NTNU
Samfunnsforskning AS

TECHNICAL NOTE 97.03-I 2

Literature Study: Plant genetics

Prepared by/Préparé par Irene Karoliussen
Reference/Réference (C90329)
Issue/Edition 2
Revision/Révision 0
Date of issue/Date d'édition 14.12.2009

APPROVAL

Title *Titre*	Plant genetics	Issue *Edition*	2	Revision *Révision*	

Author *Auteur*	Irene Karoliussen	Date *Date*	14 December 2009

Approved by *Approuvé par*		Date *Date*	

CHANGE LOG

Issue/*Edition*	Revision/*Révision*	Status/*Statut*	Date/*Date*

Distribution List

Name/Nom	Company/Société	Quantity/Quantité
Marina Zabrodina	NTNU Sam.forsk. AS/CIRiS, Norway	1
Knut Fossum	NTNU Sam.forsk. AS/CIRiS, Norway	1
Christel Paille	ESA-TEC	1

Table of contents

1. Introduction

For a plant to grow and develop normally, the Genome provides all information needed. Every organism on Earth has this unique "blueprint" and the discovery of genes have brought insight in two of the biggest mysteries of biology: what makes a species what it is, and what causes variation within a species. In order to have a healthy plant development and reproduction, the genetic machinery must operate without flaws. Plant evolution has developed certain strategies to protect the genetic material and under normal circumstances on Earth, this repair machinery will be adequate to protect the plants. But we know that a number of environmental factors can influence the genome and induce both damages, DNA and chromosome mutations as well as alter the gene expression levels.

2. Objective of WP 200, Plant Genetics

The objective of this WP (Work Package) is to assemble the relevant knowledge within space plant research emphasizing gene expression, genetic aberrations and mutations. From this information a conclusion will be made, and recommendations for future work will be presented.

Focus will be on potential effects of the Moon/Mars physical factors **space radiation, varying gravity** (including sedimentation, droplet sedimentation, isothermal settling, and natural convection)**, magnetic field** and eventual **combined effects** of these factors.
The following assumptions are made in order to limit the vast number of factors: optimal control of temperature, light, pressure (1 bar/ 1000 hPa), water and gas supply/composition. In addition we assume optimal root support, and adequate water and nutrients supply.

For further information on the particular conditions found on the Moon and Mars, see CEAS paper "Literature Study of Higher Plants in Space for MELiSSA (LiRHiPliSME) - Input to the MELiSSA Phase II project" (Kittang *et al*., 2009).

3. Plant reproduction and genetic stability

Plants can reproduce either sexually, which involves male or female plant organs or they can reproduce asexually with rhizomes, fragmentation or budding. In contrast to most animals, many plants species are aneuploid, which means they have an abnormal number of chromosomes (extra or missing). Polyploidy is pervasive in plants and some estimates suggest that 30-80% of living plant species are polyploid. Polyploidy occurs when there are more than two (diploid, 2*n*) homologous sets of chromosomes. Polyploidy types are labeled

according to the number of chromosome sets in the nucleus; from triploid (3 sets) to dodecaploid (12 sets).

The faithful segregation of replicated chromosomes during mitosis is central to maintain a genetic stability. Two nuclear transport factors, Nup98 and Rae1, which normally regulate transport of macromolecules into and out of the nucleus through nuclear pores, have now been found to perform a novel function in mitosis as a defense against chromosome mis segregation. An anaphase inhibitor called securin is targeted for proteolysis by the anaphase-promoting complex when sister chromatid separation becomes due. Nup98 and Rae1 act by preventing the premature degradation of securin, and in their absence the cells become aneuploid, either gaining or loosing extra chromosomes (Jeganathan *et al*., 2005)

3.1 Genotoxic stress in plants and repair mechanisms.

Plant cells are constantly exposed to environmental agents that inflict damage to DNA and cause genotoxic stress, which can reduce plant genome stability, growth and productivity. Plants are most affected by solar UV-B radiation which damage DNA by introducing dimers. These are mainly the photoproducts cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs). Reactive oxygen species (ROS) are also produced in the process, which adds yet another source of genotoxic stress. This stress activates the cellular DNA damage responses and the cell goes into cell cycle arrest to allow the cell to repair the damage. Since genomic stability is fundamental to ensure plant diversity and productivity, these repair mechanisms are essential. The bulky dimers are repaired by photoactivation and require CDP or 6-6PP specific photolyases. In addition there are light independent repair mechanisms such as nucleotide excision repair (NER) and base excision repair (BER) (Tuteja *et al*., 2008). There is no doubt that the primary plant response to UV radiation is shielding, such as pigments (esp. carotenoids) and wax production (cuticula). Also mismatch repair genes (MSH2) were discovered in Arabidopsis and homologues to those were found in *E.coli* and yeast (Vonarx *et al,* .1998).

3.2 Gene expression

Gene expression commonly refers to the entire process by which the information encoded in a particular gene is decoded into a particular protein. Regulation at any of the various steps (transcription, splicing, translation and modification) in this process could lead to a differential gene expression in different cell types or developmental stages in response to external conditions. Gene regulation gives the cell control over structure and function, and is the basis for cellular differentiation, morphogenesis and the versatility and adaptability of any organism. Every function in the living cell depends on proteins (Lodish *et al*., 2000). Gene expression is among other things regulated via DNA cytosine methylation. This process plays an important role in maintaining genome stability and controlling the gene

expression levels. In plants, the degree and spectrum of cytosine methylation occurs at CG (cytosine-guanine) and also at CNG (cytosine-nucleotide-guanine) and asymmetric sites (Ou *et al*., 2008).

3.3 Chromosome and DNA aberrations

Chromosomal aberrations are disruptions in the normal chromosomal content of a cell, and they produce changes in whole chromosomes (more than one gene). The aberrations can be: chromosome clumping, contraction, stickiness, paling, fragmentation, dissolution, chromosome and chromatid bridges.

DNA aberrations include one gene and can be single (SSB) and double DNA strand breaks (DSB), adducts, base modifications, covalent joining between adjacent bases and intra (and inter) strand cross-linking. Both chromosomal and DNA aberrations can have three outcomes:

1. They can be repaired if they are within threshold levels where the DNA repair mechanism operates
2. They can lead to programmed cell death (apoptosis)
3. They can result in mutations, which can lead to heritable changes in the DNA sequence.

3.4 Mutations

Mutation is the process whereby genes change from one allelic form to another. Mutations can lead to loss of a function of a gene or to a new function. Mutations that occur in germ line cells can be transmitted to progeny, but somatic mutations cannot. Genes mutate randomly, at any time and in any cell of the organism (Griffiths *et al*., 1999).

3.4.1 Chromosome and DNA mutations

Chromosome mutations include parts of or the whole chromosome, which means several genes. Four different types can occur;

- Deletion – loss of part of a chromosome
- Duplication – extra copies of a part of a chromosome, this can lead to polyploidy.
- Inversion – reversed direction of a part of a chromosome
- Translocation – part of a chromosome breaks off and attaches to another chromosome

Mutation at the DNA level includes one allele of a gene, so-called “point mutation”. In point mutations one base is altered. These alterations can be; base substitution, base addition or base deletions. Mutations have three different outcomes:

1. Silent mutations: one codon for an amino acid is replaced by another that codes for the same amino acid. The mutation has no effect on the organism.
2. Missense mutation: the codon for one amino acid is replaced by a codon for another amino acid, which can give a non-functional protein.
3. Nonsense mutation: the codon for one amino acid is replaced by a translation-termination (STOP) codon, can also give a non-functional protein (Griffiths *et al*., 1999).

4. Ground experiments

Experiments on ground where one has tried to simulate the space conditions have been done from the very beginning of the space research era. These studies should give an idea on how the space conditions would impact organisms as well as equipment meant to be sent up in space. The experiments included different devices to negate the gravitational pull, so-called clinostats. There are mainly two types, one axis clinostats and two axis (Random Positioning Machines) clinostats. In addition there have been experiments where one has tried to simulate the radiation load in space with different radiation emitters and the latest decades also with the use of heavy ion accelerators (Miller, 2003). Studies on electromagnetic and electric fields have focused primarily on strong static magnetic fields up to 5 T. Only few experiments were done to simulate the non-polar, scattered magnetic fields like the on the Moon and Mars (Figure 1), and their influence on organisms.

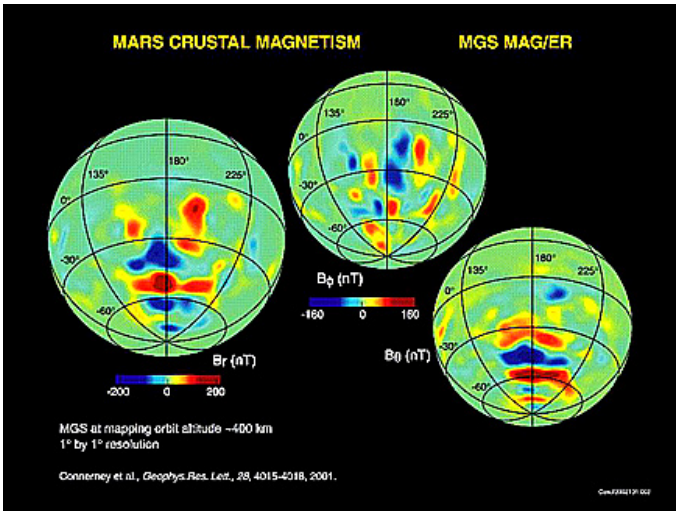

Figure 1; Mars crustal magnetism (Connerney *et al*., 2001)

4.1 Simulated gravity, Clinostats

The chosen articles presented here include effects on the gene expression level or distribution and localization of the genetic apparatus within the plant cell. Experiments done on clinostats focusing on mutation and aberration frequencies in plant cells have not been found so far.

Table 1. Results from experiments done on different types of clinostats and in microgravity (sounding rocket)

Author	Scope	**Results**				
		2-D clinostat	3-D RPM	Hypergravity	Magnetic levitation	Microgravity at space experiments
Sobol *et al*., 2005	Distribution of nucleolar subcomponents in cress (Lepidium sativum L)	Redistribution of rDNA and NopA 100 nucleolin				
Martzivanou *et al*., 2006	Gene expression in cell cultures (Arabidopsis thaliana)	No statistical difference	No statistical difference	No statistical difference (8G)		7 genes up - regulated after 6 minutes in a sounding rocket
Babbick *et al.*, 2007	Gene expression in cell cultures (Arabidopsis thaliana)	9 genes with altered expression	9 genes with altered expression	9 genes with altered expression (8G)	9 genes with altered expression	

Sobol and coworkers investigated cress (*Lepidium sativum* L) root meristematic cells in a slow rotating (2rpm) 2-D clinostat (Table 1). They found a redistribution of both ribosomal DNA and NopA100 (plant nucleolin) in nucleolar subcomponents, induced by clinorotation. The suggested effect of this was a lowering of rDNA transcripts as well as rRNA processing (Sobol et al., 2005). Martzivanou and colleagues (Table 1) did not find any significant difference in the gen-expression (about 4500 genes in the array) between *Arabidopsis* cell cultures on a 3-D clinostat, in hypergravity and the 1 G control. They found, however, an increased gene transcript level after 6 minutes in microgravity (sounding rocket). The genes involved were mainly components of early signaling chains. The following genes were up-regulated: puryvat kinase (PK), ammonium transporter (AMT 1), arginine decarboxylase (ARG-Dec), diacylglycerol (DAG), diacyl glycerol kinase

(DGK), homoserine kinase (HSK) and the transcription factor WRKY33 (Martzivanou *et al*., 2006).
Another group did, however, find significant differences in the WRKY gene expression after exposing *Arabidopsis* cell cultures to a Random Positioning Machine (3D-clinostat). They exposed cell cultures to a 2-D clinostat, a Random Positioning Machine (RPM), hypergravity (8G) and magnetic levitation. For 9 of the 12 transcripts studied, hypergravity (8G) and 2-D clinostat resulted in nearly identical responses [AGL 84, ERF 5, phosfatidylionistol-4-kinase (PI4K9), WRKYs 3, 22, 46 and 65) or mainly similar responses (ERF sub, IAA 19 and WRKY 70). The RPM and magnetic levitation also formed similar trends for ERF 5, ERF sub, IAA 19, MYB and WRKYs 3.6.22.46 and 70. The significant changes in gene expression came from the RPM and magnetic levitation with between 8-32 fold increases in the transcript level of WRKY. For WRKY 3, 6 and 75 the RMP had the highest increase and for the genes WRKY 22 and 46 magnetic levitation had the highest transcript induction (Babbick *et al*., 2007).

4.1.1 Summary clinostats

The two gene expression experiments show that it is difficult to get reproducible results using clinostats. However, it is suggested that RPM and magnetic levitation are more preferable tools to simulate microgravity than clinorotation. These experiments were performed on cell cultures rather than whole plants. We can therefore not directly relate these results to a whole plant system, which could respond in a different manner.

4.2 Radiation effects on higher plants genome

Radiation that can damage plants and act as mutagens are:

Ionizing radiation which includes:

- X rays
- Gamma rays
- UV radiation
    1. UV-C (180-290 nm) germicidal and most lethal
    2. UV-B (290-320 nm) lethal/mutagenic fraction
    3. UV-A (320-400 nm) (non-ionizing photochemical reactions)

Cosmic rays (particles) from the sun and outer space (Galactic cosmic rays) including:

- Protons, alpha (Helium nuclei) and beta particles (electrons) and neutrons
- High energy atomic nuclei (HZE). HZE particles have a higher LET (linear energy transfer) value compared to the total radiation received on the Earths surface with low LET values.

The types of DNA damage resulting from radiation are many and varied. They include adducts, cross linking between DNA bases, single (SSB) and double strand breaks (DSB) and a broad range of base damages. Ionizing radiation, such as X-rays, gamma rays and alpha particles produce single and double strand breaks. Single strand breaks dominate after exposure for X-rays, while the frequencies between SSB and DSB are more similar with alpha particles, for which the ionizing density is higher than with the X-rays (Casarett and Doulls, 2001).

Experiments studying UV radiation effects show that UV-B radiation is most dangerous for plants, since it penetrates the tissue and is absorbed by DNA and generaterating DNA dimers (CPDs) and 4-6PPs (Tuteja *et al*,. 2008). Other studies done with heavy ion accelerators reveal that high-LET radiation and gamma radiation increase both the mutation and chromosome aberration frequency linearly in maize and rice. All effects where dose-dependent up to 90-100 Gy, and high LET radiation was 2-12 times more efficient than gamma rays (Mei *et al*,. 1994). Earlier work has also proven that the aberration and mutations frequencies caused by neutron and X-rays were higher in diploids than in tetra- and hexaploids (wheat and barley). It was concluded that with increasing ploidy the radiation sensitivity decreased (Bhaskaran and Swaminathan, 1961).

4.2.1 Summary radiation effects

Ionizing as well as non-ionizing radiation can cause damage to the genetic apparatus, in the form of an increased level of mutations and various types of aberration. Studies show that with increased LET values the damages increase linearly, and heavy ions being 2-12 times more efficient for mutations and chromosome aberrations than gamma radiation.

4.3 Magnetic fields, effects on higher plants genome

Table 2. Results from three experiments done on cell cultures under various magnetic field conditions. The cited experiments were done with shielding from the Earths magnetic field.

Author	Organism and scope	Exposure	Results
Harris *et al.*, 2009	*Arabidopsis* seedling, gene expression	Static magnetic fields from 0-100 mT(shielding from the Earths magnetic field).	In 0, 50 and 500 µT, no difference in gene expression. In 100 mT the gene coding for *GST* (glutathione S-transferase) had a significantly lower expression than in the zero field (0 Tesla)

Atac *et al*., 2007	Peroxidase and total RNA levels in soybean (*Glycine max*) tissue cultures	Static magnetic fields with a flux of 2.9- 4.6 mT for 2.2 and 18.6 seconds	Peroxidase levels increased significantly in both groups (magnetic flux for 2.2 and 18.6 seconds) compared to control. Total RNA levels also increased significantly, with the highest level in the group exposed for 2.2 seconds
Pingping *et al.*, 2007	Genotoxic effects on wheat *(Triticum aestivum*) pollen mother cells	Static magnetic field. 0,1,3,5 and 7 T for 5 hours or 7 T at 1,3 and 5 hours	Below 5 T there was no significant difference in the aberration frequency compared to unexposed groups. With a field strength of 5-7 T there was an increase in aberrations, such as chromosome bridge, lagging chromosome, triple-polar segregation and micronucleus. There was no linear relationship.

Harris and coworkers (2009) measured the gene expression level of chalcone synthase (*CHS*) and *HY5*. *HY5* encodes a transcription factor element in the promoter and regulates the cryptochrome regulated genes including *CHS*. Cryptochrome is a photo-response receptor in plants and is also believed to be the magnetic field responder. The level of *GST* (glutathione S-transferase) was also studied, a gene that has protective roles in the plant (detoxification processes) and expression levels of which can indicate plant stress (Edwards *et al*., 2000). They found no significant difference in the cryptochrome regulated genes, but a significant decrease in the *GST* expression (19 %). The results indicate that there is a response to stress, but that the static magnetic fields of this magnitude (100 mT) did not induce any effect on cryptochrome genes (Table 2).

Another study found that both peroxidase and total RNA levels increased in soybean tissue cultures exposed to magnetic fluxes of 2.9-4.6 mT for 2.2 and 18.6 seconds. Peroxidase is known to be involved in many biotic and abiotic stress responses in plants, and the increase of total RNA could be due to the increase of peroxidase as well as other biosynthesis reactions in the cell (Atak *et al*., 2007). Pingping and colleagues (2007) found that a static magnetic field with strength from 5-7 T induced aberrations in wheat pollen mother cells.

4.3.1 Summary magnetic fields

These results indicate that strong magnetic fields (5-7 T) on top of geomagnetic fields and with shielding of the magnetic field are potentially genotoxic (aberrations), and medium weak magnetic fields (mT) can influence on stress response (*GST*) genes in plant cells. However, the experiments are performed by using cell cultures and in static magnetic fields. How the whole plant will react under the magnetic conditions on the Moon and

Mars (highly variable, non polar and very weak fields) is difficult to interpret from experiments done with static and much stronger fields.

5. Space flight experiments

5.1 Space effects on gene expression levels

The publications described in Table 3, 4 and 5 are chosen based on the following criteria: They include experiments on whole plant systems, as opposed to cell cultures. The environmental conditions within the space vehicle are controlled and monitored.

The papers referred to in Table 3 present results describing the "space effects" i.e. the combined effects of radiation, microgravity and magnetic fields. Publications that focus on the gene expression levels in flight in microgravity, space radiation and magnetic fields alone have not yet been found.

Table 3. Results from space experiments using four different plant species and the effects on the gene expression level.

Author and year	Vehicle	Scope	Organism	Duration	Results
Paul *et al*., 2005	Columbia STS-93, PGC (Plant Growth Chambers)	Gene expression	*Arabidopsis thaliana* plants	5 days	Most of the 21.000 genes in the array were unaffected by spaceflight.182 genes were altered more than 4-fold. Two sets of genes were altered by more than 10 fold.
Ou *et al*., 2008	Long March 2 spaceship	Gene expression and DNA methylation	Rice (*Oryza sativa* L) seeds exposed, later germinated on Earth	18 days	Changes in gene expression, both up- and down-regulation in 6 transposable elements (TEs) and 11 cellular genes. Increased methylation pattern in flight, heritable to progeny.

Sugimoto *et al.*, 2008	ISS, Lada greenhouse	Gene expression levels in defense/stress response genes	Barley (*Hordeum vulgare* L) seeds germinated and grown on ISS	26 days	ROS (reactive oxygen species) reducing proteins (antioxidant) were both up- and down-regulated as well as unaffected by flight
Visscher *et al.*, 2009	Mir, swetoblock M unit	Gene expression in wheat grown for 3 generations after flight	Wheat (*Triticum aestivum*) plants	167 days	No statistical differences in gene expression levels in three generations of wheat after flight.

Paul and coworkers (2005; Table 3) found that 182 genes were altered after spaceflight with a 4 fold (both induction and repression), and only 50 of them were expressed from moderate to high levels. Two sets of genes were differently expressed (distinguished by a common regulatory or metabolic function within the set) by at least a 10 fold. The genes that were noticeable repressed included genes for Photosystem II type I chlorophyll *alb* binding (CAB) proteins. Other genes that were down- regulated were F-box (transcription factor) proteins and a protein involved in senescence. The genes mostly induced by spaceflight were heat shock proteins (HSPs) with both small and large molecular weight (HSP 22 and HSP 17.6). The down-regulation of CAB genes remains unexplained, except that spaceflight is being reported to influence photosynthesis. Heat shock proteins react both on temperature as well as different environmental stress factors (e.g drought and nitrogen deficiency), so the up-regulation of these genes in flight could be a result of such stress. In the described experiment (Paul *et al.,* 2005) only one DNA array chip was used, resulting in a rather insufficient statistical basis.

The gene expression level in rice was altered in spaceflight (Ou *et al*., 2008). Of the 6 TEs (transposable elements) studied, 4 (mping, Osr2, Osr23 and Osr36) were not expressed in ground control plants. Of the 11 cellular genes, 4 (Yf25, Receptor kinase, Mismatch repair and Deacetylase) were not expressed on ground. Four genes (S3, Homebox, SNF-FZ14 and Hsp70) showed significant alterations in flight. All TEs and 7 cellular genes had alterations in cytosine methylation, all being hypermethylated. The level and pattern of methylation are influenced by stress conditions such as cold, drought and heavy metal contamination. A proposed function for cytosine methylation in eukaryotes is to serve as a genome defense system. Given the uniqueness of the spaceflight environment, it is likely that cytosine methylation is affected. Results also revealed that on an individual basis, there was no correspondence between alteration in methylation and alteration in gene expression, TE or cellular genes. The methylation pattern was heritable to progeny, the gene expression levels

were not. However, the numbers of loci investigated were small, so one can not exclude the possibility of a relationship in both methylation and transcription.

Sugimoto and colleagues (2008; Table 3) found that the stress response genes CAT (catalase), PR13 (pathogenesis-related proteins) and PAL (phenylalanine ammonia-lyase) were induced in flight. APX (ascorbate peroxidase) and PR1a and PR1b were decreased. SOD (superoxide dismutase) and GST (gluthatione S-tranferase) were not changed compared to the control. AOR (ascorbate oxireductase) and PHGPX (hydroperoxide gluthatione peroxidase) were not detected in both flight and ground. In the expression of pathogenesis-related genes (PR), PR13 was up-regulated and PR1a and PR1b decreased. Lipid transfer proteins (LTP), responsible for environmental changes, were not detected. Of 17 defense/stress response genes, only 3 were induced in space-grown barley. The results suggest that plants grown in the Lada greenhouse on ISS are not damaged by oxidative stress induced by space factors. However, these samples were fixed on ground; one can therefore not exclude the potential secondary effects of transporting the plants to ground.

In the last publication presented in Table 3, wheat plants were flown 167 days on Mir and formed viable seeds which were transferred back on the ground. These seeds were grown here for three additional generations. Visscher and coworkers (2009) found no alterations in the genetic expression pattern in the wheat plant three generations from growth in space. This suggests that exposure to a spaceflight environment in Low Earth orbit does not cause significant heritable changes in gene expression patterns (10263 oligonucleotide probes in the array). One can not make this statement for the rest of the genome that was not investigated in this experiment (Visscher *et al.*, 2009).

Other studies have investigated the relationship between expression of alcohol dehydrogenase (ADH) and hypoxia in *Arabidopsis thaliana* plants grown in space. The control plants showed no evidence of ADH expression in roots or shoots. All plants in flight had an ADH expression in the distal regions of primary roots, many with a dramatic accumulation (Paul *et al.*, 2001).

5.1.1 Summary effects of space flight on the gene expression level

Considering the experiments presented in Table 3, one of the experiments gave no statistical difference in the expression of the genes between space and ground (Visscher *et al*., 2009) The other experiments showed that there were both an up- and down-regulation of genes in space flight, including defense response genes such as catalase, Hsp and superoxide dismutase. It is therefore not clear how the space environment influences the gene expression for oxidative stress response in higher plants in space vehicles in Low Earth orbit. However, there were indications of an increase in the DNA methylation in space and this hypermethylation was proven to be heritable to progeny.

5.2 Space effects on aberration ratio

Studies performed with focus on DNA and chromosome aberration in plant cells have been concentrating on the factor known to induce such damages also on Earth, namely the radiation load. There is less work done on the aberration frequency in plant cells that could originate from microgravity alone. However, there are experiments done on human cells (blood lymphocytes), indicating that microgravity does not modify the yield of chromosome aberrations induced by high-energy protons. In other words, there was no synergism between radiation and microgravity (Manti *et al.*, 2005). Even though human and plant cells have some features in common, these results can not be directly transferred to plant cells. However, since the plants have a cell wall we can assume that they would be even more protected.

The publications presented in Table 4 are focusing on how the space radiation load affects the aberration frequency in plant cells. No papers have been found describing the effects of either magnetic fields or microgravity, neither how these factors alone or in combination influence the aberration ratio in plants.

5.2.1 Radiation effects

Table 4. Results from of experiments investigating the space radiation effect on genetic aberrations in plants.

Author and year	Vehicle and hardware	Scope	Organism	Duration	Results
Gaubin *et al.*, 1983	Biosatellite Cosmos 1129, plastic support in containers	Determine the HZE particle flux. Genetic aberration	Lettuce (*Lactuca sativa)* and tobacco (*Nicotiana tabacum*) seeds	19 days	Higher incidence of single chromosome aberrations, with maximum damage outside the spacecraft
Anikeeva *et al.*, 1983	Salyut 6	Genetic aberration	*Arabidopsis thaliana* and *Crepis capillaris* seeds	49, 223 and 827 days	Accumulation of genetic damage in embryonic meristem cells.

Nevzgodina *et al*., 1989	Salyut 6 and 7, container inside crew module	Radiation effects on the gene aberration frequency	Lettuce (*Lactuca sativa)* seeds	40-457 days	Hit seeds had more aberrations, increasing over time.

Earlier work done by Gaubin and coworkers (1983; Table 4) showed that lettuce and tobacco seeds flown (both in and outside) the Biosatellite Cosmos 1129 for 19 days had a higher incidence of genetic aberrations than in the control plants. Lettuce seeds had a higher frequency of single chromosome aberrations, with a maximum damage on the seeds kept outside. The most pronounced effect occurred when the root meristem cells were hit. The seedlings developed from the tobacco seeds hit by HZE particles had the highest percentage of chromosomal abnormalities.(23.4 %) compared to the non hit seeds (11.7 %) and ground control which had 7.9 % (Gaubin *et al*., 1983).

Anikeeva and colleagues (1983) demonstrated that space flight resulted in the accumulation of genetic damage in embryonic meristem cells in seeds of *Arabidopsis thaliana* and *Crepis capillaries.* The seeds were flown onboard the orbital station Salyut 6. Flight cells had 0.16, 1.04 and 13.3 % aberrations compared to 0.13, 0.45 and 3.46 % aberrations in control cells(on ground) after 49, 223 and 827 days respectively (Anikeeva et al., 1983). Yet another experiment on Salyut 6 and 7, using lettuce, showed a higher incidence of aberrations and increasing damage ratio with extended flight periods. These results are in accordance with data found by Gaubin and colleagues (Nevzgodina *et al*., 1989).

The series of spaceflight experiments, both outside the geomagnetic field (Apollo 16 or 17) or in Earth orbit (Biostack on ASTP, Biobloc on Cosmos 782, 1129, and Salyut 7) clearly demonstrate an increase in multiple chromosome aberrations in higher plants such as *Lactuca sativa* and anomalies in *Nicotiana tabacum* seeds. There seems to be differences in the two groups, lunar (more damages) vs. Earth orbit. Nevertheless it demonstrated that the hit of a single HZE particle can create damages to plant seeds (Horneck, 1994). Experiments conducted on Soyuz, and Salyut 5, 6 and 7, revealed a higher incidence of aberrations in actively developing systems (*Arabidopsis* seedlings) than dormant seeds. This can be explained by two reasons: in resting seeds the cells are affected in g1 phase, while in germinating cells, all cell cycle phases occur and can be affected by radiation. On the other side, weightlessness can cause repair suppression which is more significant in active than resting systems (Kostina *et al.,* 1984).

5.2.2 Summary: radiation effects on the aberration frequency

The conclusion of these experiments must be that the radiation load is the most important factor in space flights that causes a higher genetic aberration ratio. Dormant seeds are more resistant to aberrations than actively developing systems. There might be synergistic effects between radiation and microgravity; however, it is only possible to separate the effects of these two factors by using an in-flight control on a centrifuge, but this has not been done so far.

5.3 Space effects and mutation

Literatures presented in Table 5 represent work done on the radiation load in space and mutation frequency in plants. Studies that focus on the effect of magnetic fields and microgravity separately or in combination and how they impact on the mutation frequency, have not yet been found.

5.3.1 Radiation effects on the mutation frequency

Table 5. Space radiation effects on the mutation frequency in plants

Author and year	Vehicle	Scope	Organism	Duration	Results
Zimmermann *et al.*, 1996	SL-1, LDEF, IML, D2, ERA, BION 8, 9 and 10 comparative studies	Radiation effects on the gene mutation frequency	*Arabidopsis thaliana* seeds	Short term missions (11-13 days) and long term missions (2107 days)	Mutation frequencies highest for the seeds in Earth tray LDEF (Control)
Kranz *et al*., 1990	Kosmos 1887 satellite	Radiation effects on the gene mutation frequency	*Arabidopsis thaliana* seeds	13 days	Seeds with HZE hits had the highest mutation frequency inside the spacecraft

Zimmermann and coworkers (1996; Table 5) compared the mutation frequencies in *Arabidopsis thaliana* seeds from several short and long term missions (SL-1, LDEF, IML, D2, ERA, BION 8, 9 and 10). The long term mission LDEF (2107 days) had an

approximately absorbed dose of 1000 μGy d^{-1} compared to the BION 8,9 and 10 (11-13 days) which had a absorbed dose of 170-270 μGy d^{-1} . The survival of the seeds was 77 % in LDEF (2107 days) and 75 % in Bion 10 (11.5 days). On the shorter D2 mission (10days) the seed survival was 34 %, but the seeds had been pre-irridated with gamma rays before flight. The mutation frequencies were highest for the Earth tray LDEF (the mutation frequency of flight LDEF seeds were not determined), around 23, compared to the short term missions which had a frequency from 0.45 to 16.4 (Zimmermann *et al*., 1996). This result indicates that seeds on ground can accumulate mutations over time which exceeds the mutation frequency on short term missions in space. The quite similar survival rates between long and short term missions indicate that seeds can survive for a long period and are still viable.

Studies performed by Kranz and colleagues (1990; Table 5) demonstrated that *Arabidopsis thaliana* seeds kept both inside and outside the Kosmos satellite, the seeds that were hit with HZE particles had a higher mutation frequency than the seeds not hit by HZE particles. The highest mutation frequency was for the seeds outside that were hit with HZE particles. However, they also found that the seeds outside the spacecraft (not shielded) that were not hit by HZE particles also had a high mutation frequency. The explanation for this could be that in space there is additional radiation besides HZE-particles, such as protons, fast neutrons and secondary radiation. In addition the seeds placed outside have less shielding and are exposed to space vacuum (Kranz *et al.,* 1990).

5.3.2 Combined effects on the mutation frequency.

Table 6 presents a summary of articles in which the "space flight effects" on the mutation frequency in plants were studied. With "space flight effects" we understand the total collection of factors present, like launch vibration, radiation, microgravity, magnetic field and others.

Table 6. Three experiments presenting the space effects on the mutation frequency in plants.

Author and year	Vehicle	Scope	Organism	Duration	Results
Nechitailo *et al*., 2005	Mir space station	Morphology, polymorphism and gene mutation frequency	Tomato (*Solanum lycopersicum*) seeds	6 years	29 DNA bands were polymorphic, polymorphism being 10.8 %.

Sychev *et al*., 2007	Mir, Lada greenhouse	Morphology and polymorphism	Pea (*Pisum sativum*) plants	70, 73, 76, 76 and 73 days	No genetic polymorphism was found after 4 consecutive generations on Mir.
Li *et al*., 2007	Recoverable satellite JB-1	Gene mutations from space flight	Rice mutants (*Oryza sativa*)	15 days	Mutations prefer to occur at polymorphic regions.

Nechitailo and colleagues (2005) investigated the mutation frequency in tomato seeds kept on Mir for 6 years. The seeds were germinated and grown on Earth after flight. Forty primers were used to amplify genomic DNA by using the RAPD method (Random amplified polymorphic DNA). In total 269 DNA bands were produced, from which 29 DNA bands were polymorphic with a polymorphism being 10.8 %. The DNA mutation frequency was in 5 individual plants 8.4, 3.2, 2.8, 6.0 and 9.2 %. The mutation frequency in the control plants were 5 x 0 and 0.4 %, respectively (Nechitailo *et al*., 2005).

Another study on the space station Mir investigated the space effects on morphology and DNA polymorphism in pea plants (polymorphism is defined as the discontinuous variation of alleles; different alleles can give different phenotypes). Four consecutive generations of peas were grown in the Lada greenhouse, and there was no genetic polymorphism found in the pea plants in flight from the 1st generation (Sychev *et al.,* 2007). Since they used pea plants instead of seeds, one could expect a higher incidence of polymorphism, since active developing cells are more vulnerable than dormant seeds. However, this experiment lasted only for 70 to 76 days, compared to 6 years in the tomato seeds experiment.

Li and coworkers (2006) sent dry rice seeds on a recoverable satellite for 15 days into space. The radiation dose was 2.65 mGy. Recovered seeds were sown and grown with their parallel ground control. Three phenotypic mutants (mutant as a consequence of being in space) were chosen. These stable mutants were used for AFLP (Amplified Fragment Length Polymorphism) analyses. The three strains had a mutation rate of 4.3, 6.9 and 0.4 %, meaning that the mutation degree was different among the rice plants with a high variation frequency and wide variation range. This is consistent with the findings of Nechitailo and colleagues (2005) in tomato seeds. Of the observed mutation sites in two of the strains, 75.9 % and 84.9 % occurred in polymorphic sites with the remainder in conserved regions. Only 5 mutations were found in the last strain, but they found that they were 100 % in polymorphic sites. These results indicate that certain regions in the rice genome are potential “hotspots” for mutations induced by the space environment (Li *et al.,*

2007). Hotspots are the DNA clusters that are easily changed by the physical and chemical factors, and the contrasting concept “cold spots” are usually conserved in the genome. This may be of great importance in radiation protection in the plant genome.

5.3.3 Summary of radiation and the combined effects on mutation frequencies in plants

Radiation studies using *Arabidopsis thaliana* seeds showed that seeds hit with HZE particles had the highest mutation frequency, both inside and outside the space vehicle. The hit HZE seeds outside with the highest mutation frequency. The explanations for this could be that outside there are several factors contributing to the radiation load, such as secondary radiation and other types of radiation than HZE particles (Zimmermann *et al*., 1996). Three different experiments reported that there were 1) no genetic polymorphism in pea plants grown 76 days on Mir 2) 10.8 % polymorphism in tomato plants derived from seeds kept 6 years on Mir and 3) studies on rice show that mutations occur in polymorphic “hotspots” within the genome (Table 6).

6. Conclusions

There is no clear relationship between the findings from 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.

6.1 Conclusion; gene expression in plants

In one study wheat seeds were kept in space for 167 days. The seeds were sowed on ground after flight, and the gene expression from three generations of plants was analyzed. No statistical differences between the groups were found. These results suggest that exposure of wheat seeds to low Earth orbit does not cause any significant heritable alterations in the gene expression patterns. This indicates that some types of seeds can be rather resistant to the effects of space. Other experiments, however, observed in rice seeds there were both an up- and down-regulation of some defense response genes such as catalase and superoxide dismutase and no differences in other plant defense response genes. These enzymes react to environmental stress with a steady increase in their expression quantity until their threshold levels are reached. If the stressors increase above the threshold level, the enzymes capacity is overwhelmed and the expression of the enzymes is repressed. From these various results it is difficult to suggest how the oxidative stress level in higher plants will be influenced

under space conditions. Another experiment using rice seeds showed both alterations in gene expression and DNA methylation. The level of methylation is influenced by environmental stress and is suggested to serve as a genome defense system. There was no apparent linkage between the methylation level and the gene expression. This implies that gene expression level can be altered under space conditions, but not necessarily in connection to the methylation degree. It is unclear which factor/s in space produces this up-regulation in DNA methylation. However, the results on both wheat and rice seeds suggest that wheat seeds are more resistant to the space conditions than rice seeds.

Arabidopsis plants had an altered gene expression after 5 days in flight. Of 21 000 genes analyzed, 182 genes were altered with a 4 –fold and 2 sets of genes with a 10-fold expression.
Among the repressed genes, one set were genes linked to the photosynthetic apparatus. This could be a result of the observed altered photosynthetic output under space conditions. The genes most induced were heat shock proteins (Hsp), which are known to respond to different stresses such as heat, inflammation, hypoxia and toxins. We can conclude that there are factors in space that will stress the *Arabidopsis* plant and result in an induction in the stress genes level after 5 days in flight. Whether it is microgravity or radiation alone or a synergy between them and other factors responsible for this changed expression level remains unknown. It is also not known how the gene expression level in *Arabidopsis* would be affected after longer flights. It could be an increase, decrease or it could over time result in normal gene expression level, the latter as a consequence of adaption.

Studies done on clinostats have given ambiguous results. In one experiment there was a comparison between different clinostats, hypergravity and microgravity. The test organism was *Arabidopsis* cell cultures. They found an up-regulation of 7 genes in microgravity after 6 minutes, among them enzymes involved in glucolysis (Pyruvat kinase), amino acid metabolism (arginine decarboxylase) and WRKY (transcription factor) genes. The WRKY proteins play important roles in various processes unique to plants, including disease resistance, seed germination and plant development.

There were no alterations after exposure to either of the clinostats - or hypergravity. Yet other studies found gene expression alterations in *Arabidopsis* cell cultures in both hypergravity and on 2-D and 3-D clinostats, among them an increase in WRKY expression levels. These findings are rather difficult to interpret, although it seems that 3-D clinostats are more suited to simulate microgravity. However, these results also indicate that *Arabidopsis* cell cultures can be sensitive to various gravity levels simulated on ground and thereby react with an induction in plant disease resistance genes. If a similar gene expression would occur in a whole plant system, is unclear.

Studies on how magnetic fields influence the gene expression level have been done on ground, and alterations in *Arabidopsis* gene expression were found above 100 mT. There

was no difference in lower magnetic fields (0-500 μT). These experiments however, have been done with rather strong and static fields, very unlike what we will find in space, on the Moon and Mars. It is therefore difficult to make any conclusions from those results.

6.2 Conclusion; aberration frequency

There are a vast number of ground experiments that have proven both the range and type of damages caused by radiation in plant and animal cells. The types of damage include adducts, single (SSB) and double strand breaks (DSB) and cross linking between bases. Studies using maize and rice show that high-LET (Linear Energy Transfer) values give more damages than low-LET values, and high- LET heavy ion radiation gave 2-12 times more damages than gamma radiation. Experiments have also established the fact that with increasing ploidy, there will be a decrease in aberration frequencies. Magnetic field studies on ground have demonstrated that below 5 T (Tesla) there was no difference in the aberration frequency compared to unexposed groups. However, by using exposures between 5-7 T there was an increase in aberrations such as chromosome bridge, lagging chromosomes, triple-polar segregation and formation of micronucleus. Five to seven Tesla are a very high level, not corresponding to magnetic levels either in low Earth orbit (0.00005 Tesla) or beyond the geomagnetic shield. These results are therefore of low value for this particular research area (space).

Many radiation experiments have been done in space were HZE dosimeters have measured the total hit of HZE particles and the connection of hits to the aberration frequency in plant cells. These experiments clearly demonstrate that seeds such as lettuce and tobacco hit with HZE particles have an increased level of single chromosome aberrations. On the other side, seeds that were not hit with HZE particles outside the space vehicle also had a high degree of damages. This indicates that other factors beyond HZE particles were responsible for the damages, such as secondary radiation, vacuum or microgravity. The space flight was followed by an increase in morphological deformations of the seedlings (atrophy of cotyledons, roots and stems), and the seeds hit by HZE particles with the highest percentage of abnormalities. Other experiments have found an accumulation of aberrations in the embryonic meristem in *Arabidopsis* and smooth hawks beard (*Crepis capillaris*) seeds, but only after an extended period (827 days) in space. These aberrations lead to an increase in sterile seeds and a delayed germination compared to the control. It is also confirmed that actively developing systems have a higher incidence of aberrations than dormant ones (seeds), and the degree of damages increases with longer periods in flight. To conclude, there is no doubt that the radiation levels in space give aberrations to various degrees depending on the plants stage in lifecycle and duration of the flight. These aberrations can give lethal mutations, silent mutations or the mutant cells can be eliminated in the course of plant generative development. It is known that the process determining aging is defined by the reduction of cellular repair activity, which again is believed to be

influenced by microgravity. This suggests there might be synergistic effects between radiation and microgravity. However it is difficult to separate the effects of these two factors.

6.3 Conclusion; mutation frequency

Comparative studies were done between short and long term missions and the radiation impact on *Arabidopsis* seeds. The results showed that the Earth control seeds (long term mission) had a higher mutation ratio compared to seeds in short term space missions. The seeds that were hit with HZE particles had the highest mutation rate (22 %). These results are in accordance to other studies done on the aberration frequency, which display a similar pattern where more aberrations are found in cells hit with HZE particles. However, a high frequency of aberrations does not automatically give a high frequency of mutations, as aberrations can be repaired or the individual radiation hit cell can die (apoptosis). An increased level of mutations does not necessary result in physiological or morphological alterations in the plant, since mutations can be "silent" which means that they have no effect (chapter 3.4.1).
Studies on how space effects influence the mutation frequency reveals that on tomato seeds flown for 6 years the polymorphism was 10.8 %. The morphology and anatomy of the tomato plants derived from the space flown seeds was affected as well, with a change in seedling height and in the cell ultra structure. However, in another experiment tomato seeds were flown for 7 years in space, and these seeds produced normal tomato plants in any aspect (personal communication, Mike Dixon). Other experiments on pea plants, grown for 4 consecutive generations, showed no changes in either morphology all four generations or genetic polymorphism in the 1st generation. These results are in contrast with the fact that active developing plants are known to be more sensitive to space factors than dormant seeds. However, the seeds were kept in Mir for 6 years in contrast to 76 days for the whole plants, giving a possible accumulation of mutations in the seeds over time. Or these variable results could be due to differences in the sensitivity to space effects within the two plant species. Other interesting studies indicate that space induced mutations in rice appeared to accumulate at polymorphic regions within the genome. The regions were called "hotspots", which can be explained as DNA clusters that are easily changed by physical and chemical factors. This may be of great importance in the protection of the plant genome in future space flights. Magnetic field research has not been focusing on the mutation level in plants that will be exposed to the highly variable magnetic fields in space, or on the Moon and Mars.

7. Future work

It is important for the planning of missions to the Moon and Mars to understand how the gene expression level, DNA methylation, aberration and mutation frequency in plants respond to very low and non static magnetic fields (outside Earths geomagnetic shielding) the total space radiation load and graded gravity levels. The total radiation load can be defined as the sum of HZE particles, all other radiation types (e.g. gamma, UV) as well as secondary radiation (bremsstrahlung) originating from the deceleration of particles. Studies with graded gravity levels will give us an understanding of how plants will react in environments with low gravity (0.16 and 0.37 G), as the one we will find on the Moon and Mars, and how they will cope with microgravity during the transport phase.

7.1 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 genetic studies (including gene expression, aberration and mutation frequency) are needed on the ground;

- Genetic studies including the combination of various magnetic field (MF) strengths (shielding from Earths geomagnetic and weaker MF) and simulated microgravity on 3-D RPM machines.
- Genetic studies including the combination of MF (shielding from Earths geomagnetic and weak MF), and heavy ion radiation on 3-D RPM machines.
- The studies should include several species; both dicot and monocot plant species should be represented.

7.2 Future work in space

Since it is difficult to make proper simulations on ground, experiments in space are necessary and of major importance to get reliable results relevant for the planning of the Moon and Mars missions. The studies on radiation and aberration frequencies are numerous. We have therefore a lot of knowledge about the radiation impact on plant cells. However, we have less information on the combination of radiation, microgravity and magnetic fields. We don't know whether these factors will act in a synergistic, antagonistic or additive way on the plants genetic material, or if there is no relation at all between them.

We also need to understand how the genetic apparatus reacts to graded gravity levels and how the total radiation load affects various plant species.

Experiments using different plant species (including monocots and dicots) are preferable, because this will clarify which species are sensitive and resistant to space conditions. Fixation procedures should be done in flight. Plants and seeds are to prefer before cell cultures, as cell cultures might react in a different manner than a whole plant system. The following types of studies on gene expression, aberration and mutation frequency in plants should be conducted:

- Genetic studies should be done with plants and as well as seeds kept in graded gravity conditions by the use of centrifuges onboard
- Clarify gene expression levels, DNA methylation, aberration and mutation ratio in plants sent beyond low Earth orbit (outside the geomagnetic shield)
- Studies of the gene expression levels, DNA methylation, aberration ratio and mutation frequency in plants kept for longer periods in space, to investigate potential adaption responses
- Clarification of the factors in space that are most efficient in altering the gene expression and DNA methylation pattern in plants
- Clarify if polymorphic "hotspots" are a universal treat among edible plant species
- Clarify if there are synergistic, additive or antagonistic relations between space radiation, microgravity and magnetic fields affecting the genetic machinery in plants
- Clarify how the total space radiation load affects the genetic apparatus in higher plants

References

1 Anikeeva, I. D., Kostina, L. N. & Vaulina, E. N. Experiments with air-dried seeds of *Arabidopsis thaliana* (L) heynh. and *Crepis capillaris* (L) wallr., aboard Salyut 6. *Advances in Space Research* **3**, 129-133 (1983).

2 Atak, Ç., Çelik, Ö., Olgun, A., Alikamanoğlu, S. & Rzakoulieva, A. Effect of magnetic field on peroxidase activities of soybean culture. *Biotechnology and Biotechnological Equipment* **21,** 166-171 (2007).

3 Babbick, M. Dijkstra C, Larkin O.J., Anthony P., Davey M. R., Power J. B., Lowe K.C., Cogoli Greuter M. & Hampp R. Expression of transcription factors after short-term exposure of Arabidopsis thaliana cell cultures to hypergravity and simulated microgravity (2-D/3-D clinorotation, magnetic levitation). *Advances in Space Research* **39**, 1182-1189 (2007).

4 Bhaskaran, S. & Swaminathan, M. S. Chromosome aberrations, changes in DNA content and frequency and spectrum of mutations induced by x-rays and neutrons in polyploids. *Radiation Botany* **1**, 166-181 (1961).

5 Casarett & Doulls. Toxicology, the basic science of poisons. *McGraw-Hill, New York* , 924-925 (2001).

6 Connerney, J. E. P., Acuña, M. H., Wasilewski, P. J., Kletetschka, G., Ness, N. F., Lin, R. P. & Mitchell, D. L. The Global Magnetic Field of Mars and Implications for Crustal Evolution. *Geophysical Research Letters* **28**, 4015-4018 (2001).

7 Edwards, R., Dixon, D. P. & Walbot, V. Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. *Trends in plant science* **5** (2000).

8 Gaubin, Y., Planel, H., Gasset, G., Pianezzi, B., Clegg, J., Kovalev, E. E., Nevzgodina, L. V., Maximova, E. N., Miller, A. T. & Delpoux, M. Results on Artemia cysts, lettuce and tobacco seeds in the Biobloc 4 experiment flown aboard the soviet biosatellite Cosmos 1129. *Advances in Space Research* **3**, 135-140 (1983).

9 Griffiths, A. J. F., Miller, J. H., Suzuki, D. T., Lewontin, R. C. & Gelbart, W. M. An introduction to genetic analysis. *W.H Freeman, New York*. 464-475 (1999).

10 Harris, S.-R., Henbest, K. B., Maeda, K., Pannell, J. R., Timmel, C. R., Hore, P. J. & Okamoto, H. Effect of magnetic fields on cryptochrome-dependent responses in *Arabidopsis thaliana*. *Journal of the Royal Society* 1-13(2009).

11 Horneck, G. HZE particle effects in space. *Acta Astronautica* **11**. 749-755 (1994).

12 Jeganathan, K. B., Malureanu, L. & Deursen, J. M. v. The Rae1–Nup98 complex prevents aneuploidy by inhibiting securin degradation. *Nature* **483**, 1036-1039 (2005).

13 Kittang, A.-I., Wolff, S., Coelho L.H., Karoliussen I., Fossum, K.R.,Iversen, T.-H. Literature Study of Higher Plants in Space for MELiSSA(LiRHiPliSME) - Input to the MELiSSA Phase II project. CEAS Space Journal – Submitted (2009).

14 Kostina, L., Anikeeva, I., Vaulina, E. The influence of space flight factors on viability and mutability of plants. Advances in Space Research **4**. 65-75 (1984).

15 Kranz, A. R., Bork, U., Bücker, H. & Reitz, G. Biological damage induced by ionizing cosmic rays in dry *Arabidopsis* seeds. *Nucl. Tracks. Radiat. Meas.* **17**, 155-165 (1990).

16 Li, Y., Liu, M., Cheng, Z. & Sun, Y. Space environment induced mutations prefer to occur at polymorphic sites of rice genomes. *Advances in Space Research* **40**, 523-527 (2007).

17 Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D. & Darnell, J. Molecular cell biology. Editor: Sara Tenney. Publisher: *W.H Freeman and Company*, *New York* (2008).

18 Manti, L., Durante, M., Cirrone, G.A.P., Grossi, G., Lattuada, M., Pugliese, M., Sabini, M. G., Scampoli, P., Valastro, L. & Gialanella, G. Modelled microgravity does not modify the yield of chromosome aberrations induced by high-energy protons in human lymphocytes. *International Journal of Radiation Biology* **81**, 147-155 (2005).

19 Martzivanou, M., Babbick, M., Cogoli-Greuter, M. & Hampp, R. Microgravity-related changes in gene expression after short-term exposure of *Arabidopsis thaliana* cell cultures. *Protoplasma* **229**, 155-162 (2006).

[20] Mei, M., Deng, H., Lu, Y., Zhuang, C., Liu, Z., Qiu, Q., Qiu, Y., & Yang, T.C. Mutagenic effects of heavy ion radiation in plants. *Advances in Space Research* **14**, 363-372 (1994).

[21] Miller, J. Proton and heavy ion accelerator facilities for space radiation research. *Gravitational and Space Biology Bulletin* **16,** 19-28 (2003).

[22] Nechitailo, G. S., Jinying, L., Huai, X., Yi, P., Chongqin, T. & Min, L. Influence of long term exposure to space flight on tomato seeds. *Advances in Space Research* **36**, 1329-1333 (2005).

[23] Nevzgodina, L. V., Maksimova, E. N. & Kaminskaya, E. V. Effects of prolonged exposure of lettuce seeds to HZE particles on orbital stations. *Advances in Space Research* **9**, 53-58 (1989).

[24] Ou, X., long L., Zhang, Y., Xue, Y., Liu, J., Li, X. & Liu, B. Spaceflight induces both transient and heritable alterations in DNA methylation and gene expression in rice (*Oryza sativa* L.). *Mutation research* **662**, 44-53 (2008).

[25] Paul, A-L., Daugherty, C. J., Bihn, E. A., Chapman, D. K., Norwood, K. L. L. & Ferl, R. J. Transgene expression patterns indicate that spaceflight affects stress signal perception and transduction in Arabidopsis. *Plant Physiology* **126,** 613-621 (2001).

[26] Paul, A.-L., Popp, M. P., Gurley, W. B., Guy, C., Norwood, K. L. & Ferl, R. L. Arabidopsis gene expression patterns are altered during spaceflight. *Advances in Space Research* **36**, 1175-1181 (2005).

[27] Pingping, Z., Ruochun, Y., Zhiyou, C., Lifang, W. & Zengliang, Y. Genotoxic effects of superconducting static magnetic fields (SMFs) on wheat (*Triticum aestivum*) pollen mother cells (PMCs). *Plasma Science and Technology* **9**, 241-247 (2007).

[28] Sobol, M. A., Gonzalez-Camacho, F., Rodrıguez-Vilarino, V., Kordyum, E. L. & Medina, F. J. Clinorotation influences rDNA and NopA100 localization in nucleoli. *Advances in Space Research* **36**, 1254-1262 (2005).

[29] Sugimoto, M., Shagimardanova, E., Gusev, O., Levinskikh, M., Sychev, V. & Grogoriev, A. Gene Expression of Barley Grown in Space. *Space Utilization Re*search **24,** 412-414 (2008).

30 Sychev, V. N., Levinskikh, M. A., Gostimsky, S. A., Bingham, G. E. & Podolsky, I. G. Spaceflight effects on consecutive generations of peas grown onboard the Russian segment of the International Space Station. *Acta Astronautica* **60**, 426-432 (2007).

31 Tuteja, N., Ahmad, P., Panda, B. B. & Tuteja, R. Genotoxic stress in plants: Shedding light on DNA damage, repair and DNA repair helicases. *Mutation research* (2008).

32 Visscher, A. M., Paul, A. L., Kirst, M., Alling, A. K., Silverstone, S., Nechitailo, G., Nelson, M., Dempster, W. F., Van Thillo, M., Allen, J. P. & Ferl, R. J. Effects of a spaceflight environment on heritable changes in wheat gene expression. *Astrobiology* **9,** 359-367 (2009).

33 Vonarx, E. J., Mitchell, H. L., Karthikeyan, R., Chatterjee, I. & Kunz, B. A. DNA repair in higher plants. *Mutation Research* **400**, 187-200 (1998).

34 Zimmermann, M. W., Gartenbach, K. E., Kranz, A. R., Baican, B., Schopper, E., Heilmann, C. & Reitz, G. Recent results of comparative radiobiological experiments with short and long term expositions of *Arabidopsis* seed embryos. *Advances in Space Research* **18**, 205-213 (1996).