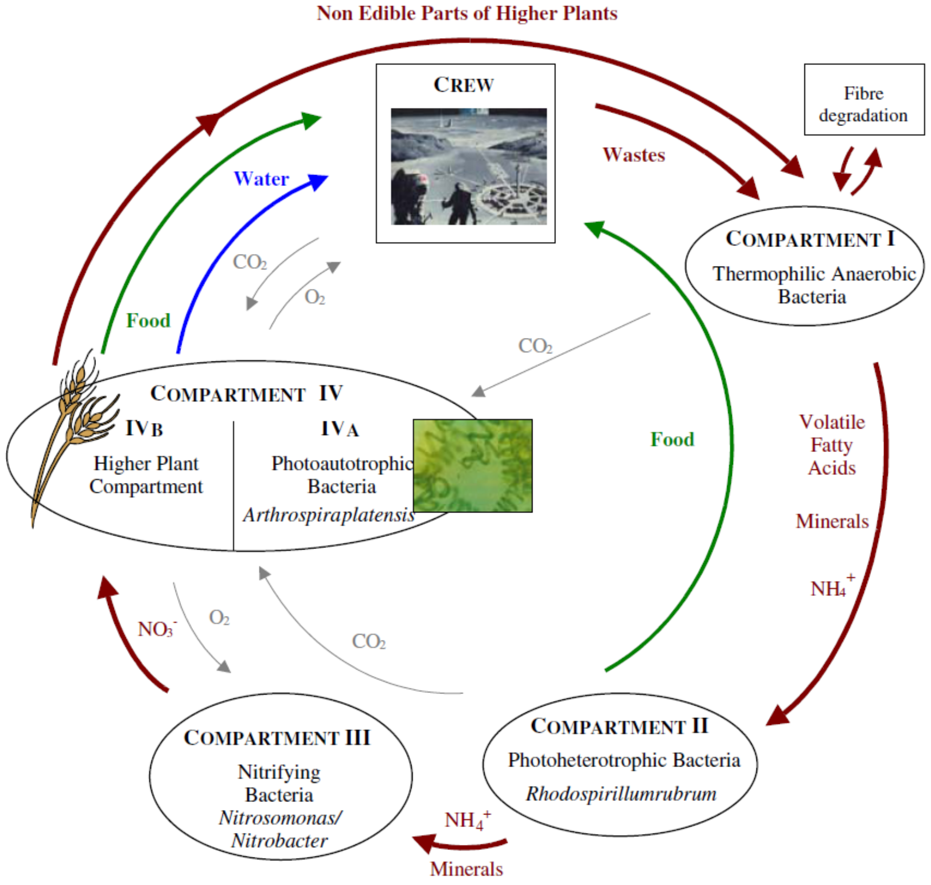
The main goal of this chapter is, based on findings concerning changed plant performance under space conditions (reduced gravity, radiation and lower magnetic field) to consider if one might expect an impact on the mass balance in controlled bioregenerative life support systems (BLSS). In this context all parts of the whole plant are equally important, both the edible but also the non-edible parts, i.e. the macronutrients (proteins, lipids and especially carbohydrates), minerals, and fibres.

For long-term interplanetary manned missions or establishment of planetary bases in the future resupply of oxygen, water, and food from Earth is very costly. Life Support Systems for these kinds of missions must be regenerative as much as possible. In Advanced Life Support (ALS) systems with bioregenerative components, plant photosynthesis would be used to produce O_2 and food, while removing CO_2 at the same time. Much of the plant biomass would be non-edible and hence must be considered in waste management. This waste could be oxidized (e.g. incinerated or aerobically digested) to resupply CO_2 to the plants, but this would not be needed unless the system would be highly closed with regard to food (Wheeler, 2003).

Regardless of system closure, high harvest index crops (i.e. crops with a high edible to total biomass ratio) would increase food production per unit greenhouse area and O_2 yields for systems where waste biomass is oxidized to recycle CO_2 (Wheeler, 2003).

The Micro Ecological Life Support System Alternative (MELiSSA; see below) is a model system for an advanced life support system based on different microbial species and higher plants (Gòdia *et al.*, 2002).

The objectives are atmospheric management, water management, food production and storage waste management. Edible biomass is to be recovered from wastes and carbon dioxide using light as an energy source for photosynthesis.



MELiSSA ADVANCED LOOP CONCEPT

Dynamic models of MELiSSA compartments including the higher plant compartment are discussed by Farges *et al*. (2008). The MELiSSA system is designed for producing food, water and oxygen by reprocessing a maximal part of the wastes produced by a human crew and by the higher plant chamber. The summary of the quantitative requirements gives approximately 20 kg of different compounds per person per day to be produced and eliminated for complete recycling (Farges *et al*., 2008).

The recycling efficiencies of C, H, O, N, S and P elements were determined and compared for the MELiSSA loop with and without the Higher Plant Chamber (Gros *et al*., 2003). With the Higher Plant Chamber system too much oxygen (170% of the system requirement) is produced, and carbon dioxide must be imported. In addition the Higher Plant Chamber produces a lot of waste (non-edible biomass). It was therefore suggested to oxidize the non-edible biomass in order to produce the carbon dioxide required for plant growth. Up to 2003 the ability of the MELiSSA compartments to transform the vegetable waste had not been studied, therefore, the physico-chemical process was added. When 80% of the organic waste was oxidized, the recycling efficiency levels were above 95% (Gros *et al*., 2003). Thus, the oxidation of the non-edible plants biomass is important for the recycling efficiency of the loop.

At the same time, the addition of an aerobic bioreactor can make the system oxygen deficient (Czupalla *et al.,* 2005).

A partition of the non-edible biomass can be used for nutrients recycling. Approximately 50% of the total nutrient requirement of hydroponically-grown potatoes was recycled using the water soluble fraction of non-edible potato biomass (Mackowiak *et al.,* 1996).

Tikhomirov *et al*. (2003a, b) suggested re-involvement of non-edible plant biomass in the system cycling by "biological combustion" (Tikhomirov *et al*., 2003b) in a special heterotrophic block involving Californian worms, mushrooms and straw. The block processes plant wastes to produce soil-like substrate, on which plants are grown (Tikhomirov *et al*., 2003a). The O_2/CO_2 balance was good, and an almost complete closure was achieved for these gases cycles, water and nitrogen (Tikhomirov *et al.,* 2003b).

However, such an approach can result in reduction of plant productivity. When radish was grown on the soil-like substrate with added non-edible biomass of carrot and beet, the productivity of the radish plants was reduced. This could be caused by both addition of considerable amounts of organic matter and the presence of compounds inhibiting plant growth in the added inedible biomass (Tikhomirov *et al*., 2008).

The flow of all elements is an important issue of sustainable life support systems.

Wheeler (2003) analysed how the carbon balance in a bioregenerative life support system depends on the degree of system closure, different waste management and the crop harvest index. The analyses are based on total biomass productivity of 32 g dry mass per m^2 per day, which was taken from findings from the NASA's studies (Wheeler *et al*., 1996).

Waters *et al.* (2004) calculated the carbon gain of beet (*Beta vulgaris*) for a life support system.

Tako *et al*. (2008) analysed carbon, oxygen, and water circulation in a closed environment, the Closed Ecology Experiment Facilities (CEEF) with two humans, two goats and 23 crops during one week (3 times) in 2005.

Arai *et al.* (2003) measured the nitrogen content in rice plants and in the nutrient solution of a plant cultivation bed to trace the material balance in CEEF.

Cycling of mineral elements (P, Mg, S, K, Na, Ca, N) in the ‘‘human–algae–higher plants’’ system is discussed by Tikhomirov *et al.* (2007).

Sodium (Na^+), fluoride (F^-), and iodide (I^-) were examined using crops in hydroponic systems. Lettuce, radish, spinach, and beet were used to study Na^+ uptake and Na^+ tolerance (Mackowiak *et al.,* 2009). Of these, sodium (Na) may be the major challenge of element cycling in a BLSS.

Sodium is essential for human metabolism at the macronutrient level. It has been shown to function only as a micronutrient for some plants, with very limited uptake in most plants. In general, Na is non-essential to higher plants, but it is beneficial to several plant species, including spinach, chard, and beet. These crops do not only tolerate Na but they can substitute Na for potassium (K), which results in a higher Na accumulation in the edible biomass (Subbarao *et al*., 1999). It is possible to achieve Na concentrations up to 100 g/kg dry weight in the edible part of Na-tolerant crops (Subbarao *et al.,* 2000).

One possible solution to the sodium problem might be use of halophytes, in particular the *Salicornia europaea* plants, capable of accumulating NaCl up to 50% of dry matter according to the reference data. At the same time, the aboveground part of this plant is fully edible. The *S. europaea* productivity, biochemical and mineral composition were investigated when grown under close to optimal BLSS vegetative component conditions (Tikhomirova *et al*., 2008).

In another study human wastes turned into a mineralized form after physicochemical processing and entered the plant nutrient solution, which was periodically desalinated by electrodialysis. The salt concentrate obtained could be used in the human nutrition through NaCl extraction (Zolotukhin *et al*., 2005).

All studies of the mass balance aspect were performed on terrestrial BLSS prototypes. As it is not yet known if plant performance will be adequate in space or on Moon or Mars and crop productivity can only be approximated, and the results in space may be very different from those on Earth.

6. Conclusions

It seems that higher plants provided with adequate ventilation and automated water/nutrient delivery systems would fairly easily adapt to space conditions. Controlled environment chambers must also provide programmable level of light, air temperature, relative humidity, and carbon dioxide to sustain plant growth. As there exists gravity on planetary surfaces (e.g. on the Moon or on Mars), nutrient delivery and water recycling may be less difficult there than in systems operated in microgravity.

One can not yet conclude whether plants can be successfully grown under microgravity over multiple generations because of partly contradictory results. Space factors affect the genome through DNA damage, and chromosome mutations, as well as alter gene expression levels. Though plants were shown to grow in space even during several consecutive generations, it is not yet known if the plant genome will manage to remain stable. The radiation aspect is also important on planetary surfaces since geomagnetic shielding is lacking there. On the other hand, the reduced magnetic field might improve the plant growth on Moon and Mars.

There are indications that under space conditions carbohydrate, protein and lipid content may be changed. However, the statistical basis for this observation is insufficient.

Under conditions different from Earth one can expect changes in secondary metabolites production and possibly in edible/non-edible plant mass ratio. These changes may be of consequence for the plants’ role as a food supply and for the mass balance in a closed life support system. It should be also remembered that some processes that run well in open systems might work differently in closed systems.

7. Future work

The higher plant chamber is designed for food production, air revitalisation and water purification in closed life support systems. But – before plants can really support such systems, it will be important to understand how well plants will perform under conditions of microgravity, reduced gravity, at higher radiation levels and in a lower magnetic field. As a baseline it is foreseen to provide optimal control of temperature, light, atmospheric pressure (1000 hPa), gas composition as well as optimal root support, water availability, and nutrient supply. In case the pressure in the plant growth compartment is reduced for resource budget reasons (e.g. 700 hPa), studies in addition to the investigations described below have to be made. Provision of optimal light will be dependent of mission constraints such as available power or sunlight.

Given the abovementioned conditions the following future investigations are recommended:

On the Earth:

- Studies of the effect of radiation on water transport and nutrient transport in the plant
- Studies of the effect of protons and high energy particles on photosynthesis and water and nutrient transport
- Studies of the effect of geomagnetic field shielding on nutrient uptake and plant metabolism
- Studies of the combined effects of simulated weightlessness, radiation and magnetic shielding
- Studies of the potential effect of changes in the cell wall on the edible/non-edible ratio
- Studies of the potential effects of changes in secondary metabolites production on the food quality

In space:

- Studies of the effect of graded (fractional) gravity conditions on plant growth
- Studies of the effect of space radiation on and beyond low Earth orbit on plant growth
- Studies of the effect of space conditions on the cell wall development
- Studies of the potential adaptation responses using long duration plant experiments in space (several consecutive generations), including graded gravity conditions.

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