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Full Length Research Paper

Effect of space flight factors on alfalfa seeds

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To explore the effect of space flight factors on the early development of alfalfa seedling, dry seeds were placed onboard a satellite for a 15-day flight. After retrieval, the ultra structure of seed coat and the chemical content of seed were tested, followed by tests for germinate ability, seedling growth, and mitotic and chromosome aberrations. Results showed that space flight factors have both positive and negative effects on alfalfa seeds. Positive effects include: (1) A 6.2% increase in germinate potential and (2) an 80% decrease in the number of hard seed in flight seeds. Meanwhile, negative effects included a decrease of 3.0 and 33.2% in the index of germination and vigor of flight seeds, respectively, which may be partly due to the inhibition of cell mitotic (26% less than ground control) and root growth (29.0% less than ground control) after the space flight. Moreover, the DNA and Ca^{2+} content of alfalfa seeds increased after the space flight, while the reserve energy content of alfalfa seeds, such as saccharine and fatty acid, decreased after the space flight. Conclusively, space flight factors accelerate the germination process of alfalfa seeds but restrain the root from growing due to chromosomal damage and abnormal mitosis induced by cosmic radiation.

Key words: Alfalfa, space flight factors, germination, chromosome aberration.

INTRODUCTION

Since the beginning of human space exploration, the responses of higher plants to space conditions have been explored because they serve a central component in controlled ecological life support systems. Numerous reports have shown that the exposure of dry seeds to space flight factors have various biological effects, such as morphological changes (Yu et al., 2007), alteration in cell mitosis and organelle shape (Jiao et al., 2004), chromosome aberration (Ren et al., 2008), gene mutation (Li et al., 2007), and even gene expression changes (Cheng et al., 2007). Moreover, many stable mutants with valuable traits such as improved biomass yield or quality (Ma et al., 2007), biotic or abiotic stress tolerance (Xiao et al., 2008), and change in heading date (Wei et al., 2006) have been selected and used in developing new cultivars (Cyranoski, 2001). However, most of these research focused mainly on food crops such as rice (Wei et al., 2006a; Cheng et al., 2007), wheat (Gu and Shen 1989), and tomato (Hammond et al., 1998). In addition, research related to forage was rarely reported, thus limiting the application of space flight-induced mutation on forage breeding.

With high biomass yield and nutrition, alfalfa is one of the most important forage in the world. Researches have shown that space flight factors caused significant changes in the amylase pattern of alfalfa leaves (Xu et al., 1999). In addition, the total amino acid content of alfalfa leaves was also observed to increase after experiencing space flight (Xu et al., 1999). In this paper, the germinate ability and chromosome aberration of flight seeds were tested, then the ultra structure of seed coat and the chemical content of seed were analyzed. The results of these tests would help us understand the primary effect of space flight factors on alfalfa seeds, which will boost the application research of space flight factors induced mutation on alfalfa breeding in the future.

MATERIALS AND METHODS

Experimental materials

Alfalfa seeds were provided by the Grassland Research Institute,

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Chinese Academy of Agricultural Sciences.

Space flight treatment

The selected seeds were grouped into two portions: One was kept at 7℃ for ground control; the other was prepared for space flight. The seeds were enclosed in cloth bags and placed aboard a Shijian-8 seed breeding satellite for a 15 days flight from September 9 to 24 in 2006. The flight conditions were as follows: The distances for perigee and apogee were 180 and 469 km, respectively; orbit obliquity was 63º, and cabin temperatures at flight stage were 7.21- 20.72℃. Heavy particle rate was 4.44 particles/cm 2·days. Average space radiation dose for plant seeds at linear energy transfer (LET) space was 4.79 mGy.

Measured indices

Germination test

The flight seeds and control were germinated on top paper (TP) at a temperature of 20℃. Indexes such as germinate rate, number of hard seeds, and germinate potential were counted following the procedure described by ISTA (2005). Both flight and ground control included 4 replications of 50 seeds.

Observation of mitotic and chromosome aberrations

Dry seeds were germinated following the conditions used in the germinate test. The roots were clipped and treated with the buffer (ethanol: acetic acid = 3:1) for 20 h. Next, it was dyed for 48 h. The cell tips were observed and pictured with a Sony DSC - F828 electron microscope. Indexes such as mitotic index, rate of chromosome aberrations, and rate of nuclei aberrations were tested. The mitotic index (MI) was calculated by the ratio of the number of mitotic cells to the total number of observed cells. The rate of chromosome aberration was calculated by the ratio of the number of cells with chromosome aberrations to the total number of observed cells. The rate of nucleic aberration was calculated by the ratio of the number of cells with nucleic aberrations to the total number of observed cells.

Ultra structure analysis

Dry seeds were fixed and sprayed gold in a vacuum environment. The seeds were then observed and pictures were taken using the electron microscope KYKY-2800B SEM.

Roman spectroscopy analysis

The seeds were analyzed by the Fourier transform infrared raman spectroscopy E55+FRA106 (Bruker Company, German). The wave length was 514.5 nm with a measurement range of 4000 to 100cm –1.

Data processing Experimental

Experimental data were analyzed using an Independent samples T-test program in SPSS12.0 statistical software. The spectroscopy data were analyzed by the Analysis-Spectroscopy program of OriginPro 8.0. The spectroscopy were classified following the report by Edwards et al. (2005).

RESULTS

Effect of space flight factors on the germinate ability of alfalfa seeds

The percentage of hard seeds in flight seeds decreases 80% than that of the ground control. Meanwhile, the germination potential of flight seeds increased by 6.2% compared with that in the ground control (Table 1), which indicated that seed germination was stimulated during the early stage (1- 4 d) after space flight. However, there was no significant difference in germination rate between the space flight and its ground control, indicating that, unlike traditional physical mutation, space mutation just injured the seeds so slightly that there was little lethal mutation and most of the seeds could grow normally.

In addition, the indexes of germination and vigor decreased 3.0 and 33.2%, respectively, which may be due to the inhibition of root growth (29.0%) after the space flight. Seedlings also developed from flight seeds showed damages such as etiolating, cotyledon indentation, and cotyledon curl to different extents, with the total percenttage of 18%. There were few abnormal cotyledons observed in the control group (Table 1).

Effect of space flight factors on the epidermal structure of alfalfa seeds

The control seed's surface coating was covered with an evenly distributed convex. Few holes were also observed on the surface of the ground seeds (Figure 1A). However, the situation was quite different for the flight seed. The surface of the flight seed coat was covered with irregular-shaped tubers. Moreover, obvious holes were found on the seed coat (Figure 1B).

Effect of space flight factors on the chromosome of alfalfa seeds

Space flight factors showed great mutated effect on cell nucleolus, which subsequently stimulated cell mitosis. The mitotic index of seedlings developed from flight seeds decreased about 26.9% less compared with the ground control (Table 2). Moreover, the rate of nucleolus aberrations (that is, single micronucleus or multi micronucleus) increased significantly in flight seeds (1.49%) more than in the ground control (0.35%). In addition, the rate of three kinds of chromosome aberrations (that is, bridge, fragment, and conglutination) increased significantly.

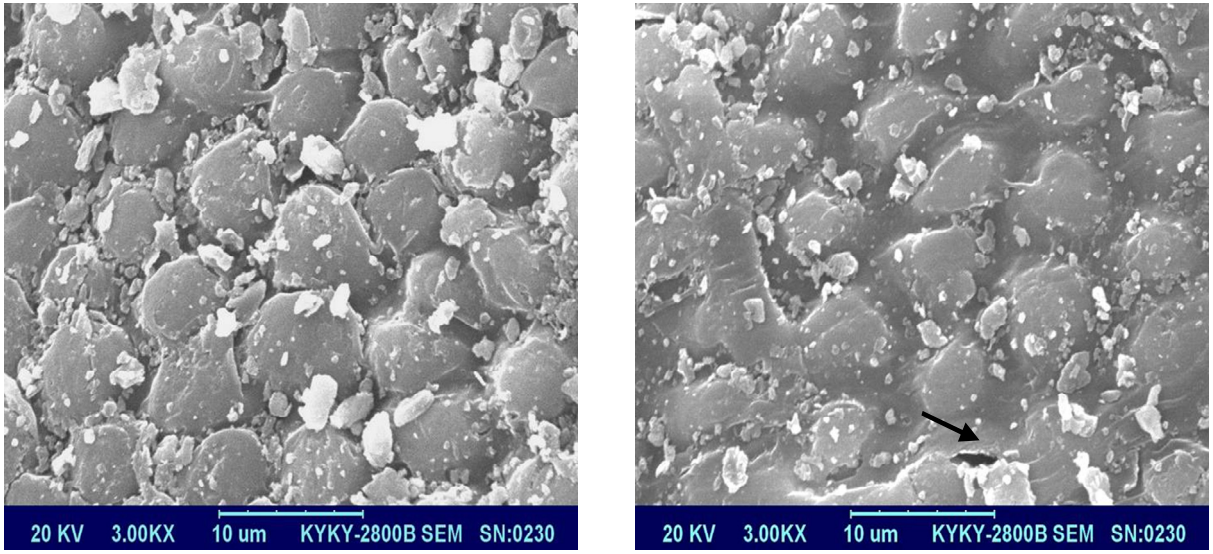
Effect of space flight factors on the chemical component of alfalfa seeds

Results showed that the intensity of two peaks (358 and

Table 1. Effect of space flight factors on the germinate ability of alfalfa seeds.

Treatment	Number of hard seeds	Germination potential (%)	Percentage of germination(%)	Length of stem (cm)	Length of root (cm)	Index of germination	Index of vigor	Percentage of abnormal cotyledon (%)
Ground control	1.66*± 1.52	67.5± 8.66	95.00 ± 5.00	2.35 ± 0.39	3.24**± 1.09	69.05*± 8.05	473.36**±33.44	18.02** ± 1.24
Space flight	0.33 ± 0.58	71.7*± 2.88	96.67 ± 3.82	2.64±0.76	2.30±0.81	66.98± 4.15	316.14±22.18	0.00 ± 0.00

*,** Indicate significant difference at the level of 0.05 and 0.01, respectively.



A B

Figure 1. Ultra structure Comparison of alfalfa seed coat (magnified with 3000diameters. **A** is ground seed coat; **B** is flight seeds. The arrows indicate the hole which has been made by the cosmic radiation particles during the space flight.

Table 2. Effect of space flight factors on cell nucleus and chromosome of alfalfa seeds.

Treatment	Index of mitotic (%)	Single micronucleus (%)	Multi micronucleus (%)	Total rate of cell aberrations (%)	Chromosome bridge	Chromosome fragment	Conglutination	Rate of chromosome aberration (%)
Ground control	18.17*±4.32	0.24±0.01	0.11±0.25	0.35±0.32	0.23±1.25	0.44±0.32	0.04±0.15	0.71±0.23
Space flight	13.28±5.86	0.88*±0.03	0.61*±0.44	1.49**±0.56	0.72**±0.87	0.81*±0.15	0.12**±0.44	1.65**±0.56

*, ** Indicate significant difference at the level of 0.05 and 0.01, respectively.

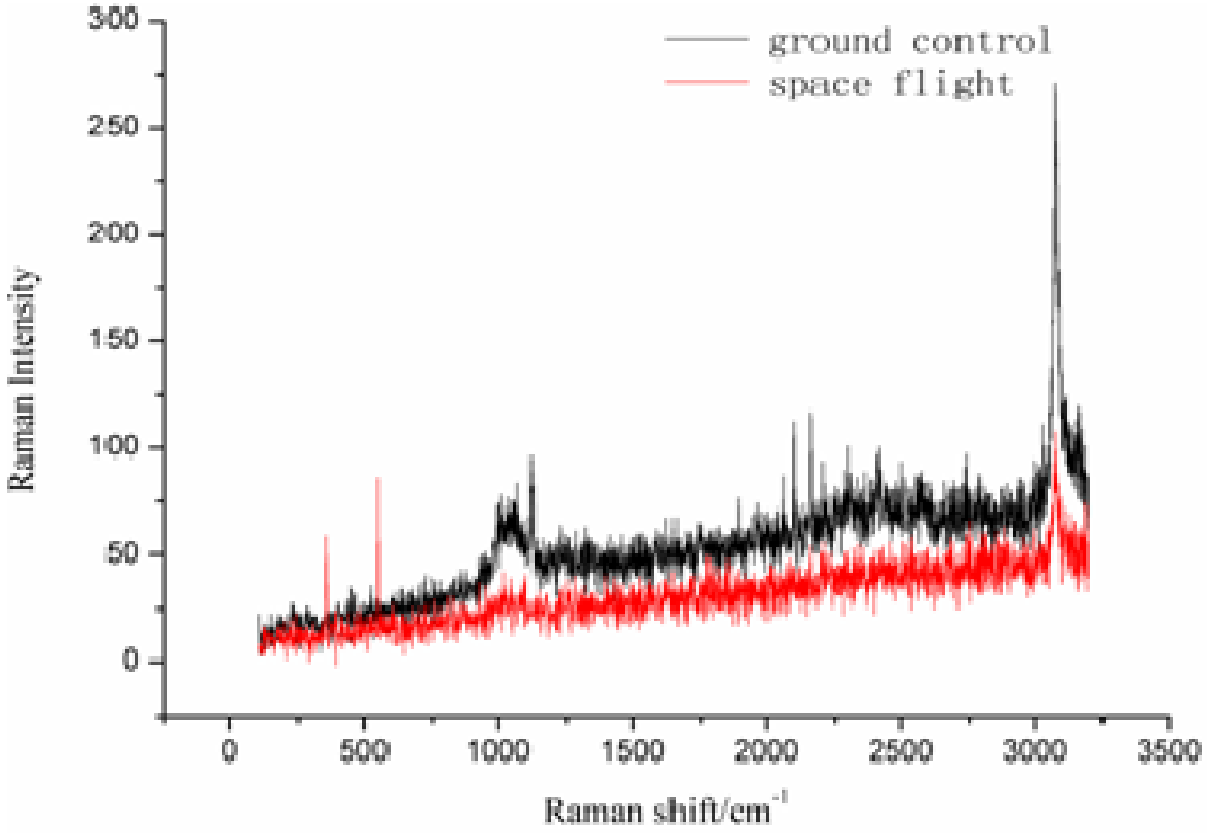


Figure 2. Comparative analysis of Raman spectroscopy between the space flight and ground control.

Table 3. Classification of Raman spectroscopy and their biology function.

Raman Shift/cm^{-1}	Tentative assignment	Possible materials/functions	State
358	Calcium	Cell signal transmission	Up-regulating
553	δ(N_1-C_2-N_2) Thymine	Deoxyribonucleic acid synthesis	Up-regulating
612	V(C-C) Fatty acid chain	Unsaturated fatty acid	Down-regulating
814	V(C-C) Fatty acid chain	Unsaturated fatty acid	Down-regulating
1122	V(C-O) Saccharide	Energy reserve	Down-regulating
1531	V(C=C)	Carotenoids or coloring matter	Down-regulating
1743	V(C=O) Ester	Fatty acids	Down-regulating

553 cm^{-1}) of space flight seeds had been increased and the intensity of four peaks (814, 1122, 1531 and 1743 cm^{-1}) of space flight seeds were decreased when compared with its ground control (Figure 2). Based on Raman spectroscopy classification, the increased peaks of 358and 553 cm^{-1} are related to DNA and Ca^{2+}, respectively (Table 3), which meant that the DNA and Ca^{2+} content of alfalfa seeds had increased after space flight. The decreased peaks of 814, 1122, and 1743 cm^{-1} are related to saccharine and fatty acid, respectively, which mean that the reserve energy content of alfalfa seeds had decreased after the space flight.

DISCUSSION

Seed dormancy is regarded as the failure of an intact viable seed to complete germination under favorable conditions (Bewley, 1997). It is well known that alfalfa seeds belong to coat-enhanced dormancy, which indicates that alfalfa seeds are prevented from germination because the embryo is constrained by its surrounding structures, especially the seed coat. The seed coat's compact structure prevents water and oxygen from entering the interior of the seeds, a feature often termed as "impermeability" (Rolston et al., 1978). The imperme-

ability of alfalfa is attributed to the thick cell wall area in the outer end of its palisade tissue (Lute, 1928). Many factors such as radiation (Nelson et al., 1968), heating (Ellis and Palmer, 1973), and freezing (Lunden and Kineh, 1957) could reduce the impermeable seed content of alfalfa.

In this research, we first reported that the number of alfalfa hard seeds decreased after being exposed to a space environment. It was speculated that etch-holes, which may be induced by high energy cosmic particles during space flight, may provide additional channels for water and oxygen to enter the interior of the seed and activate the germination of alfalfa seed. This speculation also explained why the germination potential of flight seeds was higher than that in the ground control. The following analysis of epidermal structure provided direct proof for the speculation.

Due to the bombardment of high-energy cosmic particles, the epidermal structure of flight seeds showed significant etch-figure and etch-holes. Moreover, we speculated that these etch-holes might provide additional channels, which may bring on two points: (I) these channels might allow more cosmic particles to enter the interior of the seed, which would subsequently induce greater DNA damage and mutation effect. A previous report supported this deduction indirectly. The mutation effect of the seeds which had experienced space flight twice was much greater than that of the seeds which had experienced space flight only once (Xie et al., 2004). One of the possible reasons may be that etch-holes formed during the first flight provided additional channels for the cosmic particles going deep into the seed during the second space flight; (II) these channels allow water and oxygen into the interior of seeds more easily than those without these channels, which may result in the nutritional and epidermis layers becoming more porous in the space-exposed seeds than on the earth-based control seeds. The porous nutritional region may allow the seeds to receive necessary nutrients and liquids more rapidly, thus enabling the seedling to grow at a faster rate as well as to reduce the number of hard seeds indicated by this research.

Spectroscopy analysis showed that there were two changes in the chemical component after the space flight. One was the increased DNA content, which may be due to two reasons: (1) The activation of a repair mechanism for DNA damage which induced space flight factors (especially cosmic radiation), and (2) the syntheses and duplication process of DNA induced by cell mitosis. The other was an increase in free Ca^{2+} content. Ca^{2+} is implicated as a messenger in coupling various environmental stimuli, such as gravity and response (Poovaiah et al., 1987). It may also be stimulated by two factors: (1) The requirement of signal transduction by the DNA repairing process, and (2) the complexity of gravity conditions during the space flight, especially the hypergravity which occurred when the satellite was launched. Recent researches in A*rabidopsis thaliana* have provided additional proof. Toyota (2007a, b) found that both cytoplasmic free calcium concentration and intracellular calcium concentration in Arabidopsis seedlings increased significantly when gravity was changed. Decrease of the energy materials such as saccharide and fatty acid may be explained by the energy consumption both by the repair process of DNA damage and by the process of DNA syntheses.

Space flight factors not only alter cell growth and mitotic, but also induce various chromosome aberrations such as micronuclei, chromosomal bridges, fragments, and laggards. In this research, cell mitosis was found to decrease significantly, whereas, abnormal nucleic and chromosomal aberrations increased after the alfalfa seeds were previously exposed to the space environment. Similar findings were previously reported in Crepis capillaries (Kostina et al., 1984), wheat (Gu and Shen, 1989), and *Platycodon grandiflorum* (Gao et al., 2001) after space flight. The principal reason for the induced aber-rations of the chromosomes of root tip cells was usually thought to be the occasionally large amount of energy deposited by the highly ionizing heavy nuclei of cosmic rays (Gao et al., 2001). Although a similar effect on cell could be induced by a low dose of radiation, the frequency of chromosome aberrations and MI in seeds flown in space may be much higher than those in seeds irradiated by low does (2.0 mGy) of radiations on the ground, such as carbon, neon, and iron ions (with different LET values of 13.3KeV/um, 31KeV/um, and 500KeV/um, respectively) (Wei et al., 2006b).

Combining our findings with those of former reports, a model (Figure 3) was proposed to depict diagrammatically what happen to alfalfa seeds during and after space flight. First, cosmic particles bombard the seed during the space flight, which brings in the presence of etch-figures and etch-holes. Secondly, the cosmic particles pass through etch-holes and go inside the seed which results in DNA damage and chromosomal aberration. Thirdly, the repair mechanism for DNA damage is activated. However, the presence of microgravity or other flight factors interrupt the signals transduction by Ca^{2+} flux and inhibits the efficiency of DNA repair which result in the presence of nucleolus and chromosome aberration. Finally, induced cell mitosis engenders physiological changes such as abnormal cotyledon, increase of germination potential, and inhibition of root growth. Most of these damages resulting from chromosome aberrations will be eliminated in the first post-flight generation, but a few of the damages resulting from gene mutations and micro-aberrations will be preserved and inherited by the following generations (Konstina et al., 1984).

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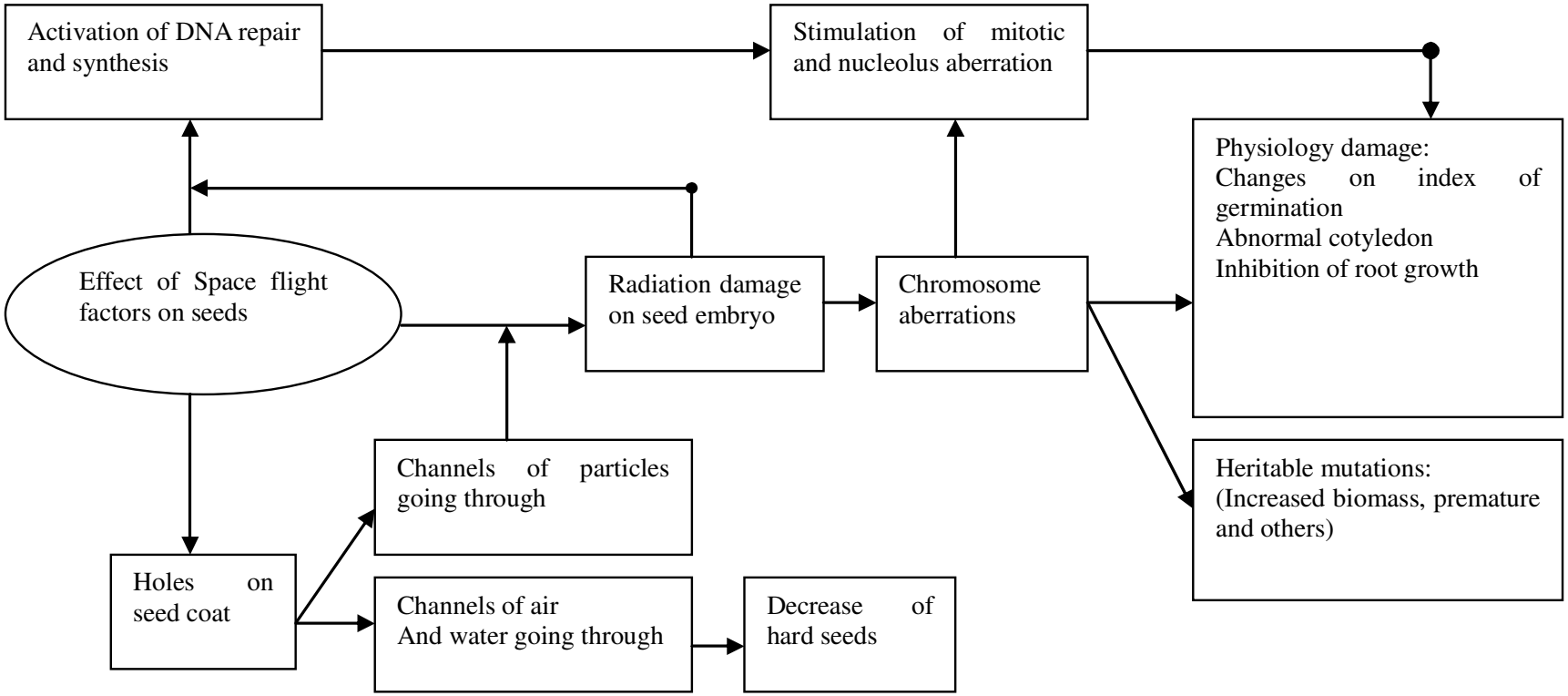

Figure 3. Speculated function model of mutation effect induced by space flight factors.

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