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Article

Effects of space flight on DNA mutation and secondary metabolites of licorice (*Glycyrrhiza uralensis* Fisch.)

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Licorice (*Glycyrrhiza uralensis* Fisch.) seeds were flown on a recoverable satellite for 18 days(the average radiation dose in the flight recovery module was 0.102 mGy/d, the distance from flight apogee to earth was 350 km, gravity 10^{-6}). After returning to earth, the seeds were germinated and grown to maturity. The parallel ground-based seeds were also planted under the same conditions. The leaves of licorice were used for inter-simple sequence repeat (ISSR) analysis and the two main secondary metabolites in one-year-old roots were analyzed by high performance liquid chromatography (HPLC). Among 22 random primers used in this experiment, 6 primers generated different DNA band types. Analysis of HPLC showed that the content of glycyrrhizic acid (GA) and liquiritin (LQ) in the roots from seeds flown in space was respectively 2.19, 1.18 times higher than that of the control group. The results demonstrated that the extraterrestrial environment induced mutagenic effects on licorice and affected its secondary metabolites. These changes indicated that extraterrestrial orbit is possible means of breeding of licorice so as to preserve this endangered medicinal plant.

licorice, space flight, ISSR, DNA mutation, secondary metabolites

Licorice (*Glycyrrhiza uralensis* Fisch.) is a medicinal herb which has been internationally used as a remedy since the beginning of recorded history[1]. The two principal active secondary metabolites in licorice roots are glycyrrhizic acid (GA, including glycyrrhetinic acid, hydrolyzed products of GA, GCA) and liquiritin (LQ) (structure shown in Figure 1). GA has anti-inflammatory activity and is well known for its clinical applications in the treatment of the spleen, sore throats, bronchitis and liver disease[2]. GA has been reported to inhibit the replication of the SARS-associated coronavirus[3]. LQ possess various biological activities including anti-allergic and anti-tussive activities[4]. Over-collection of wild licorice plants in China resulted in a decline in wild resources and induced desertification of the habitat. Thus, cultivation of licorice was a substitute for the wild resources[5]. The low germination rate and content of active components in cultivated licorice limit its large-scale cultivation[6,7].

Spaceflight mutation unites space technology, biotechnology and agricultural breeding technology. High radiation, microgravity and other space factors may cause hereditary changes in plants and thereby create new breeds[8].

In the present work, wild licorice seeds were carried by a recoverable satellite for 18 days' space flight. The ISSR technique was used to study the genetic variation of licorice after space flight. The contents of GA (including GCA) and LQ in their one-year roots were analyzed by HPLC and compared with a control.

1 Materials and methods

1.1 Plant materials

Wild licorice seeds (*Glycyrrhiza uralensis* Fisch.) were obtained from the Tacheng district of Xinjiang Autono-

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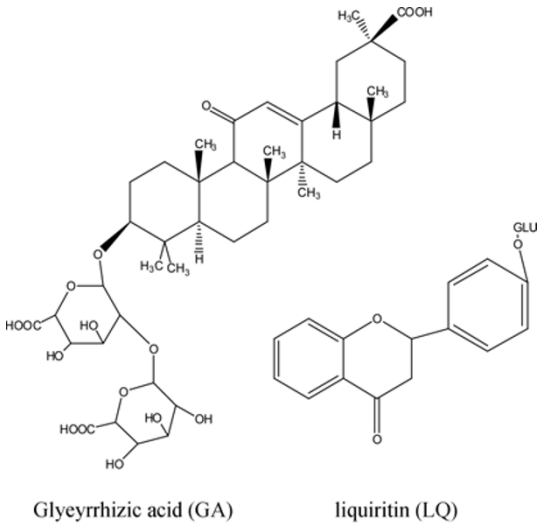

Figure 1 Structure of GA and LQ.

mous Region, China and carried by the 18th recoverable satellite for 18 d (the average radiation dose in the flight recovery module was 0.102 mGy/d, the distance from flight apogee to earth was 350 km, gravity 10^{-6}), The control ground-based licorice seeds were stored at 25℃ under dry conditions. After the space flight, both the seeds sent into outer space and the ground-based control group were sown in the ground. Fresh leaves were collected for ISSR analysis. Their one-year-old roots were used for HPLC analysis.

1.2 Chemicals

All primers used for ISSR analysis were purchased from Sangon Biological Engineering Technology and Service Co., Ltd (Shanghai, China). Taq DNA, 10×PCR buffer and dNTPs for ISSR were all purchased from TaKaRa Biotechnology (Dalian, China) Co., Ltd. GA and LQ standard references for HPLC were purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products, China).

1.3 Genomic DNA extraction

Genomic DNA was extracted from fresh licorice leaves. The extraction procedure was according to Paterson et al. (1993)[9].

1.4 ISSR reactions

The PCR reaction was carried out in a 25 μL volume of a mixture containing 10 μL template DNA (10.5 ng), 2.5 μL of 10× PCR buffer (including Mg^{2+}), 2 μL of dNTPs (10 mmol/L), 1 μL of primer (10 μmol/L), 0.25 μL of Taq DNA polymerase (5 U/μL) and 9.25 μL ddH_2O.

The reaction was performed in a thermocycler (Model TC-412, U.K.). The amplification program included a preliminary denaturing step at 94℃ for 5 min, followed by 40 cycles, each of which had a denaturing step at 94℃ for 45 s, an annealing at 53℃ for 60 s, and an extension step at 72℃ for 90 s. After the last cycle the samples were stored for 7 min at 72℃.

The PCR amplification products were tested by electrophoresis on 1.0% agarose gels containing ethidium bromide in 0.5×Tris-borate buffer. The ISSR fragments were visualized and analyzed by an imaging system (UVP Inc. USA).

1.5 HPLC analysis

(1) Liquid chromatographic conditions. The analysis was performed on an Agilent 1100 liquid chromatograph system (equipped with vacuum degasser, quaternary gradient pump, UV detection, controlled by HP Chemstation software. Palo Alto, CA, USA). A SUPELCOILTM LC_{18} column (4.6 mm×250 mm, 5 μm) was used. For GA, the analysis was employed by gradient elution beginning with a mobile phase composition of 65∶35 (methanol∶1% aqueous acetic acid, V/V) and gradually changed to 90∶10 after 30 min and then maintained for 5 min. For LQ, the mobile phase was employed by acetonitrile and 0.5% aqueous acetic acid (1∶4). The detection wavelength was respectively set at 254 nm and 276 nm for GA and LQ. The flow rate was maintained at 1 mL/min.

(2) Preparation of sample solutions. Licorice roots were powdered by an electrical blender. The powder sample (1.00 g) was extracted in an ultrasonic bath with 60 mL of methanol for 30 min. The resulting solution was evaporated to dryness in vacuo. The residue was transferred into a 5 mL volumetric flask with fresh methanol and filtered through a 0.22 μm membrane before injecting 10 μL samples.

2 Results

2.1 Polymorphisms detected by ISSR

In this study, 22 ISSR primers were used to amplify the genomic DNA of the control licorice plants and plants from exterrestrically orbited seeds in order to screen polymorphic primers. 16 primers generated the same DNA band type, and 6 generated different DNA band types between the control and exterrestrically orbited licorice plants. Table 1 shows the polymorphic ISSR primers sequence.

Table 1 Six polymorphic ISSR primers and their sequence

Primer number	Primer sequence (5′—3′)
T13761	CAC ACA CAC ACA CAC AG
T13762	GTG TGT GTG TGT GTGTC
T13763	AGA GAG AGA GAG AGA GC
T13764	GAG AGA GAG AGA GAG AA
T13765	ATG ATG ATG ATG C
T13766	CCCTCCCTCCCTCCCT

Each primer amplified different numbers of DNA bands from 5 to 11. 22 ISSR primers amplified 132 bands, 28 bands were found to be polymorphic with 21.2% polymorphism. The results of amplification by selected primers is shown in Figure 2.

Compared with the control, plants from seeds carried in the space station generated different band types. It indicates that primer T13761 amplified eight bands (Figure 2), among which 4 bands were polymorphic. Compared with type of plants from seeds orbited in outer space, the control group lacked two bands of 600 bp and 650 bp. The control group had a band of 350 bp which was absent in the type of plants from the space orbited seeds. Primer T13765 generated ten bands, among which three bands were polymorphic. Compared with the control group, plants from seeds orbited in space had two additional 350 bp bands and lacked a 150 bp band.

2.2 Analysis of the contents of GA and LQ by HPLC

To study the influence of space flight on secondary metabolites of licorice, HPLC methods were applied to the determination of the content of main secondary metabolites in one-year-old licorice roots. Representative chromatograms are shown in Figures 3 and 4. The contents of GA and LQ in the roots were summarized in Table 2. The results showed that the contents of GA and LQ in roots from plant seeds orbited in an exterrestrically environment were approximately 2.19 and 1.18 times higher than that of the ground control group with significant differences.

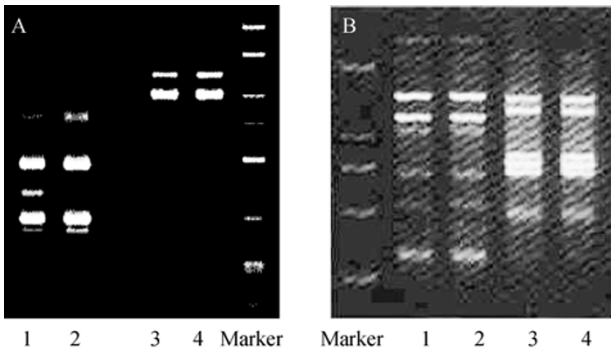

Figure 2 ISSR patterns of control licorice plants and outer space orbited seed plants generated by primers T13761 and T13765. 1, 2 mean the ground control group; 3, 4 mean the space fight group. M represents Gene Ruler^{TM}100 bp DNA Ladder Plus marker.

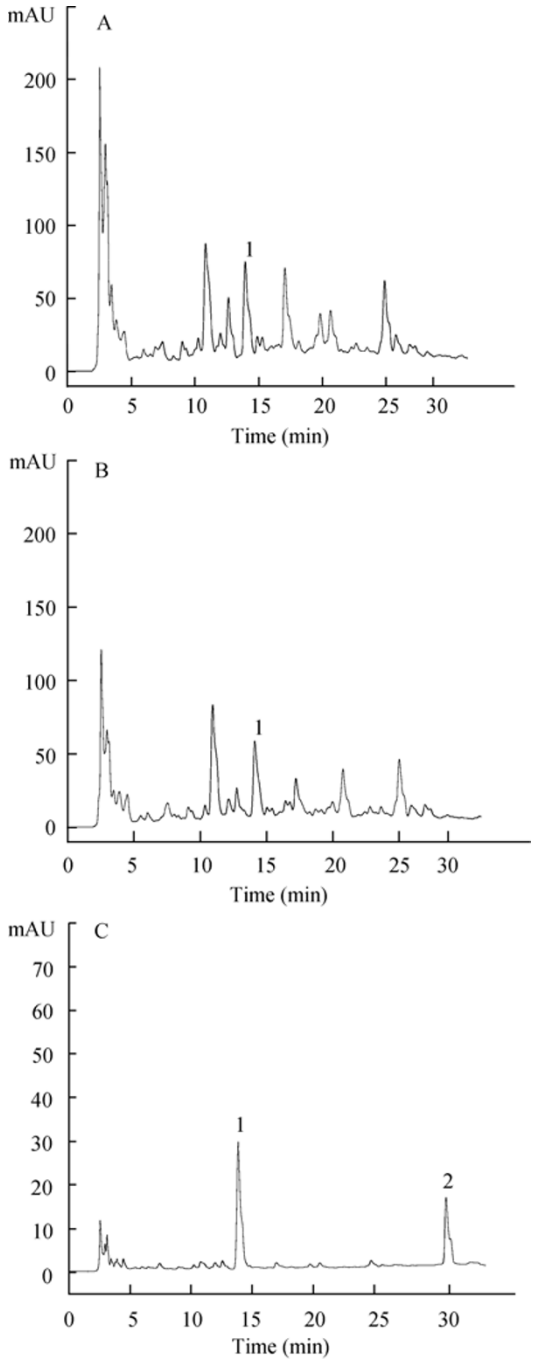

Figure 3 Comeprehensive HPLC chromatograms of GA. A, roots of ground control group; B, roots of plants from outer space orbited seeds; C, standard solution; 1, GA; 2, GCA (Glycyrrhetinic acid, hydrolyzed products of GA).

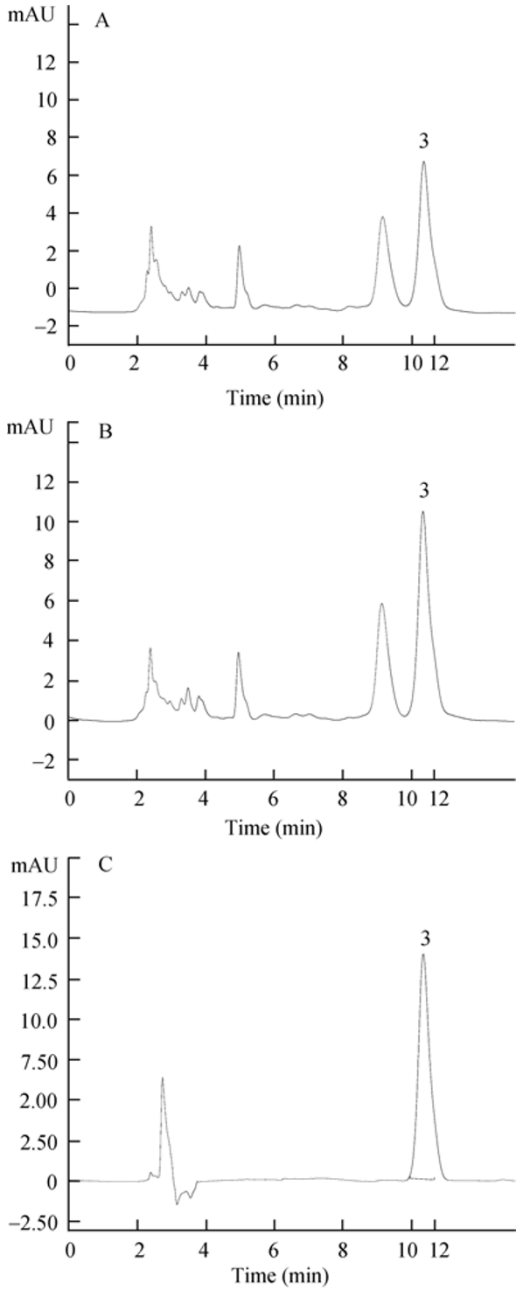

Figure 4 Comprehensive HPLC chromatograms of LQ., A, roots of ground control group; B, roots of plants from outer space orbited seeds; C, standard solution; 3, LQ.

3 Discussion

Licorice, which belongs to the leguminosae family, is used in almost all herbal preparations in China. Wild licorice mainly grows in arid and semi-arid regions where it contributes to long-term soil maintenance[10]. Over-collection of wild licorice plants is one of the factors inducing desertification in western China. In 2000, the Chinese government imposed restrictions on the collection of wild licorice so as to conserve the natural environment. With the increasing demand for licorice and the decline of wild licorice resources, cultivated licorice roots have attracted attention as an additional source. However, there remain various issues concerning licorice cultivation, such as the low content of secondary metabolites in cultivated licorice roots and the low germination rate of licorice seeds. Space science provides novel, unique research opportunities. Weightlessness, high radiation, high charge and high energy (HZE) particles may cause DNA mutation and hereditary changes in plants[11].

Many space flight experiments have been conducted on tomatoes, potatoes and fungi with results showing morphological variation and DNA mutation[12,13]. Gao investigated the influence of space flight on the medicinal plants *Platycodon grandiflorus,* and *Agastache rugosa* utilizing an electron microscope. The experiments indicated that the plants' ultrastructure changed after the space flight[14,15].

Our former study of the physiological and biological variation of exterrestrically orbitedlicorice seeds showed that they have a higher germination rate and POD, CAT enzyme activities under drought stress, which indicated that they have higher drought resistance than the ground control group. In this study, DNA mutation of outer space orbited licorice was verified by ISSR analysis, to assess genetic variation.

Table 2 Contents of GA and LQ in licorice roots (n=3) [a)]

Samples	Contents of main metabolite products (mg/g)		
	GA	GCA	LQ
Plants grown from outer space orbited seeds	3.98±0.19*	Nd	6.92±0.96*
Ground control group	1.82±0.48	Nd	5.86±0.34

a) *: Significance at the 0.05 probability level, P<0.05; each value represents mean±SD (n=3); Nd means not detected.

Secondary metabolite products are the main active components in natural drugs or medicinal plants, but there have been relatively few reports about the effects of space flight on secondary metabolites[16]. In this experiment, HPLC analysis demonstrated that the contents of GA and LQ in the experimental licorice roots were higher than that in the ground control group. It is thus apparent that the biosynthesis of GA and LQ changed after space flight. This means that space flight may affect functional gene expression of secondary metabolism in licorice.

For a better understanding of the mechanism of mutation induced by space flight, further experiments will be necessary to study the mutational events for several generations after orbital space flight, as well as cloning and sequencing the correlated genome.

4 Conclusion

In our study, licorice seeds were carried by a recoverable satellite and subsequently planted. This research represents the first study of the genetic variation of licorice after orbital space flight utilizing the ISSR technique. The results demonstrate that plants from exterrestrically orbited seeds generated different band types. Because we used the same type of licorice seeds in the space group and in the control one the effects of flights were the main factors on the licorice genetic mutation. Jiang et al.[17] have studied the effects of space flight on rice seeds, which demonstrated the effects of orbital space flight in causing the first generation mutation. The second generation generates the most broad spectrum separation in characteristics, and in the third generation these variations were transmitted to future generations achieving basic stability.

This is also the first report of the influence of secondary metabolites after space flight. HPLC data showed that the contents of GA and LQ in roots from outer space orbited seeds are significantly higher than that of control group. This research partially accounts for the molecular mechanism of space mutation in medicinal plants. Therefore, it may be concluded that space mutagenesis breeding is an efficient approach to medicinal plant breeding.

There have been studies showing that orbital space flight genetic mutations are inheritable yielding measurable economic benefits[18], e.g. improved wheat, barley, rice and tomatoes. Space mutation breeding produces enriched germplasm resources which accelerate breeding time[19]. Security is not an issue because it does not introduce new genes.

1 Cristina F, Michael E, Eugenio R, et al. A history of the therapeutic use of liquorice in Europe. J Ethnopharmacol, 2005, 99: 317—324
2 Suzuki H, Ohta Y, Kakino T, et al. Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis. Asian Med J, 1983, 26: 423—438
3 Cinatl J, Morgenstern B, Bauer G, et al. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. The Lancet, 2003, 361: 2045—2046
4 Taniguchi C, Homma M, Takano O, et al. Pharmacological effects of urinary products obtained after treatment with Saiboku-To, a herbal medicine for bronchial asthma, on type IV allergic reaction. Planta Med, 2000, 66: 607—611
5 Shibano M, Kawase S. Basic studies for cultivation of Glycyrrhiza uralensis. Nature Medicines, 2000, 54: 70—74
6 Fenwick J, Lutomski C N. Liquorice, *Glycyrrhiza glabra* L.—Composition, uses and analysis. Food Chem, 1990, 38: 119—143
7 Yutaka Y, Takami M, Ikuo S, et al. Pharmaceutical Evaluation of *Glycyrrhiza uralensis* Roots Cultivated in Eastern Nei Menggu of China. Biol Pharm Bull, 2003, 26: 1144—1149
8 Mashinsky A L, Nechitailo G S. Results and prospects of studying the gravitationally sensitive systems of plants under conditions of space flight. Space Res, 2001, 39: 1—12
9 Paterson A, Brubaker C, Wendel J. A rapid method for extraction of (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. Plant Mol Biol Rep, 1993, 11: 122—127
10 Qiu M X, Liu J Q. Utilization and protection of the licorice grassland resource in the arid region of China. J Nat Resour, 1993, 8: 314—321
11 Belyavskaya N A. Biological effects due to weak magnetic field on plants, Tsitologiya, 2004, 34: 1566—1574
12 Nechitailo G S, Lu J Y, Xue H, et al. Influence of long term exposure to space flight on tomato seeds. Adv Space Res, 2005, 36: 1329—1333
13 Jian J Q, Rong C M, Xiang D C, et al. Analysis of genetic variation in ganoderma lucidum after space flight. Adv Space Res, 2003, 31: 1617—1622
14 Gao W Y, Lei F, Paek K Y, et al. Spaceflight of platycodon grandiflorum seeds changes ultrastructure of pedicel and style cells. J Kor Soc Hort Sci, 2000, 41: 545—549
15 Gao W Y, Shu P Z, Xue L, et al. Effects of spaceflight on medicinal plant Agastache rugosa. China J Chin Mate Med, 1999, 24: 203—205
16 Gao W Y, Jia W, Xiao P G. Utilization of spaceflight technology on medicinal plant study. China J Chin Mate Med, 2004, 29: 611—614
17 Jiang X C, Li J G, Chen F Y, et al. The effects of space on the rice heredity which were carried in the recoverable satellite "8885". Chin Sci Bull, 1991,36: 1820—1824
18 Jiang X C. The progression and prospect of the space mutation breeding on crops. Aerospace China, 1997, 2: 5—8
19 Jia J H, Wang B. Progress in space mutagenesis breeding. Acta Agricul Nucl Sin, 1999, 13: 187—192