Space Radiation Systems Biology Research in SJ-10 Satellite

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Abstract Space radiation biology is one of the important parts of space biology, mainly focusing on the mechanisms of biological effects and genetic variations in organisms induced by space radiation. Space radiation biological effects cannot be described by a simple dose-effects relationship due to the complexity of space radiation environment. The diversity of space radiation induced biological mechanisms and the uncertainty of the synergetic interaction between space radiation and microgravity should be considered from the views of multidimensional and systematic ways. The system biology approaches should be used to study the relationships between the space radiation qualities and the space radiation induced biological effects, which are to find the main mechanisms and the key stressors to induce the mutagenic effects. This study investigates the space radiation qualities and the corresponding biological effects with the aid of SJ-10 satellite. The approaches of data mining and system biology are used to analyze these datasets. In the experiment, biological materials (*O. sativa* seeds, *A. thaliana* seeds and *C. elegans*) were located in three different bio-radiation boxes to obtain three distinct radiation environments inside the satellite. The absorbed dose, absorbed dose rate, linear energy transfer, and dose equivalent were measured with the use of active and passive radiation detectors. The biological samples irradiated by the space radiation within the satellite were harvested. After recovery of the satellite, by applying phenotypic and physio-

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logical analysis as well as system biology analysis such as genome epigenetic and proteomic scanning to biological samples. The biological changes under different radiation qualities will be analyzed and relevancy between biological effects and radiation parameters will be studied.

Abbreviations

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BRB	Bio-radiation boxes
ced-1	Cell death abnormality protein 1
CR-39	Columbia Resin #39
DDR	DNA damage response
DE	Differentially expressed
DSB	DNA double-strand breaks
dys-1	Dystrophin-1
GCRs	Galactic cosmic rays
GO	Gene Ontology
HIMAC	Heavy Ion Medical Accelerator in Chiba
HZE	High charge and high energy heavy ions
IQR	Inter Quantile Range
LEO	Low earth orbit
LET	Linear energy transfer
pgm	Phosphoglucomutase
PIN2	Auxin efflux carrier component 2
rad-51	DNA recombination and repair protein
RF	Random forest
SAA	South Atlantic anomaly
SEPs	Solar energetic particles
SITEL	Silicon Telescope
SNDE	Slow Neutron Dose Equivalent
SVM	Support vector machine
TEs	Transposable elements
TLD	Thermoluminescent dosimeters
ttg	Transducin/WD40 repeat-like superfamily protein
unc-54	Myosin-4

1 Introduction

Space radiation biology is one of the important parts of space biology, mainly focusing on the mechanisms of biological effects and genetic variations in organisms induced by space radiation. The main sources of space radiation in low earth orbit (LEO) include galactic cosmic rays (GCRs), solar energetic particles (SEPs), geomagnetic trapped belts, and albedo particles (Chancellor et al. 2014). GCRs originate from space outside the Solar System where high energetic particles are involved. GCR particles consist of 98% baryons and 2% electrons, and the baryonic component is composed of 85% protons, 14% alpha particles and 1% heavy ions (Lebel et al. 2011). High charge and high energy heavy ions are termed as HZEs. Although the fluxes of HZE particles in GCR are low their contribution to the radiation dose equivalent is high compared to those from other types of radiations, because their radiation quality factors are large due to their high linear energy transfer (LET) (Hada et al. 2007). In addition, HZE particles are difficult to be shielded due to their long range. Therefore, HZE particles are considered as the main factors to induce the biological effects in LEO.

SEPs emitted by the Sun are mainly protons with the energy ranges from 10 to 1000 MeV. SEPs are observed as random events, and the occurrence frequency and intensity of SPEs are related to the solar-cycle phase, typically high in the peak years of the solar activity (Cucinotta et al. 2013). The geomagnetic trapped belts or radiation belts, also called Van Allen belts, are the main regions of the bounded energetic particles originated from the interactions of GCRs and SEPs with the Earth's magnetic fields and the atmosphere, including the inner belt and outer belt. The inner belt is formed mainly by protons, while the outer belt is mainly consisted by electrons. The radiation belts extend over a region from 200 km to about 75,000 km around the geomagnetic equator. Of special importance for LEO is the so-called South Atlantic anomaly (SAA) where the radiation belt extends down to 200 km, which is due to an 11° inclination of the Earth's magnetic dipole axis from the rotation axis. The radiation received is remarkably increased when spacecrafts pass through the SAA. Albedo particles are mainly protons and neutrons scattered from the Earth's atmosphere.

All the primary radiation particles from different sources can produce a variety of secondary particles, such as secondary heavy ions, light ions, neutrons and gamma rays. Through nuclear reactions and bremsstrahlung when they pass through the material of spacecraft and the instruments and equipment inside the spacecraft (Cucinotta et al. 2011). In general, these complex particles in spacecraft can firstly induce the biological damage of genetic material, such as DNA double-strand breaks (DSB), gene mutations (Bessou et al. 1998) and chromosomal aberrations (George et al. 2001), then cause the cellular dysfunction and even cell inactivation, which can lead to a series of pathogenic risks, including carcinogenesis (Durante and Cucinotta 2008), degenerative tissue effects [such as cataracts (Cucinotta et al. 2001), heart disease (Preston et al. 2003), acute radiation syndromes (Durante 2014)] and the damage to the central nervous system (Cucinotta et al. 2014). Therefore, space radiation are

generally considered to be one of the most important risk factors in space environment (Durante and Cucinotta 2008), and the potential for adverse health effects of radiation will limit further development of long-duration space missions (Cucinotta et al. 2013).

2 Foundations of This Research

In early time, the dry seeds were mainly used to study the biological effects and their mechanisms induced by space radiation; and the phenotypes of plants grown from the carried dry seeds were observed after recoverable satellites or spacecrafts were landed from space flight. With the development of the experimental methods and equipments, the biological effects induced by space environment in some model animals, e.g., chromosomal aberration, functional genomics and its methylation were also investigated in our lab. These studies indicated that the anti-stress mechanism can be activated by space radiation, and cause a series of biological effects in model organisms. In addition, the biological effects can also be affected by the persistent microgravity environment, suggesting that the biological effects and mechanisms induced by the space environment are very complicated.

We have sent 50 varieties of seeds of *Oryza sativa* (L.) on board recoverable satellites (e.g., "JB-1", "20th and 21th recoverable satellites") and spacecrafts (e.g., "Shenzhou-3", "Shenzhou-4", and "Shenzhou-6") since 1996. After landing from spaceflight, the seeds of *O. sativa* (L.) were planted standard in the laboratory and field, and then the phenotype variations, cytological effects, the characteristics of genomic mutations, and the change of protein profiling and genomic methylation were further studied from the levels of phenotype, cytology and molecule, etc.

For example, we have found that the mutation rates of phenotype in ten varieties of *O. sativa* (L.) after boarding Shenzhou-3 flight were significantly increased, which showed that different varieties of *O. sativa* (L.) have different sensitivities to space radiation environment. Furthermore, we have researched the phenotype variations of plant height, effective spike number and leaf color in the second generation of *O. sativa* (L.) carried in Shenzhou-3, and the results showed that the mutation rate ranged from 0.05 to 0.52% (Yu et al. 2007), which indicates that space radiation can induce a large widely variations in phenotypic characters. In addition, the inheritable effects could also be observed in these results. In order to compare the difference between the biological effects induced by space radiation and γ-rays, we have investigated the induced effects in the 24 varieties of var. *japonica* and var. *indica* of *O. sativa* (L.) with different maturation periods, which were exposed in Shenzhou-4 spacecrafts and γ-rays simulated in the ground, respectively. In the level of plant height and ripening rate, the result showed that the biological effects caused by space radiation in different varieties of var. *japonica* were equivalent to the mutagenic effect induced by 10–50 Gy γ-rays, and the characters of stimulated effects were obviously observed in the different varieties of *O. sativa* (L.) (Wang et al. 2006; Wei et al. 2006b; Xu. 2000). Furthermore, most of the varieties insensitive to γ-rays were also not sensitive to

space radiation either, while half of the varieties sensitive to space radiation were also sensitive to γ-rays. The phenotypic results of second generation mutation indicated that space and γ-ray radiations could also induce the mutations in plant height and heading stage, while there was a significant difference in mutation frequency of phenotypes between them (Wei et al. 2006b). All these results indicated that the mutation mechanisms of space radiation were different from those of γ-rays in the ground due to the differences in particle type and energy, radiation dose and dose rate, etc. between two kinds of radiation conditions.

To study the mutagenic effects and mechanisms of space radiation, we put dry seeds of *Zea mays* (L), heterozygous for Lw_1/lw_1 alleles (The mutation of Lw_1 gene will lead to yellow stripes on leaves, which can be used as an indicator of mutagenic effects of space radiation.), sandwiched biostack between nuclear track detectors aboard "JB-1" satellite for 15 days (Mei et al. 1998). After landing, the radiation doses and the corresponding mutations of morphology and molecule were detected, and the results showed that the radiation dose and dose-rate, measured by LiF detector, were 2.656 mGy and 0.177 mGy/d respectively. And, the flux of radiation particles with $Z \geq 3$ was 29/cm^2, and the average LET was about 0.5 keV/μm, which means that the majority particles penetrated the shielding of the satellite are high energy protons (Mei et al. 1998). In addition, the results also showed that the phenotypic mutations of yellow stripes were associated with the hit numbers of radiation particles in the *Z. mays* (L), while further study is needed for a better understanding of the nature mechanisms (Mei et al. 1998).

Furthermore, we have studied the phenotypic mutation, chromosomal aberration and mitotic index of root tip meristem in 9 varieties of *O. sativa* (L.), which were exposed in "20th recoverable satellite" for 21 days. And the same variety seeds were irradiated with the same radiation dose as spaceflight (2 mGy) but different LETs by Heavy Ion Medical Accelerator in Chiba (HIMAC). The results showed that the mutagenic effects on the above indexes caused by space radiation were significantly higher than those of Iron ions radiations (500 keV/μm), Neon ions radiations (31 keV/μm) and Carbon ions radiations (13.3 keV/μm) (Wei et al. 2006a). Similarly, the chromosomal aberration and mitotic index in different varieties of *O. sativa* (L.) experienced in Shenzhou-4 were also significantly higher than those of the treatment in the ground (Wei et al. 2007), which suggests that space environment can stimulate the processes of mitosis and halt the cell division at metaphase. And, the chromosome aberration of *O. sativa* (L.) in the space environment was higher than those of γ-rays and heavy ions radiations in the ground (Wei et al. 2006a), which indicates that the mutation mechanisms induced by different kinds of radiation might be different.

To reveal the genetic variation mechanisms of *O. sativa* (L.) induced by space radiation environment on the levels of phenotype and cell, we have found some differentially expressed genes in the stable mutants of *O. sativa* (L.) (e.g. 972-4), in which some can also be identified as new resistance genes (Li et al. 2004; Zhang et al. 2011). In addition, the method of amplified fragment length polymorphism (AFLP) was used to analyze the characteristics of genome mutations of *O. sativa* (L.) after Shenzhou-3 spaceflight. In the second generation mutation of *O. sativa*

(L.) experienced Shenzhou-3 mission, we have found that the genome mutation rate ranged from 1.7 to 6.2% in the 479 polymorphism loci (Yu et al. 2007). We compared the characteristics of the genome mutations in the three different mutants induced by spaceflight and other 11 cultivars of *O. sativa* (L.) and found that the mutated sites in the three mutations were 75.9, 84.9 and 100% in the polymorphic regions of interspecific genome, indicating that there was a preference in the presence of genome for the mutation effects induced by space radiation. That is, there are "hot spot" regions in the genomic mutations caused by space radiation (Li et al. 2007).

We analyzed the protein expression profiles of *O. sativa* (L.) experienced "JB-1 satellites", "Shenzhou-6 spacecraft", and "20th and 21th recoverable satellites", for investigation of protein expression profiles of rice plants after spaceflights and the hereditary capacity in the offspring plants, clustering and principal component analysis were performed to summarize and compare the protein expression variations between the space samples and their corresponding ground controls in different spaceflights and in 1st and 2nd generations. Results indicated that protein expression profiles were changed after seed space environment exposures onboard the satellite or space craft, but there were variations in the degree of difference in different types of flights: the longer the flight duration lasted, which meant that rice seeds were affected by space radiation environment heavier, the greater differences existed in protein expression profiles. Distribution of biological processes of differentially expressed proteins indicated that physiological and biochemical changes of rice cells were induced by space environment. The biological processes impacted in 1st generation plants after flights were nucleic acid metabolic process, light reaction, proteolysis, defense response to fungus, response to stress with amino acid and derivative metabolism. Alterations of protein expression profiles in 1st generation would resume or recover in the corresponding 2nd generation plants; the degree of recovery was associated with the sensitivity of the rice variety to environment. Changes of proteins involving in biological processes such as cell wall organization, nucleic acid metabolic process, proteolysis, RNA-dependent DNA replication, amino acid and derivative metabolism, pentose-phosphate shunt, response to stress tended to recover in 2nd generation plants; while seed maturation, protein folding, glycolysis, lipid biosynthetic process, glycogen biosynthetic process and tricarboxylic acid cycle related proteins only differentially expressed in 2nd generation plants. Therefore, the biological effects of space environment could be reflected in protein expression level (Ma et al. 2007; Wang et al. 2008).

To analyze the relationship between the genomic and epigenetic changes, we analyzed the genomic methylation in *O. sativa* (L.), which have been exposed in the spaceflight and heavy ions simulated radiation on the ground. The results showed that space flight and heavy ion simulated radiation could cause the changes of genetic methylation in genomic DNA, and the changes of methylation were more pronounced in the contemporary phenotypic variants (Shi et al. 2009, 2014b). The special sites could also be observed in the changed regions of methylation (Shi et al. 2009, 2014b). Most importantly, the polymorphisms in DNA methylation caused by space flight and heavy ionization radiation were associated with the polymorphisms of their corresponding genomic sequences. That is, a higher degree of methylation will lead

to a higher change of the corresponding genomic mutations. However, determination of the sequences of the polymorphic sites showed that the distribution of the site of methylation changes was significantly different from those of genomic changes. For example, the changes of methylation were occurred in the coding region, while the changes of the genomic sequence were occurred in the repetitive sequence region. Further studies focused on the corresponding gene expressions of the methylation changes in the coding region of genome, and the results showed that the expressions of the nucleotide metabolism, molecular chaperone family and heat shock protein related genes were changed, which preliminary revealed that methylation changes induced by space environment may be associated to genome instability (Shi et al. 2014a).

The similar results can be found in the studies of Ou et al., which also showed that the genomic methylation and the expressions of the corresponding gene were changed in *O. sativa* (L.) after spaceflight. The expression of 6 transposable elements (TEs) and 11 genes were also identified, and the changes of the DNA methylation of TEs were generally occurred at the CG and CNG sites, whereas the changes of the DNA methylation of other genes were generally occurred only at the CNG sites (Ou et al. 2009). Our studies also indicated that the carbon ion radiations can induce the polymorphism changes of DNA methylation in *O. sativa* (L.), the level of DNA demethylation was significantly higher than that of DNA methylation, and the changes of the DNA methylation were general in the CNG sites (Zhao et al. 2016a).

These results give rise to a new question that why such a significant biological effects were induced by the extremely low dose (mGy order of magnitude) of space radiation in the LEO. It is still unclear that whether it is caused by the hit of HZE or the persistence of microgravity in space. To study the synergistic effect of space radiation and microgravity on organisms, dauer larvae of *Caenorhabditis elegans* (L), including *dys-1* (dystrophin-1) mutant, *ced-1* (cell death abnormality protein 1) mutant, and wild-type, were divided into nine groups and put into the special SIMBOX devices in Shenzhou-8, and they were exposed to three different conditions: spaceflight, spaceflight control (in a 1 × g centrifugal device), and ground control. During 7 h after the landing of the re-entry vehicle, the mRNA (Gao et al. 2015a) and miRNA (Xu et al. 2014) expression profiles were performed enrichment analyses by distribution characteristics, biological processes and signal pathways, with particular attention given to DNA damage response (DDR) processes (Xu et al. 2014). The results showed that the spaceflight synergistic environment increased the quantity and significance of differentially expressed genes and miRNAs than space radiation environment in wild-type *C. elegans* (L). And compared the *dys-1* mutant and *ced-1* mutant with the wild-type, we found that the limited transcriptional differences were detected between the microgravity and non-microgravity environments in the gravity-sensing defective *dys-1* mutants, and the radiation-sensing mutant *ced-1* had an enhanced radiation response. Results indicated that, microgravity, depending on gravity sensor, enhanced the DNA damage response in the presence of space radiation, and probably play a vital role on DDR during short-duration spaceflight (Gao et al. 2015b).

In brief, the previous results have indicated that space radiation and microgravity environment are the main stress factors to cause the biological effects in the model plants and animals. And the mutagenic effects including phenotypic variations and cytological effects are associated with genomic mutations in the regions of "hot spots", transcriptome and proteomic changes, indicating the changes were involved with epigenetic regulations.

3 Significance of This Research

It can be seen from the above discussions that the space radiation biological effects cannot be described by a simple dose-effects relationship due to the complexity of space radiation environment, the diversity of space radiation induced biological mechanisms, and the uncertainty of the synergetic interaction between space radiation and microgravity, etc., and should be considered from the perspective of multidimensional and systematic views of point. Firstly the qualitative and quantitative analysis of the space radiation quality on bio-samples should be analyzed to obtain the data for incentives analysis. Secondly, the biological effects induced by space environment will be detected on molecular, cellular and phenotypic levels, but whether it can lead to damage or repair, depends on complex organisms function of molecular network regulation. Therefore, the biological mechanism of space radiation induced effects is very complex, which is involved in different levels and dimensions. And then, the system biology approaches should be used to study the relationships between the space radiation qualities and the space radiation induced biological effects, which are to find the main mechanisms and the key stressors to induce the mutagenic effects. In addition, this will also contribute to discover the space environment sensitive biomarkers to provide the basic data for further space radiation risk assessment and early risk warning in the long-term space missions.

This study investigates the space radiation qualities and the corresponding biological effects with the aid of SJ-10 satellite by integrating the space radiation detection technology. The approaches of data mining and system biology are used to analyze these datasets from the point of genetics and epigenetics, reveal the space radiation environment of biological genetic variation and damage mechanisms. Therefore, the following description includes two main aspects: "project design and implementation" and "progress of the research on space radiation systems biology". And the first one will be depicted in detail on "project design", "selection of space radiation detectors and model organisms", and "manufacture of bio-radiation box". The later one will be composed of "methods and results of space radiation measurements", "space radiation induced biological effects", and "analysis for space radiation systems biology researches".

4 Project Design and Implementation

4.1 Project Design

The research of this project focuses on the space radiation systems biology, as shown in Fig. 1. The various integration of radiation measurement unit and model organism unit results in three different bio-radiation boxes (BRB). They could provide comprehensive information of the space radiation environment and the corresponding biological effects at different levels, which could be quantitatively analyzed. Data integration and the corresponding analysis of systems biology are carried out so as to systematically describe the mechanisms of the biological effects of space radiation and its relationship with the space radiation quality.

4.2 Selection of Space Radiation Detectors and Model Organisms

4.2.1 Selection of Space Radiation Detectors

In order to measure the space radiation completely received by the model organisms, the space radiation measurement module for this research is combined of active detectors and passive detectors, which are listed in Table 1. The active detectors

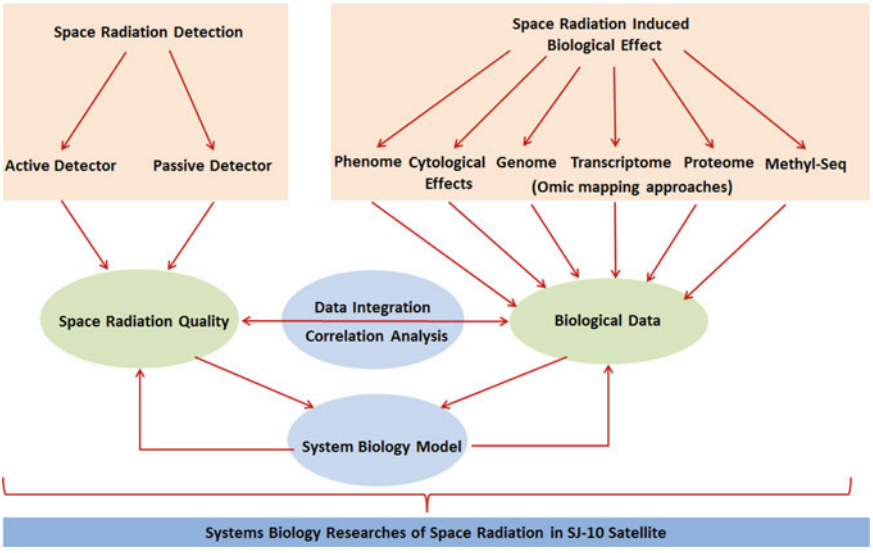

Fig. 1 Framework of the research on space radiation systems biology

Table 1 Space radiation detection modules and its function used in this project

Space radiation detection modules		Types of radiation measured	LET (keV/μm)	Dose	Dose equivalent	Flux
Passive detectors	TLD	Proton, electron, X-ray	/	√	/	/
	CR-39	High LET radiation	≥5	√	√	√
Active detectors	SITEL	Proton and heavy ions	0.1–230	√	√	√
	SNDE	Neutron with energy from 0.025 eV to 10 MeV	/	/	√	√

Note The symbol of "√" indicates that the detector have such a function, while the symbol of "/" indicates that the detector do not yet have such a function

include the Slow Neutron Dose Equivalent (SNDE) detector and the Silicon Telescope (SITEL). The SNDE detector is designed to measure the radiation equivalent dose rate of neutrons in space, with the ^{3}He gas proportional counter. In order to moderate the fast neutrons, the ^{3}He counter is in the center of a polyethylene sphere with a diameter of 15 cm. The SNDE detector could measure the neutron dose equivalent rate in the range of 1 μSv/h–10 mSv/h. The SITEL detector consists of two silicon semiconductor detectors, which have a thickness of 300 μm and a diameter of 26 mm. From the energy loss of the particles in the silicon detector, the LET values of the particles could be calculated. The detected LET spectrum is from dosimeters (TLD) and Columbia Resin #39 (CR-39) plastic nuclear track detectors. In order to accurately and comprehensively measure the radiation quality for radiation received by the model organisms, the TLD and CR-39 detectors were placed together with the organisms.

4.2.2 Selection of Model Organisms

O. sativa var. *japonica* (L), *Arabidopsis thaliana* (L) (*col*) and *C. elegans* (L) were selected in this research by considering the different mechanisms of space radiation biological effects between inter-species (model plant and animal), and monocotyledon and dicotyledons. Because of the different genetic background of each variety in the same species, six varieties of *O. sativa* var. *japonica* (L) seeds were carried on SJ-10 satellite, including Nipponbare, Koshihikari, Zhenzhuhong, Dongnong416 (DN416), Dongnong423 (DN423) and 433. In addition, the seeds of the wild type of *A. thaliana* (L) (*col*) and 3 mutants were also carried on SJ-10 satellite for studying the different mechanisms of biological effects induced by radiation and microgravity, as shown in Table 2.

Table 2 Cultivars of model varieties and their functions selected in this study

No.	Varieties	Cultivars	Characteristic
1	*O. sativa* var. *japonica* (L)	Nipponbare	Model variety of *O. sativa* var. *japonica* (L)
2		Koshihikari	Sensitive to space environment
3		Zhenzhuhong	Sluggish to space environment
4		Dongnong 416	Sluggish to space environment
5		Dongnong 423	Sensitive to space environment
6		433	Compact panicle varieties
7	*Arabidopsis thaliana* (L)	Columbia (*col*)	Model varieties of *Arabidopsis thaliana* (L)
8		PIN2 mutant	Mutant of auxin transport protein, which affects to the mechanism of plant response to gravity
9		*pgm* mutant	Lack mutant of starch grains, which affects to the gravitropism of the root
10		*ttg* mutant	Mutant of the blocked anthocyanin synthesis

Fresh seeds were harvested in 2015 for spaceflight and ground-control, and 200 main spikes were selected for each variety. Three copies of seeds were taken from each of the main spike to detect the germination rate and purity identification, where the following conditions should be required: the germination rate was greater than 98%, while the purity identification that was detected by the AFLP molecular marker should be less than 1%. These seeds which meet the above conditions were randomly divided into 6 copies: three copies for ground-control, three copies for spaceflight. And ground-control and spaceflight copies were placed into the BRB-A, -B, and -C, respectively.

To further compare the mechanism difference of space radiation biological effects in the model animal, N2 (wild-type) strain, two microgravity perception mutant strains (*dys-1*: anti-muscle atrophy protein defect, *unc-54*: Myosin-4, myosin heavy chain structural defect) and two radiation perception mutant strains (*ced-1*: apoptotic defect, *rad-51*: DNA damage repair defect) were selected and carried on SJ-10 satellite, as shown in Table 3. More details about the cultivation, propagation, synchronization for *C. elegans* (L) can be found in our previous publications (Gao et al. 2015a, b, c; Xu et al. 2014).

Table 3 Strains of *C. elegans* (L) and their functions selected in this study

No.	ID.	Strains of *C. elegans* (L)	Function
1	N2	Wild type	Model variety
2	LS292	*dys-1*	Anti-muscle atrophy defect
3	AM141	*unc-54*	Myosin heavy chain structural defect
4	MT4930	*ced-1*	Apoptotic defect
5	VC1873	*rad-51*	DNA damage repair defect

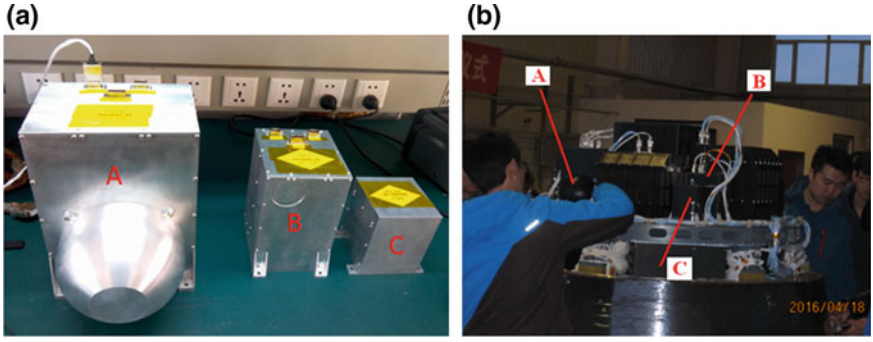

Fig. 2 The BRB and their corresponding arrangement in the satellite

4.3 Manufacture of BRB

The hardware of this research consists of three payloads, i.e., BRB-A, -B and -C, as shown in Fig. 2a. The surface material of the BRBs is aluminum, which has an average thickness of 2.5 mm. In each BRB, both the space radiation measurement module and the model organism module are included.

4.3.1 Space Radiation Detection Module

The radiation measurement module in each bio-radiation box is a combination of different radiation detectors, as shown in Table 4. The passive detectors, TLD and CR-39, are placed within each of the three BRBs. In the BRB-A, the measurement unit also includes both SNDE and SITEL active detectors. Only the SITEL active detector is in the BRB-B, and there is no active detector in BRB-C.

Table 4 Arrangement of the radiation detectors in BRBs

Radiation detectors	Passive detectors	Active detectors	
	TLD+CR-39	SITEL	SNDE
Bio-radiation box A	√	√	√
Bio-radiation box B	√	√	/
Bio-radiation box C	√	/	/

Note The symbol of "√" indicates that the bio-radiation box have such detectors, while the symbol of "/" indicates that the bio-radiation box do not yet have such detectors

4.3.2 Model Organisms Culture Modules

There are three types of model organism units: the sandwich-like biostacks (Fig. 3a, b), the seed bags and the *C. elegans* (L) container (Fig. 3c, d). Biostacks and seed bags are placed within each BRB. The *C. elegans* (L) container is placed only in the BRB-C. The biostacks are designed to provide the radiation information of heavy ions which hit the plant seeds. In the biostacks, the plant seeds are reserved in the organic material and fixed using non-toxic glue. Above the plant seeds are two layers of CR-39 detector, which could measure the heavy ions that hit the seeds. In each biostack, besides CR-39 detector, there are also four TLD detectors, of which two are TLD-600s and two TLD-700s. During the experiment, a total number of 576 rice seeds and a number of 4800 *A. thaliana* (L) seeds are in the biostacks. In the seed bags, there are different varieties of rice seeds, as well as radiation detectors of TLD and CR-39. The *C. elegans* (L) container is divided into eight small rooms. Each room contains a different type of *C. elegans* (L). Above the rooms there are four CR-39 detectors.

After the matching test under rigorous quality control, the model organisms were placed in the respective model organism unit of the BRB-A, -B and -C. The BRBs were then installed in re-entry capsule of SJ-10 satellite. The look direction of BRB-A, -B and -C is -Y Axis, -Z axis and +X axis of the satellite respectively (Fig. 3b). The altitude of SJ-10's orbit is 252 km and the inclination angle is 42°. After the flight on orbit of 12.5 days, the three boxes including the radiation measurement modules and the model organism modules were returned to the laboratories. From the radiation measurement modules, space radiation quantities such as LET spectrum, radiation absorbed dose, dose equivalent, et al. could be achieved. The contributions to the radiation qualities from different types of particles (protons, heavy ions and neutrons, etc.) were also studied. The biological phenotype, functional genome, genome methylation, and proteome of the model organisms would be fully analyzed.

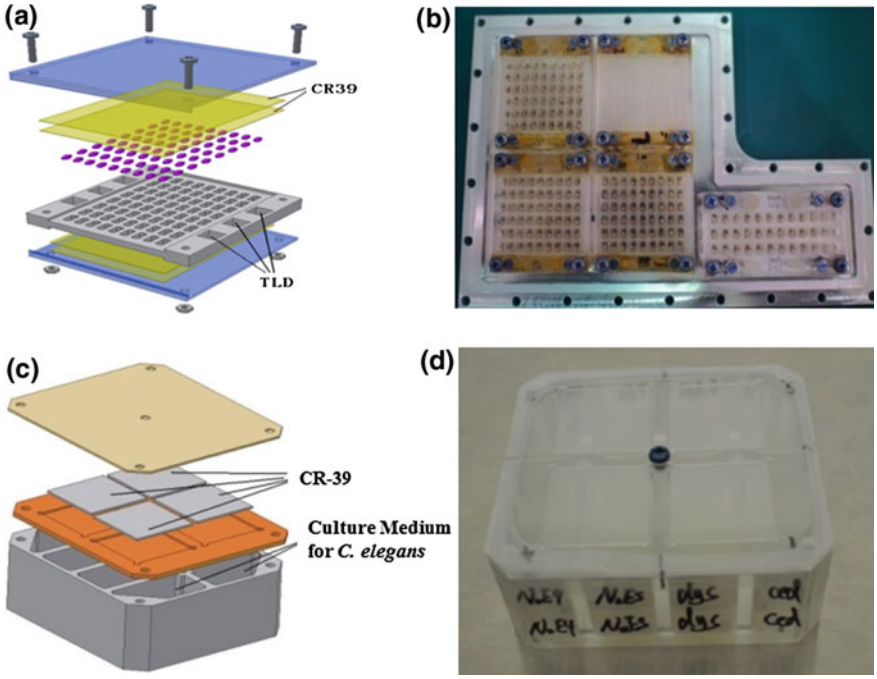

Fig. 3 The biostack (**a**, **b**) and the *C. elegans* (L) container (**c**, **d**)

5 Progress of the Research on Space Radiation Systems Biology

5.1 Methods and Results of Space Radiation Measurements

5.1.1 LET Methods of Space Radiation Detection

The LET values of the GCR heavy ions, the radiation physical quantities and the radiation spectrum have not been accurately measured in previous studies of space biological effects. The CR-39 nuclear track detectors in this research of SJ-10 and LET methods were used for the first time to achieve these measurements. These results would be useful for quantitatively studying of the relationships between the biological effects and LET values.

Detailed approaches to the LET spectrum of CR-39 detectors in radiation measurements can be found in the related papers (O'Sullivan et al. 1999; Reitz et al. 2009; Zhou 2012; Zhou et al. 2006a, b, 2007, 2008, 2009, 2010, 2011). The key processes of the LET spectrum method include: design of the CR-39 detector stack, LET calibrations with the heavy ions and protons generated by accelerators, space radiation exposure of the detector stack, detector stack recovery and chemical etch-

ing, microscopic scan and identification of the nuclear tracks and data acquisition, data analysis and processing, accurate calculation of the LET value for each particle event and the generating of differential/integral LET spectrum. The HZE particles can be obtained from the coincident tracks during the scan, and the distance between the HZE particle and the biological sample can also be accurately measured.

5.1.2 Preliminary Results of Space Radiation Measured in SJ-10

According to the number of GCR heavy ions hitting on the seeds of *O. sativa* (L), the total of 576 seeds in the biostacks is divided into nine groups, and the seed number of each group is shown in Fig. 4a. Result shows that, among the 576 rice seeds, about 9.9% of rice seeds were not hit by heavy ions. About 18.2% of the seeds were hit once, 23.1% of the seeds were hit twice, and about 48.8% of the seeds were hit no less than three times. It's also found that there are seven rice seeds, each of which is hit by eight heavy ions.

Figure 4b shows the LET spectrum measured by SITEL and CR-39 detectors. The LET spectrum obtained is in the range from 0.1 to 1700 keV/μm. The LET spectrum of 10–230 keV/um from the SITEL active detector agrees well with those from CR-39 passive detectors. The results has been published on "Journal of Geophysical Research: Space Physics" (Zhou et al. 2018). In addition, the radiation absorbed dose rate and dose equivalent rate were 0.072 mGy/d and 0.162 mSv/d, respectively, which were lower than the absorbed dose rate of 0.2–0.3 mGy/d and dose equivalent rate of 0.4–0.6 mSv/d on the international space station. This is mainly due to the fact that the fluxes of the Galactic cosmic rays and the radiation belt particles are decreased in the orbit of SJ-10, compared to those for the International Space Station (orbit altitude: 370 km/403 km).

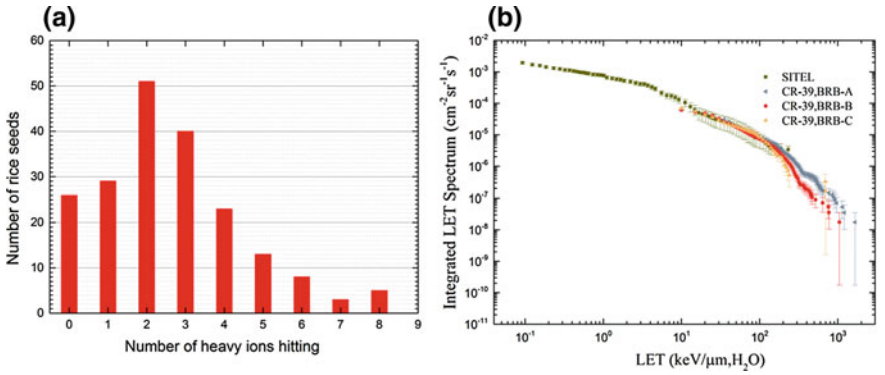

Fig. 4 **a** The distribution of the rice seed numbers according to the number of heavy ions hitting on the seeds; **b** the LET spectrums achieved from the SITEL and CR-39 detectors in the BRBs

5.2 *Biological Effects Induced by Space Radiation*

5.2.1 Biological Effects in Different Varieties of *O. sativa* (L)

In order to observe the biological effects induced by space radiation on *O. sativa* (L) at different growth stages, different varieties of *O. sativa* (L) seeds from seed packets undergone SJ-10 flight, including Nipponbare, Koshihikari, Zhenzhuhong, DN416 and DN423 were grown in the artificial climate room after spaceflight. The deionized water was used at 25 °C for 4 days to measure the germination rate. The Yoshida medium was used for rice cultivation in artificial climate chamber (25 °C, 12 h light, 60% relative humidity, 3000 lx of the light intensity) till the three-leaf stage for further studies. And these biosamples were used to analyze the changes of phenotype, antioxidant enzyme systems and the expressions of genes and proteins. For example, as the phenotype of the Nipponbare of *O. sativa* var. *japonica* (L), the results showed that there was no significant change in seed germination after spaceflight ($p > 0.05$). But, the heights of the seedlings in spaceflight were higher than those in ground-control. And the heights of seedlings were higher significantly than those of the ground-control group after the fourth day ($p < 0.05$), as shown in Fig. 5, indicating that spaceflight has stimulation effect to the height of seedling stage of *O. sativa* (L).

In addition, the phenotypes of the *O. sativa* (L) were observed from the four-leaf stage to the mature stage when growing in the fields. The seeds from seed packets undergone SJ-10 flight were firstly sprouted at 25 °C after 48 h for germination, and

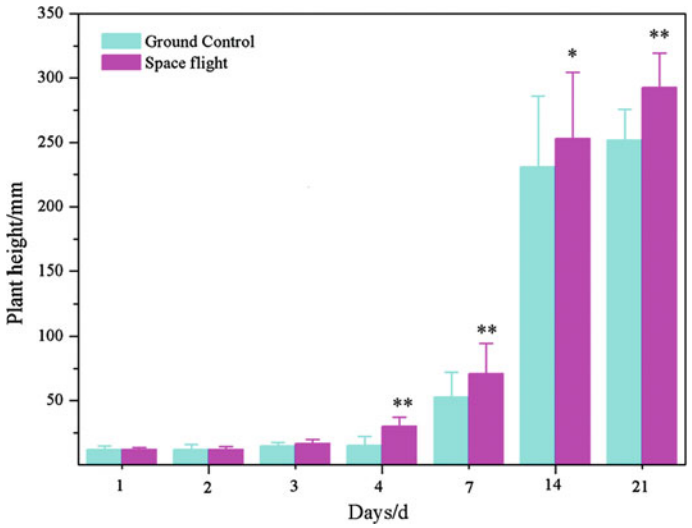

Fig. 5 The dynamic curve of the height of the rice induced by spaceflight in SJ-10 satellite. The data was depicted in the mean ± standard deviation

then planted in special discs. Finally, the seedlings were transplanted to the field on May 30th, 2016. DN416 and DN423 were planted in Wu Chang, and Nipponbare, Koshihikari and Zhenzhuhong were planted in Dong Gang, Dandong. More than 500 seeds of each varieties of *O. sativa* (L) were cultivated in the field.

The results showed that the germination rate of the six varieties of *O. sativa* (L) seeds was not significantly changed ($p > 0.05$). Thirty plants were randomly selected, and the plant heights of *O. sativa* (L) and Fv/Fm of photosynthesis parameters were measured at four-leaf stage. The results showed that the height of Nipponbare and DN416 in the spaceflight group were lower than those of in ground control, indicating the inhibition of growing. The plant heights of Koshihikari, Zhenzhuhong and DN423 in spaceflight group were higher than those of in ground control, indicating the stimulation of growing. And the Fv/Fm of photosynthetic parameters was changed in different varieties, suggesting the stimulation and inhibition effects were both existed in this study. The stimulation and inhibition phenomenon still existed on the height of plant and photosynthetic parameters at heading stage. The phenotype, including the number of tillers and effective tillers, the height of plant, the length of spike and awn, the number of grain, immature grain and filled grains, the rate of filled grains and the shape of spikes etc., were also measured at mature stage on Nipponbare, Koshihikari, Zhenzhuhong and 433. These results indicated that, except for the number of filled grains and the rate of filled grains, the other phenotypes were mainly inhibited. In addition, the contrary phenotypic changes between the seedlings and the field plants might be due to the different culture environments and growing stages.

All these results demonstrated that the influence of the phenotypic traits induced by spaceflight is persistent, and related to the growth stage of *O. sativa* (L): the stimulation effect at three-leaf stage, the coexistence of the stimulation and inhibition effects from the four-leaf stage to the heading stage and the growth inhibition effects at mature stage. Further, the cytological effects, such as the chromosome aberration and mitotic index, should also be observed. And the sequencing of the genome, methylation of DNA, and transcriptome, combining with proteomic researches will be detected in further work to explore molecular basis of these changes. The seeds of *O. sativa* (L) that were hit by HZE were chosen for the analysis of biological effects in the further work too.

The seeds of contemporary rice were tested for successive generations. On the phenotypic level, it was found that the number of tillers and effective tillers in the F1 generation of rice was significantly reduced, while the length and grain number of spike presented both growth stimulation and physiological inhibition between different varieties. At the same time, we also found a small number of contemporary suspected phenotypic mutant strains, such as the next generation of rice with the mutation of plant height and mature period showed stable inheritance, as shown in Fig. 6. The mutation of the rice growth period in the spaceflight seed showed that the next generation showed the characteristic of early-maturing mutation. The hereditable changes in phenotypic level can clear shown mutagenesis effect of the spaceflight mutation on rice genetic, and mutation frequency of offspring was obviously higher than that of contemporary. The genome instability cause by the space

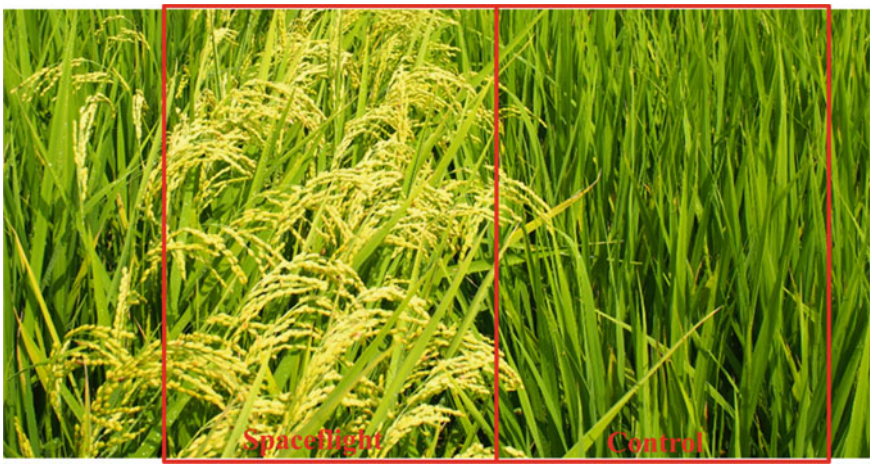

Fig. 6 The hereditable early-maturing mutation of rice seeds offspring after spaceflight

environment was strong evidence of radiation effects. It lays a foundation for the discovery of the biological effect mechanism of space radiation from the molecular level.

5.2.2 Biological Effects in Different Strains of *C. elegans* (L)

After landing to the ground, the survival ability, reproduction ability and locomotion ability were followed during life-cycle for five strains of *C. elegans* (L). Furthermore, by comparing the proteomic changes of the microgravity or radiation defect mutants with that of the wild type *C. elegans* (L) under the conditions of spaceflight and ground, the regulated functions induced by space environment, including the longevity, oviposition, muscle contraction, DNA damage repair process, etc., the mechanism and regulation pathway of the synergistic effects between microgravity and radiation are analyzed in detail.

For the protein profiles, a total of 5,000 *C. elegans* (L) were used as one sample to extract total protein, and then the protein profiles were analyzed by LC-MS/MS. And three experimental replicates were conducted for each treatment. The results showed that a total of 24,407 peptides and 3,343 proteins were identified. The intensities of 3343 proteins were plotted into clustered heat maps, as shown in Fig. 7a. It is obvious to find that the expression patterns were divided into two clusters for the treatments of ground control and space flight, indicating that the proteome of the wild type *C. elegans* (L) was significantly changed after spaceflight. In addition, we selected the differentially expressed proteins by the filtering conditions of the fold-change (spaceflight/ground control), which is greater than 2 or less than 0.5 with $p < 0.05$ (Significant B analysis). The results showed that a total of 175 proteins

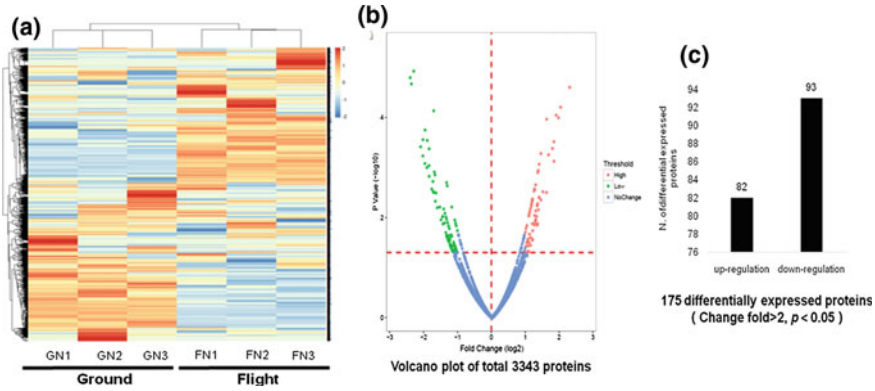

Fig. 7 Protein expression profiles of *wild-type C. elegans* (L) in SJ-10 satellite

were identified as significant changes, in which 82 proteins were up-regulated and 93 proteins were down-regulated, respectively (Fig. 7b, c). The regulated pathways will be further analyzed and discussed for the synergetic biological effects induced by space radiations and microgravity.

5.3 Systems Biological Analysis of Space Radiation Effects

The spaceflight environment can significantly induce the biological responses on the levels of genes and proteins, which are acquired from "omics" technologies, including genomics, transcriptomics, proteomics, etc. At present, "omics" technology is now extensively used to mine potential biomarkers in space biology researches. The characteristics of "omics" datasets obtained directly after spaceflight are always much smaller than the features to be investigated, which can be termed as small samples with big data.

In such cases, the key question is how to mine the sensitive biomarkers of space radiation environment from the high-throughput data. Currently, at least two different strategies can be adopted to deal with this situation (Von Heydebreck et al. 2004). One strategy attempts to use relevant biological knowledge to reduce the set of genes to a manageable number, while the other ignores the dependencies between genes and analyses the data gene-by-gene. In order to select the special genes involved in the biological responses (DNA damage responses, apoptotic gene expression, and miRNAs expression, etc.) of spaceflight environments for Shenzhou-8 mission, our previous studies mainly focused on the first strategy (Gao et al. 2015a, b). By integrated analysis of miRNA and mRNA, we have found that microgravity probably enhanced the biological responses in the presence of space radiation, and suggested a possible synergistic interaction between space radiation and microgravity (Gao et al. 2015a, b; Xu et al. 2014). However, we do not know what kinds of genes are

important and should be studied due to the lack of understanding of the function or pathway of these genes.

Different techniques have been proposed and implemented to select the differentially expressed (DE) genes based on the second strategy (Mutch et al. 2002). Historically, the first method used to identify DE genes was the "fold change", which is performed through a simple fold change cutoff, typically between 1.8 and 3.0 (Aittokallio et al. 2003; Tarca et al. 2006). However, the choice of threshold has a certain degree of arbitrariness, which may give rise to both false negative and false positive results (Mutch et al. 2002). We have ever used the traditional method of fold change to detect the DE genes in the microarray datasets in our previous studies, while the defects in this method are obvious. For example, some genes with small fold-change may have important biological functions, such as transcription factors (Aittokallio et al. 2003; Mutch et al. 2002; Tarca et al. 2006). In addition, the traditional method does not consider the background noise and variability of microarray datasets (Mutch et al. 2002).

In order to overcome the deficiency of the "fold change", the feature selection techniques have been proposed to meet the challenges of biomarkers screening (Saeys et al. 2007). These techniques can be divided into three categories: filter methods, wrapper methods and embedded methods. Each of them possesses advantages as well as disadvantages. Briefly, the filter methods [such as Inter Quantile Range (IQR) (Cordero et al. 2007), *t*-test (Jafari and Azuaje 2006), analysis of variance (ANOVA) (Nadon and Shoemaker 2002), Wilcoxon rank sum (Thomas et al. 2001), etc.] identify the relevance of features by looking only at the intrinsic properties of the data, i.e., the method of "gene-by-gene", which are computationally simple, fast and independent of the classification algorithm (Saeys et al. 2007). However, the main flaws of filter methods are that they ignore the feature dependencies, which may lead to worse classification performance. Wrapper methods [such as sequential search (Inza et al. 2004), genetic algorithms (Ooi and Tan 2003), etc.] embed the model hypothesis search within the feature subset search, while the common drawback of these methods is that they all have a higher risk of over-fitting and computational cost than filter

Fig. 8 Overlapping features based on the feature sets generated by IQR and ANOVA algorithms, reprinted from ref. Zhao et al. (2016b), copyright 2016, with permission from Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

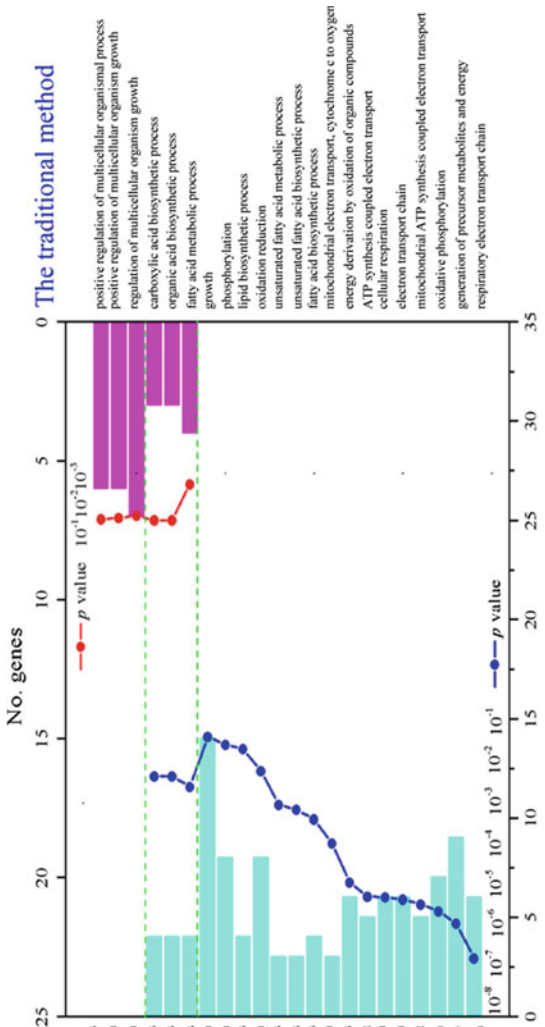

Fig. 9 Biological process of differential expression genes obtained from the traditional method of fold change and the present method of IQR and ANOVA, reprinted from ref. Zhao et al. (2016b), copyright 2016, with permission from Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. Differential expression genes were annotated by GO using DAVID software, GO terms with computed *P* values less than 0.05 were considered as significantly enriched

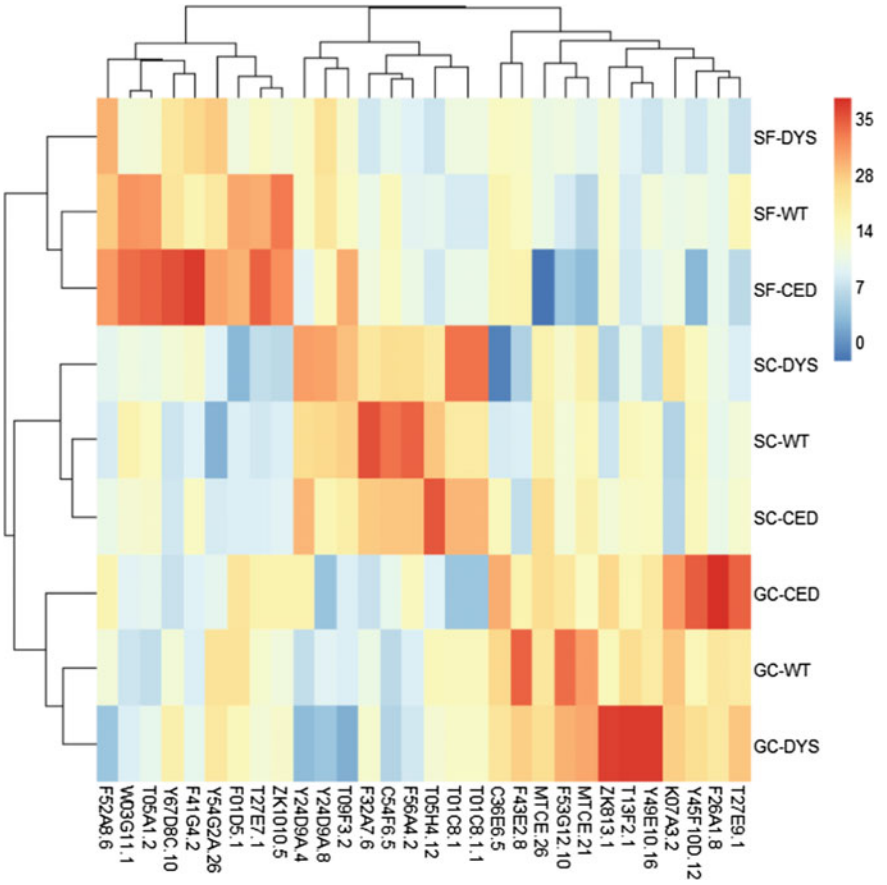

Fig. 10 Heat map of the top 30 ranking based on random forest algorithm, reprinted from ref. Zhao et al. (2016b), copyright 2016, with permission from Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

techniques (Saeys et al. 2007). In addition, the embedded methods (such as Random forest (RF) (Diaz-Uriarte and de Andres 2006), Support vector machine (SVM) (Guyon et al. 2002) take an optimal subset of features to build into the classifier construction, while at the same time being far less computationally intensive than wrapper methods (Saeys et al. 2007).

To identify the potential biomarkers associated with space flight, a combined algorithm, which integrates the feature selection techniques, was used to deal with the microarray datasets of *C. elegans* (L) obtained in the Shenzhou-8 mission (Zhao et al. 2016b). A total of 86 DE genes in responses to space synthetic environment or space radiation environment were identified by two filter methods (IQR and ANOVA algorithms) (Fig. 8). Compared with the ground control treatment, this algorithm can select more functional genes in responses to spaceflight environment. Gene Ontology

(GO) annotation and functional enrichment analyses indicated that these DE genes mainly related to oxidative phosphorylation (Fig. 9), suggesting that the combined use of these algorithms may make the feature selection more reliable and robust. Furthermore, the results of RF algorithm and clustering analysis (Fig. 10) showed that 17 genes can be regarded as potential biomarkers associated with spaceflight due to their high sensitivity to space flight environment. And the synergetic biological effects are likely to exist between space radiation and microgravity.

The combined algorithm, which integrates the feature selection techniques, will be used to analyze the high-throughput profiles of *O. sativa* (L) and *C. elegans* (L) experienced SJ-10 flight to select the biomarkers, which can reflect the space radiation environment. And these results will be compared with the previous conclusions obtained from the Shenzhou-8 mission to confirm the space radiation sensitive biomarkers. Based on these, the correlation analysis between space radiation qualities and the corresponding sensitive biomarkers will be further conducted to mine the key factors or main mechanism of the biological effects induced by space radiation. And the systems biology model will be established for space radiation risk assessment and early risk warning, as shown in Fig. 10.

6 Conclusions

Based on the comprehensive review to the research background, foundation, and significance, this project was designed and implemented by putting the space radiation detectors and model organisms into three BRBs inside the SJ-10 satellite. The space radiation quantities and LET spectra were precisely measured, and the biological effects for *O. sativa* (L) and *C. elegans* (L) were observed after landing to the ground. The further in-depth studies will focus on the system biology approaches, which will be used to mine the biomarkers sensitive to the space radiation and the key factors or main mechanisms of the biological effects induced by space radiation.

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