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1. Introduction

The unique feature and characteristics of a plant are expressed through its phenotype that is its form and appearance or spoken scientifically, its morphology. Morphology refers to form, structure and configuration of an organism and includes shape, colour, structure and pattern. The morphology and anatomy of an organism decide its functions, e.g. the structure and colour of a flower is developed in a particular way to attract insects, and in that way ensures the fertilization. If a morphological modification of this flower should occur, like a loss of colour, then the fertilization and in this way the reproductive success of this particular individual could be at risk (Miller *et al.,* 2009).

It is known that environmental factors can alter the plant morphology, and this will again influence the physiological function of the plant. In plant cultivation it is therefore important to ensure such conditions for the plants, so that they develop a normal morphology and anatomy. This will have influences on the functionality, health and the nutritional uptake not to mention; the **quality of the edible** parts.

2. Objective of WP 220.

The objective of WP 220 is to present the existing knowledge about how the physical factors in space will influence plants morphology, anatomy and development. From this knowledge a conclusion will be made, and recommendations for further work will be presented.

The main focus will be on potential effects of the physical factors **space radiation, varying gravity, magnetic field** and potential **combined effects** of these factors.

The following assumptions have been made in order to limit the vast number of factors: optimal control of temperature, light, pressure (1 atm), water and gas supply/composition. In addition we assume optimal root support, and adequate water and nutrients supply will be the basis for the evaluation of the essential factors.

3. Plant morphology

The science of morphology is subdivided in two distinct branches: *anatomy* which is the study of the structure of the internal organs of an organism, and *eidonomy* (usually called morphology), which is the study of the external appearance of an organism. In plant morphology both the vegetative structures of a plant (leaves, stems and roots) as well as the reproductive structures (flowers, seeds, sori and capsules) are studied.

4. Plant anatomy

Plant anatomy or *phytotomy* is the general term describing the study of the internal structure of plants. Figure 1 shows one example of the anatomy of a leaf.

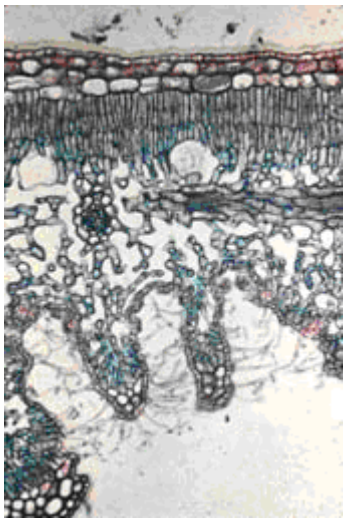

Figure 1. Leaf anatomy; Oleander leaf with stomata crypts, thick cuticle and multiple epidermis (Curtis, Lersten, and Nowak, 2002).

4.1 Cell structures

The internal structures in a plant cell include various organelles with different functions. In addition the plant cell has an inner supportive structure, the cytoskeleton. It plays a crucial role in the organization of the polarity of the cell, the cell division process and the direction of plant growth. The cytoskeleton consists of three types of fiber: microfilaments, intermediate filaments and microtubules. These cytoskeleton fibers are well ordered polymers built from small protein subunits (Lodish *et al*., 2008).

The microfilament *actin* is one of the major components in the cytoskeleton which are important for cell growth and cell mobility. Actin is also expected to play major role in gravity perception in the plant roots. The actin filaments are necessary for myosin-mediated movements of secretory vesicles, which contain precursors for the cell membrane and cell wall material. Actin filaments are also important for the cell-plate positioning and the orientation of these planes are crucial in directing of cell growth and establishing new growth axes. Actin is also required for extension of tricome branches and the expansion of elongating hypocotyl cells.

Microtubules are, like actin filaments, key components in determining cell shape and growth. Cortical microtubules are essential for the anisotropic properties of diffuse growth in expanding cells. Mutants e.g. *MOR*1 (Microtubule Organization 1) have cell shaping defects resulting in left handed twisting of organs, isotropic cell expansion and impaired root-hair polarity. However, their mitotic and cytokinetic microtubule arrays are not affected by this mutation (Martin *et al*., 2001).

Movable amyloplasts - also called *statoliths* - are cell organelles thought to be involved in graviperception associated with gravitropism. Amyloplasts in graviperceptive cells (statocytes) will sediment in the direction of the gravitational vector into the distal part of the cell, while the nucleus is located in the proximal part (Kordyum and Guikema, 2001). Experiments have shown that *Arabidopsis thaliana* plants with a mutation in *pgm* (phosphoglucomutase) and *sgr*1 (shoot gravitropism 1) show no or a reduced gravitropic response and they lack sedimentable amyloplasts (Hoson *et al.,* 2005). In shoots sedimenting amyloplasts have been observed in several kinds of tissues, including coleoptiles in monocots and in hypocotyls and inflorescence stems of dicots. Genetic analysis has revealed that the *endodermis* with its movable amyloplasts is the graviperceptive tissue in *Arabidopsis* shoots (Morita and Tasaka, 2004).

Vacuoles in plant cells are multifunctional and one of the most variable organelles. Together with plasma membranes and cell walls, vacuoles are involved in the control of both cell volume and turgor mechanisms within the cell. The shape and size of the vacuoles will vary over the cell lifetime and are determined by tissue and species distinctive characteristics. The vacuole occupies about 90 % of the cell volume in mature parenchyma cells. The turgor pressure in cells is determined by the following parameters: presence of solutes in the vacuole that keep the water potential below the surroundings of the vacuole, and inwardly directed hydrostatic pressure exerted by the stretched cell wall. Vacuoles are also involved in the plant defense responses to wounding, as well as environmental and biotic stress factors (Klymchuk *et al.,* 2003). Experiments have clarified that there is a correlation between vacuoles and the amyloplast sedimentation in plant cells, indicating that gravity controls both the development of the vacuoles and the features of the sedimentation process (Kato *et al.,* 2002).

4.2 The cell wall

The cell wall is a complex extracellular matrix surrounding each plant cell. It has a variety of functions: it determines the cell shape, the cell stability, protects the cell against pathogens such as vira and bacteria and the tensile strength of the wall allows the plant cell to develop turgor pressure. The cell wall of elongating cells is still elastic, a property that is lost in fully differentiated cells. There is a clear structural and functional difference between the primary and the secondary cell wall. Components for the *primary* cell wall are deposited during cell growth and the common wall develops normally between the two daughter cells during early telophase.The major components of the primary cell wall are polysaccharides (cellulose), cellulose-binding hemicelluloses and pectins. The primary wall needs to be mechanically strong to avoid cell rupture under the turgor pressure; at the same time it should be sufficiently extensible to permit cell expansion. Components for the *secondary* cell walls are deposited after the termination of the cell growth. In this way the secondary wall obtains mechanical stability in order to develop to specialized cells such as e.g. trachea in the xylem tissue. The secondary walls include major components like cellulose and hemicelluloses, and are often impregnated with lignins. The plant cell wall in general also contains hundreds of different proteins and some are important in restructuring the cell wall. These specific restructuring proteins are proline-rich proteins, glycine-rich proteins and hydroxyproline-rich glycoproteins, also called extensins. Extensins may act as regulators of cell wall expansion and they are a link between the cell wall and the plasma membrane (plasmalemma). The extensins will be induced under tensile stress and strong mechanical pressure (Martin *et al.,* 2001).

Primary cell walls are subdivided into Type I and Type II. Type 1 is present in all dicotyledons, gymnosperms and most non-graminaceous monocotyledons. Type II is typical for *Poaceae* and a few other monocotyledons. Type II cell walls are composed of small amounts of pectin and a high amount 1,3-1,4-β-glucan and xylan instead of xyloglucan (Konno *et al*., 2008).

Primary cell walls are adapted to withstand tensile stress while secondary cell walls also need to withstand compressive stress. One strategy to compensate for the compressive stress on the tissue has to be taken by the turgor pressure within the cell. By that the cell walls are permanently maintained in tension and are adapted also for the tensile functions – see Figures 2 and 3.

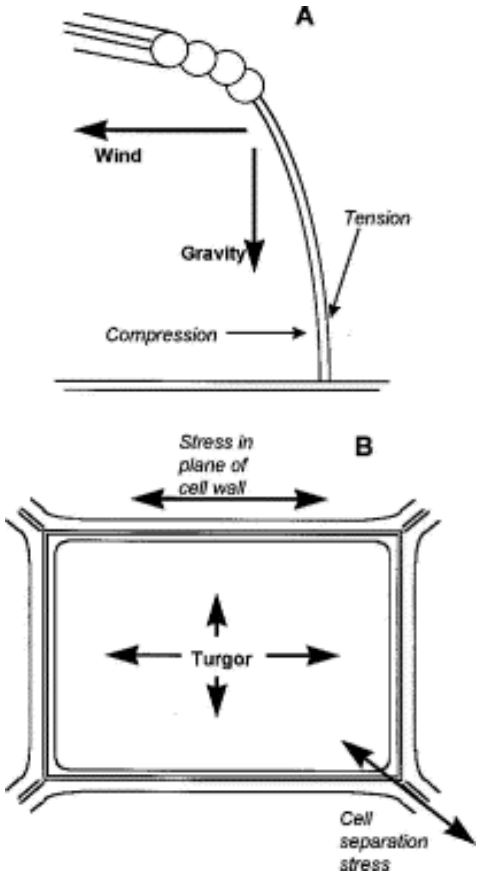

Figure 2.
A. Environmental stress on the whole plants. Originating from compressive and tensile stress on the stems.
B. Stress induced on cell walls by turgor pressure (Jarvis and McCann, 2000).

Figure 3. Spiral primary cell wall in *Rubus* (Lersten and Curtis, 1976)

5. Plant development

5.1 Vegetative growth

In the field of plant morphology also the pattern of **development**, i.e. the process by which structures originate and mature as a plant grows, is in focus. While animals produce all life lasting body parts in the early phases in their life, plants constantly produce new tissues and structures throughout their life from meristems (undifferentiated cell tissue). A living plant always has embryonic tissues and the totipotential capacity. The way in which new structures mature as they are produced may be affected by the stage in the plants life when they begin to develop, as well as by the environmental conditions to which the structures are exposed. Polar growth or directional expansion in the plant occur by two main processes: tip growth and diffuse growth. Diffuse growth is cell extension that is dispersed over the entire cell surface, along one axis and in a polar manner. This process is driven by turgor pressure and constrained by microfibrils. The other process, tip growth, occurs in pollen tubes and root hairs of higher plants. Polar extension in tip growth is driven by local insertions from secretory vesicles. These vesicles add a new plasma membrane and secrete enzymes and cell wall precursors at the apical growth site. Intact cortical microtubules are required to stabilize growth in tip growing cells. The microtubules stabilize growth by controlling the movement of a tip-focused cytoplasmic Ca^{2+} gradient.

Cells may use a combination of both tip and diffuse growth in order to get their final shape. To regulate the cell expansion, the cell must control the deposition and spatial organization of cellulose and rearrange bonds. This will allow the cell wall to yield or resist under turgor pressure (Martin *et al*., 2001).

5.2 Fertilization and embryonic development

Fertilization in flowering plants (angiosperms) is a complex multi-step mechanism, starting by pollination followed by pollen tube growth and ending by the caryogamy (fusion of two pronuclei). The fertilization includes two sperm cells and two female gametes, and two fertilization events take place. The egg is activated by many cellular processes including protein synthesis and the cell cycle. The calcium theory of egg activation proposes that an increase in concentration of cytosolic Ca^{2+} is responsible, at least in part, for signaling the development of the egg.

Pollination is the transfer of a male nucleus containing unit (male gametophyte) from the male organ, the anther, to the receptive female organ, the stigma surface of the pistil. The

pollen tube serves as a pathway for sperm cells on their course to the embryo sac. Various mechanisms are involved in the pollen tube guidance, including chemo-attraction, mechanical guidance, growth stimulation and adhesion. Growth of the pollen tubes is also proven to be regulated by spatial and temporal variations in the level of cytosolic Ca^{2+} (Dumas and Gaude, 2006). In *Arabidopsis*, pollen tube attractant candidates might be proteins encoded by genes expressed in the synergids and secreted into the filiform apparatus. Once the pollen tube has reached the embryo sac, the pollen tube ceases growth and discharges its contents through interaction with the synergids. When the two sperm cells reach the female gamete, specific recognition signals take place, leading to plasmogamy (gamete membrane fusion) (Berger *et al*., 2008).

Embryogenesis begins after ovule fertilization and transforms a single-cell zygote into a multicellular individual. When compared to animals, the mature embryo of higher plants is relatively simple. It involves basic body polarities with the primary shoot meristem (SAM) and the root meristem (RM). The development of the embryo is accomplished through the coordination between two processes; pattern formation and cell fate determination on one side and tissue growth on the other. Early stages in the development involve the apical-basal and the radical axes. Auxin has through molecular analyses been proven to have an important role in the apical-basal axis (Wu *et al*., 2007).

6. Ground experiments

Experiments on ground have been done from the very beginning of the space research era, trying to simulate the space conditions. These studies should give an idea on how the space conditions would impact organisms as well as equipment meant to be sent up into space. The experiments have included different devices to compensate the gravitational pull, so-called clinostats. There are mainly two types, one axis clinostats (2-D) and two axis (Random Positioning Machines, 3-D) clinostats. In addition there were experiments were one tried to simulate the radiation load in space with different radiation emitters and heavy ion accelerators. Studies of the effects of electromagnetic and electric fields have focused primarily on strong static magnetic fields up to 5 T. Experiments including simulations of the non-polar, scattered magnetic fields like the ones found on the Moon and Mars (Figure 4) and their influence on organisms, are scarce.

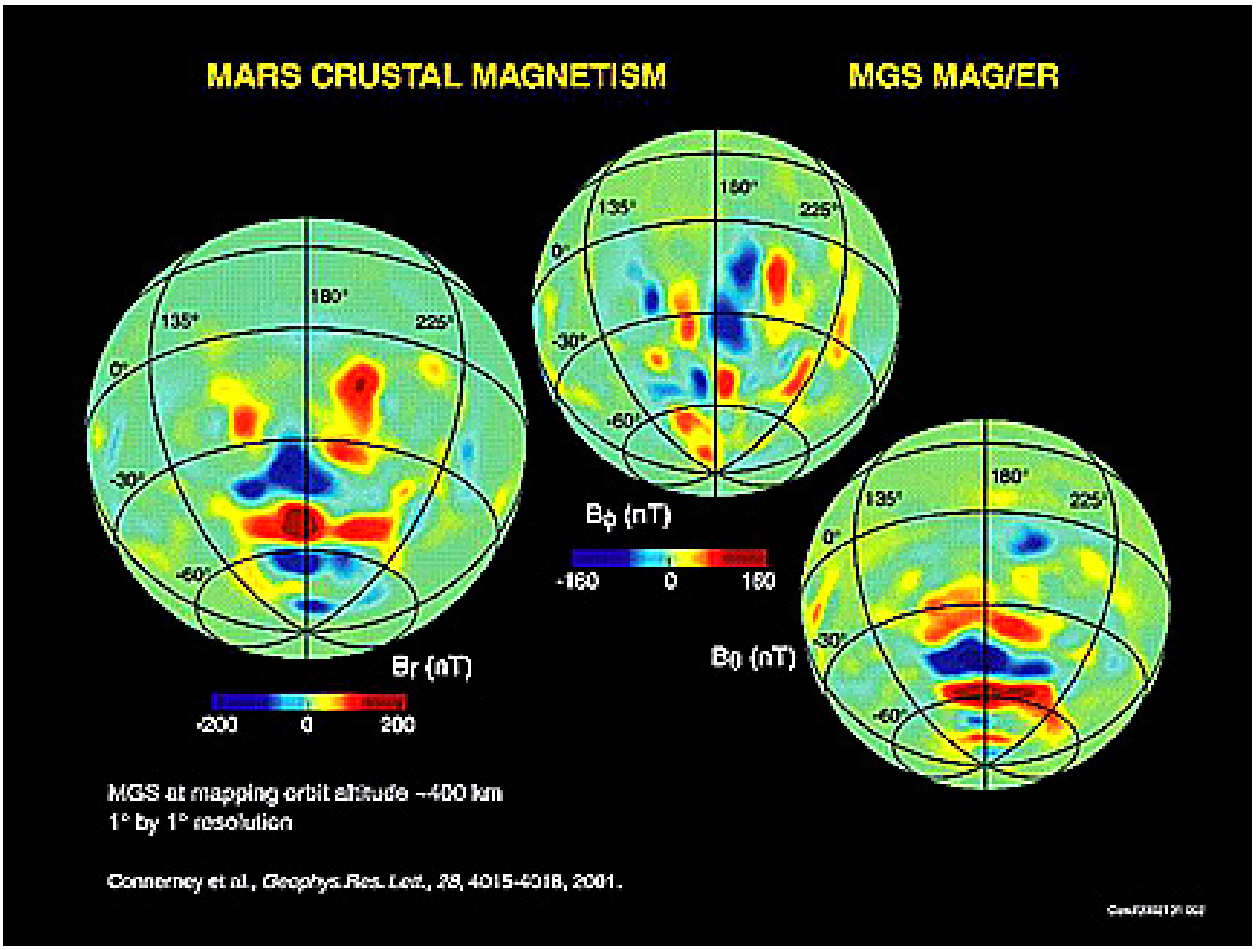

Figure 4. Mars crustal magnetism (Connerney *et al*., 2001**)**

6.1 Clinostats

Results from articles presented in the following sections include experiments done on 2-D and 3-D clinostats, in hypergravity and microgravity. All articles include experiments on whole plants, and both monocot and dicot plants are represented.

Clinostats, effects on morphology and vegetative growth

Hilarie *et al*. (1996) found a decrease in shoot length and an increase in root length of soybean seedlings grown on a 2-D clinostat. Plants exposed to clinorotation also produced twice as much ethylene gas over a period of 7 days. These results are in contradiction with the results from De Micco *et al*. (2005), who found no differences in soybeans kept on a clinostat, with regard to the length, fresh and dry weight of the seedlings (Table 1). This might be explained by the use of different hardware in the two experiments. In the first experiment (Hilarie *et al.*, 1996) BRIC (Biological Research in Canister) ground hardware was used and in the second the seeds were kept in cotton cloths on Plexiglas supports, attached to the clinostat. It is known that if plant material is kept in closed compartments with no or reduced ventilation, ethylene gas can accumulate within the compartment. Ethylene is proven to influence growth parameters and inhibit growth in meristems of roots and shoots apparently because the gas hinders auxin transport. An increased production of ethylene can also delay the cell differentiation process, so that previously expanding cells grow to enormous size (Burg, 1973). These factors could explain why there was a decrease in shoots and increase in roots in the experiment performed in BRIC canisters and not in the experiment performed by De Micco and coworkers (2005), where the seedlings were not encapsulated. One can not rule out that other factors related to the clinorotation may have induced these effects.

Another experiment done by Shimazu *et al*. (2001) using pea and maize seedlings in a closed Plant Growth Chamber kept in darkness, showed that the emergence of the 3rd internodes in pea was promoted, while the 1st internodes and the total length were inhibited. In maize seedlings the growth of coleoptiles showed little difference, but growth of mesocotyls was suppressed and the emergence of the leaf out of coleoptile was promoted. It was concluded that the decrease in cell wall extensibility and osmotic potential in the cell sap, might have contributed to the inhibition of the mesocotyls. This is in accordance with the fact that vacuoles and cell walls are involved in controlling the cell volume and turgor pressure (Sections 4.1-4.2). The results from this experiment are more

reliable than the previously mentioned, as they are conducted on a 3-D clinostat which is proven to be the best equipment to simulate microgravity. The results are also verified by the work of Ueda *et al*. (1999) who found a good correlation between a decrease in cell wall extensibility and the epicotyl growth of etiolated pea seedlings and the suppressed mesocotyl of maize under actual space conditions.

Table 1. Results from three articles presenting the effects on plant morphology. The experiments are performed on 2-D and 3-D clinostats.

Author	Organism	Exposure	Results
Hilarie *et al*., 1996	Soybean (*Glycine max*) seedlings	2-D clinostat	Decrease in shoot length (33 %) and increase in root length (doubling). Shoots had a larger diameter whereas roots had a smaller cross section. A doubling of the ethylene production after 7 days.
Shimazu *et al.*, 2001	Pea *(Pisum sativum*) and maize (*Zea mays* L) seedlings	3-D clinostat	Clinostat rotation promoted emergence of 3rd internodes in pea, and slightly inhibited the 1st internodes and the total length. In maize elongation growth was less affected, but elongation of mesocotyls was suppressed and the emergence of leaf promoted.
De Micco *et al*., 2005	Soybean (*Glycine max*) seedlings	2-D clinostat	No difference measured in length, fresh and dry weight. Vascular differentiation increased by clinostat rotation.

Clinostats, effects on anatomy (cell structures and cell wall)

Lorenzi and Perbal (1990) investigated the position of the statocyte nucleus in various gravity simulating equipments and under microgravity conditions in space (Table 2). They found that the average area and radius of the nuclei were not different between the exposures. The estimated average radius of the nucleus was 4.14 µm. In the ground control cells (1 G), the nuclear membrane was almost in contact with the plasmalemma of the proximal cell wall. In statocytes grown on a clinostat, there was a distance of 0.76 µm (vertical rotation) and 0.47 µm (horizontal rotation) between these membranes. In microgravity the nucleus was most displaced, with a distance of 0.98 µm between the nucleus to the proximal cell wall. The nucleus in the 1 G space control was also displaced by 0.70 µm. This was explained by the fact that the samples were subjected to microgravity

for 15 minutes before chemical fixation. The results show that both on clinostats and under microgravity conditions the nucleus will be displaced, and the location of the nucleus was identical in statocytes grown in microgravity and on a vertical clinostat. The displacement of the nucleu could be due to a relaxation of the actin filaments, since the nuclei is attached to this type of cytoskeleton microfibrils. The results also demonstrated that relative high G-forces (50 G) were necessary to detach the nucleus from the proximal cell wall. This indicates that the association between the actin filaments and the nucleus must be very strong.

Hoson *et al.* (1998) found that the statocytes in cress and maize rotating on a 3-D clinostat had a normal development in the root cap. However, in coleoptiles the number and size of the amyloplasts were decreased. Maize coleoptiles that were grown in normal gravity after cultivation on the 3-D clinostat from 24 hours, showed the same response as those grown on a clinostat for the whole growth period. This could indicate that a gravity stimulus in the early phase may be necessary to develop normal shoot statocytes (Hoson *et al.*, 1998).

Another study investigated the actin microfilament arrangement in root cap statocytes and in peripheral root tissues (epidermis and cortex cells) of the transition zone in beet seedlings (Table 2). Cells and statocytes in the transition zone represent the postmitotic cells originated from root meristems and they are specialized in graviperception (root cap) and gravireaction (transistion zone). There was no difference in either arrangement or structure of the actin filaments in cortex and apical cells (root cap meristem). However, there was an increase in the cytoplasmic microfilament network in the cortex cells in the transition zone. Microtubules were also found to be disorganized within the transition zone. This suggests that the increases in actin filaments in this zone are induced to counteract the distortion in microtubules and strengthen the cell (Shevchenko and Kordyum, 2005).

Shevchenko (1999) also found that meristem cells in clinorotated *Beta vulgaris* had both longitudinally and transverse arrangement of microtubules as opposed to a transverse arrangement in the control. Shevchenko *et al*. (2007) investigated the interrelation between microtubules and microfilaments with *Arabidopsis thaliana* plants on a 2-D clinostat. They found some indications that anisotropic growth requires a different interrelation between microtubules and microfilaments than isotropic growth, but that anisotropic growth is mostly driven by microtubules (Shevchenko *et al.*, 2007).

Table 2. Results from seven articles presenting anatomical results of plant experiments done on 2-D, 3-D clinostats, hyper and microgravity.

Author	Organism	Exposure	Results
Lorenzi and Perbal, 1990	Lentil roots (*Lens culinaris*)	2-D clinostat (both horizontal and vertical), 1 G control on ground, 1 G control in space and microgravity	The nucleus in statocytes was displaced in both microgravity and on clinostat. The nucleus had the same average area and radius in all exposures.
Hoson *et al*., 1998	Cress (*Lepidium sativum*) seeds and maize (*Zea mays*) caryopsis	3-D clinostat	In cress and maize roots there was a normal development of statocytes with amyloplast statoliths in the root cap. In maize coleoptiles, the number and size of the amyloplasts were decreased.
Shevchenko, 1999	*Beta vulgaris* seedlings	2-D clinostat	In clinorotated meristematic cells, the microtubules were longitudinally oriented in addition to the transverse arrangement.
Shimazu *et al.,* 2001	Pea (*Pisum sativum*) and maize (*Zea mays* L)	3-D clinostat	Decrease in osmotic concentration of cell sap in the 1st internode in pea seedlings. Total osmotic solutes in 1st and 3rd internodes were decreased and increased, respectively.
Shevchenko and Kordyum, 2005	Beet *(Beta vulgaris*) seedlings	2-D clinostat	There was no difference in actin cytoskeleton structure or arrangement in the root cap meristem cells, but an increase in cytoplasmic actin in cortex cells in the transition zone. The arrangement of cortical microtubules was distorted in the transition zone.
Wakabayashi *et al*., 2005	Wheat (*Triticum aestivum*) caryopses	Hypergravity (300 G)	Hypergravity increased the net amount of cell wall polysaccharides, but reduced the shoot elongation.
Shevchenko *et al*., 2007	*Arabidopis thaliana* plants	2-D clinostat	No difference in microtubule organization between the clinorotated plants and control. Anisotropic growth is driven mostly by microtubules.

Shimazu and coworkers (2001) did a study with pea and maize seedlings grown on a 3-D clinostat. They found that clinorotation reduced the osmotic concentration of the cell cap in both the coleoptiles and the mesocotyls in maize. In pea the osmotic concentration was reduced in the 1st but not the 2nd and the 3rd internodes. Cell wall extensibilities of the 1st and 3rd internodes of pea were significantly lower and higher respectively compared to control. The changes found in the cell wall correlated to growth of each organ in pea and maize (see Table 2). This indicate that growth and development was controlled by gravity and growth responses of plants in simulated microgravity are regulated by both turgor pressure in cells and the cell wall mechanical properties (Shimazu *et al.,* 2001).

Wakabayashi *et al* (2005; Table 2) found that wheat seedlings exposed to hypergravity (300 G) for three days increased the net amount of cell wall polysaccharides such as hemicellulose and cellulose but reduced the shoot elongation. In addition also the sugar components arabinose and xylose were increased. Cell wall phenolic constituents such as ferulic acid (FA) and diferulic acid (DFA) and the enzyme phenylalanine ammonia-lyase (PAL) were also increased. This suggested that the increased activity of PAL induce the production of FA and DFA, all being antioxidants triggered by stress conditions. The increase in cell wall constituents may be the way for plants to strengthen their cell walls and to sustain the structures against gravitational stimuli (Wakabyashi *et al.,* 2005).

Clinostats, effects on reproduction and embryo development

De Micco *et al*. (2005) evaluated pollen germination rates and pollen tube growth in 4 different species on a 2-D clinostat (Table 3). They found that the percent pollen germination was not affected in apple, apricot, and almond and increased in broad bean. In apple, there was normal pollen tube development but with following perturbations, i.e. the two migrating sperm cells were blocked by callose plugs or one sperm cell was blocked by callose plugs. In almond, 70 % of the cases the pollen tubes either had only one sperm cell or no sperm cell counted. In broad bean sperms sometimes migrated to the tube tip, but remained there. The metabolism of callose (plant polysaccharide necessary in pollen migration) endured the perturbations in *Prunus* species and *Malus domestica,* because the callose was spread throughout the pollen tube as if a plug was obstructing the tube. In apple the callose plugs were longer and in apricot clinorotation caused callose spreading and incomplete plug development. The result indicates that even if there is normal pollen germination during clinorotation, various perturbations in the pollen tube development can impair the gametophyte growth. Despite the fact that there was some normal development, the overall fertilization success was reduced due to the aberrations in the pollen tubes and it

is likely that the sperm cells would not have been able to perform fertilization. This experiment showed that all species investigated had some degree of perturbations in the pollen tube development during simulated microgravity, although the degree varied between the species (De Micco *et al.*, 2005).

Table 3. Results from four articles presenting plant experiments done on 2-D clinostats and in hypergravity, focusing on reproduction and development.

Author	Organism	Exposure	Results
De Micco *et al*., 2005	Apricot (*Prunus armeniaca*), almond (*Pruus dulcis*), apple *(Malus domestica*) and broad bean (*Vicia faba*)	2-D clinostat	Simulated gravity affects pollen tube development. Pollen germination in apple, apricot and almond was not affected, but in bean an increase in percent germination
De Micco *et al*., 2006	*Prunus persica, Prunus avium, Prunus domestica, Prunus communis* and *Brassica rapa*	2-D clinostat.	Pollen germination not affected in either species. In all species except *P. domestica* there were differences in sperm cell formation and migration
Musgrave *et al*., 2009 a	*Arabidopis thaliana*	Hypergravity (1-4 G)	At 4-G the flowers self-pollinated, but did not produce seeds. Pollen growth tubes into the stigmas was curtailed in 4 –G.
Musgrave *et al*., 2009 b	*Brassica rapa*	Hypergravity (1-4 G)	There were no adverse effects of hypergravity on flowering, fertilization or embryogenesis. *Brassica* embryos from both 2-G and 4-G were larger than the controls.

The same authors conducted another study in 2006 by exposing woody and herbaceous species (*Prunus persica, Prunus avium, Prunus domestica, Prunus communis* and *Brassica rapa*) to clinorotation on a 2-D clinostat. There were no differences found in the pollen germination rates in either species. All species had different alterations in the nuclei formation and sperm cell migration, except *P. domestica*. The alteration was similar to the previous experiment (De Micco *et al.*, 2005); 1 or 2 sperm cells blocked by callose plugs, sperm cells remaining in the grain or no sperm cells detected. Again, it is clear that clinorotation can induce perturbations in the male gametophyte development (De Micco *et al*., 2006).

Musgrave *et al.* (2009 a) exposed *Arabidopsis* plants to various levels of hypergravity, from 1 to 4-G (Table 3). Plants grown in hypergravity produced comparable number of flowers buds when compared to the 1-G control. There was no difference in progressing of flowers to seed pods between plants at 1 and 2-G, but in 4-G there were changes. The flowers in 4-G were produced at normal rate, but few developed siliques, many of these were empty. The pollen germination and viability was normal in 4-G, but the development of the pollen tube spanned a range of morphologies, with crumbled tubes predominating. These abnormalities were also seen in 3-G. In 3-G there was also a flattening of the pollen tube and a significant increase in tube length which also occurred at low levels (1.3-G). The suggested reason for these results was that hypergravity influenced tip growth in pollen tubes. It is the turgor pressure in addition to active turnover of microfilaments and microtubules that drives the tip growth. It has been shown that the cytoskeleton-mediated endocytotic retrieval of the plasma membrane during tip growth is inhibited by hypergravity, and this type of retrieval may well have occurred in the pollen tube.

In another study, Musgrave *et al*. (2009 b) did a similar experiment but with *Brassica rapa* both *in vivo* and *in vitro* (with a silique culture system that permits maturation of seed *in vitro*). In this experiment there were no adverse effects of hypergravity on flowering, fertilization or embryogenesis. In both systems (*in vivo* and *in vitro*) the hypergravity embryos from both 2-G and 4-G treatments were larger than the controls. These results indicate that *Brassica rapa* can have a normal seed production in hypergravity as opposed to *Arabidopsis thaliana,* which had pollen tube alterations beginning at 3-G. The *Brassica* seeds were also more developed in hypergravity as indicated by the storage reserve composition. This is contradictory to experiments in microgravity, in the same model system, where seeds showed a delayed development.

From these two experiments one can conclude that gravity can have strong effects on seed production over a narrow range of G levels, although it seems to be species dependent (Musgrave *et al*., 2009 b).

Summary clinostats

Soybean seedlings kept on 2-D clinostats showed no difference in one experiment (De Micco *et al*., 2005) and alterations in shoot and roots in another experiment (Hilarie *et al.,* 1996). These results are difficult to interpret, but can be due to hardware and accumulation of ethylene gas, which is known to inhibit growth and differentiation in plant cells. In peas and maize kept on a 3-D clinostat (Shimazu *et al*., 2001) growth of the pea internodes were either promoted or inhibited. In maize the coleoptiles were not affected, but mesocotyl growth was suppressed. These effects are due to changes in cell wall and turgor pressure, also verified under microgravity conditions.

In lentil roots the nuclei in statocytes are displaced, both in microgravity and on a 2-D clinostat. The displacement is due to a relaxation of the actin filaments and the association between the actin filament and the nucleus has been proven to be very strong (Lorenzi and Perbal, 1990). Statocytes in cress and maize had a normal development in roots when exposed to a 3-D clinostat, but in the coleoptiles the number and size of amyloplasts were decreased (Hoson *et al*., 1998). Other maize investigations have demonstrated that actin filaments are normally arranged in cortex and apical cells, but an increase is seen in actin filaments in the transition zone where also disorganization in microtubules was found (Shevchenko and Kordyum, 2005). Microtubules will be arranged both longitudinally as well as transversely in clinorotated *Beta vulgaris* seedlings (Schevchenko, 1999), and anisotropic growth appears to be driven mostly by microtubules and not by microfilaments (Shevchenko *et al.,* 2007). Hypergravity (300 G) increased the net volume of cell wall polysaccharides, cell wall phenolics and PAL (phenylalanine ammonia-lyase), most likely to strengthen the cell wall against gravitational stimuli (Wakabayashi *et al*., 2005). Pea and maize seedlings on a 3-D clinostat had alterations in osmotic concentration in the cell cap and cell wall extensibilities, both correlating to alterations in growth (Shimazu *et al.,* 2001).

Pollen germination in apple, apricot and almond is not affected on a 2-D clinostat but increased in broad bean. There were, however, various perturbations in the pollen tube development that could impair the gametophyte growth and fertilization process. The same results were found for *Prunus persica, Prunus avium, Prunus domestica, Prunus communis* while *Brassica rapa* were not affected by clinorotation on a 2-D clinostat. Clinorotation can thereby induce perturbations in the male gametophyte development (De Micco *et al.,* 2005, 2006). Hypergravity induces changes in *Arabidopsis* in 4-G, with crumbled pollen tubes., but *Brassica* had normal pollen tubes at 4-G. *Brassica* seeds were larger and more developed in hypergravity as opposed to *Brassica* seeds in microgravity which showed a delayed development (Musgrave *et al.*, 2009 a, 2009 b).

6.2 Radiation effects on higher plants

For more information on the various radiation sources found in space - see TN 97-03 (Plant genetics).

Radiation effects on morphology and vegetative growth

Experiments on ground have revealed that low doses of gamma rays (1-5 Gy) did not cause any morphological changes in the phenotype. A higher dose (50 Gy), however, inhibited seedling growth significantly (Figure 5). This was thought to be a consequence of the radiation because the plant cell growth went into arrest (Wi *et al.,* 2007).

Figure 5. The phenotypes of the control (left) and 50 Gy-treated *Arabidopsis* seedlings (right) at day 6 after gamma irradiation. Bar = 5 cm (Wi *et al.*, 2007).

Other experiments with a relatively low (realistic) UV-B dose in combination with high PAR (photosynthetically active radiation) levels, have shown that plant morphology is more sensitive to UV-B radiation than biomass production in a variety of grasses like wheat, rice and *Calamagrostis epigeios*. In *Bromus catharticus* the plant stem, leaf and total dry weight increased slightly with increasing UV-B radiation up to 90 % of ambient UV-B. Plant height and number of tillers increased at lower levels, but decreased at 90 % of ambient UV-B) (Deckmyn and Impens, 1998). Another experiment demonstrated that UV-C radiation (10-50 kJ/m^2) induces apoptotic responses in *Arabidopsis* cells (Danon and Gallois, 1998).

Radiation effects on anatomy

Experiments with *Arabidopis thaliana* have shown that chloroplasts are extremely sensitive to gamma radiation compared to other cell organelles; in particular the thylakoids became heavily swollen (Wi *et al.*, 2007). In fruits with chloroplasts found in hypodermis at harvest time, 1 kGy (=1000 Gy) altered the thylakoids structure significantly. Gamma radiation has also been shown to inhibit grana development in bean and barley. Mitochondria react to gamma irradiation with a temporal promoted activity, followed by an increase in resistance to irradiation and /or capacity for radiation repair. Mitochondria of 5 kGy irradiated cherries were less dense than control and the irradiation induced disorganization of matrix and cristae. The cell wall consists of pectic substances, derived from an esterification of galacturon acid residues. Gamma irradiation from 1kGy will increase the activity of polygalacturonase and pectin methyl esterase significantly, and this can result in a degradation of pectin in the cell wall. Supplemental UV-B radiation on top of ambient UV-B radiation damages the chloroplasts, manifested by a dilation of thylakoids and a

disintegration of the double membrane envelope surrounding the chloroplast (Kovács and Keresztes, 2002).

Radiation effects on development

Experiments have shown that reproductive growth is much more sensitive to UV-B radiation than vegetative growth, and the number and dry weight of *Bromus catharticus* seeds almost doubled in a high UV-B field (Deckmyn and Impens, 1998). Other studies have shown that gamma- and beta-radiation were equally effective in reducing seedling growth in both dormant and germinating barley **(***Hordeum distichum***)** seeds. Fission neutron radiation induced a 40 times higher LD_{50} (LD=Lethal dose) level of damage than either beta- or gamma-radiation in dormant seeds e, but only 6-8 times higher damage in germinating seeds. The germinating seeds were 5.0, 29.3 and 30.0 times more sensitive to neutron, beta- and gamma-radiation, respectively, than the dormant seeds at the (damage) D_{50} level. These values also decreased with increasing dose and level of damage (Conger *et al.*, 1972). In durum wheat (*Triticum durum*) the percentage of fertilization was lower in the beta- and gamma-irradiated male and female gamete groups than in the control group. Some stimulation in the development of the pro-embryo has been observed 72 h after pollination when the female gametes were irradiated. In contrast there was a reduction in pro-embryo development observed at 72 and 120 h in the irradiated male gamete group. The endosperm was more advanced in its development in the irradiated male gamete group when compared with both irradiated female and control groups. These observations showed that beta- and gamma-irradiation of male gametes produced more deleterious effects on the development of the embryos than irradiation of the female gametes (Donini and Hussain, 1968).

In a recent experiment six alpine meadow species were grown under reduced, ambient and increased (simulating 9 % ozone depletion 2900 meter above see level) UV-B light. Increased UV-B light significantly reduced pollen germination and pollen tube growth in *Vicia angustifolia* compared to the control. Pollen germination of *Poa annua* and *Polygonum aviculare* as well as pollen tube growth of *Poa annua* and *Malva sinensis* were significantly enhanced by an increased UV-B radiation compared with the control. Meanwhile, no significant effect was found in pollen germination and pollen tube growth in *Plantago depressa* and *Elsholtzia densa* by increased UV-B radiation compared to the control. There was no significant negative effect found in pollen germination and pollen tube growth after exposure to reduced UV-B radiation in either of the species. The results showed that pollen in some species were relatively resistant to UV-B radiation, and there was clearly a species-specific effect with both positive and negative responses to the treatments (Wang *et al.*, 2006).

6.3 Magnetic field, morphological and anatomical effects on higher plants

Several experiments with seedlings of different species placed in weak magnetic fields (WMF, with the geomagnetic field screened off) have shown that the growth of primary roots is inhibited in comparison with the control. The cell reproductive cycle slows down, due to the expansion of the G1 (first growth phase in the cell division cycle) phase in many species, among them broad bean (*Vicia faba*), while other phases of the cell cycle remain rather stable (Rapley and Rowland, 1998).It has also been shown that roots can disintegrate in a WMF. In one study by Negishi *et al.* (1999) using pea (*Pisum sativum*) they found that epicotyls were elongated in a WMF compared to the control. Yet another study found the opposite, that WMF caused a significant growth inhibition in sugar beet, pea and wheat seedlings (Sytnik *et al.*, 1984).

At the ultrastructural level, experiments have revealed alterations in distribution of chromatin, developments of lytic compartments (vacuoles) and reduction of phytoferritin in plastids of pea roots in a WMF. Mitochondria were found to be very sensitive to WMFs, their size and relative volume increased, while the cristae were reduced (Belyavskaya, 2002). Other experiments demonstrated that the cells of pea seedlings had an increased osmotic pressure in a WMF.

Developmental studies revealed that germination of seeds of pea (*Pisum sativum*), lentil (*Lens culinaris*) and flax (*Linum usitatissimum*) was unaffected by WMF (1 nT) (Davidkov *et al.*, 1981). In the majority of the experiments a delay in seed germination was observed, but in pea both inhibition and stimulation of seed germination occured (Negishi *et al.*, 1999).

Magnetic field and combined effects

In other studies plants were exposed to a weak magnetic field and a normal magnetic field in 1 G (control) and zero magnetic field (shielding from the geomagnetic field) and a randomized magnetic field on a 3-D clinostat. The results showed that during 1-G condition, pea epicotyls became longer in the WMF than in the normal geomagnetic field. There was no significant difference between the seedling growth under the randomized magnetic field and the zero magnetic field on the 3-D clinostat (Yamashita *et al.*, 2004).

Earlier work done by Mericle *et al.* (1966) gave evidence for an interaction between ionizing radiation (X-rays) and magnetic fields. There was a synergistic effect associated with a delay in the development of the root, shoot and coleoptile. There was also a reversed role of the magnetic field in the growth of irradiated versus non-irradiated material. When the magnetic field was applied alone it inhibited growth, but when applied during germination of pre-irradiated grains it stimulated growth (Mericle *et al.*, 1966). Similar

results were found in another study, where cell cultures exposed to a magnetic field after gamma irradiation, had a significantly shorter regeneration period than the group not exposed to the magnetic field. This suggests that magnetic fields can diminish the radiation damage in plant cells (Alikamanoglu *et al*., 2007).

7. Space experiments

In some space experiments the scope has been to investigate how one factor, such as the radiation load influences higher plant's morphology. But usually most experiments done in space do not allow a clear distinction between the effects originating from radiation, magnetic fields, gravity or other stimuli like vibrations. The plant response is therefore the total sum of effects, thus phrased "space effects".

7.1 Space effects on morphology and vegetative development

The Biostack I and II experiments were done on Apollo 16 and 17, respectively (Table 4). The biological effectiveness of HZE particles was investigated in seven biological systems in resting state, among them *Arabidopsis thaliana* and *Vicia faba* seeds. It is suggested that irreparable biological damage caused by HZE particles is an all or nothing situation where the event occurs when the energy of the HZE particle exceeds a certain value. Otherwise inactivation or reparable damage occurs. On its path, the HZE particle has its maximum energy loss in the Bragg peak, shortly before it stops. Stopping particles are called "enders". In the Biostack experiment "enders" of Z=10 were found, which is only found as you leave the Earths protecting geomagnetic field. The radiation load in the Apollo 16 and 17 was therefore higher and with more energized particles than in low Earth orbit and one could suspect a higher rate of biological damage. However, they found that the growth of the *Vicia faba* radiculae and germination rate of *Arabidopsis thaliana* seeds that were hit by HZE particles was not significantly influenced. There was, however, an increase in multicaulous plants (multiple stems) (Bücker and Horneck, 1975).

Table 4. Results from six experiments focusing on morphology and vegetative development

Author	Organism	Duration	Vehicle/mission	Scope	Result
Bücker and Horneck, 1975	*Arabidopsis thaliana* and *Vicia faba* seeds	11 and 12 days	Apollo 16 and Apollo 17, Biostack	Morphology and development, HZE radiation	Increase of multicaulous plants in seeds hit by HZE particles
Kranz *et al.*, 1990	Dry *Arabidopsis* seeds	13 days	Kosmos 1887 Biosatellite, Biorack 1/0 container	Morphology and development, Ionizing radiation	Shorter radicle from seeds flown outside the spacecraft. No difference in radicle length from seeds hit by HZE particles, compared to ground
Legue *et al.*, 1996	Lentil seedlings (*Lens culinaris* L.)	4-29 hours	IML-2, Spacelab	Morphology and development, microgravity	Root growth was not affected by reduced gravity.
Cook *et al.*, 1998	Potato (*Solanum tuberosum*) explant	16 days	STS-73	Morphology and development, microgravity	No difference in size and shape of potato explants
Levine and Piastuch, 2005	Etiolated soybeans (*Glycine max*) germinated in flight	5 days	STS-87	Morphology, space factors	No significant differences on either cotyledons or hypocotyls. Longer primary roots, higher root fresh weight.
Sychev *et al.*, 2007	Pea (*Pisum sativum*) plants	4 x ~ 70 days	Lada greenhouse, ISS	Morphology and genetics, space factors	No morphological difference between flight and ground plants

Kranz *et al.* (1990) (Table 4) investigated the biological damage induced by ionizing cosmic rays in *Arabidopsis* seeds (Biorack experiments). They found that damages increased significantly in orbit at several biological endpoints, especially germination and flowering (see Section 7.3; Reproduction and development), with maximum damage for HZE-hit seeds. Of the seeds flown outside the vehicle, the length of the radicle was shorter than from the seeds kept inside the space vehicle. However, the radicle length of HZE hit seeds was not shorter than the backup seeds (control). Since the plant growth characters are also affected for flown but not hit seeds, additional effects of the space environment must be considered. Protons, fast neutrons and disintegrating stars form a source of additional radiation damage, which could give the same effect as HZE particles alone (Kranz *et al.*, 1990).

Legué and co-workers (1996) investigated lentil roots under the following conditions; 1) in microgravity, 2) on an in-flight 1-G centrifuge, 3) in microgravity and placed on a 1G centrifuge after 4 hours, 4) on the 1G centrifuge and placed in microgravity for 4 hours. They found that root length and growth were similar in all 4 samples after 29 hours of growth. However, this was a very short time experiment, and this should be taken into consideration when interpreting the results with respect to radiation effects.

Potato explants consisting of a leaf, an axilliary bud, and a small stem were sent to space in the STS-73 mission (Table 4). At the end of the 16 days mission, tubers were present on each explant. The size and the shape of the explants were similar to the ground control tubers (Cook *et al.,* 1998). Levine and Piastuch (2005) found no differences in the morphology in either cotyledons or hypocotyls of etiolated soybeans germinated in space (Table 4). However, they found that the primary roots were significantly longer in space and the total root production (measured as fresh weight) was increased compared to the ground controls.

Sychev *et al.,* (2007) investigated the effects on four consecutive generations of peas grown on the ISS (International Space Station), with each ontogenetic cycle lasting from 73-76 days. There were no morphological differences between the groups from flight related to ground control. In other space experiments on STS-95, Hoson *et al*. (1999) found a slight promotion of growth of etiolated hypocotyls of *Arabidopsis* and etiolated coleoptiles of rice seedlings. Schulze *et al.* (1992) reported no significant differences in either fresh or dry weight between maize plants grown under 1-G and under microgravity conditions in space.

Summary - morphology and vegetative development

Space experiments reveal that some morphological alterations are found in plants exposed to space as well as in plants derived from seeds kept in space. The Apollo missions (Biostack I and II) had an additional radiation load, since these missions were outside the Earths protective geomagnetic field. In these experiments, the frequency of multicaulous *Arabidodpsis* plants was higher in seeds hit with HZE particles, but the growth of *Vicia faba* radicles was not affected. Yet, other studies found significantly shorter radicles in *Arabidopsis* plants derived from seeds kept outside the space vehicle. However, the radicles in plants from seeds kept inside but hit with HZE particles was not different from the control. In soybeans there were no difference in either cotyledons or hypocotyls, but the primary roots were significantly longer in the space flown seedlings. Other experiments showed no difference in root length in lentil seedlings after exposure to various gravity levels during a 29 hour period. Potato explants developed normally with no morphological alterations in microgravity and so did pea plants grown for 4 consecutive generations on the ISS.

7.2 Space effects on anatomy (cell structures, organelles and cell wall)

Space effects on cell structures and organelles

The articles presented in the following include experiments examining the cell organelles and the cell wall.

Table 5. Results from seven experiments in microgravity focusing on cell structures and organelles.

Author	Organism	Duration	Vehicle/mission	Scope	Result
Lorenzi and Perbal, 1990	Lentil roots, *Lens culinaris*	25.5 hours	STS-61-A, Spacelab D1	Cell structure, microgravity	Displacement of the nucleus in statocytes are caused by a relaxation of the cytoskeleton (actin filaments) in microgravity
Guisinger and Kiss, 1999	*Arabidodpsis thaliana* seedlings. Columella cells	65 hours	STS-84, (Biorack) 1G control in flight	Cell structure, microgravity	Cell size not affected. Amyloplasts show random distribution. Nuclei, ER and mitochondria show normal structure and location. Starch content decreased (due to ethylen)
Kordyum and Guiekema, 2001	*Brassica rapa* seeds	15 days	STS-87	Amyloplasts in root statocytes, microgravity	Normal cap formation in root. In microgravity the amyloplasts did not sediment and had a random distribution in the cell
Kordyum, 2003	Review			Calcium signalling	Redistribution of Ca^{2+} ions and an increase in the intracellular concentration in microgravity
Klymchuk *et al*., 2003	Soybean (*Glycine max*) seedlings	6 days	STS-87	Vacuoles, microgravity	Progressive vacuolization in the root apex cells, more in columella cells. Plasmolysis in columella cells fixed in solutions with high osmolarity.
Nechitailo *et al.*, 2005	Tomato (*Solanum lycopersicum)* plants derived from tomato seeds flown 6 years	6 years	Mir	Cell walls, cell organelles, space factors	Cells in the leaves disorderly arranged. Chloroplast larger with loose structure. Starch grains per chloroplast increased.
Stutte *et al*., 2006	Wheat (*Triticum aestivum* L.) seedlings	21 days	ISS	Cell organelles, microgravity	Chloroplasts were ovoid in shape and thylakoids had a greater packing density.

Lorenzi and Perbal (1990) (Table 5) found that the nuclei in lentil statocytes were displaced under microgravity (also on a clinostat). The distance between the nuclear membrane and the proximal cell wall was 0.87 µm in microgravity, compared to in 1 G (control) where the nuclear membrane was almost in contact with the proximal cell wall. The suggested cause for this nuclear displacement observed in microgravity, was connected to the relaxation of the cytoskeleton (actin filaments).

Guisinger and Kiss (1999) studied the ultrastructure of root cap columella cells of *Arabidopsis thaliana* in microgravity using a 1-G control centrifuge in flight. The relative volume of mitochondria in microgravity was not statistically different from the 1-G flight control, but the relative mitochondria volume in the 1G control on ground was statistically different from the flight control. Neither the distribution of nuclei nor the endoplasmic reticulum (ER) was affected by microgravity, but in the second story cells in the cap there was a decrease in starch levels. This decrease of starch was thought to be a consequence of an ethylene gas build-up within the plant chambers. Amyloplasts did not sediment towards the basal cell wall, but had a random distribution in the statocytes under the microgravity conditions. The amyloplasts in the 1-G flight control, sedimented in a similar manner as in the 1-G control on ground (Guisinger and Kiss, 1999).

These findings are in accordance with the results obtained by Kordyum and Guiekema (2001) and Klymchuk *et al.* (2003) as shown in Table 5. In these experiments they found that in *Brassica rapa* and soybean (*Glycine max*) cells the amyloplasts had a random distribution in the absence of a gravitational vector.

According to the gravity decompensation theory, the following events are expected to happen in non-gravisensing cells exposed to altered gravitational levels: alterations in the physicochemical properties of the membrane → changes in membrane permeability and ion transport → physiological responses. The responses are thought to be induced by the cytoskeleton via the Ca^{2+} messenger system. Experiments have shown that there is redistribution and an increase in the intracellular Ca^{2+} concentration during altered gravity, suggesting that Ca^{2+} signalling plays a fundamental role in cellular biochemical regulation in microgravity (Kordyum, 2003; Iversen *et al.*, 1992). Vacuoles have been shown to increase in root apex cells of soybean flown for 6 days in microgravity and there was also an increase in plasmolysis in cells fixed in solutions with high osmolarity (Klymchuk *et al.*, 2003).

Tomato plants (Table 5) derived from seeds kept in space for 6 years, had disorderly arranged cells and larger chloroplasts with more starch grains per chloroplast than in the control group (Nechitailo *et al.*, 2005). These results contradict those of a similar experiment were tomato seeds were kept for 7 years in space and no anatomical differences

were found in the plants thereafter (Mike Dixon, personal communication). From other experiments it has also been reported that a decrease in starch levels in *Arabidopsis* plants was observed (Guisinger and Kiss, 1999). However, in studies performed by Stutte *et al.* (2006) during the PESTO experiment there were no differences in the starch level or the gene expression of gravity naïve wheat plants kept 21 days on ISS (Table 5). They did however, find that the chloroplasts in wheat had a different shape in microgravity compared to the Earth control samples and the thylakoids were more densely packed (Stutte *et al.*, 2006).

Space effects on plant cell walls

The effect of a space environment on cell walls has not been examined to the same extent. As seen in Table 6, only two articles focusing on this issue are included in this section.

Table 6. Results from two experiments examining the plant cell walls

Author	Organism	Duration	Vehicle/mission	Scope	Result
Nedukha, 1996	Durum wheat (*Triticum durum*), *Impatiens balsamina* and *Funaria hygrometrica*	16 days	Svetoblock, Mir	Cell wall structure, microgravity	Thinner cell wall, 10 x less wax nipples. Fixation 24-36 hours after landing. *Funaria*: 3 times thinner cell wall after 96 days in orbit (Salyut)
Hoson *et al.*, 2001	Rice (*Oryza sativa* L.) and *Arabidopsis thaliana* L. w.t. Columbia and cv.*etrl-1*	68.5, 91.5 and 136 hours	STS-95	Cell wall metabolism, microgravity	A decrease in cell wall constituents (polysaccharides) and increase in cell wall extensibility (irreversibly) in microgravity

Earlier experiments have shown that long term flights can cause considerable changes in cell wall (3 times thinner) in *Funaria hygrometrica* protonema exposed to microgravity for 96 days on Salyut orbital station. These results were not reproduced with moss protonema in a short-time experiment on a clinostat, but were evident after a long time on the clinostat (30 days). At this stage, thinning, loosening, and lysis of cell walls (Sytnik *et al.*, 1984) was observed. Nedukha (1996) found that the cell walls of *Triticum durum* and *Impatiens balsamina* were thinner after a short flight period (Table 6). The number of wax nipples in *T. durum* was found to be 10 times lower and their size two times smaller than in the

ground control. A possible cause for these results may be a decreased synthesis of polysaccharides and a lysis of cellulose microfibrilles in the cuticle leading to an increase of cuticular transpiration. Changes in the cell wall were accompanied by a decrease in cell size, the occurrence of starch and swelling of thylakoids in chloroplasts. However, the fixation of the *T. durum* plants was done 24 hours post-flight and for the *I. balsamina* plants 36 hours after flight, making the results rather insecure.

Hoson *et al.* (2001) examined growth regulation mechanisms and the composition of the cell wall in the monocotyledon rice and the dicotyledon *Arabidopsis* seedlings during the STS-95 mission. The elongation was significantly increased in both rice and *Arabidopsis* hypocotyls. The total amount of cell wall polysaccharides was not affected, but there was a decrease in cell wall polysaccharides per unit length in space grown coleoptiles. This was connected to the irreversible extensibility of the cell wall observed in the space grown plants. No difference was found between the three cell wall fractions (pectins, hemicellulose and cellulose) that were investigated. Spontaneous curvature (automorphogenesis) of the coleoptiles in space was also found, which was basically the same as growing the plants on a 3-D clinostat (Hoson *et al.,* 2001) Automorphogenesis is further discussed in TN 97-07 (Plant movements).

Summary - cell organelles and cell walls

Plant cell organelles are not profoundly affected by microgravity and the results from the space experiments indicate that ER (endoplasmic reticulum), mitochondria and nuclei are not altered during exposure to microgravity. However, amyloplasts, chloroplasts and vacuoles seem to be affected by the microgravity conditions. For example amyloplasts do not sediment to the cell basal wall, but have a random distribution in microgravity. Vacuoles in soybean were found to have an increased size and chloroplasts in wheat had a more ovoid shape in microgravity. Microgravity can result in thinner plant cell walls, with a decrease in cell wall constituents (polysaccharides) and with an increased extensibility, which is irreversible.

7.3. Reproduction and development

Under this heading totally eight articles have been selected (Table 7) where higher plant reproduction and embryo development have been the main focus. Experiments with seeds and seedlings were examined, including both dicot and monocot plants. The experiments have been performed on satellites, in space craft, and on space stations, varying in periods from 68 hours to 69 months.

Table 7. Results from eight experiments focusing on reproduction and development

Author	Organism	Duration	Vehicle/mission	Scope	Result
Merkys *et al.*, 1984	*Arabidopsis thaliana* plants	69 days	Salyut 7	Plant embryogenesis, space flight factors	42 % of the plants produced seeds.
Kranz *et al.*, 1990	*Arabidopsis thaliana* seeds	13 days	Kosmos 1887 satellite	Germination and survival, ionizing cosmic rays	Later germination and flowering frequency reduced in seeds from flight (germinated on ground).
Planel *et al.*, 1994	Tobacco (*Nicotiana tabacum* L.) and rice (*Oryza sativa* L.) seeds	69 months	STS-41C	Germination and survival. Space flight factors	Tobacco; seeds kept in monolayers died, but those kept in bulk survived. Germination rate 22 % compared to approx. 80 % in the control. Germination rate of rice seeds was dependent on the variety.
Musgrave *et al.*, 1997	*Arabidopsis thaliana* plants	6, 10 and 11 days	CHROMEX-03 CHROMEX-04 CHROMEX-05	Reproduction, space factors	In the 1st experiment, both male and female reproductive development aborted. Applied CO_2 and sugar in the following exp. Experiment 2: normal development, but no pollination. With air-flow provided in the 3rd experiment large siliques with seeds were obtained.
Kordyum, 1998	*Arabidopsis thaliana*	Review		Reproduction, microgravity	Sterility of androecium and gynoecium, may be due to hormones or high levels of ethylene gas.
Kuang *et al.*, 2000 Popova *et al.*, 2009	*Brassica rapa* plants	16 days, plants 13 days old at launch	STS-87, Plant growth facility	Pollination and embryo development, microgravity	No stage of reproductive development in *Brassica* is absolutely dependent upon gravity, also embryo development in general. Number of buds and flowers differed from ground control.
Roux *et al.*, 2002	Dormant spores of the fern *Ceratopteris richardii*	68 hours	STS-93	Development in early embryo, microgravity	The gravity-directed polarity development of the spore was normal even in microgravity, followed by a normal cell division.
Salisbury *et a*l., 2003	Super dwarf wheat (*Triticum aestivum*) plants	90, 123 days	Svet growth chamber, Mir	Reproduction, space factors	1. Experiment; no heads 2. Experiment; heads, but no seeds (abnormal pollen grains) may be due to increased levels of ethylene.

Kranz *et al*. (1990) (Table 7) investigated the biological damage ionizing cosmic radiation could have on *Arabidopsis* seeds, exposed both inside and outside a space vehicle. They concluded that the seeds flown in space, and particularly those that were hit with HZE particles had a delayed germination and the frequency of flowering plants was significantly reduced. The frequency of lethal embryos both inside and outside the space vehicle was significantly higher than in the ground control, with the highest frequency on the seeds outside hit with HZE particles. Since plant growth characteristics are also affected for flown but non-HZE-hit seeds, additional effects caused by space factors have to be considered (Kranz *et al.,* 1990). Similar results were obtained in an experiment done by Planel *et al.* (1994) where they studied the influence of long duration space exposure (69 months) to tobacco and rice seeds. The seeds received a total radiation load of 3.75 Gy, and they were shielded against UV-radiation during the period. The results revealed that all tobacco seeds died when kept in a monolayer. However, the seeds kept in bulk survived, but they had a delayed germination and germination rate was 22% compared to approx. 80 % in control, followed by death of seedlings after germination. In rice, the germination rate varied from 60-100 % in the rice var. Cigalon to 0 % in the var. Delta. In this experiment all the controls kept at DLR (German Aerospace Centre) (both var. Cigalon and Delta) died, and of the controls (embryos) kept in Montpellier laboratory germination was 92 % before flight and 6 % six years later for the Delta variety. These findings suggest that one can find huge differences in sensitivity between varieties within one species. However, one can not make any strong conclusions regarding the rice results, since the majority of the control plants were dead (Planel *et al.,* 1994).

In another experiment the gravity directed polarity development in early embryo (fern spores) was investigated (Roux *et al.,* 2002). Migration of the nucleus from the centre of the cell and downwards in a linear fashion about 25 µm from the center to periphery, initiates an asymmetric cell division that results in a two-cell gametophyte. The results showed that the nuclear movement, which appears to be gravity directed on Earth, still occurs in microgravity. The direction of the movement in microgravity is random, and not preferentially directed by signals such as light or mechanical vibrations. This was also found on the clinostat. After the migration of the nucleus, a normal cell division was initiated with a normal prothallus at one end and a rhizoid on the other.

Early plant reproduction experiments (Table 7) performed with dwarf wheat in 1995 on the Mir station demonstrated that the plants either developed no heads or heads with no seeds, as a consequence of abnormal pollen grains. It was suspected that major problems with the hardware and the presence of ethylene gas were the cause of space induced sterility of the plants (Salisbury *et al.,* 2003). This conclusion is supported by Kordyum (1998) claiming

that sterility of both androecium and gynoecium, may be due to hormones, inadequate nutrition or ethylene gas.

The first experiment where one was able to produce viable seeds in space was performed during the long term space experiment onboard Salyut 7 orbital station in 1983 (Table 7). Viable seeds of *Arabidopsis thaliana* were obtained in 5 plants (22 pods) while 2 plants formed 11 sterile seedless pods. However, the experiment proved that growth, flowering and embryo development were possible in microgravity (Merkys *et al.,* 1984). Later, Musgrave *et al.* (1997) did three experiments (CHROMEX-03, 04 and 05) where they studied the plant reproduction process in space. In the CHROMEX-03 experiment, *Arabidopsis* plants were grown in closed plant growth chambers (PGCs), and both female and male gametophyte development aborted at an early stage in the flight group. In CHROMEX-04, carbon dioxide enrichment was provided to the closed PGCs and reproductive development proceeded normally up to the pollination stage. Then there was an obstacle to the pollen transfer in the spaceflight group. In CHROMEX-05, an air-exchange system was used to provide the PGCs with cabin air. Under these conditions, the spaceflight plants had a reproductive development comparable to the ground control and seeds were produced (Musgrave *et al.,* 1997).

Recent experiments done by Kuang *et al.* (2000) showed that all developmental milestones occurred normally in *Brassica rapa* during a 16 days spaceflight (STS-87) (Table 7). It appears that no stage of the reproductive development is absolutely dependent on gravity, but some of the embryos had a lower developmental rate than on ground. There could be some qualitative differences in seed vigor that may result from physical changes in the microenvironment around the seed (Kuang *et al.,* 2000). Popova *et al.* (2009) did other investigations within the same experiment (STS-87) and found that the pollen viability was normal in space, but the quantity of buds and flowers in *Brassica rapa* plants differed relative to the ground control. The number of buds was lower than in the ground control; in contrast the number of flowers was higher than in the ground control. This suggests an acceleration of flowering of plants under spaceflight conditions (Popova *et al.,* 2009)

Summary - reproduction and development

Arabidopsis seeds exposed to space radiation showed higher lethality and a lower germination and flowering frequency than seeds on ground, with the seeds hit directly with HZE particles most dramatically affected. Tobacco and rice seeds kept for an extended period in space (69 months) also showed a lower germination ratio after flight. In rice, there were differences in the sensitivity between the various cultivars, var. Delta being the most sensitive. Migration of the nucleus followed by cell division in the gametophyte in fern spores was normal in space microgravity, even though the movement of the nuclei was

random and not downwards as on the Earth. From several experiments it is concluded that sterility of male and female plant organs can be due to inadequate nutrition and increased levels of ethylene gas. The first successful experiment producing viable seeds in space was performed on Salyut 7 in 1983. Later the three CHROMEX experiments clarified that with addition of carbon dioxide and a continuous air flow through the plant chambers, a reproductive development comparable to the appearance under ground conditions is possible. Developmental milestones are normal in *Brassica rapa* plants kept in space, although there are some differences in the quantity of buds (fewer than on ground) and flowers (more than on ground) produced.

8. Conclusions

There is no clear relationship between the space environment and plant morphology based on several space experiments. This can be a result of the difficulties of getting reproducible outcomes in space, which again is connected to factors like choice of hardware, vehicle, and duration. Other factors are the limited amount of individual plants tested (due to lack of space) in each experiment, and the use of different handling and fixation techniques.

8.1 Conclusion; Morphology

The few experiments on plants that have been done outside the shielding effect of Earths magnetic field, were on the Apollo 16 and 17 missions. These missions experienced an increased level of radiation, and also received “enders” (stopping HZE particles) of Z=10, similar to the conditions one will find on missions to the Moon and Mars. Under these conditions one can suspect an enhancement of biological damage in plant material. During the Apollo 16 and 17 *Arabidopsis thaliana* and *Vicia faba* seeds were kept onboard for 10 and 11 days and were germinated when returned back to the ground. There was no significant impact on the plants growth parameters, even for the seeds directly hit with HZE particles. However, there was an enhanced frequency of multicaulous *Arabidopsis* plants, suggesting an increased level of mutations. Since the mutation was similar in all affected plants, it is likely that one particular spot in the seed was susceptible to HZE radiation, such as the cells of the vegetation cone. The fact that *Vicia faba* plants were not affected and that *Arabidopsis* had only minor morphological deviations, indicates that plant seeds are quite resilient to radiation or that intact cells replace the destroyed cells. Radiobiological experiments in LEO (Low Earth Orbit) where *Arabidopsis* seeds were kept both inside and outside the space vehicle revealed a lower growth found only in the plants derived from the seeds flown on the outside. The radicle length of plants from seeds kept inside the vehicle were normal compared to the ground control. These findings suggest that the shielding of the space vehicle is sufficient to prevent morphological damages, and that

the seeds kept outside are damaged due to increased levels of several types of radiation in combination with the space vacuum. The results are in accordance with the results obtained in radiation experiments done on Earth, where the radiation level must be very high (50 Gy of gamma radiation) in order to create an effect on the morphology in *Arabidopsis thaliana* (see Section 6.2). Other experiments on ground have shown that plant morphology is more sensitive to UV-B radiation than biomass production in species like wheat and rice. However, UV-B radiation is usually not a problem in space experiments, since the space vehicle has to be shielded against this type of radiation due to the presence of a crew.

Several space experiments have revealed that the total sum of factors such as microgravity, magnetic fields and radiation, will have little or no impact on the plant morphology. Lentil and soybean seedlings in short term experiments (4 hours to 16 days) and long term experiments using peas (70 days) showed plants with no alterations in morphology or growth parameters in either of the species. However, from some space experiments a promotion of *Arabidopsis* hypocotyls and coleoptiles of rice and an inhibition of shoot growth in pea and maize seedlings has been reported. Ground experiments on 2-D clinostats have shown both growth inhibition and promotion in soybean shoots and roots, respectively. Other similar experiments using soybean showed no difference in morphology between the clinorotated compared to control with regard to the length, fresh and dry weight of the seedlings. Experiments on 3-D clinostats showed that the emergence of the 3rd internodes in pea was promoted, while the growth of the 1st internode and the total length were inhibited. In maize seedlings the growth of coleoptiles was only little affected, but the growth of mesocotyls was suppressed. It was concluded from this experiment that the decrease in cell wall extensibility and the changed osmotic potential in the cell sap were responsible for the inhibition. This is consistent with the knowledge that vacuoles and cell walls are involved in cell growth through regulation of cell volume and turgor pressure. Why the cited experiments revealed such conflicting results both in space and on ground is difficult to explain. It could be a result of different hardware (build-up of ethylene gas within canisters), different experiment setup, the combination of several known and unknown space factors and the use of different types of clinostats (2-D vs. 3-D) on ground.

Literature dealing with space experiments investigating how the magnetic fields influence the morphological level in plants has not yet been found. Experiments on ground with magnetic fields have given contradictory results. Several experiments on plants exposed to weak magnetic fields (WMF) have shown that the cell cycle slows down, and that growth inhibition of primary roots (broad bean) and significant growth inhibition of sugar beet, pea and wheat seedlings occur.

In other experiments where pea plants have been exposed to WMF, normal magnetic fields and zero magnetic fields, it was found that pea epicotyls were longer when exposed to WMF, compared to the normal geomagnetic field. There was no significant difference

between the seedling growth under the randomized magnetic field and the zero magnetic fields. These various results could be due to different hardware or variation in sensitivity between species and perhaps also between the different cultivars within one species.

In studies of combined effects on ground, there is evidence for an interaction between ionizing radiation and magnetic fields with a synergistic effect associated with a growth delay in root, shoot and coleoptiles. However, when a magnetic field was applied alone the result was a growth inhibition and when applied on pre-irradiated material during germination it was found stimulatory. Similar results are found in other experiments, where cell cultures exposed to magnetic fields after irradiation had a significantly shorter regeneration period. These outcomes suggest that magnetic fields can diminish or hinder the radiation damage in plant cells and might have an important protective role.

Taken together, there is little or no impact on the morphology of plants in either short or long term (one generation period) experiments. There is also no apparent difference in sensitivity between monocotyledons (e.g. rice) or dicotyledons (e.g. peas). These results can be explained by the fact that damage and/or alterations first occur at the cellular level, and a substantial amount of deviations on the cellular level has to take place in order to affect the morphology. However, it is not clear whether there will be an accumulation of the effects over time giving a later effect on the morphological level, or if the plants will adapt and maintain a normal morphology and vegetative development.

8.2 Conclusion; Anatomy

At the anatomical level the deviations in plants exposed to microgravity as well as on clinostats, have mainly been found in specialized root cap cells - the statocytes. Within these cells there are amyloplasts (plastids) that have a gravity dependent sedimentation, and this distribution becomes random in plants exposed to microgravity. Statocytes are therefore suspected to be graviperceptional cells in plants due to the occurrence of moveable statoliths.

In one space experiment the results showed that nuclei, endoplasmic reticulum (ER) and mitochondria are normal in *Arabidopsis* seedlings flown for 65 hours. This must be considered as a short term experiment performed on a model plant. This means that knowledge about the long term impact on these organelles as well as in other plant species is scarce. Mitochondria are shown to react on gamma radiation with a temporal decrease in activity followed by an increase in the resistance to irradiation. This could explain why mitochondria seem to be less sensitive to space conditions. However, mitochondria have also been shown to be very sensitive to weak magnetic fields, with their size and relative volume increased while the cristae membranes decreased. This indicates that the development of the mitochondria could be influenced in flights to the Moon or Mars, where the magnetic fields are highly variable compared to the conditions on the Earth.

Other studies have shown that there is a redistribution of Ca^{2+} ions and an increase of the intracellular Ca^{2+} concentration. These responses are thought to be induced by the cytoskeleton via the calcium messenger system. The responses will induce changes on the physicochemical level, proving the fundamental role Ca^{2+} ions have in biochemical regulation in microgravity. It has been suggested that actin filaments are associated with both the nucleus and the plasma membrane and generates a tension between them. Results are in good agreement with this hypothesis since the displacement of the nucleus in lentil statocytes in microgravity could be induced by a relaxation of the cytoskeleton. On ground in clinostats (both horizontal and vertical), there was also found a displacement of the nucleus in statocytes, although it was less pronounced compared to the displacement found in microgravity. Other experiments on 2-D clinostats showed that there was no difference in the actin cytoskeleton structure or arrangement in the beet seedlings root cap meristem, but an increase of the cytoplasmic actin in the cortex cells. However, the microtubules in the beet seedlings were oriented longitudinally in addition to the transverse arrangement. In *Arabidopsis* plants there were no difference in the microtubule organization. Since these experiments are done on 2-D clinostats, which are considered to be poor microgravity simulators, it is difficult to draw any conclusions.

In soybean flown for 6 days, the vacuoles were increased and there were also an increase in plasmolysis in cells with high osmolarity. Since vacuoles are involved in plant defense systems, the increase could be due to the environmental stress plants experience in space. Vacuoles are also shown to be influenced by weak magnetic fields. Vacuoles are along with the plasmalemma and cell walls controlling both cell volume and turgor pressure,, which are critical to the plants stability as well as development. The result of an unbalance in this system (osmolarity and turgor pressure) could be plasmolysis, which again can lead to complete collapse of the cell wall. Since vacuoles are involved in the sedimentation process of amyloplasts, microgravity is the most likely candidate responsible for the deviations found in vacuoles in flight.

Other experiments documented alterations in chloroplasts in tomato plants derived from seeds kept 6 years in space and in wheat seedlings kept 32 days on the ISS. The chloroplasts in the tomato leaves were larger, and in wheat they were more ovoid with a greater packing density of the thylakoids. Since chloroplasts capture light energy and the thylakoids are the site of the light dependent reaction in photosynthesis, a reduction in the starch metabolism might be the result. A reduced starch content is found in plant cells exposed to microgravity. However, this reduction in the starch content may have been connected to increased ethylene levels rather than microgravity. Alterations in chloroplasts could also have been be induced by radiation, since chloroplasts are shown to be extremely sensitive to gamma radiation compared to other organelles.

Plant cell walls in durum wheat (*Triticum durum*), *Impatiens balsamina* and *Funaria hygrometrica* became thinner when exposed to space for 16 days. In *F. hygrometrica* the cell wall was 3 times thinner after 96 days in orbit. Other space experiments demonstrated a decrease in rice cell wall constituents and an irreversible increase in the cell wall extensibility. The opposite was found in ground experiments with hypergravity, where cell walls had an increased level of polysaccharides. This is suggested to be the plant cell strategy to be able to withstand the increased pressure. Cell walls determine the cell shape and stability and allow cells to develop turgor pressure. Alterations in the cell wall can therefore have major impacts on both the plant's stability and growth. Since the cell wall of fully differentiated cells has lost its elasticity, an irreversible extensibility of the cell wall can hinder the cell differentiation and as such the development of plants. The deviation (thinning) in the cell walls could have been induced by space radiation. It is known that gamma irradiation will increase the activity of polygalacturonase and methyl esterase, which again will degrade pectin and result in a thinner cell wall. However, the space experiments were performed only over a short time period which must be taken into consideration when interpreting the results with respect to radiation load.

Judging from the existing experiments, space conditions seem to have some influence on the anatomy of cell organelles and cell walls in plants. It is difficult to interpret which single factor is the most important, although it is reasonable to believe that the sum of all factors (radiation, gravity, magnetic fields) contributes to the observed effects. It is possible that the plant cells over a longer period of time will adapt to these conditions, or alternatively the deviations will accumulate and influence on a higher morphological and physiological level. These are questions still to be answered.

8.3 Conclusion; Development

Results indicate that the space environments can be lethal and/or reduce the germination frequency in *Arabidopsis* seeds, with the seeds directly hit with HZE particles will have the worst outcome. Similar results were obtained for rice and tobacco seeds kept for 69 months in space. In rice, there were differences in sensitivity between the two cultivars and the var. Delta was the most sensitive. Tobacco seeds also had a delayed germination (22%) compared to the ground control plants (80 %). In this specific experiment the majority of the control seeds died, which therefore makes it difficult to draw a conclusion. However, it is beyond doubt that the increased radiation levels in space and particularly the type found outside the geomagnetic shield will inhibit the germination process to some degree.

Since the seeds that were not directly hit with HZE particles also showed a reduced germination, there is reason to believe that other types of radiation (e.g. secondary radiation) and additional space factors also will be responsible for the lower germination rate. These findings are in accordance with experiments on ground were generative growth

was found to be much more sensitive to UV-radiation than vegetative growth. Gamma- and beta- radiation were equally effective in reducing seedling growth in both dormant and germinating barley seeds. Neutron radiation is 40 times more damaging than gamma- and beta-radiation on dormant seeds and 6-8 times more damaging on germinating seeds. The level of damage increases with increasing radiation load, as observed in space. Based on observations on ground one can also conclude that radiation on male gametes produced more severe effects on the development of the embryo than radiation on the female gametes. This is in accordance with the observations that male gametes are also the most sensitive organism in gravitational studies. However, pollen in some species is relatively resistant to UV-B radiation. There is clearly a species-specific effect, which can give both positive and negative responses to UV-B radiation. This species-specific variation in sensitivity is most likely the case for other types of radiation as well.

Based on results from several experiments one can conclude that sterility of male and female plant organs can probably be caused by inadequate nutrition, problems with the hardware and increased levels of ethylene gas. The first experiment to be successful in producing viable seeds was performed on Salyut 7 in 1983 and 22 pods with seeds were achieved in 5 plants, while 2 plants produced 11 sterile pods without seeds. The experiment was important, however, because it demonstrated that a normal plant reproduction process in space was possible. Around ten years later (1991-95) the three CHROMEX experiments identified the obstacles as well as established the basis for necessary requirements in order to have a successful plant reproduction process in space. The conclusion these experiments gave the basis for was that with the addition of carbon dioxide and with a continuous air - flow through the plant chambers the plants were able to produce viable seeds. These results verified the hypothesis that insufficient nutrition and buildup of ethylene gas will disturb or inhibit the reproduction process.

In experiments done on ground using 2-D clinostats there were in principle no perturbations in the germination in apple, apricot and almond. However, in apple and almond some pertubations were detected in the pollen tube development, with lacking sperm cells or blocking of the pollen tubes. In the woody and herbaceous species *Prunus persica, Prunus avium, Prunus domestica, Prunus communis* and *Brassica rapa* there was no difference in pollen germination but some disturbances in the pollen tube development in all except *P. domestica*. Hypergravity levels from around 3-G induced certain alterations in *Arabidopsis* such as sterile siliques and abnormal pollen tubes. The suggested reason for this is that hypergravity influences tip growth in pollen tubes, which again is driven by turgor pressure and the cytoskeleton characteristics. Hypergravity has been shown to induce a retrieval of actin filaments, which may be the reason for the abnormal pollen development. However, other studies have shown that hypergravity had no adverse effect on flowering, fertilization or embryogenesis. This indicates that in both clinostat rotation and under hypergravity conditions the reproduction success is species dependent, and the

plants reproduction system is adjusted within a narrow range of gravity levels like the ones we have on Earth. This does not change the fact that plants are able to reproduce under both microgravity and hypergravity, but with a slightly reduced reproductive success.

There are few investigations done on how magnetic fields influence the reproductive process in plants. The ones performed indicate that in some species the germination process is unaffected. Other studies report a delay in the germination and in e.g. pea there were both an inhibition and promotion of the seed germination. The results are therefore inconclusive.

Plants can reproduce in space with the adequate hardware and optimal environmental conditions. Since it is proven that generative tissue is more sensitive to radiation than vegetative tissue, it is possible that different hardware or arrangements are necessary in handling the various tissues in space. There are still many questions to be answered; how the reproduction system functions over longer periods of time, the impact of the individual space factors such as magnetic fields, the possibility of synergistic or antagonistic effects of the various space factors on the reproductive system and the important species-specific variation.

9.0 Further work

It is important for the planning of missions to the Moon and Mars to understand how the morphology, anatomy and reproduction in plants respond to low magnetic levels (outside the Earth's magnetic field) the total space radiation load and graded gravity levels. With the total radiation load one understands the sum of HZE particles, all other radiation types (e.g. gamma, UV) as well as secondary radiation (Bremsstrahlung) originating from the decceleration of particles. Studies with graded gravity levels will improve our understanding of how plants will react in environments with low gravity (0.16 and 0.37 G), as are found on the Moon and Mars.

9.1 Future work on ground

Ground experiments would give some indications of how plants will react under the actual space conditions, although the work performed must be understood as preliminary studies and that the interpretation of the results can not replace results obtained from real space studies. The following types of morphological, anatomical and developmental studies (including vegetative and generative development) are needed on the ground;

- Morphological plant studies including the combination of magnetic field (MF) (shielding from Earths geomagnetic and lower MF) using 3-D RPM machines
- Anatomical studies including the combination of MF (shielding from geomagnetic and lower MF), and heavy ion radiation using 3-D RPM machines
- Developmental studies were focus should be on the fertilization process with the combination of MF, heavy ion radiation performed using 3-D RPM machines.
- The studies should include several species; both dicot and monocot plant species should be represented

9.2 Future work in space

Since it is difficult to make proper simulations on ground, experiments in space are necessary to get reliable results relevant for the planning of the Moon and Mars missions. The studies on plant morphology in space are numerous and it is concluded that with the proper shielding and hardware, there is less impact on the morphological level. However, deviations will first appear on the anatomical and cellular level before they eventually manifest themselves at the higher morphological level. It is therefore important to focus in future studies on the plant anatomy. Radiation effects on the genetic apparatus are well documented, but it is still inconclusive how different types of radiation will have an effect on cell organelles and cell walls. References to experiments performed using plant

microfilaments and microtubules have not been found, only experiments done on ground. There is also little knowledge about how the combination of space factors affects plant anatomy and reproductive development. It is not known whether these factors will act in a synergistic, antagonistic or additive way or if there are no relationship at all between them. One also needs to understand how the plant anatomy is affected by graded gravity levels and how the total radiation load affects various plant species. There is also a need to verify the results from developmental studies under graded gravity conditions, including more plant species (preferably edible).

Experiments using different plant species (including monocots and dicots) are preferable, because this will clarify which species are sensitive or resistant to space conditions. Fixation procedures should be done during flight. Plants and seeds are to be preferred before cell cultures, as cell cultures might react in a different manner than a whole plant system. The following types of studies on plant morphology, anatomy and reproductive development should be performed;

- Anatomical studies should be done with plants and seeds exposed to graded gravity conditions by the use of centrifuges onboard a space vehicle. This is important on order to investigate the conditions both under transport to the Moon and Mars (microgravity) and during cultivation where there will be some gravity present.
- Clarify the impact on anatomy and reproductive development in **plants** sent beyond low Earth orbit, including the use of centrifuges onboard to simulate graded gravity levels outside the geomagnetic shield
- Studies of the anatomy and reproductive development in plants kept for longer periods in space, to investigate potential adaptation responses
- Clarification of the factors in space that are most efficient in altering the anatomy and reproductive development in plants
- Clarify if accumulations of deviations on the anatomical level will become operative on the higher morphological level
- Clarify if there are synergistic, additive or antagonistic relations between space radiation, microgravity, graded gravity and magnetic fields affecting the anatomy and reproductive development in plants
- Clarify if those deviations on the anatomical and morphological level have an impact on the quality and quantity of the edible parts of the plant.

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