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Determination of chemical changes in Isatis indigotica seeds carried after Chinese first spaceship with FTIR and 2D-IR correlation spectroscopy

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Abstract

Spaceflight represents a complex environmental condition. Space mutagenesis breeding has achieved and marked certain results over the years. This method was employed in our previous studies in order to obtain improved germplasm of Isatis indigotica. This study is to determine the chemical changes in I. indigotica seeds carried after Chinese first spaceship (Shenzhou I). Fourier transform infrared (FTIR), second derivative and two-dimensional infrared (2DIR) correlation spectroscopy were used in analysis. Not much differences between the two spectra were found except the peaks in the range of 1500–1200 cm⁻¹ which was about 7 cm⁻¹ different and indicated the absorption could be initialed from different bonds. SP4 showed different derivative compared with C4 in the second derivative spectra of 1200–800 cm⁻¹. The stronger signal of 2DIR in SP4 indicated the protein content of the seed was changed after spaceflight. It is concluded that spaceflight provided an extreme condition that caused changes of chemical properties in I. indigotica.

Introduction

Radix Isatidis (Banlangen in Chinese) is the dried root of Isatis indigotica Fort., one of the most popular Chinese traditional medicine and is officially listed in the Chinese Pharmacopoeia (edition 2010) [1]. Radix Isatidis has broad therapeutic activities including anti-virus, anti-bacterial, anti-endotoxic, anti-inflammatory, anti-tumor and immune regulatory efficacy [2–4]. Its finished products are used clinically to prevent and treat influenza, tonsillitis and malignant infectious diseases, especially severe acute respiratory syndrome (SARS) and H1N1-influenza [5–8].

Germplasm degeneration of cultivated medicinal plants has been a growing problem. The degenerate medicinal plants are easily suffered from pests, diseases and the production and quality declined [9]. As one of the famous medicinal plants, the germplasm of I. indigotica faced the similar problem. Therefore methods should be used to keep desirable traits of I. indigotica. One of such methods is the application of aerospace condition. Aerospace has different environmental conditions from the earth with respect to gravity,
Fig. 1. FTIR spectra of SP4 and C4 in the range of 4000–400 cm\(^{-1}\).

Fig. 2. Second derivative spectra of SP4 and C4 from 1800 to 1200 cm\(^{-1}\).
Fig. 3. Second derivative spectra of SP4 and C4 from 1200 to 800 cm⁻¹.

Fig. 4. Second derivative spectra of SP4 and C4 from 800 to 400 cm⁻¹.
radiation and magnetic field. Aerospace conditions was used as an effective method of genetic changes to create new germplasms over years [10]. China’s space mutagenesis breeding program began in 1987 and has created a series of new germplasms and generated significant economic benefits. This series of pioneering research has won universal recognition [11,12].

Space mutagenesis breeding is employed in our previous studies. China developed its first spaceship Shenzhou I in 1999. The seeds of *I. indigotica* were chosen for spaceflight aboard Shenzhou I. After returning to the earth, the seeds (labeled as SP1) and the ground control without spaceflight (labeled as C1) were cultivated for next generations at the experimental plot of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. A plant from SP1 showed significant phenotypical changes and its seeds were chosen and labeled as SP2 [13]. Seeds produced from C1 were labeled as C2 accordingly. In following years, continuing cultivations were done and SP4 and C4 were obtained. This study is to evaluate the effectiveness of space mutagenesis breeding by analyzed the internal chemical changes of *I. indigotica* seeds with FTIR, second derivative and 2DIR correlation spectroscopy.

**Materials and methods**

*Samples and preparations*

SP4: the fourth generation of the seeds of *I. indigotica* SP1 after 12 h of spaceflight aboard Shenzhou I. C4: the fourth generation of the seeds of *I. indigotica* C1.

Compared with SP1, C1 was kept at normal ambient condition as the control. Both SP4 and C4 were pulverized into fine powder using Fritsch Universal Cutting Mill with sieve cassette 1.00 mm Trapezoidal perforation. Achieved powder were sieved using Retsch test sieve 200 mm DIA + 50 mm.

**Apparatus**

Perkin–Elmer Spectrum GX Fourier-transform infrared (FTIR) spectrometer with an attached DTGS (deuterated triglycine sulfate) detector was used as the main equipment for the whole experiment. The dynamic FTIR spectra were recorded with the above spectrometer combined with a portable, programmable temperature controller (Model 50-886, Love Control) with a controllable range from 20°C to 120°C. The thermal dependent 2D-IR spectra were obtained from the dynamic spectra series using 2D-IR correlation analysis software developed by the Analysis Center of Tsinghua University, PR China.

**Procedures**

The powder (0.01 g) was mixed evenly with 0.09 g of KBr crystal. The mixture was ground and pressed into a tablet with a pressure of not more than 10 psi (pounds per square inch). In this experiment, various ratios of SP4 and C4 were attempted several times. The best transmission was when the difference between the highest and lowest transmission value was about 80 and was chosen for further analysis. Later on, the sample tablet was put into the temperature-controlled pool and the FTIR spectra were

![Fig. 5. Synchronous 2D-FTIR correlation spectra of SP4 and C4 in the region of 1800–1400 cm⁻¹.](image-url)
recorded in situ. These results were interpreted for 2DIR correlation spectroscopy. Dynamic FTIR spectra were obtained after 32 scans and were collected by varying the temperature from 50°C to 120°C at an interval of 10°C.

Results and discussions

Comparison of the 1D-FTIR spectra

Fig. 1 showed the overall pattern of both spectra which indicated the basis absorption of mid infrared for the raw material of *I. indigotica*. The spectra were compared with those in the report on “The Compilation of 2D-IR Chinese Herbal Medicines” [14]. Both the spectra showed two strong peaks at 2925 cm\(^{-1}\) and 2854 cm\(^{-1}\) respectively which were assigned to methylene (CH\(_2\)) in their asymmetric and symmetric stretch vibration absorption for alkaloids, steroids and triterpenoids plant content [15]. Most of the peaks bound together and overlaid but the characteristic peaks were clearly visible in the range of 1800 cm\(^{-1}\) to 500 cm\(^{-1}\). Hence, the interpretation have to be based on raw material. There are 5 major peaks with two strong peaks achieving absorbance >0.3 and accompanied by a few peaks which could be combined. The peak at 1745 cm\(^{-1}\) on both spectra exhibited similar intensity for the content of aldehydes, ketones and carbonyl stretching vibration. On the other hand, peak at 1656 cm\(^{-1}\) on both spectra was considered as the absorption of O—H bond bending vibration, C=O stretching vibration of flavonoids and NH\(_2\) of amino acids.

Not much differences of the two spectra was found except the peaks in the range of 1500–1200 cm\(^{-1}\) which was about 7 cm\(^{-1}\) different within both spectra (1435.96–1429.15 cm\(^{-1}\) and 1265.53–1258.34 cm\(^{-1}\)), indicating the absorption could be initiated from different bonds. The region between 1429.15 cm\(^{-1}\) and 1265.53 cm\(^{-1}\) in SP4 spectrum was closer, whereby region between 1435.96 cm\(^{-1}\) and 1268.34 cm\(^{-1}\) expanded wider in C4 spectrum. The peaks around 1429 cm\(^{-1}\) and 1435 cm\(^{-1}\) were attributed to C—H bond bending in methylene as well as O—H bond in-plane bending vibration from flavonoids and amide peak. In addition, peaks around 1265 cm\(^{-1}\) and 1258 cm\(^{-1}\) were attributed to C=O bond of flavonoids, steroidal and triterpenes. In the range of 1200–1020 cm\(^{-1}\), the absorption peaks were considered strong and most of them were attributed to organic anhydrides, ethers, sugars as C—O bond of glycosides and C—N bond of amine.

Comparison of second derivative spectra

Fig. 2 showed the second derivative spectra of SP4 and the control C4. The important regions were shown in the highlighted boxes. The overall picture showed peaks in the spectrum of SP4 were sharper in the two highlighted zones, especially the peak at 1747 cm\(^{-1}\) which showed stronger than that at 1745 cm\(^{-1}\) of C4 in intensity. The region around 1500 cm\(^{-1}\) had double peaks at around 1544 cm\(^{-1}\) in C4 but a single peak at 1537 cm\(^{-1}\) in SP4. This scenario occurred oppositely for the single peak at 1512 cm\(^{-1}\) in C4 and double peaks around 1516 cm\(^{-1}\) in SP4.

Fig. 6. Synchronous 2D-FTIR correlation spectra of SP4 and C4 in the region of 1400–800 cm\(^{-1}\).
Fig. 3 continued the description on the second derivative in the range of 1200–800 cm\(^{-1}\) which consequently played the crucial role of the fingerprint of these samples. The figure was conveniently divided into 3 regions for discussion. First region was observed the peak at 1188 cm\(^{-1}\) in SP4 which disappeared in C4. The peak at 1166 cm\(^{-1}\) in SP4 might be assigned similar absorption with the peak at 1162 cm\(^{-1}\) in C4. The similarity of this region in these two spectra also included derivative in the peak at 1108 cm\(^{-1}\); peaks at 1105 cm\(^{-1}\) and 1079 cm\(^{-1}\) of C4 was derived from the peak at 1108 cm\(^{-1}\) in the first derivative spectrum. Both were separated by 26 cm\(^{-1}\). However, peaks at 1111 cm\(^{-1}\) and 1076 cm\(^{-1}\) in SP4 were separated by 35 cm\(^{-1}\). SP4 showed different pattern of derivative to C4. As mentioned earlier, the absorption in the range of 1200–1020 cm\(^{-1}\) is attributed for organic anhydrides, ethers and sugars. A report for determination of salicylic acid, syringic acid, benzoic acid and anthranilic acid in *Radix Isatidis* by Wang et al. [16] showed a similar evidence. The derivative from the peaks from 1057 cm\(^{-1}\) to 984 cm\(^{-1}\) in C4 showed slight changes and the peak at 984 cm\(^{-1}\) obviously sharper in SP4. Most of the peaks in the third region of C4 were not as sharp as in those of SP4.

Fig. 4 showed the second derivative spectra in the range of 800–400 cm\(^{-1}\). The lower wavenumber of this range below 500 cm\(^{-1}\) showed fewer big and sharp peaks due to the possibility of noise. The region focused on around 600 cm\(^{-1}\) as the huge peak was specifically located in this region on 1D spectra. The small peaks in the highlighted area could be differentiated by the pattern of absorption in C4 and they are considered constantly low and steady. While in SP4, the scenario changed as the peaks fluctuated. However, most of the peaks showed weak intensity. The peak at 666 cm\(^{-1}\) in SP4 was about twice as high as that at 667 cm\(^{-1}\) in C4. Nakanishi and Solomon [17] reported that the wavelength of absorption in the 700–600 cm\(^{-1}\) referred to the bending of C–H where its overtone may appear around 1250 cm\(^{-1}\). Compared with 1256 cm\(^{-1}\) in the spectrum of C4 and SP4, diagnostic value is limited.

### 2DIR spectra analysis

**Synchronous 2D-FTIR correlation spectra of I. indigotica seeds in the region of 1800–1400 cm\(^{-1}\)**

Two spectra could be differentiated in the range 1800–1400 cm\(^{-1}\) in term of their intensity and color (Fig. 5). SP4 could eliminate the absorption of infrared because its whole synchronous spectrum was not as strong as that of C4. The signal caused by spaceflight could be weaker after the seeds cultivated with several generations. The exceptions are the peaks at 1700–1600 cm\(^{-1}\) which were colored red and showed strong intensity. On the other hand, the single auto-peak at 1580 cm\(^{-1}\) in C4 is weakly correlated with the cross-peak compared with SP4. For SP4, the correlation
square formed at 1650 cm\(^{-1}\) were correlated strongly to the cross-peak at (1650, 1550), (1550, 1650) and 1550 cm\(^{-1}\). The auto-peak spectrum showed the actual sharpness of the peaks. Both peaks were referred to amide I (1650 cm\(^{-1}\)) and amide II (1550 cm\(^{-1}\)) and the stronger signal of 2DIR in SP4 indicated the protein content of the seed was changed after spaceflight. Auto peaks at 1495 and 1550 cm\(^{-1}\) in SP4 were sharper than those in C4. The double peaks of SP4 achieved different level as the peak close to 1700 cm\(^{-1}\) was lower. Even the double peaks in C4 were not the same level but the peak close to 1700 cm\(^{-1}\) was slightly higher. The crystal-like illustration of the 3D-IR spectrum C4 clearly showed different level of absorption. The red spot on the top was the specific scope which is smaller in SP4. The four different direction corners of SP4 contained peaks with similar height and bigger than those of C4. Nevertheless the four different direction corners of SP4 contained peaks with similar height and were larger than those of C4.

Synchronous 2D-FTIR correlation spectra of \textit{I. indigotica} seeds in the region of 1400–800 cm\(^{-1}\)

The region of 1400–800 cm\(^{-1}\) showed unique and dissimilar pattern of 2DIR spectrum in Fig. 5. The main and higher peaks disseminated to four corners which were separated to two clusters and the highest auto peak was located in the upper right of both spectra. Therefore the main differences of both spectra was the correlation square formed by auto peak at 950 cm\(^{-1}\), crosspeak (950, 1250), auto peak at 1250 cm\(^{-1}\) and crosspeak (1250, 950). By comparison, the intensity of spectrum SP4 of the four main peaks was moderate. Fig. 6 enhanced the comparison which showed that the auto peak from 820 to 1100 cm\(^{-1}\) in C4 was about one time higher than that in SP4. The scenario contradicted with a auto peak at 1200 cm\(^{-1}\) in SP4 which was slightly higher than that in C4. Both spectra in the 3D-FTIR obviously enhanced the performance of differences especially within this range of wave length. The four auto peaks in red top shape pointed four directions in C4 but only a single auto peak with red top shape in SP4. The comparison showed that the characteristic fingerprint of \textit{I. indigotica} of SP4 has stronger absorption.

Synchronous 2D-FTIR correlation spectra of \textit{I. indigotica} seeds in the region of 800–400 cm\(^{-1}\)

There is not much differences of the spectra within the region of 800–400 cm\(^{-1}\) (Fig. 7). The most interesting could be the dark red and the highest auto peak at 730 cm\(^{-1}\) in both spectra. The 2D-FTIR showed the strong single peak at 730 cm\(^{-1}\). The comparison indicated that there was not much change in saccharides contents between SP4 and C4.

Conclusions

The internal chemical changes of SP4 and C4 were analyzed with FTIR, second derivative and 2DIR correlation spectroscopy.  
Under the 1D FTIR spectra, the sample SP4 spectrum has similar absorption IR peaks with that of C4. The second derivative revealed the differences of two spectra. In the range of 1800–1200 cm\(^{-1}\), the peaks of spectrum C4 was stronger and sharper than that of SP4. The 2DIR spectra of two samples were comparable in the range of 1800–1400 cm\(^{-1}\). Amide II absorption of SP4 was not preserved as that of C4, but its lower intensity indicated that mutation caused by spaceflight affected the protein structure of the plant and was stable after cultivation with a few generations. Second derivative spectra in the range of 1200–800 cm\(^{-1}\) showed different spectra of two samples. The 2DIR spectrum in the range of 1400–800 cm\(^{-1}\) of C4 was stronger than that of SP4 in the main correlation square. Albeit the range of 800–400 cm\(^{-1}\) of second derivative showed the C4 spectrum contained sharper peaks than that of SP4. 2DIR correlation spectroscopy enhanced and confirmed that there are limited changes between SP4 and C4. In conclusion, spaceflight provided an extreme condition that caused chemical changes in \textit{I. indigotica} and the changes could be determined with FTIR and 2DIR correlation spectroscopy even after cultivation with several generations.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.01.048.

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