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The Effects of Spaceflight on Soluble Protein, Isoperoxidase, and Genomic DNA in Ural Licorice (*Glycyrrhiza uralensis* Fisch.)

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We investigated the effects of weightlessness, and ionizing radiation plus weightlessness, on changes in the levels of soluble protein, isoperoxidase, and genomic DNA, respectively, in a medicinal plant-ural licorice (*Glycyrrhiza uralensis*)-after a 15-d spaceflight in a recoverable satellite. Both the weightlessness samples (Ws) and the ionizing radiation plus weightlessness samples (IR/Ws) showed increases in soluble protein content or peroxidase activity, compared with the ground control (Gc). Moreover, the increased isoperoxidase activity for the IR/Ws group was significantly greater than for the Ws, compared with the controls. Likewise, distinctive RAPD profiles were generated among the Ws, the IR/Ws, and the Gc. The Ws and IR/Ws yielded 66 and 78 polymorphic RAPD fragments, respectively, based on bulk template DNA, along with 19 selected primers. Therefore, weightlessness alone can trigger genomic alterations, to some extent, and may even result in modulation of gene expression, whereas ionizing radiation would probably enhance the effect of weightlessness.

Keywords: genomic alteration, ionizing radiation, RAPD fingerprinting, recoverable satellite, weightlessness

The effects of spaceflight exposure have been studied extensively in the growth, reproductive development, morphology, physiology, and cytogenetics of test plant materials (Facius et al., 1990; Levine and Krikorian, 1992; Kuang et al., 1996a, 1996b; Peterson et al., 1997). However, little is known about genomic alterations and changes in the base(s) and/or sequence(s) of genomic DNA of test plant cells under spaceflight conditions.

Random amplified polymorphic DNA (RAPD), and protein and isozyme analyses are the most widely employed techniques for DNA and protein fingerprinting, respectively (Sambrook et al., 1989; Reiter et al., 1992). In general, protein and isozyme studies demonstrate genetic changes in coding and non-coding regions of the genome. Likewise, the RAPD technique has been widely used to detect plant genomic alterations during development or under stress environments (Lamboy, 1994; Chen et al., 1997).

The effects of exposure in a space-travel environment are primarily induced by the dual functions of weightlessness and ionizing radiation; few experiments have estimated the effect of weightlessness or ionizing radiation alone. In the current study, we hypothesized that those two main stresses would trigger varying genomic alterations in test plants, and might even result in changes in gene expression. Such changes could be characterized by PCR-based DNA marker, protein, and isozyme analysis techniques. In 1996, we were offered an opportunity to send plant materials on a 15-d spaceflight by the Chinese Space Academy. We chose ural licorice (*Glycyrrhiza uralensis* Fisch.), an important medicinal plant used for thousands of years in Asian traditional medicine for treating mild or moderate cases of hypocorticosteroidism, or Addison's disease (Huang, 1993). In this experiment, we sent the seeds into space, recovered and divided them, grew them in a greenhouse, and then investigated differences in soluble protein, isoperoxidase, and genomic DNA among the various test groups.

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MATERIALS AND METHODS

Plant Materials

Ural licorice (*G. uralensis* Fisch.) is distributed mainly in East Asia, especially China, and in Europe.

Our seeds were collected from the Medicinal Plant Garden at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. They had been pooled from individual plants that were genetically homogenous. The seeds were glued to a board to form a seed biostack, as described by Li et al. (1996). The seed biostack was then loaded into a small, hermetically sealed biocabin constructed of an all-sealed aluminum alloy. This biocabin contained a self-regulating oxygen generator that used KO_2 and LiOH (Li et al., 1996). The biocabin was carried on board the recoverable Chinese satellite (FSW-2-3), which was placed in a 356-km-high orbit for a 15-d spaceflight in October 1996. During the spaceflight, a second group of seeds (ground controls) was placed in an incubator that remained on Earth.

Seeds were recovered after the spaceflight and photographed. They were viewed with a microscope (XST-2A; 200× or 400× magnification) to ensure the presence of particle trajectories derived from cosmic heavy ions, and then divided into two groups: Ws (weightlessness, i.e., seeds without particle trajectories), and IR/Ws (ionizing radiation plus weightlessness, i.e., seeds with particle trajectories). Both the spaceflight seeds and the Gc (Ground control) seeds were then sown separately in pots (20 × 25 cm) containing loamy soil (pH 6.0). They were grown in a greenhouse at 25°C, under lights (2,000-3,000 μmol m^{-1} s^{-1}), with an adequate water supply. Leaves were sampled three days after the start of the full-bloom stage.

Determination of Peroxidase and Soluble Protein

Leaf samples were collected from 10 plants (approximately 0.15 g fresh weight per plant) in the Ws, IR/Ws, and Gc groups. They were separately homogenized in 0.2 M Tris-HCl buffer (pH 7.0) on ice. The homogenates were then centrifuged for 15 min at 20,000*g* at 4°C. Peroxidase activity in the supernatants was determined spectrophotometrically in a mixture containing the enzyme source, 3.4 mM guaiacol as the substrate, 0.9 mM H_2O_2, and 50 mM sodium phosphorate buffer (pH 6.0). The increase in absorbance was measured at 470 nm with a UV/Vis spectrophotometer (KONTRON, Swiss). Activity was expressed in OD_{470} min^{-1} g^{-1}. The protein content was determined as described by Bollag and Edelstein (1991). Each measurement was repeated five times, and standard errors were calculated for mean values.

DNA Isolation and RAPD Analysis

Leaf materials were sampled as described above. They were disinfected for 30 s in 75% ethanol, followed by several rinses in sterile water, then dried with sterile filter paper, frozen in liquid nitrogen, and ground into fine powder in a mortar and pestle. Genomic DNA was isolated according to Fu et al. (1998). Isolated and purified DNA was then dissolved in 100 μL of dd H_2O.

The 65 pre-screened primers used in this analysis were obtained from Professor F. Sala (University of Milan, Italy) and Operon Technologies, Inc. (USA). The *Taq* DNA polymerase, 10× *Taq* buffer, and dNTP were from Promega (USA). DNA amplification was performed in a Perkin-Elmer 480 thermal cycler (USA). The 25-μL reactions consisted of 1× *Taq* buffer [10 mM Tris-HCl (pH 8.3), 5 mM KCl, 1.5 mM $MgCl_2$, and 0.1% gelatin], 0.2 mM dNTP, 1.0 U *Taq* polymerase, 2 μM of primer, and 0.5 ng, 5.0 ng, or 50.0 ng of template DNA overlaid with mineral oil. The three concentrations of template DNA were used to ensure that the observed differences in banding patterns were not due to variations in template-DNA concentrations. The thermal cycler was programmed as described by Fu et al. (1998). Ten microliters of PCR products were resolved by electrophoresis on 2.5% agarose gels (GIBCO BRL, USA), run at 4.0 V/cm in 1× TAE, and photographed using a UV light source.

The 65 primers were screened on bulk template DNA from the Ws, IR/Ws, and Gc groups. This was done to identify primers that were capable of generating repeatable patterns of a minimum of four clear bands and at least one scoreable polymorphic band. Negative controls (i.e., template DNA replaced with ddH_2O in an amplification reaction) were used in each PCR reaction to monitor possible contamination. After suitable primers were identified, each amplification reaction was repeated twice, and the reproducible banding patterns were recorded.

RESULTS

Soluble Protein Content and Peroxidase Isozyme Activity

Soluble protein content and peroxidase isozyme activity changed significantly for ural licorice samples that had undergone either weightlessness or ionizing radiation plus weightlessness (Fig. 1, A and B). Com-

pared with Gc, the soluble protein contents increased 1.77-fold and 4.46-fold under Ws and IR/Ws, respectively. The maximum level of soluble protein was 106 mg g^{-1} DW (IR/Ws treatment, Fig. 1A).

In contrast, isoperoxidase activity increased significantly under IR/Ws rather than under Ws. Plants grown from seed under IR/Ws had an activity of 0.124 OD 470 min^{-1} g^{-1}, a 6.13-fold increase over the controls. However, the isoperoxidase activity of the Ws group was similar to that of Gc, with an increase of only 0.30-fold over the Gc (Fig. 1B). Therefore, ionizing radiation was more important than weightlessness for inducing changes in isoperoxidase activity. Weightlessness perhaps increased the synthesis of other enzymes or other soluble proteins, while ionizing radiation may have enhanced the effect of weightlessness.

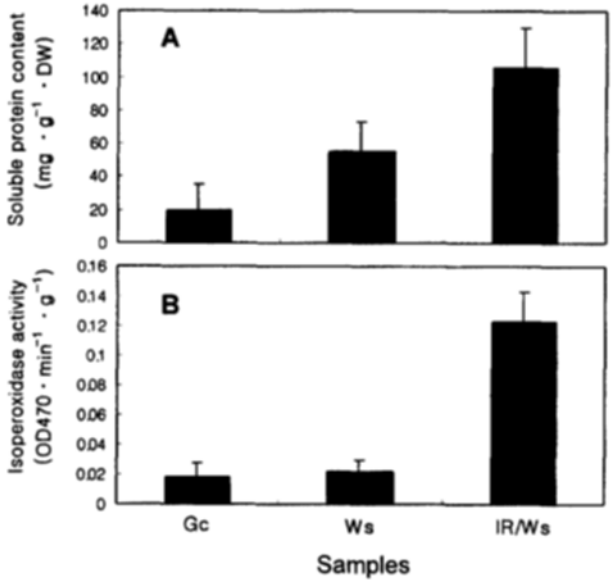

Figure 1. Soluble protein and isoperoxidase measurements of ground-control and spaceflight samples. Gc, ground control; Ws, weightless; IR/Ws, ionizing radiation plus weightlessness. **A.** soluble protein content. **B.** isoperoxidase activity. The error bars indicated the SE of five repeated values.

RAPD Fingerprints

The 65 pre-screened primers varied from 10 to 20 bases in length. From these, 19 were selected (thirteen 20-mer and six 10-mer) for further RAPD analysis (Table 1). These produced distinct differences in RAPD polymorphic fragments for each DNA sample

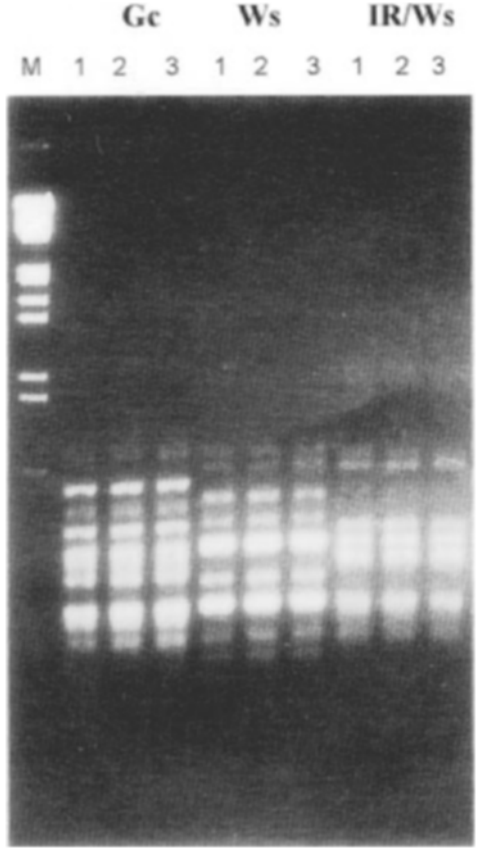

Figure 2. RAPD profiles of DNA samples from Ws, IR/Ws, and Gc, using primer chl 19. Lanes 1, 2, and 3: 0.5 ng, 5 ng, and 50 ng of template DNA, respectively; M: λ DNA HindIII/EcoRI marker. Gc, ground control sample; Ws, weightless sample; IR/Ws, ionizing radiation plus weightlessness sample.

Table 1. Name and sequence of 19 selected primers in this study.

Name	Sequence (5′ → 3′)	Name	Sequence (5′ → 3′)
chl 2	AATGCGTTGAGGCGCAGCAG	chl 30	CAGGCAATCCTAAACTCTCT
chl 10	TTCTTCTCCTACCAGTATCG	chl 23A	ACGCTGTACCTAGAGGATAG
chl 12	CGGAAGCAATTTGCTTGGCT	chl 3N	AAGGTATTCGTTCGGTTGCG
chl 13	GCAATTACTATGGCTCGGCA	OPI-6	AAGGCGGCAG
chl 15	CCTTTACGCTTTGCCGAGAT	OPI-7	CAGCGACAAG
chl 16	CTCCCTCATGATTCTTGGGA	OPI-9	TGGAGAGCAG
chl 19	GAGGATATGTTCGCCGTCTT	OPI-11	ACATGCCGTG
chl 21	AGAAGCATCCCAAAAGCGTC	OPI-12	AGAGGGCACA
chl 25	TCGAGTCCTCTTCAAGGCAT	OPI-13	CTGGGGCTGA
chl 26	CAATTCGAGGATCCAGAGAC		

Table 2. Total RAPD bands and polymorphic (poly) RAPD bands shown by the Ws and the IR/WS samples, compared with the Gc samples[z].

Primer	Gc	Ws		IR/Ws		Primer	Gc	Ws		IR/Ws	
		Total*	Poly**	Total*	Poly**			Total*	Poly**	Total*	Poly**
chl 2	6	7	4	7	5	chl 30	9	10	4	6	3
chl 10	14	12	4	12	5	chl 23A	12	9	4	11	6
chl 12	9	8	5	11	5	chl 3N	7	8	3	8	3
chl 13	12	11	3	2	3	OPI-6	6	6	2	5	4
chl 15	8	6	2	6	4	OPI-7	9	9	5	10	5
chl 16	10	8	5	8	3	OPI-9	10	8	4	11	4
chl 19	8	8	3	6	4	OPI-11	8	10	3	10	4
chl 21	14	13	3	13	3	OPI-12	8	8	3	12	5
chl 25	6	6	4	5	6	OPI-13	9	8	2	6	4
chl26	10	9	3	8	2						
Total***	175	164	66	167	78						

*All bands (present) per primer; **polymorphic (present and absent) bands per primer; ***the sum total of DNA bands with 19 primers.
[z]Gc, ground control sample; Ws, weightless sample; IR/Ws, ionizing radiation plus weightlessness sample.

(Fig. 2). Using bulk template DNA of the 10 samples from spaceflight-exposed plants, we obtained 66 and 78 polymorphic fragments for the Ws and the IR/Ws, respectively, compared with Gc (Table 2). The amplified products ranged from approximately 200 to 1600 bp. In each amplification reaction with a selected primer, three template DNA concentrations (0.5 ng, 5 ng, and 50 ng) were examined (Fig. 2). Any differences between samples that did not reproduce at the three DNA concentrations were not scored. DNA concentrations ≤ 0.05 or ≥ 100 ng per 25 µL of amplification reaction usually resulted in a low number of amplified bands and unstable fragment patterns, and, hence, also were not scored.

Differences in band number and intensity were reproducibly observed between the two space samples (Ws and IR/Ws) and the Gc, as well as between Ws and IR/Ws (Table 2 and Fig. 2).

DISCUSSION

Stress can trigger genomic alterations, which would be inherited if not repaired in a timely manner by the intrinsic repair mechanisms of organisms (McClintock, 1984). Previous studies have suggested that weightlessness and ionizing radiation are the two main types of stresses that confront biological organisms during spaceflight. To test this hypothesis, we looked for distinct differences in soluble protein content (Fig. 1A), isozyme activity (Fig. 1B), and RAPD fingerprints (Table 2 and Fig. 2,) between two groups of spaceflight samples and the ground control. Our spaceflight plants showed changes in genomic DNA and related gene expression. Likewise, the effects of the two space stresses were manifested in the different levels of soluble protein content (Fig. 1A), isoperoxidase activity (Fig. 1B), and RAPD polymorphism (Table 2).

Alcohol dehydrogenase (ADH) activity was altered in the root cells of *Arabidopsis thaliana* by spaceflight exposure (Porterfield et al., 1997). In addition, peroxidase activity and the amount of protein were lower in spaceflighted rapeseed (*Brassica napus* L. cv Niklas) and carrot (*Daucus carota* L. cv Nobo) than in their ground controls (Rasmussen et al., 1992). However, these effects seemed to be induced only by ionizing radiation plus weightlessness.

In our study, however, both the effect of ionizing radiation plus weightlessness and that of weightlessness alone were examined. Here, spaceflight exposure increased isoperoxidase activity and soluble protein content (Fig. 1, A and B), as well as the generation of repeatable polymorphic bands (Table 2). This demonstrates that weightlessness alone could somewhat trigger genomic alterations and result in increased gene expression, while ionizing radiation would enhance the effect of weightlessness. The decreasing effect reported by Rasmussen et al. (1992) may have been because they used protoplasts, which are prone to injury during spaceflight.

In spaceflight tests of medicinal plants *Platycodon grandiflorum* Jacq. and *Carthamus tinctorius* L. (data not shown), the stress responses differed for isoperoxidase activity and RAPD profiles. For example, both the Ws and the IR/Ws of *P. grandiflorum* showed higher levels of isoperoxidase activity and soluble pro-

tein production than those of *C. tinctorius* (data not shown). Therefore, different species could produce different genomic alterations in a space environment and, hence, lead to different gene expression. These differences probably depend mainly on spaceflight duration, the variety of protective measures for flight materials, and their different physiological status. Further work will focus on the stable inheritance of genomic alterations triggered by a spaceflight environment. This information will be useful for future marker-assisted space medicinal plant breeding.

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