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RESEARCH ARTICLE

Genomic polymorphism in consecutive generation rice plants from seeds on board a spaceship and their relationship with space HZE particles

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Abstract Dry rice seeds (*Oryza sativa*, subspecies *indica*) were sandwiched between nuclear track detectors, aboard the Chinese spaceship Shenzhou-3 for seven days. The seeds were recovered and the genomic polymorphism in 201 plants developed from these seeds was studied using random amplified polymorphism DNA analysis. When compared with plants from ground-based control seeds, the genomic polymorphic bands were amplified in 30.2% of the plants from the seeds exposed in space. The results for sequencing and SNP analyses of the polymorphic bands verified the single nucleotide variations in these plants. Genomic polymorphisms in the consecutive generations of individual plants of the seeds from space were also discovered. Seven seeds receiving hits of HZE (high atomic number and high energy) particles from space were selected for further analyses and variable genomic polymorphisms were detected in all plants that developed from these seeds. Among them, the embryos of three seeds were hit at least once, and mutants with significant changes in agronomic traits were only found in later generations of these seeds. This result implies that the HZE particles of space are effective in inducing the changes of plant genome of inherited phenotypes.

Keywords Rice seeds, Genomic polymorphism, On board spaceship, HZE particles in space

1 Introduction

The dry seeds of various kinds of plants have been placed on board high-altitude balloons, satellites or space ships. They were recovered and then grown for generations. Several stable mutants with promising traits were selected from the progenies of rice, wheat, vegetable, etc., as reported by Chinese scientists in recent years. The mechanism of the mutagenic effect induced by the space environment has also been explored. The molecular markers, such as random amplified polymorphism DNA (RAPD), simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP) have been used to analyze the changes of genomes in stable mutant lines of rice, green bean, and maize induced by the environment in space (Xing et al., 1995; Wang et al., 1996; Mei et al., 1998; Zhou et al., 2001; Yi et al., 2002). However, most of the publications in this field focused on the genomic polymorphism in mutants and only a few addressed the genomic changes in the consecutive generations from space-exposed seeds. The causes of these kinds of mutant induction are even more complicated because of the multiple factors present in space environment. Among these are high energy, high Z-ionizing particles (HZE) and micro-gravity, which have been identified to be the effective factors to the growth, development and inheritable variation in a number of biological systems. Furthermore, the results from studies on space radiobiology reported by Li et al. (1997), Horneck (1992) and Mei et al. (1998) demonstrated that HZE might be the main cause for inducing mutation in space.

A seed box, with dry rice seeds sandwiched between CR-39 nuclear track detectors, was placed on board the spaceship Shenzhou-3 to determine the relationship between the induced mutation and space radiation in this study. The results of the genomic changes in the consecutive generations from the space-borne seeds with molecular marker analyses, as well as the relationship among the HZE particle hitting the changes in genome, and variation in phenotypes were studied here.

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2 Materials and methods

2.1 Rice materials and boarding conditions

Four hundred and sixteen dry seeds (13% water content) of Qinghuazhan, a stable rice strain (*Oryza sativa*, subspecies *indica*), were placed aboard the Chinese spaceship Shenzhou-3 for seven days at an orbit of 42.2°, 198/338 km. The recovered seeds were grown in the experimental field station of the South China Agricultural University with ground-based seeds as control. The plants with variation in morphological traits were selected from the first, second and third generation plants, and observed for another 3–4 generations to obtain the stable mutant lines (The data for mutants selection will published elsewhere).

DNA for genomic analysis was extracted from the fresh leaves of plants developed from recovered seeds and their consecutive generations, as well as from the ground-based controls.

2.2 Isolation of genomic DNA

The CTAB method described by Murray and Thompson (1980) was used to isolate the genomic rice DNA.

2.3 Polymorphism analysis of genome

2.3.1 Random amplified polymorphism DNA analysis

Random primers with 10 mer (Operon Technologies USA) were used for RAPD analysis. The reaction conditions for amplifications according to Williams et al. (1990) were as follows: total volume for reaction 25 μL; pre-denatured 94°C, 60 s; denatured 94°C, 30 s; annealed 36°C, 30 s, extension 72°C, 90 s, 35 circles, and finally extension for 8 min at 72°C. The amplified products were separated with argolose gel (1.5%) electrophoresis systems, stained with EB, and were detected using Dot-gel I system (Bio Rad, USA). For each primer/sample combination, at least three repeats were conducted to verify the results of amplification.

2.3.2 Single-nucleotide polymorphism (SNP) analysis

Single-nucleotide polymorphism analysis (Hayashi et al., 2004) was performed for individual products that displayed different patterns between plants from space-borne seeds and ground-based control seeds obtained in RAPD analyses. The specific products were cloned, and both ends were sequenced and used to design the primers of sequence characterized amplified region (SCAR). The amplified products from these primers were sequenced. DNA from plants of space-borne seeds and ground-based control seeds used as templates were also sequenced. According to the differences in sequences, the new primers were designed to detect the existence of SNP. For SNP detection, two rounds of amplification were conducted. The primers of SCAR were used for the first reaction with 30 circles and the products of this reaction were then diluted 100 times and used as templates for the second reaction with primers designed for SNP detection with nine circles.

2.4 Measurement of HZE particle hitting from space on rice seeds

The seed box with the dry rice seeds was sandwiched between CR-39 nuclear track detectors that were designed for and placed on board the spaceship Shenzhou-3 for seven days. The tracks of HZE particles on the detectors were observed by etched with NaOH after recovery from Shenzhou-3 and the parts of seeds receiving hits from HZE were identified, as described by Xie et al. (2005). The physical parameters of the particles were deduced based on the ground-calibration experiments with accelerators (Wei et al., 2005).

3 Results

3.1 Phenotypic variation and genomic polymorphism in the plants developed from the space-borne rice seeds

The comparison between plants developed from 416 seeds on board the spaceship and the ground-based control seeds indicated that the growth and fertility for most of the plants from flown seeds were normal. However, variations in plant height, fertility, number of tillers, length of spikelet, and heating date were found in 41 plants, 9.9% of the total plants observed, as shown in Table 1. Among them, dwarfism was the major variation occurring in 5.5% of the total plants observed.

Table 1 Morphological changes in plants developed for rice seeds on board Shenzhou-3

Traits changed	For seeds on board spaceship			For ground-control seeds[a] (trait)
	Number of plants	Variation	Name of representative plant (trait)	
Dwarfing	23	60–72 cm[b]	I-L-15 (60 cm)	(84.8 ± 1.1) cm
Higher plant	4	97.5–104.5 cm[b]	I-B-16 (97.5 cm)	(84.8 ± 1.1) cm
Lower fertility	8	12.4%–49.4%[b]	I-L-1 (12.4%)	(83.6 ± 1.8)%
No. of tillers increased	3	19–24[b]	II-L-15 (24)	8.7 ± 0.4
Larger spikelet	2	25.1–25.2[b]	I-B-11 (25.2 cm)	(19.9 ± 0.3) cm
Later heading	1	Nine days later than control		

[a] Average of 30 plants ± standard error

[b] The results of t test ($P < 0.05$) indicated the significances of difference between the plants from flown seed and those from ground control seeds

Random amplified polymorphism DNA analysis was conducted using 24 random primers and the genomic DNA of 201 plants, from space-borne seeds as templates, and detected 189 loci. The number of loci for each primer was 4–15 and the fragment lengths were around 400–2000 bp. When compared with those from ground-based control seeds, 74 polymorphic loci with fewer or more bands were detected. A number of polymorphic bands were amplified in 19 of the 24 primers observed and the polymorphisms for different primers were varied. For example, polymorphic bands were detected in 31 plants, 15.42% of the total plants observed, when using primer OPM-10, as shown in Fig. 1, eight polymorphic bands were found in one individual plant when using primer OPN-11. The amplified bands (products) from different primers were inferred to represent various loci in the genome. Therefore, the various amplification results might reflect the genomic variations in different regions of plants from the space-borne seeds.

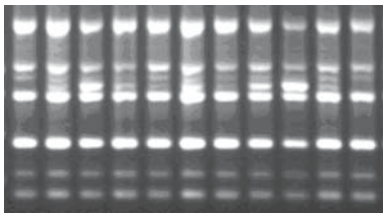

Fig. 1 RAPD amplification of the plants developed from space-borne rice seeds
The random primers used: OP-M10; templates-DNA of the plants; 1–10 developed from individual rice seeds on board Shenzhou-3 (I-C1–I-C10), CK plants from ground control seeds

Table 2 summarizes the genomic variations for individual plants from flown seeds. When compared with ground-control, the polymorphic bands were amplified from genomic DNA of 61 plants (30.2%) among 201 plants observed. The number of polymorphic loci varied from 1 to 25. Forty-two plants (20.8%) had only one polymorphic locus and 13 plants with more than three polymorphic loci suggesting that the space environment might induce a range of variation in rice genome.

Table 2 Genomic polymorphism of plants developed from recovered rice seeds after on board Shenzhou-3*

Number of polymorphic loci	1	2	3–5	>5	Sum
Number of plants with polymorphic loci	42	6	7	6	61
Number of plants with polymorphic loci/number of plants screened	20.8%	3.3%	3.5%	2.35%	30.2%

*Detection with RAPD analysis. Twenty-four random primers were used in PCR, and plants from 201 on board seeds were screened. DNA of plants developed from ground control seed used as reference (CK).

For verification of the variations that occurred in the genome of plants from the space-borne seeds, further analyses with polymorphic bands were performed. For example, OPS-19-B11, a polymorphic band with a size of 763 bp, was amplified when the DNA of plant from a space-borne seed I-B11 was used as a template and OPS-19 as primer (Fig. 2(a)). This band was cloned and sequenced. The sequencing comparison with National Center for Biotechnology Information (NCBI, USA) database shows that the similarity of this fragment to OSJNBa0056L25, a BAC clone located on rice chromosome 4, was estimated to be 99%. Sequence characterized amplified region primers were designed according to the flanking sequence and the amplified products for DNA from I-B11 and CK were of the same size (891 bp), as shown in lane 1 and 2 in Fig. 2(b). The results of these product sequencings identified the existence of 12 single base mutations in the genome of plant from seed I-B11, when compared to CK. Single-nucleotide polymorphism analysis was performed with the forward primer designed according to the sequence of CK (QHZ) at the flanking region of two mutated loci, 244 (T-C) and 366 (A-G), and the same reverse primer as the SCAR analysis. The results shown in Fig. 2(b) indicated that the amplified products only appeared for QHZ (lane 3 and 5), but not for I-B11 (lane 4 and 6). Single-nucleotide polymorphism analyses were also performed for the other specific amplification bands such as OPN-9-B16 and OPB-13-A4 with similar results obtained. This verified the occurrences of single base mutation in the genomes of plants developed from space-borne seeds.

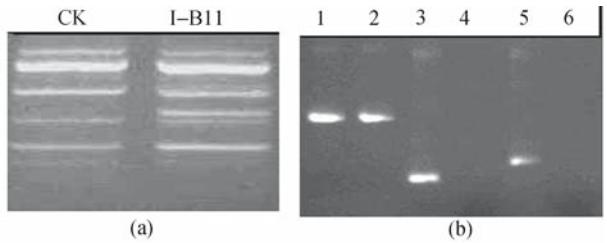

Fig. 2 Polymorphism of genome DNA between plants from seed I-B11 (on board Shenzhou-3) and ground-based controls (CK)
(a) RAPD with OPS19 as primer
(b) SNP detection, templates for lanes 1,3,5-DNA from CK; for lanes 2,4,6-DNA from I-B11
Primers for lanes 1, 2-SCAR primers; primers for lanes 3, 4-SNPA244 and SCAR-R; primers for lanes 5 and 6-SNPA366 and SCAR-R (see the text for details)

3.2 Genomic polymorphism in the consecutive generations from space-borne seeds

To understand the genomic changes in the various generations grown from the space-borne seeds, RAPD analyses were performed on plants from I-B11 and I-B15, two rice seeds recovered from spaceship, and their consecutive generations with 29 random primers and detecting 121 loci. The results, as shown in Fig. 3 and Table 3, indicated that the polymorphisms for individual plants from different space-borne seeds, as well as their progenies, were varied when compared to those from ground-based control seeds. Taking

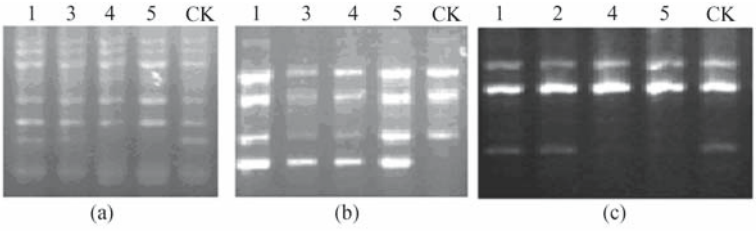

Fig. 3 RAPD amplifications using DNA from consecutive generations of rice seeds on board spaceship
(a), (b) results for seed I-B11; (c) results for seed I-B51
Templates: DNA from consecutive generations (the number above each land: No. of generation); Primers used: (a) OP-A10, (b) OP-M10, (c) OP-A03

Table 3 Genomic polymorphism of consecutive generations*

Name of plant material	Generations	Polymorphic loci	Polymorphism/%	Same polymorphic loci as those found in the first generation	Additional polymorphic loci (compare to those of the first generation)
I-B11	1	9	7.43	–	–
I-B11-27-X[a)]	3	8	6.61	6	2
I-B11-27-6-1	4	9	7.43	6	3
I-B11-27-6-1-X	5	9	7.43	6	3
I-B15	1	2	1.65	–	–
I-B15-X	2	2	1.65	2	0
I-B15-10-X	3	4	3.31	1	3
I-B15-10-7-1	4	4	3.31	1	3

*With RAPD analyses; 29 primers used and a total of 121 loci detected
[a)]X is the mixture of DNA from more than one plants in this generation used as templates
DNA of plants developed from ground-based control seed was used as reference

I-B11 as an example, among nine loci found to be polymorphic in the first generation, six polymorphic loci were also detected in the plants from the third, fourth and fifth generations (Fig. 3(a)). The other 2–3 polymorphic loci might not be detectable in the following generations (Fig. 3(b), lane 3 and 4). The studies in field planting showed that the plant from seed I-B11 was shorter than that of the wild type (QHZ), and one of its progenies selected from the second generation displayed a variation of trait with an earlier heading date of 8–10 days as compared to QHZ. This variation was stable and inheritable to the sixth generation as observed in this study. Although not all the changes in the genome in the first generation were inherited by the subsequent generations, as mentioned above, the changes in the later generation tended to be more stable and inheritable as indicated by the same polymorphic loci found in the fourth and fifth generations. The observation for seed I-B15 presented a different kind of result. The polymorphism was low for the first generation, but increased from the third generation (Table 3 and Fig. 3(c)). Correspondingly, no trait variation was observed in the plant from seed I-B15 and the mutant with larger grains (the weight of 100 grain 25% higher than that of QHZ) was selected from the third generation and was noted to be stable and inheritable to the sixth generation as noted in this study. These results demonstrate that the processes of genomic damages and repairs after seed on board are rather complex and varied for individual seeds/plants.

3.3 Relationship between hits of HZE particle on seeds and the variation in the genome and phenotype of plants developed from these seeds

The field observation, polymorphism analysis of genome and the radiation detection of space-borne seeds (Xie et al., 2005) were conducted by two groups in this study with part of the results summarized in Table 4. The plants from seven seeds receiving hits during the space flight were used for RAPD analysis with 70 random primers. As shown in Table 4, both the embryos and endosperms of seeds I-B11, I-B15, I-B16 and I-K6 were hit by HZE particles and the changes in genomes were detected in the plants from these seeds in the range of 4.5%–8.0%. Furthermore, the mutants with phenotype changes in plant height, heading day as well as grain weight were selected from the later generations of I-B11, I-B15 and I-B16 (data for mutants' selection are not shown in this paper). The results from further identification of ions hitting seeds (personal communication with Professor Wei Z, 2006) indicated that the embryo of seed I-B11 was hit by Cr ions with the energy of 103.2 MeV/u, and the seed I-B15 was hit by Mg ions with the energy of 608 MeV/u.

Genomic change was also found in the plants from the other four seeds which only had hits on the endosperms. No significant phenotype changes were found in their later generations.

Table 4 Space-borne rice seeds hit by HZE particles from space, their genomic polymorphism*, and the morphological changes in later generations

Number of seeds	Hits of HZE particles/object	No. of primes used for RAPD analysis	Locus detected with RAPD	Polymorphic locus/total locus detected (%)	Morphological changes in later generations
I-B11	8/endosperm 2/embryo	70	426	8.0	Increased plant height, earlier heading
I-B15	10/endosperm 1/embryo	70	421	5.5	Larger grain
I-B16	1/embryo	70	424	4.5	Increased plant height
I-K6	1/embryo 1/endosperm	70	430	7.6	No phenotype change observed
I-A4	3/endosperm	70	441	6.8	No phenotype change observed
I-E7	5/endosperm	70	426	3.3	No phenotype change observed
I-A11	3/endosperm	70	419	2.1	No phenotype change observed

*Compared with DNA of plants developed from ground control seeds

4 Discussion

In previous years, the dry seeds from various kinds of plant were widely used as materials on board spacecrafts and anomalistic growth and chromosome aberrations were observed as researchers studied the biological effects of space environmental factors (Horneck, 1992). The mutants selected from the progenies of space-borne seeds and their utilization in breeding programs have been reported in recently years (Mei, 2001). The developments of molecular marker techniques, such as RAPD, AFLP, etc., provided the suitable tools for analyzing the changes of genomes. In this study, the results from RAPD analyses with 24 random primers showed that polymorphic products detected in 30% of plants developed from space-borne seeds as compared with those from the ground-control seeds. The existence of single base mutation was further verified by SNP analyses which demonstrated that the environmental factors in space did induce the changes in the genomes of space-borne seeds (Table 2), and that some of these changes remained in the consecutive generations (Table 3). The inheritable changes in the genome were the basis for the variation in the phenotypes and the mutants with variations in heading date and grain weight had been selected in this study (data for selection are not shown).

The environment in space is significantly different from that on the ground in terms of the intensities of ionizing radiation, gravity, magnetic field, etc., all of which might affect biological systems in space. The ionizing radiations in space include the galactic cosmic rays (GCR), the solar particle events (SPE), and the trapped belt radiation (TBR). The main contents are the high energy protons and alpha particles, as well as HZE particles.

A number of studies demonstrated the efficiency of HZE particles in inducing mutations in various biological systems because of their high capacity in ionization, penetration, and in damage to macro-molecules (Peterson et al., 1977; Yurov et al., 1979; Hartman et al., 2001). The polymorphism of the genomes of seven plants from the space-borne seeds that received HZE particle hits in different parts was analyzed in this study. The results showed that although the polymorphic loci were detected in all these plants, the mutation in phenotype were only observed in the progenies of seeds that received one hit of HZE particles on their embryos, which implied the importance of HZE hit on the embryo in mutation induction. The hitting ions were identified as Cr and Mg. Although they consist less than 1% of space radiation, the equivalent doses reach 1%–5% according to the estimation by NASA (http://www.spaceresearch.nasa.gov/research projects/radiaton.html). As reported by Peterson et al. (1977), the somatic mutations were observed in one maize plant developed from the seed embryo received two hits from particles with $Z > 20$. The evidence from this study and other researches implied that the HZE particles in space might be an effective factor in inducing mutation. However, more evidence from the corresponding analysis of the mutation in the progenies of space-borne seeds and the particle hits they received would be helpful in the determination of the main cause for space-induced mutations.

Hartman et al. (2001) reported that the mutation frequency at locus *fem-3* was 3.3-folds higher after eggs of *C. elegans* were brought on board the space shuttle with a radiation dose of 0.268–0.306 cGy detected by TLD, as compared to the ground-based controls. However, a similar mutation frequency was obtained by irradiating the same material with an iron ion beam from the Brookhaven National Laboratory accelerator with 2.5 Gy. The radiation dose in Shenzhou-3 was only 10 mGy in this study as measured by TLD. Why were the genetic mutations induced by such low doses of radiation in space? Although it is still an issue that cannot be solved today, two possible explanations might be proposed. The first one is the synergistic/additive interactions of multiple factors in the space environment. The misrepair/non-repair of DNA damage induced by radiation has been considered as a cause for phenotype mutation. As indicated in this study, the changes in genomes were induced by the space environment. DNA repair could have been interfered by another important space environmental factor, micro-gravity. This interference has been proposed to increase the efficiency of mutation induced by space radiation. However, this theory was challenged by the negative results obtained by Kiefer and Pross (1999). The second hypothesis is that the genomic instability may be induced by very low doses of radiation, which refers to the delay of radiation-induced effects. This kind of phenomenon was first observed in mammals. However, the

report from Zaka and his co-workers (2001) shows that a steep increase in chromosomal aberrations and the effect on plant growth during the first and second generations were observed for root tip cells from *Pisum sativum* exposed to 0.4 Gy of ^{60}Co-γ rays, which proved the existence of this phenomenon in plants. Since the genomic instability may be induced by space radiation, especially by the HZE particles, and may be related to canceration in long-term effects of space flight, it has been studied widely in recently years (Ducray and Sabatier, 1998).

The genomic changes and the phenotypic variations in the progenies of I-B15, the rice seeds whose embryos received Mg particle hits, might be used as an example for this kind of mechanism. The genomic polymorphism in the first generation plants developed from I-B15 was low (1.65%, as shown in Table 3). However, the polymorphic rates increased starting from the third generations. Also, a mutant with larger grains than that of the wild types was selected from the third generation plants and was stable and inheritable to at least the sixth generation. Therefore, the increased changes of genome and phenotype occurred in the later generations. On the other hand, the changes of genome and phenotype in the plant developed from I-B11, another rice seed whose embryo received a Cr particle hit, were significant from the first generation. The results from this study agree with the others in that there is a complicated set of biological effects caused by factors in the space environmental. There is a role, as well, of space radiation in inducing mutation. Further studies in the mechanism of induced mutation and simulated ground-based experiments will be helpful to understand the importance of space radiation in mutation induction and may provide useful data for evaluating the health effects of space radiation on humans.

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