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MELiSSA

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1. Introduction

In the context of a bioregenerative life support system on the Moon and Mars plants will play an important role. Through photosynthesis they will contribute to atmospheric purification and water regeneration for the crew, in addition to the food production. Therefore it is of basic importance to understand how gas exchange and metabolism will be affected by different levels of gravity, magnetic field and radiation and how these will affect the photosynthetic efficiency and food productivity of the plant.

The stomata play an important role in regulating the gas exchange of terrestrial plants. As we know the exchange of gases (and also the loss of water vapour) occurs through stomata. The major function of stomata is to balance the uptake of CO_2, which is essential for photosynthesis, with loss of water from the plant. Guard cells can sense variations in environmental conditions, which are followed by conformation changes in order to regulate the degree of stomata opening. The control of stomata opening and closure has been largely studied and many of the principal characteristics of this control are already well known. However, there are still some gaps that need to be better understood. The major features of the stomata control is presented below (see Figure 1.1 for summary). This outline is based mainly on the review of Roelfsema and Hedrich (2005).

The central mediators of the stomata opening are the anion and K^+ channels, localised at the plasma membrane of the guard cells. There are two types of anion channels; the rapid (R)-type (Keller *et al.,* 1989) and the slow (S)-type (Linder and Raschke, 1992). The genes encoding these plasma-membrane anion channels are still unknown and little is known about anion uptake transporters. The anion channel activation will normally depolarize the guard cells plasma membrane. This will lead to the extrusion of Cl^- anions (sometimes NO_3^- and malate) and the efflux of K^+ via outward-rectifying channels. The K^+ outward-rectifying channels are encoded by a single gene, called GORK (Hosy *et al.,* 2003). As the ions flow out, the water potential inside the guard cells will increase. In order to balance with of the apoplast, water will flow out of the guard cells leading to the stomata closure. When the anion channels are deactivated by environmental signals, Cl^- will be taken up from the apoplast by symport transport with H^+. K^+ will be taken up via the inward-rectifying K^+ channels. The K^+ channels probably assemble four subunits, where each subunit is encoded by one or more genes to form a homomeric or a hetromeric complex (Dreyer *et al.,* 1997). The genes involved in the inward-rectifying K^+ channels are KAT1, KAT2, AKT1, AKT2/3. The inward transport of ions inside the guard cells will cause the water potential to decrease. The water will then flow into the guard cells. The inflow of water will cause the guard cells to swell. The increase in volume of both guard cells causes the opening of the stomatal pores.

Generally, dry atmospheric conditions lead to stomata closure while exposure to light leads to stomata opening. In nature the plants will sometimes receive both signals at the same time. Therefore the plants have to find a way to balance between the uptake of CO_2 and the loss of water. These fine-tuning of the stomata opening may vary depending on plant species, growth conditions and developmental stage. However, the principal mechanisms involved in this process are probably the same for all plant species.

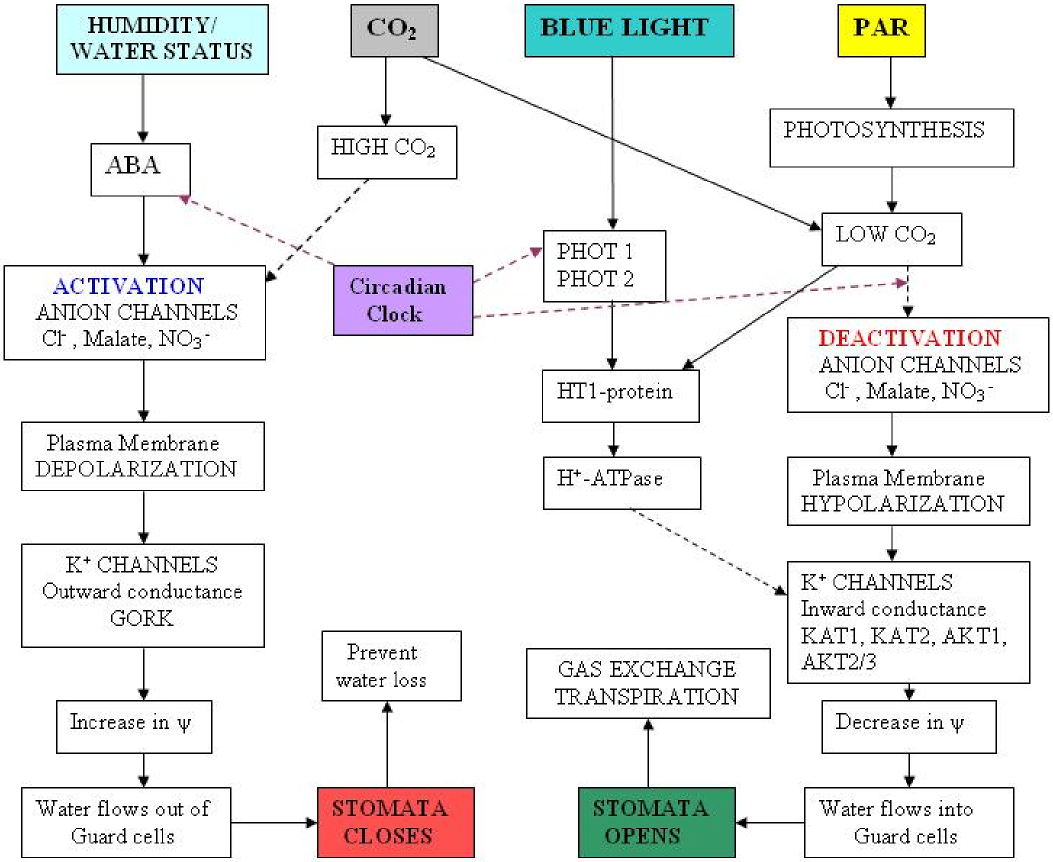

Figure 1.1 – Summary of the pathways leading to stomata closure or opening. Dry conditions and high CO_2 concentration lead to stomata closure. Blue light, low levels of CO_2 and PAR trigger stomata opening. The circadian clock is believed to balance the environmental signalling received by the plant to help the fine-tuning of stomata opening/closure. Dashed lines are accounting for the pathways that are not fully understood while the pathways covered by the black lines are better understood.

When the plant root tips sense the dry soil abscisic acid (ABA) is released from the roots into the xylem leading to activation of anion channels followed by stomata closure. The receptors on the guard cells involved in the ABA signalling are unknown. The first signal sent by ABA will create a large current through the cell membrane leading to rapid closure of the stomata. After 10 minutes the current will drop, creating only a 'preventive opening'. The stomata are maintained closed but are now more susceptible to other signals.

The photosynthetic-active radiation (PAR) will lead to a drop of the CO_2 content in the leaf tissue due to photosynthesis. Low CO_2 will lead to the deactivation of the anions channels. However, the process underlying CO_2 signalling remains unclear compared with ABA and the light pathway. Hashimoto *et al.* (2006) reported that a protein named high leaf temperature 1 (HT1) regulates stomata opening in response to low CO_2 levels. HT1 triggers the activation of H^+-ATPase that induces stomata opening. The blue light is also known to strongly stimulate stomata opening. The stomata response to blue light is due to the activity of PHOT1 and PHOT2 blue-light receptors located on the plasma membrane (Kinoshita et al., 2001). HT1 also transduces the signal from the blue-light receptors to regulate H^+-ATPase (Hashimoto *et al.,* 2006), which then actives triggers stomata opening. On the other hand high levels of CO_2 lead to the activation of the anion channels that result in stomata closure. In addition there is also the plant circadian clock that regulates the sensitivity of the stomata to extracellular signals. The response of the stomata to ABA-signalling, blue-light and low CO_2 will also depend on the phase of the circadian clock at which the signal is applied (Webb *et al.,* 2003). In addition, there are some peculiar responses of stomata when CO_2 becomes 'super-elevated' (e.g. 75000 ppm or 0.5 kPa), which can occur in space cabin environments with humans. In this environment, stomata of some plants tend to open rather than close down, and close very little at night (Wheeler *et al.,* 1999). The mechanisms behind this are still unknown.

Gas exchange is linked to metabolism via photosynthesis that is fundamental for plant survival. The photosynthetic apparatus is highly dynamic and able to respond to several environmental stimuli, including changes in the quality and quantity of incident light and the availability of CO_2 (Szabó *et al.*, 2005). During photosynthesis the photons are absorbed by antenna pigments as chlorophylls and carotenoids. The light-harvesting complex II (LHCII) is the main antenna on the photosynthetic apparatus II (PSII), here the excitation energy is transferred to the reaction centres where is converted into charge separation that will drive electron flow between PSII and PSI. This energy is transferred to NADPH and ATP. This reaction requires water oxidation and releases O_2. The final products will be carbohydrates which are usually translocated in form of sucrose from the photosynthetic to non-photosynthetic organs.

It is well-known that changes in the environment will cause modifications in the photosynthetic apparatus due to the adaption of the plant to these new surroundings. Environmental modulation impacts also both primary and secondary metabolism (Keegstra and Thomashow, 2002). It is very likely that the new physical factors that the plants will find on the Moon and Mars will affect the plants photosynthetic apparatus and metabolism. Therefore effort must be put into examining how plants perceive and respond to the multitude of signals that they will receive from their environment in space. This study will focus on three environmental factors – gravity levels, magnetic fields and radiation - while other factors like water and gas supply are considered to be resolved by technical solutions of the greenhouse providers.

In this technical note it is tried to summarize what one already knows about the effects of microgravity, magnetic fields and radiation on plant gas exchange, photosynthesis and metabolism and to outline where more research is needed to get adequate knowledge in this area to ensure that higher plants can be a reliable food source and part of advanced life support systems in space exploration.

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).

2. Microgravity effects

2.1 Space flight experiments

Several experiments in microgravity in the entire history of space plant biology showed apparently decreased levels of carbon fixation in higher plants (Porterfield, 2002). It has been documented in space experiments that changes occur in the carbohydrate content and especially in the occurrence of storage of starch grains. These results have been obtained examining leaves of pea, *Arabidopsis*, pepper and other species that have been grown in microgravity - compared with control plants grown at 1-g on Earth (Johnson and Tibbits, 1968; Abilov *et al.*, 1986; Brown *et al.*, 1993; Laurinavichius *et al.*, 1986, Aliyev *et al.,* 1985, Merkys *et al.*, 1987).

It was believed for many years that microgravity had a negative effect on plant metabolism, but more recent studies demonstrated that the hardware obviously had a great impact on the results obtained under microgravity conditions due to the indirect effects created by the microgravity environment (e.g., Monje *et al.,* 2005).

In a review Porterfield (2002) elucidated the biophysical limitations in gas exchange created by the microgravity environment. In this review he focuses on how the lack of buoyancy-driven thermal convection leads to limited diffusion between the plant and the environment. These changes in the physical behaviour of fluids due to microgravity are termed *indirect* effects of microgravity because they are not due to gravity interacting with the mass of the plant body itself.

By using a mathematical model Porterfield showed the impact of limiting gravity-dependent transport phenomena. The model clearly demonstrated that the microgravity environment induced significant limitation in the flux/efflux of fluids in the plant leaf.

Musgrave and co-workers (1998) showed in the Chromex studies that in the absence of proper ventilation plants in microgravity had a reduction of 40% in the carbohydrate content (especially glucose and starch) compared to ground controls. The differences disappeared when a continuous air flow was provided to the plants. The results were obtained with studies of *Arabidopsis thaliana* leaves and bracts from flowering stems that had been in space for periods ranging from 6 to 10 days (see Table 2.1 for details). The hardware used was the Plant Growth Unit that was provided with continuous light (67 µmol^{-2}s^{-1}) and temperature control.

Table 2.1 – Results obtained during the *Chromex-03, -04, -05* experiments of Musgrave *et al.* (1998)

Chamber configuration	Effects (compared to ground control)
Sealed chamber	• Lower carbohydrate content (total 40%) especially glucose • Higher chlorophyll concentration
Sealed chamber + CO_2	• Starch concentration was higher than in plants grown in a sealed chamber but still lower than in the ground control
Continuous air flow	• Carbohydrate and chlorophyll concentration were equivalent to the occurrence in the ground control

Starch grains from soybean (*Glycine max L*., BRIC-03) grown under microgravity conditions were on average 20-50% smaller in diameter than in the ground controls. A reduction in the amylase content was also demonstrated. The plants were grown in a closed system. On the other hand, soybean from the GENEX experiment that was provided with proper ventilation showed no difference compared to the ground controls in size, distribution, density and amylase content in the starch grains (Kuznetsov *et al.,* 2001). These experiments showed similar results as the *Chromex* experiments. However the plants were grown in canisters with no environmental control.

Potato explants (*Solanum tuberosum*, Astroculture-05) were grown in microgravity under a controlled environment and ventilation. The shape, surface texture, and size range of starch grains from both ground and microgravity grown plants were similar, but a greater percentage of smaller starch grains formed in spaceflight than on Earth (Croxdale *et al.,* 1997).

Kitaya *et al.* (2001, 2003, 2004 and 2006) tried to clarify the effects of weightlessness and reduced gravity on the heat/gas exchange between plant leaves and the ambient air by performing a series of **parabolic flights**. They performed a study to obtain adequate air circulation rates needed for promoting plant growth in a closed plant production module in space.

During the 20-25 sec of microgravity in the parabolic flight the leaf temperature of sweet potatoes and barley rapidly increased 1.9 ºC and 1.3 ºC, respectively. By raising the air velocity from 0.2 m/s to 1.0 m/s the leaf temperature of both plants were lowered. The higher air velocity around the leaves reduced differences in the leaf surface temperature at all gravity levels. It was predicted that the leaf temperature could increase up to 4 ºC under prolonged microgravity conditions. The net photosynthetic rate of barley leaves decreased 13-20% under such microgravity conditions and low air velocity (0.2 m/s). The equipment used by Kitaya and co-workers to measure leaf temperature and net photosynthetic rate is shown in Figure 2.2 and 2.3.

After measuring the single leaf net photosynthetic rate, Kitaya *et al.* (2004) decided to study the air control in a closed chamber with a large number of plants. The results showed that the net photosynthetic rates of tomato plant seedlings canopy increased with increasing air velocities (0.1 – 0.8 m/s) and CO_2 concentrations (0.4 and 0.8 mmol mol^{-1}) under microgravity conditions. Suppression of canopy photosynthesis was induced by low air velocities at 800 and at 400 µmol/mol CO_2 levels. The set up used by Kitaya and co-workers for measuring the plant canopy photosynthetic rate is illustrated in Figure 2.3 B. Experiments done on ground have also shown that air movement is important for increasing plant canopy photosynthesis. Experiments with tomato seedlings (Shibuya and Kozai, 1998) and rice seedlings (Kitaya *et al.*, 2000) demonstrated that the net photosynthetic rate of plant canopies was greater under air velocity higher than 0.1 m/s.

The results found by Kitaya *et al.* (2001, 2003, 2004 and 2006) are in accordance with the findings of Porterfield (2002) and the results of the Chromex experiments. But parabolic flight gives only a very short period of microgravity stimulation to the plants (20-25 sec) followed by a 20 sec period of a 2-g stimulation. The relevance of such short term microgravity effects compared to a long term stimulus can be discussed. It is also hard to know how much the different gravity levels can interfere with each other due to the short time frame between them. However, taken the temperature increase of almost 2 ºC of the barley leaves in only 20 seconds and the considerable decrease in temperature when the fan was used into consideration, it can at least be concluded that such experiments can be useful in the interpretation of the effects of microgravity conditions on the growth of healthy plants. It shows that the air movement around the plants must be controlled properly to enhance the heat and gas exchange. The air velocity should be above 0.2 m/s to obtain maximal gas exchange rates (Kitaya *et al.,* 2003).

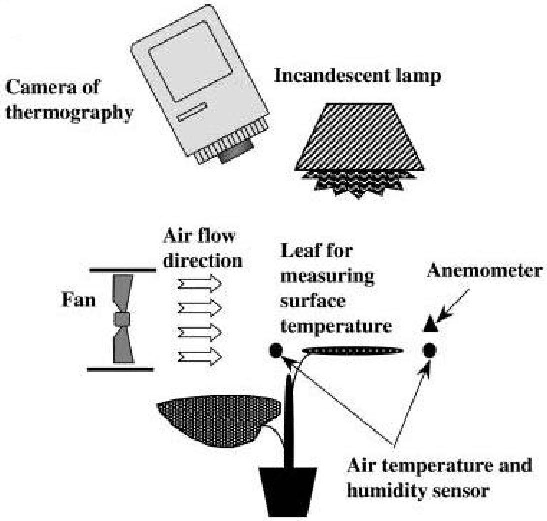

Figure 2.2- Set-up used by Kitaya and co-workers for measurements of leaf surface temperature. Illustration from Kitaya *et al*. (2001)

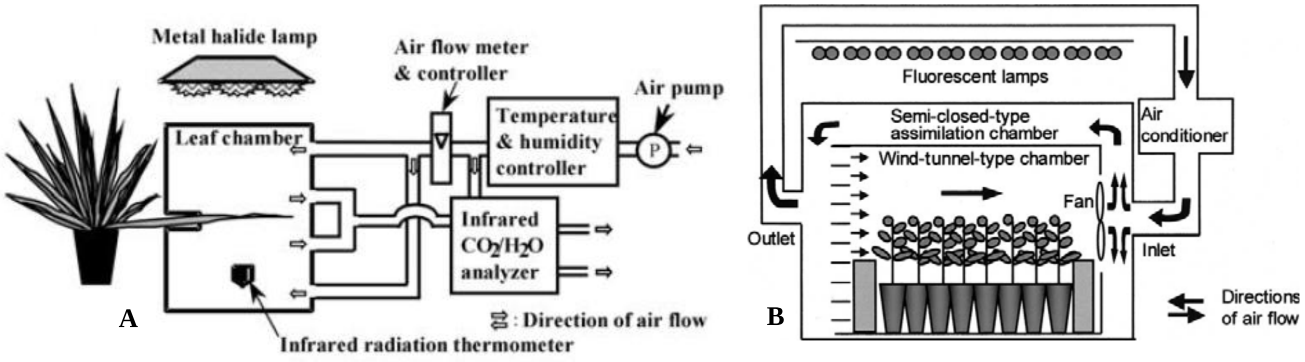

Figure 2.3 - Set-up used by Kitaya *et al.* for measurements of net photosynthetic rates of a single leaf (A) and plant canopy (B). Illustration from Kitaya *et al.*(2004 and 2006)

The Photosynthesis Experiment System Testing and Operation (PESTO) was the first* experiment test system which made it possible to directly measure photosynthesis in microgravity with relevant environmental conditions (moderate light levels, elevated CO_2, temperature and relative humidity control) (see set-up used during the PESTO experiment in Figure 1.3). Dwarf wheat plants (*Triticum aestivum*) were grown for 21 days at moderate light levels (~300 µmol $m^{-2}s^{-1}$) and elevated CO_2 (1500 µmol mol^{-1}) in the Biomass Production System onboard the ISS. The results showed that microgravity did not affect gas exchange rates (evapotranspiration and P_{net}) (Monje *et al.*, 2005). No differences were observed in the starch, soluble sugar, or lignin content of the leaves grown in microgravity or under the 1-g condition on ground (Stutte *et al.*, 2006). The leaf chlorophyll content was also similar to the content found in the 1-g control plants. The only significant effect of the microgravity conditions compared to ground control found was a reduction of 16%, 13% and 13% simultaneously in the PSI, PSII and whole chain electron activities (Stutte *et al.*, 2005). A summary of all results from the PESTO experiment is shown in Table 2.2.

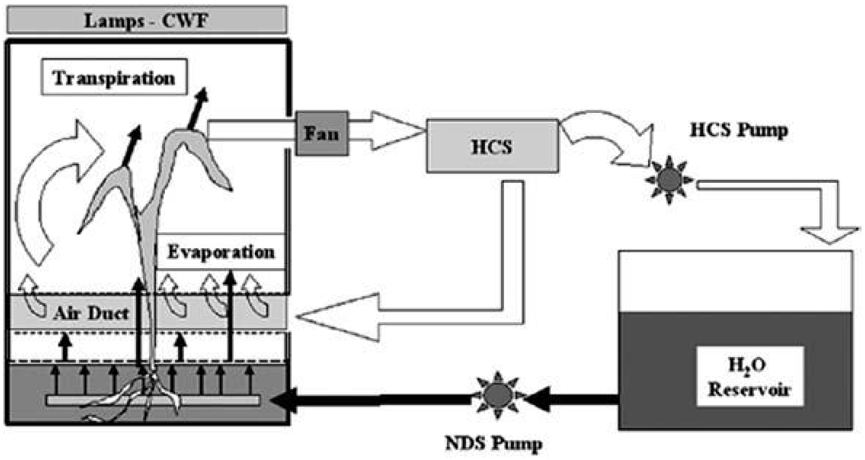

Figure 2.4 -The set-up used during the PESTO experiments for direct measurement of photosynthesis. The air and water fluxes of the humidity control (HCS) and nutrient delivery system (NDS) of the Biomass Production System. Evapotranspiration was calculated by measuring the amount of water condensed by the HCS system (PGC,) and P_{net} was daily measured by the amount of CO_2 added to maintain a constant chamber [CO_2] set-point. Illustration from Monje *et al.* (2005).

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* Probably the first demonstration of CO_2 uptake and O_2 production by plants was done by Herb Ward in 1996 on a satellite experiment with Spirodella, but he control was nowhere as good as in PESTO (Ward *et al.*, 1970).

Table 2.2 - Summary of results from Stutte, Monje and co-workers (2005): the PESTO experiment

	Microgravity grown plants* (ISS)	1-g grown plants* (ground control)	Difference (P>0.05)
Starch concentration	11.2 µg	10,9 µg	Non-significant
P_{net}	7.4 µmol $m^{-2}s^{-1}$	7.2 µmol $m^{-2}s^{-1}$	Non-significant
Dry mass	0.113 g	0.122 g	Non-significant
CO_2 compensation	121 µmol mol^{-1}	118 µmol mol^{-1}	Non-significant
Chlorophyll content	126 µg mm^{-1}	135 µg mm^{-1}	Non-significant
Photosynthetic Photon Flux	85 µmol $m^{-2}s^{-1}$	81 µmol $m^{-2}s^{-1}$	Non-significant
Evapotranspiration	~ 4.5 µmol $m^{-2}s^{-1}$	~ 4.5 µmol $m^{-2}s^{-1}$	Non-significant
PSI activity	329 µmol (15.6 % lower than control)	390 µmol	Significant
PSII activity	196 µmol (13 % lower than control)	226 µmol	Significant
Whole chain electron activity	112 µmol (13 % lower than control)	130 µmol	Significant

*The values shown in the table were obtained from 21-days-old plant tissue.

There have been only a few studies before PESTO focusing on the effects of space flight on the photosynthetic apparatus. Tripathy *et al.,* (1996) found a reduction of 28% in the PSI and PSII of isolated chloroplasts of *Triticum aestivum*. A reduction of 28% in dry mass was also found and indicating that the overall plant health had declined during the experiment. Since the authors used a hardware lacking gas flow control these photosynthetic measurements could be considered to be a symptom of artifacts. This might be explained by an increased boundary layer due to the lack of buoyancy driven convection and the declined overall plant health status, but not as consequence of decreased plant growth and carbon fixation in microgravity (Porterfield, 2002). Jiao and co-workers (1998) and Kochubey and co-workers (2004) observed similar results and an approximate 30% decrease of the PSI photochemical activity of *Brassica rapa* grown in microgravity for 14 days. Jiao (1998) also reported on a 16-30% reduction of the amount of PSII proteins in the plants grown in microgravity.

It is important to note that in the Tripathy and co-workers (1996) and Jiao's (1998) experiments the plants were grown under low light levels (50-60 µmol $m^{-2}s^{-1}$), but the PSI and PSII activity were measured at saturating light (2000 µmol $m^{-2}s^{-1}$) condition during the post flight analysis on Earth. The plant growth chamber used in all three experiments (Tripathy *et al.,* 1996; Jiao 1998 and Kochubey *et al.,* 2004) provided only limited environmental control; the plants were exposed to high humidity and fluctuating temperature levels. The hardware used by them also lacked forced ventilation making it difficult to discriminate the effects caused by microgravity and environmental stress (Stutte *et al*., 2005).

The results found in the PESTO experiment confirm the hypothesis that gas exchange and metabolism are not directly affected by microgravity but it is indirectly affected under microgravity conditions due to the lack of buoyancy driven convection forces that increase the leaf boundary layer causing a much slower diffusion limiting the transport of gases into the leaf. The interpretation of the PESTO experiments also emphasizes the importance to have a hardware with a good environmental control to avoid the secondary effects of microgravity on plant growth and development (Stutte *et al.*, 2005, Musgrave *et al.,* 1998, Monje *et al.*, 2003).

Figure 2.5 summarizes the indirect effect of microgravity on plant gas exchange and metabolism. It is obvious that a stringent environmental control is required for plant growth under reduced gravity conditions, especially proper ventilation of the leaves to prevent reduced gas exchange due to boundary layer effects. However, this is a more technical issue on the design of a greenhouse on Moon and Mars or on the ISS.

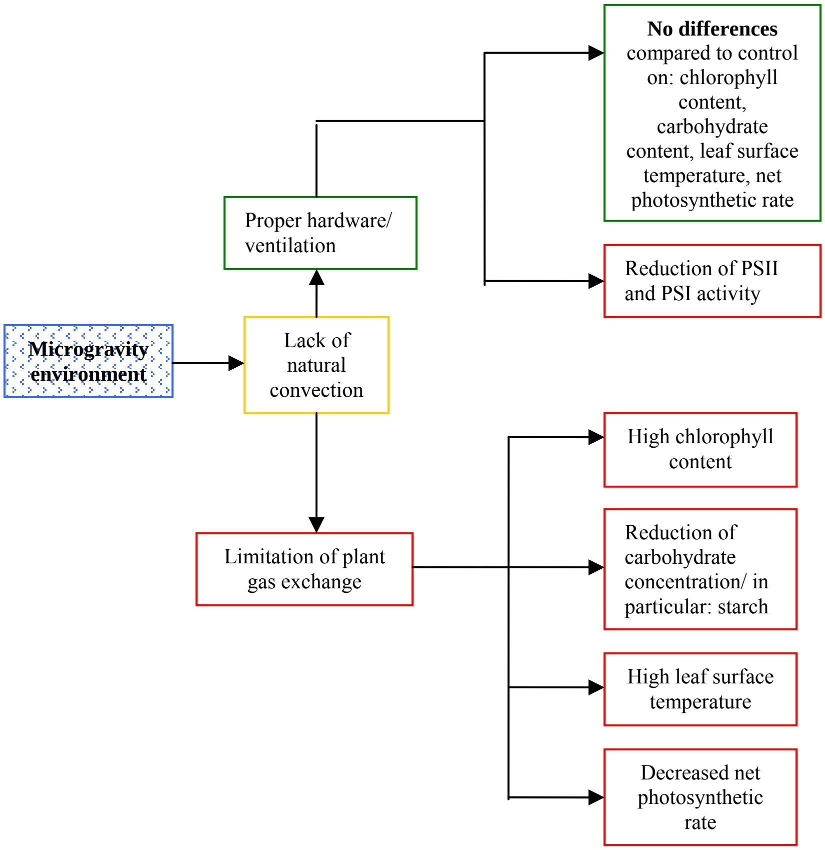

Figure 2.5. - Summary of the indirect effects of the microgravity environment on plant gas exchange and metabolism. When proper ventilation is provided to the plants, the effects disappear.

The only remaining open questions regarding the effects of microgravity on plant gas exchange and metabolism are linked to the reduction in activity of the PSII and PSI found in the PESTO experiments. Even though dry mass, chlorophyll and carbohydrate content were not affected under microgravity, PSII and PSI showed a reduction of 16% and 13% respectively, compared to the ground control. Since Jiao *et al.* (1998) and Tripathy *et al.* (1996) reported higher reduction (30%) of the PSII and PSI photosystem activity and they used even lower light intensities (4x) than the PESTO did, it could be possible that the plants had adapted to this low light. When the plant tissue was tested back on Earth much higher PPF (Photosynthetic Photon Flux) intensity (~10x) was used in this test than during growth in space ; this could be the reason of the decrease in activity found in the photosystems (Stutte *et al.,* 2005). However, more tests need to be done to clarify this unsolved problem.

2.2 Simulated microgravity

Clinostats have been used for many years in the attempt of simulating microgravity. However, the use of a clinostat as simulation tool for microgravity is still questionable. Subtle differences between true microgravity and horizontal clinostat were known as early as the 1960's (Brown, Chapman and Liv, 1974).

Kordyum and Adamchuk (1997) conducted investigations on the ultrastructure and the state of the photosynthetic apparatus in *Arabidopsis thaliana* leaf mesophyll cells at the different stages of plant development under clinorotation. It was concluded that clinorotation affects the state of photosynthetic membranes.

Brown *et al.* (1996) reported that *Pisum sativum* grown on a horizontal clinostat had greater CO_2 assimilation rates than vertically rotated or control plants. Ward and King (1978) reported increased photosynthetic and respiratory gas exchange rates in marigold plants rotated on a horizontal clinostat.

Zhao Qi *et al.* (2002) found that strawberry seedlings rotated on a clinostat for 120 h contained 47.5 % less chlorophyll. There was a reduction of 4% in the shift toward PSI for the excitation energy of the chloroplasts. However, they concluded that this difference did not affect the normal photosynthetic functions of the chloroplasts of strawberry seedlings.

Sugano *et al.* (2002) showed that the photosynthetic rate of Japanese flowering cherry did not significantly differ between the control and the 3D-clinorotated plants. The chlorophyll content, chlorophyll a/b ratio and the density of the stomata were also similar to the control. Yamada *et al.* (1993) found also similar results using the cotyledons of garden cress grown on the 3-D clinostat.

The results found here for the 3-D clinostats are in agreement with some of the real microgravity results obtained as described in Section 2.1. The 3-D clinostat is believed to be the tool that is most efficient in simulating microgravity; however control and key experiments in space are needed to prove the data from clinostats (Hoson, 1997).

3. Radiation Effects

3.1 UV- radiation

UV-B radiation

The UV-B effects on plant gas exchange and metabolism are well known. Studies using more than 300 plant species have shown that 50% of the species are considered sensitive against UV-B. Another 20-30% are considered moderately sensitive and the rest are insensitive to UV-B (Teramura *et al.*, 1990, Teramura and Sullivan, 1994). The papers that are referred on this section have no link to space research but are dealing with the increased UV-B levels on the Earth due to depletion of the ozone layer.

The damages caused by enhanced UV-B to the photosynthetic machinery have been mapped in several studies. The dominant target of UV-B is the photosystem II (PSII). Even though PSI has the same structure as PSII, the damages caused are not the same (Bornman and Teramura, 1993). Different experiments have shown that PSII can be totally inactivated after UV-B irradiation while PSI only decreased its activity by 35% (Teramura and Ziska, 1996). The process of inactivation of PSII is believed to start by degradation of D1 and D2 proteins. The D1 protein has been shown to be particularly sensitive to UV-B radiation inducing rapid D1 degradation (Greenberg *et al.,* 1989, Wilson and Greenberg, 1993). Several pathways of inactivation of PSII have been suggested; some believe that degradation occurs via the formation of plastosemiquinone anion (Q_B^-) (Jansen *et al.,* 1993); others believe that UV-B causes direct damages in the plastoquinone molecule itself (Bornman and Teramura, 1993). Renger and co-workers (1989) proposed that the UV-B radiation primarily modifies the binding site of the primary and secondary quinone receptors (Q_A and Q_B) following a blocking of pheophytin.

The UV-B radiation is also believed to directly affect the Calvin cycles, i.e. the site of carbon reduction (see figure 3.1). Several studies have shown that UV-B radiation decreases the activity and the content of Rubisco (Ribulose 1,5-biphosphate carboxylase/oxygenase) the main CO_2-fixing enzyme in C3 plants (Teramura and Ziska, 1996).

In addition the UV-B irradiation exposure affects photosynthesis indirectly by degradation of the photosynthetic pigments chlorophyll and carotenoids. These effects have been observed in different species such as beans, barley, corn, pea and soybean (Teramura and Ziska, 1996). The direct and indirect effects of enhanced UV-B on plant gas exchange and metabolism are shown in Table 3.1.

Table 3.1 – Summary of the effects of UV-B radiation on plant gas exchange and metabolism (after Hollósy , 2002 and Jansen *et al.,* 1998)

Effects of enhanced UV-B on plant gas exchange, metabolism and photosynthesis	
Direct effects	*Indirect effects*
- Inactivation of PSII and to a lesser extent PSI - Decrease in Rubisco activity and other enzymes - Decreased CO_2 fixation and O_2 evolution - Degradation of D1 and D2 proteins of PSII - Reduction in dry weight, starch and chlorophyll content	- Induction of stomatal closure (reduced gas exchange) - Changes in leaf thickness and anatomy may alter the light environment within the leaf - Changes in canopy morphology may also alter and indirectly affect the whole plant photosynthesis

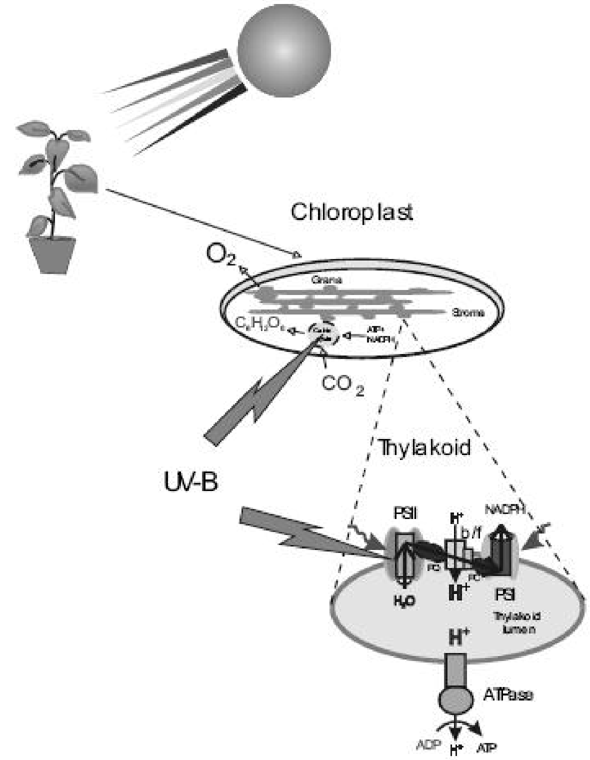

Figure 3.1 – Targets of UV-B on chloroplasts. The main targets of UV-B radiation in plant cells are the nucleic acids, the Calvin cycle enzymes and the light-energy converting photosystem II complex. Illustration from Sicora *et al.* (2006).

UV-C radiation

It is well-known that UV-C is very harmful to all organisms including plants, but little is known about the detailed effects of UV-C on plants and especially on the photosynthetic apparatus. Most of the UV-C rays do not reach the Earth's surface and are not relevant under natural conditions. Most of the studies have therefore been concentrated to examine the UV-B effects on plants.

The photosynthetic apparatus seems to be damaged by the UV-C rays (Stapleton, 1992). However, the site of the UV photoinhibition has not been conclusively determined (Mantai and Bishop, 1967, Mantai *et al.,* 1970). Large numbers of starch grains were found in sugar beet leaves after exposure to UV-C radiation, the leaf tissue showed also extensive damage after exposure for only 1.5 hours (Bornman *et al.,* 1983). Among the various cellular components, the chloroplasts appear to be most sensitive to UV-C radiation (Mantai *et al.,* 1970).

Murali and Saxe (1983) observed a considerable decrease of the photosynthetic and transpiration rate shortly after exposure to UV-C radiation (80 $\mu Wcm^{-2} nm^{-1}$) in *Spathiphyllum wallisii*. The dark respiration was significantly increased. The effects last for 2-4 days, before a complete recovery. It was suggested that the immediate effects of UV-C are caused by stomatal closure.

Jayakumar *et al.,* (1999) analyzed the chlorophyll content and photosynthetic activity of photosystem II (PSII) of *Azolla microphylla* Kaulf. after exposure to UV-C. The plants were exposed to 13 Wm^{-2}, 30 min per day for 13 days. The chlorophyll content was 48% of the control after only one day of exposure. The production of chlorophyll increased in the plants after 4 days of treatment. The concentration of Chlorophyll was close to but still lower than in the control plants after 13 days. The photosynthetic activity of PSII was measured to be slightly lower than the control samples after one day of exposure. However, despite the fact that the pigments apparently seemed to recover within a few days, the photosynthetic activity continued to decrease being only 65% of control after 13 days. Inhibition of PSI by UV-C radiation has also been reported, but its impairment was much less pronounced than that of PSII (Hollósy, 2002).

It has also been reported by Sargheim and co-workers (2008) that the total chlorophyll content of plant leaves exposed to UV-C had decreased. However, the difference to the control samples was much less than that reported by Jayakumar *et al.* (1999). After UV-C irradiation (4.77 Wm^{-2}, 8 days) the carotenoid content was slightly reduced compared to the control while the fresh weight (in shoots) and the leaf numbers were significantly reduced.

Biswal *et al.* (1997) demonstrated that UV-C radiation increases the senescence rate in maize leaves. The pigments and proteins of detached maize leaves under senescence were evaluated after exposure to 15 Wm^{-2}, 15 min per day for 3 days (rate 50 $\mu molm^{-2}s^{-1}$). The leaves showed a dramatic decrease in the chlorophyll and carotenoid content compared to the control. The decay of protein was also accelerated by the UV treatment followed by a decrease in PSII activity.

Table 3.2 - Summary of the effects of UV-C on photosynthesis

Exposure dose	Target	Effects	Reference
$9.46 \times 10^{-2} Wm^{-2}$ (1.5 h)	Sugar beet seedlings	Large number of starch grains	Borman *et al.*, 1983
$9.46 \times 10^{-2} Wm^{-2}$ (1.5 h)	Sugar beet seedlings	Leave damage	Borman *et al.*, 1983
$80 \mu Wcm^{-2}$ nm^{-1} (short exposure)	*Spathuphyllum wallisii* (whole plant)	Reduced photosynthetic and transpiration rate	Murali and Saxe 1983
13 Wm^{-2} (30 min)	*A. microphylla* Kaulf.	48% decrease in the chlorophyll content	Jayakumar *et al.*, 1999
13 Wm^{-2} (30 min per day for 13d)	*A. microphylla* Kaulf.	22% decrease in the chlorophyll content	Jayakumar *et al.*, 1999
13 Wm^{-2} (30 min per day for 13d)	*A. microphylla* Kaulf.	Reduction of 35% in the photosynthetic activity of PSII	Jayakumar *et al.*, 1999
15 Wm^{-2} (15min per day- for 3d)	*Zea mays* detached leaves	Significant decrease in chlorophyll, carotenoid and total protein content	Biswal *et al.*, 1997
15 Wm^{-2} (15min per day for 3 d)	*Zea mays* detached leaves	Severe decline in PSII activity	Biswal *et al.*, 1997
4.77 W m^{-2} (8 days)	Red pepper (whole plant)	Slight reduction in chlorophyll and carotenoid content, significant reduced number of fresh weight of leaves	Sarghein *et al.*, 2008

UV-A radiation

Since the UV-A is not absorbed by the ozone layer, approximately 95% of the UV radiation that reaches the surface of the Earth is UV-A. On Mars the UV-A environment has been measured to be 1.3 x higher than that found on the Earth surface. The effects caused by UV-A on the photosynthetic apparatus have been observed to be less dangerous than the effects caused by UV-B and UV-C. However, the available literature regarding the effects of UV-A in plants is still limited.

Even though the damage efficiency of UV-A radiation on the photosynthetic apparatus is much smaller than that of UV-B the effects caused by the two UV radiations seem to be similar. UV-A and UV-B have demonstrated to affect the electron transfer in the PSII complex both at the water-ionizing complex and at the Q_B binding site (Turcasányi and Vass, 2002).

Nayak and co-workers (2003) also reported no changes in the level of the photosynthetic pigments of senescing leaves of wheat exposed to UV-A for 1 hour during 8 days (~ 22.5

Wm^{-2} : fluence rate 75 µmol $m^{-2}s^{-1}$). However, a significant decrease in the maximal photochemical efficiency of PSII (F_v/F_m) was observed. It was suggested that D1 protein may be the most susceptible target of the UV-A radiation, due to a rise in the minimum fluorescence F_0 and the modification of the Q_B binding site after exposure. The results are similar to that found by Turcasányi and Vass (2002).

Ivanova *et al.,* (2008) demonstrated that UV-A leads mainly to damage in the PSII centers. They also found a relationship between the molecular organization of LHCII (light harvesting centre II) and the response to UV-A. The data showed that oligomeric forms of LHCII play a key role for sensitivity to UV-A radiation in the photosynthetic apparatus.

Despite most of the articles have demonstrated that UV-A damages photosystem II, some researches have observed a positive response to UV-A on higher plants. Christopher and Mullet (1994) observed that UV-A activated the expression of several chloroplast-encoded photosynthetic genes (psbD-psbC) in clusterbeans allowing the plants to maintain their capacity to synthesize D2 and CP43 that are usually damaged in light stressed plants. Gartia and co-workers (2003) showed that UV-A has the ability to protect the photosynthetic apparatus from damage caused by UV-B radiation. Plants irradiated simultaneously with UV-A and UV-B showed much less decrease in the chlorophyll and carotenoid contents as well as the maximal efficiency photochemistry of PSII, than plants irradiated only with UV-B. The plants irradiated with UV-A and UV-B had 25% more carotenoid content than after irradiation with UV-B only. Carotenoid may enhance the plant protective capacity to stabilize D1 protein. Therefore carotenoids are believed to have a special role in the ability to protect the PSII from the UV-B damage. Also providing some UV (UV-A and UV-B) seems prevent a physiological disorder called oedema or intrumescence in some plants (Lang and Tibbitts, 1983).

Sarghein *et al.* (2008) reported no significant changes in the total chlorophyll and carotenoid content of red pepper plants exposed to 5.25 Wm^{-2} day^{-1} for 15 days. On the other hand the flavonoid content increased. It is believed that flavonoid acts as an UV filter, protecting the photosynthetic apparatus from damage. The plant capability to accumulate flavonoids has been related to their UV tolerance (Gonzalez *et al*., 1998).

3.2 Gamma radiation

Despite the numerous studies on plant response to microgravity little is known about the detailed effects of ionizing radiation on the photosynthetic apparatus (Esposito *et al.,* 2006). There are indications that the gamma irradiation damages the photosynthetic system (Kovács and Keresztes, 2002; Saakov, 2003).

McCabe *et al.,* (1979) observed a 37% reduction of the photosynthetic CO_2 uptake in soybean after an irradiation dose of 41 Gy. The effects could be seen 15 min after the exposure of the plants that were also exposed to high light doses. Lower doses (1.2 Gy) also caused a significant reduction in the rates of photosynthetic CO_2 uptake. Ursino *et al.,* (1977) showed that the reduction in the photosynthetic rate in soybean was not due to the increased stomatal resistance but was caused by the radiation effects on the photosynthetic apparatus.

In some other studies from the 1960s and 1970s were reported changes in rates of photosynthesis followed by exposure to ionizing radiation in other species like pine (Bordeau *et al.,* 1964; Dawe, 1972, Hadley and Woodwel, 1965), wheat (Zill and Tolbert, 1958) and barley (Sprey, 1972).

Thiede and co-workers (1995) analyzed the carbohydrate content of the leaves, stem and roots of sunflower after exposure to 40Gy. The irradiated leaves contained 22% more starch than control plants. In contrast, the starch content in the stems was 94% lower. The levels of sucrose and hexose in the leaves and in the stem were not different from content in the control plants. Even though McCabe *et al.* (1979) found effects on photosynthesis for exposures as low as 1.2 Gy, Thiede *et al.* (1995) only observed significant effects on plants exposed to 40 Gy.

The effects of gamma radiation on the photosynthesis of young bean plants after a seed exposure of 150-200 Gy were studied by Stoeva and Bineva (2001). Two different types of cultivars were used - Plovdiv 10 and Plovdiv 11. They observed a decrease in the photosynthetic rate, transpiration rate and stomatal conductivity. The data of the pigment (chlorophyll a and b, carotenoids) concentration did not differ from the control. The irradiation dose of 150-200 Gy increased the respiration intensity and the peroxidase activity with 12% and 41 % for Plovdiv 10 and 18% and 38% for Plovdiv 11 (Stoeva *et al.,* 2001). The effects found by Stoeva *et al.* were more apparent at stronger doses; it was also found a difference in reaction between the two cultivars since Plovdiv 10 showed more resistance to gamma stress than Plovdiv 11.

Saakov (2003) performed the first detailed study of the effects induced by gamma radiation on the fine structure of the photosynthetic apparatus. Native leaves of black locust, kidney bean and a barley mutant (no chlorophyll b) were investigated after exposure to 8kGy and 12kGy. The results showed effects on protein denaturation and transition of the protein moiety of the complex to an unfolded state. The gamma-rays exposure gave also rise to a substantial dehydration of the leaves and caused an increase in the rate of transpiration. The effects caused by the gamma-radiation on the photosynthetic apparatus were in many cases similar to the inhibition of photosystem (II and I) caused by herbicide or the disorders caused by severe atmospheric drought.

Kim and co-workers (2004 and 2005) investigated the effects of low-dose irradiation in the photochemical efficiency of PSII and alterations in the photosynthetic pigments of red pepper seedlings and the whole plant. The results showed that the seedlings emerging from seeds irradiated with 2, 4 and 8 Gy (not 16 Gy) had an enhanced development although the maximum photochemical efficiency of PSII did not differ among the different levels of irradiation. Low-dose irradiation altered the content of photosynthetic pigments chlorophylls and carotenoids. But, the effects were opposite between the two different cultivars: the pigment content was higher in cv. Yomyung and lower in cv. Joheung at 14 days after irradiation. The effects on the pigment content were also correlated with the developmental stage (Kim *et al.,* 2004).

The effects of the gamma irradiation on the whole plant (red pepper) showed that growth was stimulated at 2 and 4 Gy but inhibited at 8 and 16 Gy. The chlorophyll amount did not change during the entire experiment. The carotenoid content decreased in a dose-dependent manner but it had almost fully recovered by 2 days after the irradiation. All irradiation groups showed no difference compared to the control in maximal photochemical efficiency (Fv/Fm). The non-photochemical quenching clearly decreased but had recovered to be equal to the control 2 days after the irradiation (Kim *et al.,* 2005).

Table 3.3 - Summary of the effects of gamma radiation on photosynthesis

Exposure dose gamma rays (Gy)	**Target**	**Effects**	**Reference**
Low dose (2-16)	Red pepper, seeds	Altered content of chlorophylls and carotenoids (being different among cultivars)	Kim *et al*., 2004
Low dose (2-16)	Red pepper, whole plant	Decrease in carotenoid content/ growth stimulated	Kim *et al*., 2005
41	Soybean, whole plant	Decrease of 37% in the photosynthetic rate (at high light)	McCabe *et al.*, 1979
40	Sunflower, whole plant	Increase in the starch content in leaves (22%)	Thiede *et al., 1995*
40	Sunflower, whole plant	Decrease in the starch content in the stem (94%)	Thiede *et al., 1995*
200	Beans, seeds	Reduction of the photosynthetic rate (33-38%) and the transpiration intensity (26-39%)	Stoeva and Bineva, 2001
200	Seeds, beans	Reduced stomata conductivity (34-50%)	Stoeva and Bineva, 2001
8k -12k	Kidney bean, black locust and barley mutant, native leaves	Degradation of photosynthetic apparatus, leaf dehydration	Saakov, 2003

3.3 X-ray

No relevant articles were found about the effects of X-ray radiation on plant gas exchange and metabolism.

3.4 Protons and neutrons

No relevant articles were found about the effects of proton irradiation on plant gas exchange and metabolism

3.5 Space radiation

There have been no experiments done in space (on the ISS or space shuttle) regarding the effects of **space radiation** on the photosynthetic apparatus or plant metabolism.

4. Magnetic field

4.1 Weak magnetic field (Shielding of the Earth's geomagnetic field, GMF)

The knowledge about the effects of shielding the Earth's geomagnetic field on plant photosynthesis is limited.

Kursevich and Travkin (1973) found that the respiration intensity and CO_2 emission increased by 70-100% in barley seedlings in a weak magnetic field (100 nT-0.5 mT) compared to the control (local GMF). There have also been evidences that shielding of the Earth geomagnetic field results in a decline in the contents of photosynthetic pigments such as chlorophyll a and b in kidney bean leaves (Belyavskaya, 2004).

Even though some articles are mentioning the effects of a weak magnetic field on photosynthesis the access to the publications is very restricted due to the fact that many of these publications are published in old journals and in most of the cases written in Russian.

4.2 Magnetic field on top of GMF

Several studies have observed an influence of a stationary magnetic field on plant growth. Effects of an additional magnetic field on the photosynthetic apparatus of different types of bacteria, have been reported. However, the effects of an additional magnetic field on plant photosynthesis have rarely been studied (Brettel and Sétif, 1987). In the following the results found for the effects of a magnetic field on photosynthesis are summarized. One should, however, note that most of articles were published in very low impact factor journals.

Aladjadjiyan and Zahariev (2002) investigated the influence of the stationary magnetic field (B = 150mT) on the absorption spectra of the plant photosynthetic apparatus. They found changes in the absorption spectra of all studied samples. The magnetic field decreased the role of the carotenoids and increased the contribution of chlorophyll b in the absorption. It was suggested that this could be due to changes in the pigment ratio in the chloroplasts.

Brettel and Sétif (1987) studied the effects of magnetic field on the primary reaction of photosystem I, i.e. on the formation of P-700 triplet state in higher plants. The effects of external magnetic fields on these primary reactions have been extensively studied in purple bacteria (Hoff, 1981 and 1986; Boxer *et al.,* 1983). On the other hand there are only a few studies about these effects on higher plants. Brettel and Sétif (1987) found that the amplitude of the transient decay ($t_{1/2}$~ 6μs phase) attributed to the triplet state of P-700 increased by weak magnetic fields (6mT) and decreased beyond approximately 12m T. The magnetic field showed an obvious effect on the yield of ^{3}P-700.

Jovanić and Jetović (2002) found that a magnetic field produced a substantial decrease in the chlorophyll concentration and an increase of the photosynthetic apparatus efficiency. The plants (*Phasoleus vulgaris* L.) were grown for three weeks under controlled conditions and different magnetic field intensities. The decrease in chlorophyll content was pronounced with the magnetic field strength. The photosynthetic activity increased by 28% compared to control with increasing magnetic field intensity (60mT, 100mT and 160mT).

Yano and co-workers (2004) studied the effects of a 60 Hz magnetic field on the photosynthetic uptake of CO_2. Radish seedlings were exposed to a 60 Hz, 50μT sinusoidal magnetic field (ELF MF) and a parallel 48μT static MF for 6 days or 15 days. The results showed that the CO_2 uptake was lower than those of the control exposed for both the 6 and 15 days of exposure. The seedlings had also a lower dry weight. However, since the inhibitory effect was not so strong, the effect could not be observed under short term exposure (6 days) to the magnetic field.

Table 4.1- The effects of an additional magnetic effect on plant photosynthesis

Magnetic field	Plant species	Effects	Reference
Stationary magnetic field **150mT**	*Caragana arborescens* lam., *Robinia pseudoacacia* L. and *Laburnun anagiroides* Med.	Changes in the pigments ratio (chlorophyll and carotenoids)	Aladjadjiyan and Zahariev (2002)
Static magnetic field **6mT** and **12mT**	Spinach	Increase in the transient decay of the triplet state of P-700 at low magnetic fields/decrease at higher magnetic fields	Brettel and Sétif (1987)
Static magnetic field **60mT**, **100mT** and **160mT**	Beans (*Phasoleus vulgaris*)	Decrease in chlorophyll content Increase in the photosynthetic activity (28%)	Jovanić and Jetović (2002)
Sinusoidal magnetic field 60 Hz, **0.05mT** + static magnetic field **0.048mT**	Radish (*Raphanus sativus* L. seedlings)	Lower CO_2 uptake Lower dry weight	Yano *et al.*, (2004)

5. Conclusion

Plant gas exchange, metabolism and photosynthesis seem to work properly in the microgravity environment when the plants are provided with ventilation and grown at moderate light levels. The hardware has been shown to be of great importance for the results found under microgravity conditions. Proper environmental control and a well stirred atmosphere are essential to grow healthy plants in space and to avoid the indirect effects of microgravity e.g. lack of buoyancy driven forces.

The effects of enhanced UV-B on higher plants have been intensely studied. Different studies showed that enhanced UV-B seems to be harmful to the photosynthetic apparatus, affecting different key protein and enzymes. If it supplied in the overall light spectrum, protection against high levels of UV-B will be needed. But, note that some UV-B can still be good for the plant since it increases antioxidants like anthocyanin and carotenoids (Voipio and Autio, 1995; Caldwell and Britz, 2006).

UV-C and γ-radiation seems to also be harmful to the photosynthetic apparatus but more studies need to be done to understand the extent of the damage and the effects on the whole plant. Experiments with chronic exposure need to be done, to prove if the plants are able to recover from the damages.

No studies were found that focused on the effects of protons and neutrons on plant photosynthesis and metabolism. Since protons assemble for 90% of the space radiation it is of great importance to understand which effect they have on the photosynthetic apparatus.

The effects of radiation on gas exchange, photosynthesis and metabolism have not been studied under space conditions (low Earth orbit). The plants studied in space (Section 2.1) have been exposed to higher levels of radiation occurring on the ISS or shuttle flights, compared to the controls on ground. However, none of the experiments had measured the radiation levels or discriminated it from potential microgravity effects. Space flight per se, i.e. microgravity together with space radiation in low orbit, did not affect parameters like starch and chlorophyll content, dry mass, CO_2 compensation, PPF and evapotranspiration; only significant effects on electron transport were reported.

Plant gas exchange and metabolism seem to be affected by a weak magnetic field (shielding from the Earth’s MF). More studies investigating the effects of the weak magnetic field and a static additional magnetic field on photosynthesis need do be done.

6. Recommendations for further work

Microgravity

- Experiments with **saturated light levels** need to be done. Due to the power constraint and the volume-limited flight hardware, experiments have only been done with low light intensities (Monje and Mary Musgrave - Interview). In order to clarify the decrease in photosynthetic activity of PSII and PSI as found by Monje and Stutte (2005) experiments with saturated light levels should be done.
- Larger growing areas are needed to measure the plant canopy. Gas exchange and/or photosynthetic efficiency need to be tested in space with more plants in the same area (Monje and Mary Musgrave - Interview).
- More experiments focusing on photosynthesis need do be done in space
- Experiments with fractional gravity need to be done in space, either by using a centrifuge onboard the ISS or by actually doing experiments on the Moon or Mars. Little is known about the effects of fractional gravity. However, the results found under hypergravity in combination with results on the microgravity could be used to predict the effects of fractional gravity.

Radiation

- Experiments with protons and neutrons need to be done to better understand the effects of space radiation on plant photosynthesis.
- There is also a lack of experiments regarding the effects of UV-C and gamma radiation on plant gas exchange and metabolism. There are clear indications linked to the harmful effects of these types of radiation on the photosynthetic apparatus. However, several details need to be understood better.
- Experiments in real space radiation conditions need to be done. There are no publications about experiments concerning metabolism and photosynthesis.
- The effects of chronic exposure to radiation should be addressed.

Magnetic Fields

- The static magnetic fields have already been shown to affect the photosynthetic apparatus of bacteria. More work needs to be done to understand the effects of magnetic fields (shielding and addition to the Earth’s MF) on photosynthesis of higher plants. At the moment only a few and low impact articles have been found.

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