African Journal of Biotechnology Vol. 10(29), pp. 5578-5586, 22 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2160
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Elevated level of polysaccharides in a high level UV-B tolerant cell line of *Bupleurum scorzonerifolium* Willd

Yuzhong Li[1], Jing Fan[1], Haiying Ma[1], Fei Shen[1], Ge Zhang[1], Jianguang Wang[1], Guangmin Xia[2], Duoqing Fan[3] and Suiyun Chen[1]*

[1]School of Life Sciences, Yunnan University, Kunming 650091, China.
[2]School of Life Sciences, Shandong University, Jinan, 250100, China.
[3]HongYunHongHe Tobacco (Group) Co., Ltd., 650202, China.

Accepted 26 April, 2011

A cell line of *Bupleurum scorzonerifolium* Willd with high level tolerance to UV-B radiation was obtained through exposure of the protoplasts to high doses of UV-B radiation at the intensity of 380 μW/cm^2 for 240 s. The cell line was named anti-UV-B cell line and proved to be a mutant from the original protoplast by RAPD tests and kept the genetic stability among different generations. Chemical analyses showed that, the anti-UV-B cell line contained excessively higher content of polysaccharides and relatively higher content of triterpenes than those from the wild-type plants and cell lines from unscreened protoplasts. To understand the possible contribution of polysaccharides to its high tolerance to UV-B radiation, the antioxidant activity of the polysaccharides in this cell line was evaluated by free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. The results showed a significant antioxidant effect of the polysaccharides from this cell line in a concentration-dependent manner. This indicated that, the high content of polysaccharides might account for the high tolerance to UV-B radiation of this anti-UV-B *B. scorzonerifolium* cell line. Furthermore, this cell line displayed vigorous growth ability with high content of polysaccharides and triterpenes under artificial conditions, especially in the bioreactor, indicating it could have promising application potential for industrial production of antioxidant in medical and cosmetic areas.

Key words: *Bupleurum scorzonerifolium* Willd, polysaccharides, *in vitro* culture, UV-B tolerance, antioxidant.

INTRODUCTION

Ultraviolet radiation (UV) is one of the major stresses for all plants confront. UV stress has been particularly serious since the 1970s because of the depletion of the stratospheric ozone, the primary attenuator of UV rays, resulting in an increased amount of UV-B radiation reaching the earth's surface (Solomon s, 1999). This has become a serious concern of many researchers.

UV radiation is generally divided into three categories depending on the wavelength, long wave UV-A (320 to 400 nm), medium wave UV-B (280 to 320 nm) and short wave UV-C (200 to 280 nm). Among them, UV-C is absorbed by the ozone layer; UV-A is almost harmless for it is hardly absorbed by DNA; UV-B is the most destructive for organisms because it is easily absorbed by DNA and therefore, induces many adverse biological effects (Jansen et al., 1998; Hollósy, 2002). It has been shown that, UV-B exerts its detrimental effects mainly through direct DNA damage and the production of reactive oxygen species (ROS) (Jordan, 2002; Hollósy, 2002; Brosch and Strid, 2003). The mechanisms regarding effects of UV-B radiation on organisms in the world have been extensively studied in the last decades.

Plants have developed various mechanisms to alleviate the damages caused by UV-B during long period of evolution (Cockell and Knowland, 1999; Jordan, 2002; Frohnmeyer and Staiger, 2003; Jansen et al., 1998). Generally, two strategies are commonly taken by all plant; a passive way of UV-B screening substances to absorb UV-B rays and an active way of physiological mechanisms to repair UV-induced damages via repairing the DNA damage and removing the harmful oxygen-free

*Corresponding author. E-mail:chensuiyun97@gmail.com. Tel: +86 871 5031 491. Fax: +86 871 5031491.

radicals (ROS). Due to different origin and environment, different plants have evolved their own specific mechanisms of defense against UV-B and their capacity to protect from UV-B damage is greatly different, while we do not yet understand these underlying mechanisms. Some plants, especially their mutants or cell lines, are particularly worth to be studied intensively because they show remarkable tolerance to UV-B radiation, which would provide good material for us to understand various mechanisms against UV-B in plants.

Nan-Chai-Hu, *Bupleurum scorzonerifolium* Willd, is an important Chinese herb and has been used for thousands of years in China, Japan, Korea and many other places of Asia. It is commonly used for the treatment of influenza, fever, malaria, cancer and menstrual disorders (Chinese Pharmacopoeia Commission, 2005). Numerous studies have proved various biological activities of *B. scorzonerifolium*, including anti-inflammation activity, antipyretic effects, anticonvulsant effect, hepatoprotective effects, antibacterial and antivirus effects and reduction of blood pressure, etc (Izumi et al.,1997; Benito et al.,1998). In traditional Chinese medical practice, it is not recorded for treatment or prevention of solar radiation.

Surprisingly and interestingly, the protoplasts of *B. scorzonerifolium* were found to be UV-B radiation tolerance to some extent in a previous study (Wang et al., 2005). In a pretreatment of asymmetric somatic hybridization, protoplasts of *B. scorzonerifolium* were irradiated with UV-B radiation at the intensity of 380 μW/cm^2 for 120 s, but the chromosomes of *B. scorzonerifolium* remained intact. This triggered us to further study the UV-B radiation tolerance of protoplasts of *B. scorzonerifolium*, the underlying mechanism and its potential application of medical and cosmetic areas. A UV-B radiation tolerance cell line at intensity of 380 μW/cm^2 for 240 s was selected. Chemical analyses, RAPD analysis and the biological activity of polysaccharides on the cell line were conducted. Base on the results, the mechanism on the UV-B radiation protection of *B. scorzonerifolium* and its potential application in medical and cosmetic areas was discussed.

MATERIALS AND METHODS

Selection of a high UV-B radiation tolerance cell line from callus cells of *B. scorzonerifolium*

Protoplasts of *B. scorzonerifolium* were provided by Prof. Xia GM from School of Life Sciences, Shandong University, China. These protoplasts could tolerate UV-B radiation at intensity of 380 mW/cm^2 up to 120 s (Wang et al., 2005). To get higher UV-B radiation tolerant cell lines, the protoplasts of *B. scorzonerifolium* were screened by different doses of UV-B radiation.

Monolayer protoplasts of *B. scorzonerifolium* were spread on 3 cm Petri dishes at a density of 10^6-10^7 cells/ml in P5 liquid medium (Xia and Chen, 1996) and irradiated with UV-B at an intensity of 380 mW/cm^2 for 0, 60, 120, 180, 240 and 300 s, respectively. After irradiation, the protoplast plates were covered with plate lids, sealed with tape and cultured in the dark at 25°C. After the formation of callus at 1.5 to 2.0 mm long, the callus was carefully picked from the MB2 solid medium (Xia and Chen, 1996). The calli which survived and grew vigorously after treatment with the highest level of UV-B radiation was selected. A cell line of *B. scorzonerifolium* which can endure an UV-B irradiation of intensity 380 μW/cm^2 for 240 s was named as anti-UV-B cell line and was used for later study.

The culture of the anti-UV-B cell line

Solid medium culture

The anti-UV-B line calli with a volume about 2 to 4 mm^3 were subcultured on solid MS medium (Murashige and Skoog, 1962) supplemented with different concentrations of 6-BA (at 0, 0.5, 1.0 and 1.5 mg/l) and 2, 4-D (at 0, 0.5, 1.0 and 1.5 mg/l) and five repeats were cultured for each combination of different concentrations of hormones. Cultures were grown under an illumination of 2000 to 3000 lx for 12 h/d, at 23 to 25°C. The growth rate of the cell line was tested for each combination of different concentrations of phytohormones and measured as: growth rate = ((the fresh weight-the original weight) /the original weight)× 100%. The color and the texture of the cell line were also recorded.

Liquid flask culture

Approximately 1.0 g of anti-UV-B line calli in active growth phase (that is, the 14th day after subculture), was subcultured in 250 ml flasks containing 50 ml liquid MS medium supplemented with 1.0 mg/l 2, 4-D. The flasks were cultivated on a rotary shaker under agitation of 100 to 110 rpm at 23 to 25°C in diffuse light. Every seven days, three flasks were collected to determine their fresh weight by filtration with a Büchner funnel.

Bioreactor culture

The anti-UV-B line cells were injected into 10 L bioreactor (Bioflo 110 bioreactor, U.S.A.) at an optical density of approximately 2.0% (w/v). Oxygen was supplied through filtered air at 1 v/v/m and agitation was adjusted to maintain dissolved oxygen levels above 30%. The temperature of the culture in the bioreactor was controlled at 25°C and pH was controlled at 5.5 using 10% NaOH. Every seven days, 100 ml of the suspension culture was collected and filtered with a Büchner funnel to measure the fresh weight of the cell line.

Verify UV-B resistance of subculture material

Protoplasts were isolated from the anti-UV-B line cells after 10, 50 or 100 generations of subculture on solid MS medium and irradiated with UV-B radiation at intensity of 380 mW/cm^2 for 240 s, respectively. The equivalent anti-UV-B line cells (subcultured 10, 50 and 100 generation) without UV-B irradiation were set as the control to prepare protoplasts, respectively. Then the protoplasts were cultured as above to form callus.

RAPD assay for genetic character analysis

Genomic DNAs were isolated from wild-type plant (aerial parts), the unscreened callus cells and anti-UV-B line cells (subcultured 10, 50 and 100 generations on solid MS medium) with CTAB method (Stewart and Via, 1993). RAPD analysis was conducted using

Table 1. Primers used in the RAPD analyses.

Primer	5'- 3'sequence
G10	ACAACGCGAG
A1	CAGGCCCTTC
H4	GGAAGTCGCC
A8	GTGACGTAGG
H20	GGGAGACATC
F5	CCGAATTCCC
A19	CAAACGTCGG
J19	GGACACCACT

previously selected RAPD primers (Table 1) and amplification conditions (Wang et al., 2005).

Chemical analysis

Fresh unscreened callus cells, anti-UV-B line cells (subcultured 10, 50 and 100 generation on solid MS medium) and wild-type plants (aerial parts) were collected, crushed and vacuum dried to constant weight, respectively. Polysaccharides was expressed as glucan equivalents and estimated by phenol-sulfuric acid colorimetric method (Buysse and Merckx, 1993). Saponins was expressed as Panax notoginseng saponins equivalents and estimated by vanillin-perchloric acid colorimetric method (Hui et al., 2006). Flavonoids was expressed as rutin equivalents and estimated by aluminum nitrate colorimetric method (Hui et al., 2006). Triterpenes was expressed as ursolic acid and estimated by vanillin-perchloric acid colorimetric method (Brieskorn and Briner, 1954).

Estimation of antioxidant activity of the aqueous soluble polysaccharide extracts using DPPH test

Aqueous soluble polysaccharide was extracted from approximately 50 g dry anti-UV-B line cells with 400 ml distilled water in water bath at 90°C for 2 h. The extract was filtered and centrifuged to remove the contaminants. The supernatant was concentrated by evaporation under reduced pressure and the soluble polysaccharide was precipitated with 95% alcohol. Then, the precipitation was dissolved with water and dialyzed to remove the small molecules. The dialyzed solution was freeze-dried to yield crude cell line polysaccharides (CCCP).

The antioxidant activity of CCCP was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl-2- picryl-hydrazyl (DPPH) free radical test according to the method (Brand-Williams et al., 1995) with slight modification. In brief, 2 ml of sample solution at different concentrations (1.0 to 10 mg/ml) was added to 2 ml 0.2 mM ethanol solution of DPPH. The reaction mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm. The ability of scavenging the DPPH radicals was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [(A0 - A1)/A0] \times 100 \quad (1)$$

Where, A0 is the absorbance of DPPH solution without the tested samples; A1 the absorbance of the tested samples with DPPH solution.

Statistical analysis

All results are expressed as mean ± standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA). Means were compared by Tukey's or Dunnet's test, with $p < 0.05$ being considered as statistically significant.

RESULTS

The isolation of a *B. scorzonerifolium* cell line tolerance to high level of UV-B radiation

The number of calli formed from the protoplasts of *B. scorzonerifolium* under different dose of UV-B irradiation is showed in Figure 1. In the low dose (380 μW/cm^2 for 60 s) of the UV-B radiation, the number of calli formed was not significantly different from that of the blank control. Following the increase in dose of UV-B radiation, the number of calli formed decreased and no calli was formed under the highest dose (380 μW/cm^2 for 300 s) of UV-B radiation. Therefore, one cell line was selected and named as anti-UV-B cell line as it can endure an UV-B irradiation of intensity 380 μW/cm^2 for 240 s and grow vigorously. This line was cultured for further studies.

The growth state of anti-UV-B line cells

The anti-UV-B line cells grew well on solid medium, in liquid culture condition and in bioreactor. On solid medium, the optimal condition was MS medium supplemented with 2, 4-D (Table 2). The calli tended to maintain the undifferentiated state on MS medium supplemented with 2, 4-D, but prone to differentiation on MS medium supplemented with 6-BA. The MS medium containing 1.0 mg/l 2, 4-D was most suitable for the rapid growth and propagation of the calli, on which the growth rate of calli was up to 486% after 14 days subculture.

In the suspension culture condition, the suspension anti-UV-B clone cell line had an immediate increase of the fresh weight at the very beginning of the incubation process, but showed a wave of growth at later stages, during which initially the pH value of the medium went down and rose later; accordingly cells grew slow at first but much faster in the later stages. A significantly higher biomass accumulation was recorded on day 21 (Table 3). The anti-UV-B line cells grew normally in the bioreactor as well. As the culture proceeded, great amounts of biomass were accumulated (Figure 2). On the 21st day, optimal biomass was achieved as same as the culture in flasks. Therefore, continuous culture of the cell line can be achieved in the bioreactor.

Stability of UV-B radiation tolerance of the anti-UV-B line

All protoplasts isolated from the anti-UV-B cell line after

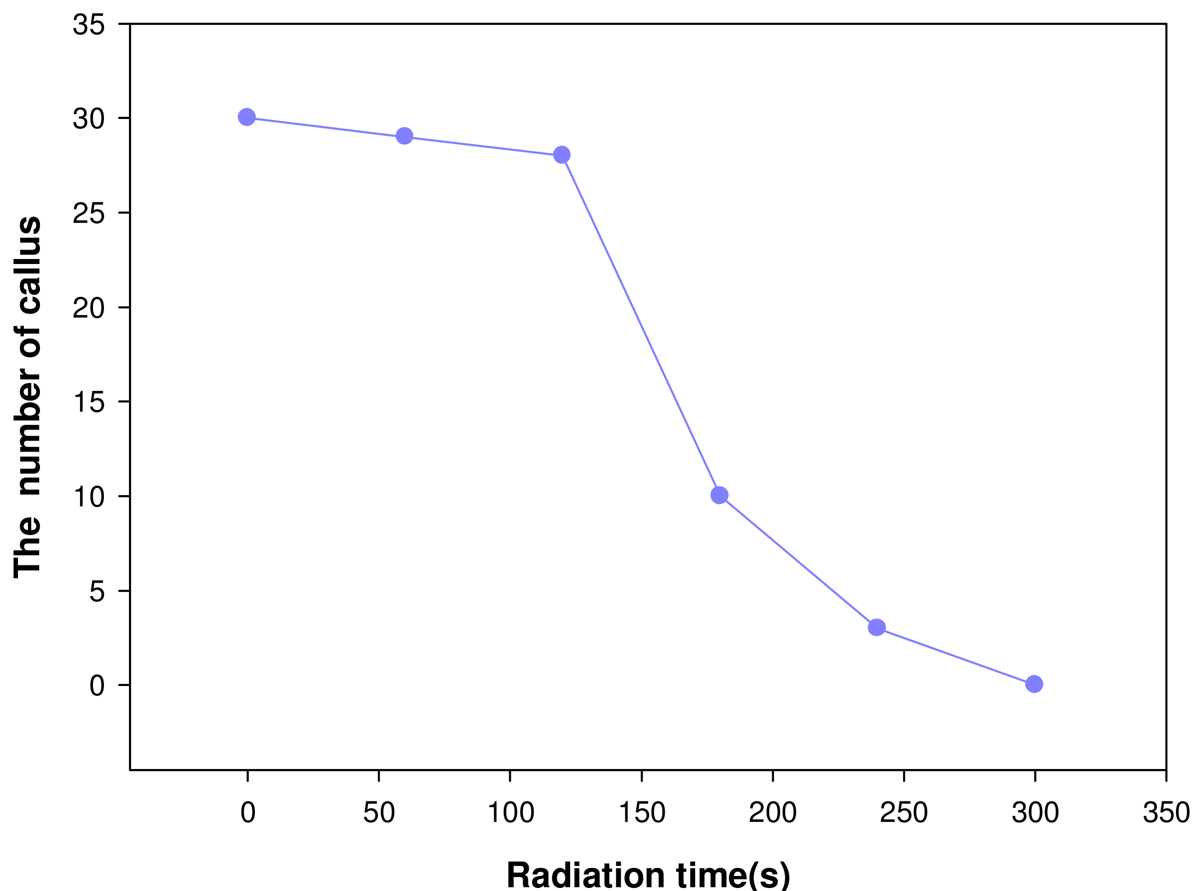

Figure 1. The number of calli formed from the protoplasts of *B. scorzonerifolium* under different time of treatment with UV-B radiation (380 μW/cm^2).

Table 2. The growth rate of the anti-UV-B cell line cultured on solid MS medium added with different 6-BA and 2, 4-D concentration.

Phytohormone (mg/l)		Growth rate (%)		Colour
6-BA	2,4-D	14th day	28th day	
0.5	0	192±10	292±11	Light yellow green
1	0	219±11	389±12	Yellow green
1.5	0	291±12	472±10	Yellow green
0	0.5	367±10	581±14	Light yellow
0	1	486±11	778±13	Yellow
0	1.5	332±13	510±12	Dark yellow
0	0	158±10	227±11	Yellow white

Results are the means ± SD of 3 different experiments running in quintuple.

10, 50 and 100 generations of subculture on solid MS medium showed their ability to tolerate the UV-B radiation at an intensity of 380 mW/cm^2 for 240 s (Figure 3) which suggested that the offspring of the anti UV-B cell line maintained the ability to resist UV-B irradiation.

RAPD analysis

Seven (G10, A1, H4, H20, F5, A19, J19) of eight primer pairs (Wang, 2004) were used to amplify RAPD bands. Different RAPD bands were amplified between the

Table 3. The fresh weight of the anti-UV-B cell line cultured in flasks.

Day	Fresh weight (g/l)	pH
0	20.0±2.1	6.0±0.2
7	26.0±2.5	5.8±0.2
14	102.5±2.2	5.3±0.1
21	112.3±3.3	5.6±0.1
28	113.4±2.4	6.1±0.2
35	114.5±2.4	6.4±0.2

Every 7 days, 3 flasks were collected to determine their fresh weight filtered with a Büchner funnel. Results are the means ± SD of 3 different experiments running in triplicate

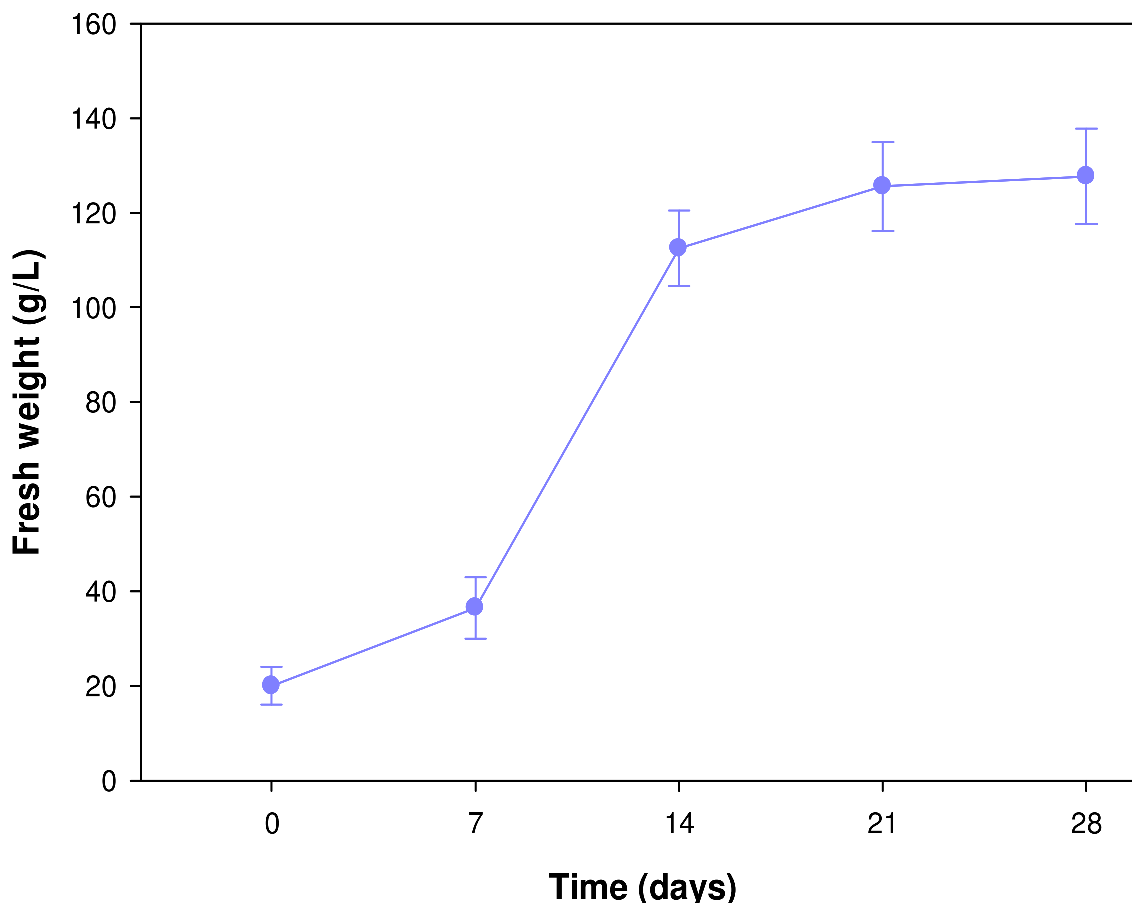

Figure 2. The growth curve of the anti-UV-B cell line cultured in bioreactor (Bioflo 110 bioreactor, U.S.A.).

unscreened callus cells of *B. scorzonerifolium* and wild-type plant (aerial parts) by three primer pairs (A1, J19, A19). Moreover, among those three primer pairs, different RAPD bands were amplified between unscreened callus cells of *B. scorzonerifolium* and anti-UV-B line cells (Figure 4). This indicated that, some somaclonal variation sites existed in the unscreened callus cells of *B. scorzonerifolium* and after selection new somaclonal variation sites appeared. However, the anti-UV-B line cells subcultured 10, 50 and 100 generations on solid MS medium all had the same amplification patterns (Figure 4), except for J19 which amplified a new band at 50 generation but disappeared at 100 generation. Thus, it was proved that those cells that survived after treatment with UV-B radiation for 240 s were genetically different from the original protoplasts and the genetic characteristics of the cell line was stable for RAPD markers used during the 5 year's subculture.

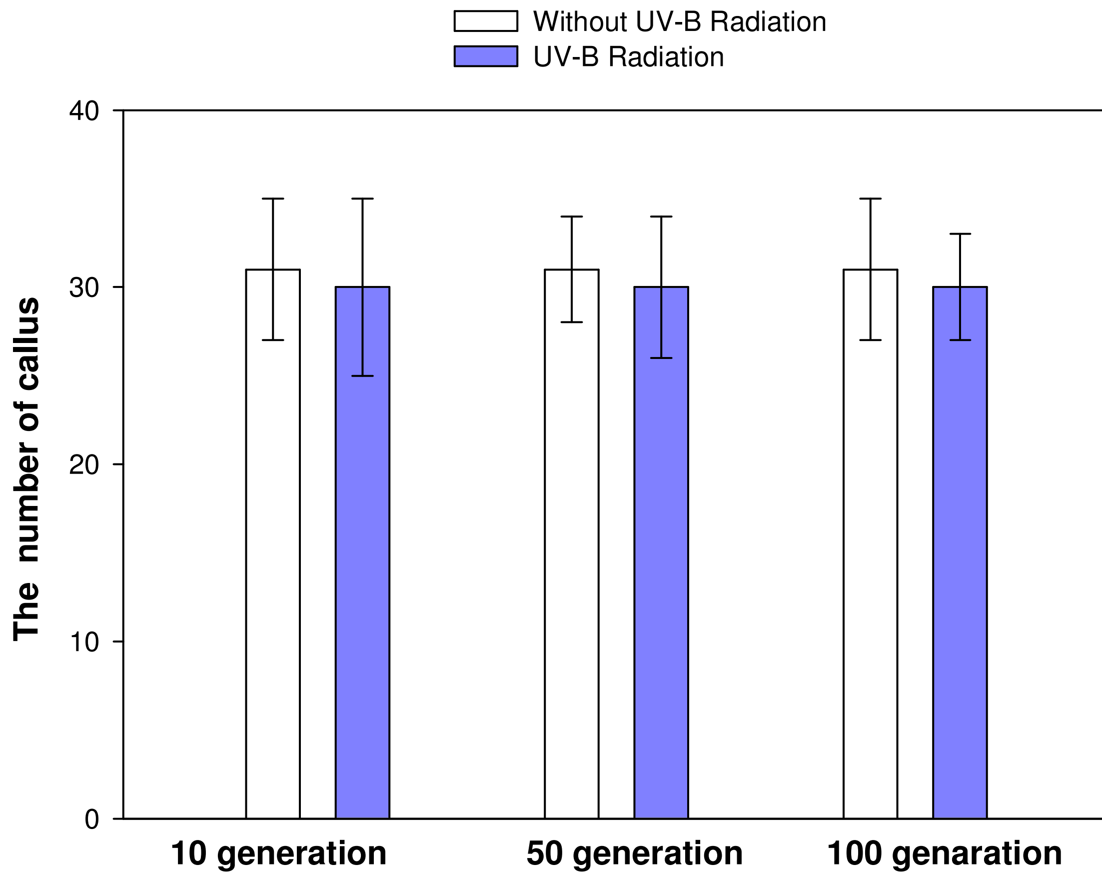

Figure 3. The number of calli formed from protoplasts of the anti-UV-B cell line after different generation of subculture.

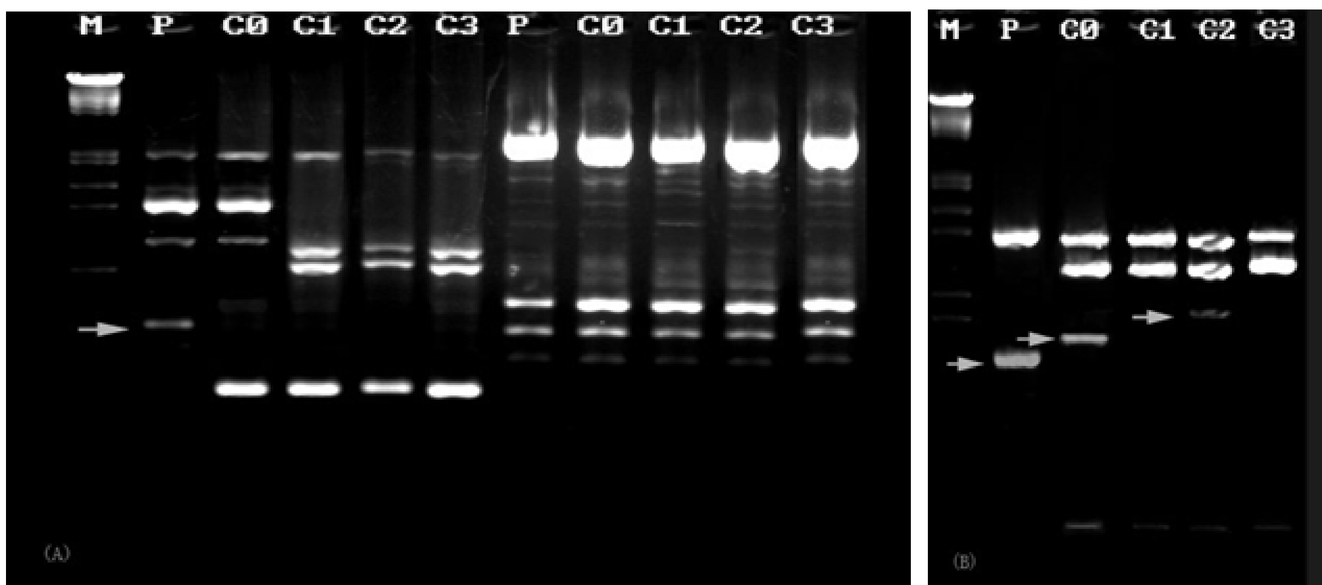

Figure 4. RAPD profile using A1, H20 and J19 primer pairs. (A), Primer pairs A1, H20 (left to right); (B), primers J19. C0, Wild-type plant (aerial parts); C1,10th generation; C2, 50th generation; C3, 100th generation; P, unselected *B.scorzonerifolium* callus; M, the lambda DNA size marker cut with *Eco*RI + *Hind*III. Arrow, the specific bands.

Table 4. Component analysis of the anti-UV-B cell line cultured at different generation.

Material	Polysaccharide (%)	Saponin (%)	Flavonoid (%)	Triterpene (%)
10th generation	2.5773±0.2943[a]	0.3438±0.0054[a]	0.5122±0.0202[a]	2.5093±0.0240[a]
50th generation	2.5763±0.2947[a]	0.3401±0.0061[a]	0.5102±0.0315[a]	2.5082±0.0320[a]
100th generation	2.5770±0.2916[a]	0.3418±0.0038[a]	0.5111±0.0517[a]	2.5097±0.0400[a]
Unscreened *B. scorzonerifolium* callus	1.0842±0.1022[b]	0.3325±0.0154[a]	0.5211±0.0620[a]	2.2712±0.0282[b]
Wild-type plant (aerial parts)	0.8968±0.1900[b]	0.5008±0.0249[b]	1.8470±0.1625[b]	1.5354±0.0292[c]

Polysaccharide was expressed as glucan equivalents; saponin was expressed as *Panax notoginseng* saponin equivalents; flavonoid was expressed as rutin equivalents; triterpene was expressed as ursolic acid. Numbers followed by the same letters were not significantly different at $p < 0.05$ /ANOVA one way (Tukey test); results are the means ± SD of 3 different experiments running in triplicate.

The content of polysaccharides, saponins, flavonoids and triterpenes

Of the subcultured anti-UV-B line cells, the quantitative determination analysis showed stable contents of polysaccharides, saponins, flavonoids and triterpenes in different generations of subculture (10th, 50th and 100th generation on solid MS medium) (Table 4). But there were different content among the anti-UVB line, unselected callus (which was actually selected once at lower UV-B irradiation before our study) and wild-type plant material. The subcutured anti-UV-B cell line had excessively high contents of polysaccharides than that from the unselected callus and wild-type plant of *B. scorzonerifolium* (aerial parts) (around 2.6% V.S. less than 1.1%) and marked higher level of triterpenes than the wild-type plant (2.5 V.S. 1.5%). The contents of saponins and flavonoids were similar in the anti-UVB line and unselected calli, but both were lower than that in the wild-type plant (0.3 V.S. 0.5%, 0.5 V.S. 1.8%, respectively) (Table 4).

Assay for antioxidant activity of water soluble polysaccharides from anti-UV-B cell line

Figure 5 demonstrates the DPPH scavenging activity caused by different concentrations of crude cell line polysaccharides (CCCP). The DPPH radical scavenging activity of CCCP reached 80.08% at 10 mg/ml and the scavenging ability of CCCP was in a concentration-dependent fashion.

DISCUSSION

In this study, a cell line of *B. scorzonerifolium* Willd with ultra-high tolerance to UV-B radiation was obtained from the protoplasts exposed to high doses of UV-B radiation at the intensity of 380 μW/cm^2 for 240 s. The chemical analysis showed that, the contents of polysaccharides were excessively accumulated in this anti-UV-B line. Additional DPPH test proved that, the aqueous soluble polysaccharides in this cell line had a significant scavenging activity of ROS in a concentration-dependent manner. As ROS play a very important role in mediating UV damage and scavenging of ROS to alleviate UV-B radiation detrimental effects (Frohnmeyer and Staiger, 2003; Jansen et al., 1998; Gao and Zhang, 2008), we postulated that the high content of polysaccharides was one of the pivotal factors that enabled this cell line of ultra-high resistance to UV-B irradiation.

The isolation and characterization of mutants or cell lines tolerant to UV-B radiation has been shown a powerful tool to learn about the mechanisms that protect cells against UV-B induced damage. The anti-UV-B cell line we obtained in this study showed higher tolerance in comparison with the wild type *B. scorzonerifolium* and other plants (Wang et al., 2005). There must have some defense system against UV-B radiation damage in the natural *B. scorzonerifolium* and some materials contributing to higher UV-B tolerance in the anti-UV-B cells. In most plants, DNA will be damaged with 380 μW/cm^2 UV-B radiation only for 30 s. In a previous study (Wang et al., 2005), the protoplasts of *B. scorzonerifolium* were found to resist UV-B radiation at such intensity for at most 120 s. However, in this study, the anti-UV-B cell line of *B. scorzonerifolium* still survived for up to 240 s and this high tolerance war also was found in the 10th, 50th and 100th generations; that is the cell clone still maintained this high UV-B tolerant property after sub-cultured through hundreds of generations.

Hard and strict experimental condition may contribute to the remarkable tolerance of the *B. scorzonerifolium* to UV-B radiation. Inconsistent with other studies on UV-B tolerant plants or mutants selection from complete plant cells (Landry et al., 1997; Bieza and Lois, 2001; Tanaka et al., 2002; Fujibe et al., 2004), in this study, the protoplasts were used. It has been shown that the cell walls and the apparatus (for example, wax and hairs) provide a physical defense to avoid UV-B radiation penetrating into the cells (Turunen and Latola, 2005); therefore, the UV-B radiation that enters into the cytoplasm is reduced. This exactly indicated that, the anti-UV-B cell line had extremely strong UV-B defense systems that can survive this harsh irradiation condition.

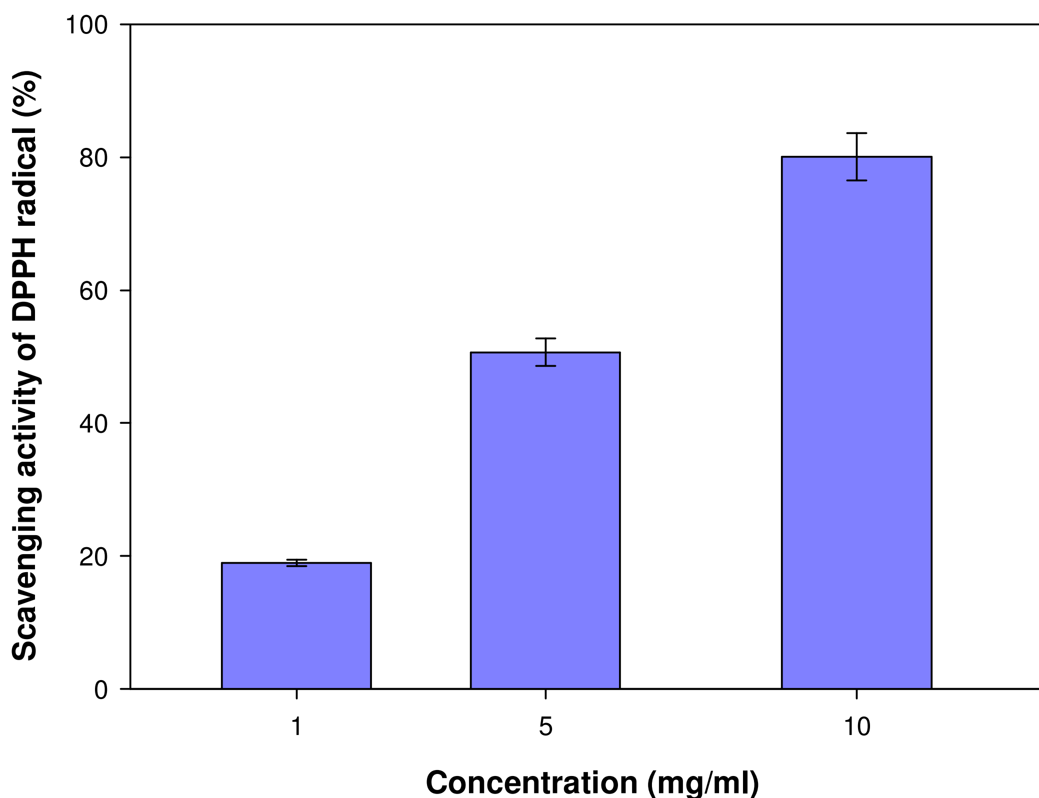

Figure 5. Scavenging ability of CCCP (crude cell line polysaccharides) against DPPH radical. Results were presented as means ± SD (n = 3).

In some studies, the callus cells were cultured in the Petri disc and the cells were treated with UV-B radiation with disc (Levall and Bornman, 1993). In this case, the UV-B radiation was also reduced by diffusion and reflection of the glass. Moreover, the uniformity of the cells was not secured in the callus cells, because the cells are multi-layered in the callus. While the upper layer cells may die from the treatment with UV-B radiation, the lower layer cells may probably survive at the same treatment condition with UV-B radiation, because they were actually exposed to low radiation. Thus, the mutant cell line selected by callus might not be genetically uniform. They might be a mixture of cells with high UV-B tolerance and those with low tolerance. In our study, monolayer protoplasts of *B. scorzonerifolium* were radiated with UV-B radiation, which had ensured the uniformity of the condition and therefore, all the protoplasts that have survived after treatment with the lethal UV-B radiation in the study possessed equally high UV-B tolerance.

As UV-B radiation can cause non- lethal mutations sometimes (Jansen et al., 1998), we speculated that some protoplasts of *B. scorzonerifolium* gained high UV-B tolerant capacity through mutation during the first treatment with UV-B radiation. The survived anti-UV-B cell line retained the mutation that were required for the tolerance to high dosage of UV-B radiation, so that in later generation all protoplasts isolated from anti-UV-B cell line can tolerate the high doses of UV-B radiation without any cell death from the 2nd treatment with UV-B radiation. These speculations were proved by RAPD test. The results of RAPD showed genetic difference between the anti-UV-B cell clone and the original protoplasts and the band patterns were steady in the protoplasts of the later generation, which means the mutation contributing to the high tolerance were genetically stable in all of the cells. A further study to characterize pertinent genes related to genetic difference between the cell line and the original protoplasts may help to elucidate the genetic mechanism underlying the high tolerance against UV-B radiation of the cell line.

In most studies on UV-B protective mechanisms, the polyphenolics, especially flavonoids were found to be the most important UV-B alleviator (Bieza and Lois, 2001), working as both UV-B absorbing compounds and antioxidants. However, in this study, the results of the chemical analysis showed excessively high contents of polysaccharides and relatively high level of triterpenes in the anti-UV-B cell line of *B. scorzonerifolium.* The contents of saponins and flavonoids were not pronouncedly accumulated. This inferred that rather than polyphenolics, the extra protection probably came from

elevated accumulation of polysaccharides and triterpenes in the mutant cells. As biological activity of triterpenes connecting with UV-B protection had not been reported and the increase in the content of triterpenes was not very remarkable in mutant cells, we proposed the triterpenes may only play a minor role in protecting the cell against the UV-B radiation. Therefore, the major UV-B alleviator in this anti-UV-B cell clone was probably polysaccharides. Polysaccharides exist widely in plants playing various roles in the cells and now more attention is paid to its antioxidant activity (Kardošováa and Machová, 2006). In this study, we used DPPH method to test the free radical scavenging activity of the polysaccharides extracted from the mutant cell clone. The results showed that, the polysaccharides had a strong capability of ROS scavenging. As there is no report of UV-B absorbing functions for polysaccharides, we postulate that the major defense mechanism of *B. scorzonerifolium* to UV-B is through the scavenging of ROS by polysaccharides. Together with a lot of studies that showed antioxidant activity of polysaccharides (Kardošováa and Machová, 2006), we suggested polysaccharides could be connected with the UV-B protective mechanism through their antioxidant activity. Notably, the contribution of triterpenes in ROS protection is also an interesting question to explore in the future study as the depletion of the ozone and increasing skin diseases occurred in the last decades. The polysaccharides extracted from this anti-UV-B cell line have direct efficacy of defense on UV-B damage caused in human keratinocytes (unpublished data, Chinese patent ZL 200610010859.6); therefore, the anti-UV-B cell line would be a good source of medicine or cosmetic material. Moreover, in the current farming production, pesticides cannot be completely avoided and other harmful residues which affect its security applications. However, the composition and content of chemical compounds in the cell line had little variable because of the single origin and genetic stability in our results. In addition, the cell line had a fast growth rate and can continuously be cultured in bioreactor under artificial conditions which can eliminate the uncertainty factor in the current farming production. Therefore, it is a potential engineer cell line for production of skin photoprotective agent in pharmaceutical industries or used as ingredient in cosmetic industries.

ACKNOWLEDGEMENT

The National Natural Science Foundation of China, no. 30760089 supported this study.

REFERENCES

Benito PB, Martínez MJA, Sen AMS, Gómez AS, Matellano LF, Contreras SS, Lanz AMD (1998). *In vivo* and *in vitro* antiinflammatory activity of saikosaponins. Life Sci. 63: 1147-1156.

Bieza K, Lois R (2001).An Arabidopsis mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. Plant Physiol. 126: 1105-1115.

Brand-Williams W, Cuvelier ME, Berset C (1995).Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 28: 25-30.

Brieskorn CH, Briner M (1954). Analysis of triterpenes. I. Colorimetric determination of total content of triterpenic acids in labiate plants. Arch. Pharm. Ber. Dtsch. Pharm. Ges. 287: 429-432.

Brosch M, Strid (2003).Molecular events following perception of ultraviolet-B radiation by plants. Physiol. Plant, 117: 1-10.

Buysse JAN, Merckx R (1993). An improved colorimetric method to quantify sugar content of plant tissue. J. Exp. Bot. 44: 1627-1629.

Chinese Pharmacopoeia Commission (2005). Pharmacopoeia of People's Republic of China. Chemical Industry Press, Beijing, China.

Cockell CS, Knowland J (1999). Ultraviolet radiation screening compounds. Biol. Rev. 74: 311-345.

Frohnmeyer H, Staiger D (2003). Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. Plant Physiol. 133: 1420-1428.

Fujibe T, Saji H, Arakawa K, Yabe N, Takeuchi Y, Yamamoto KT (2004). A methyl viologen-resistant mutant of Arabidopsis, which is allelic to ozone-sensitive rcd1, is tolerant to supplemental ultraviolet-B irradiation. Plant Physiol. 134: 275-285.

Gao Q, Zhang L (2008). Ultraviolet-B-induced oxidative stress and antioxidant defense system responses in ascorbate-deficient vtc1 mutants of Arabidopsis thaliana. J. Plant Physiol. 165: 138-148.

Hollósy F (2002).Effects of ultraviolet radiation on plant cells. Micron, 33: 179-197.

Hui R-h, Hou D-y, Li T-c, Liu X-y (2006). Simultaneous Determination of Total Flavone and Total Saponin in Gynostemma Pentaphyllum by Signal Multiplier Spectrophotometry. Spectrosc. Spect. Anal. 26: 1753-1756.

Izumi S, Ohno N, Kawakita T, Nomoto K, Yadomae T (1997). Wide range of molecular weight distribution of mitogenic substance (s) in the hot water extract of a Chinese herbal medicine, Bupleurum chinense. Biol. Pharm. Bull. 20: 759-764.

Jansen MAK, Gaba V, Greenberg BM (1998). Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci. 3: 131-135.

Jordan BR (2002). Molecular response of plant cells to UV-B stress. Funct. Plant Biol. 29: 909-916.

Kardošováa A, Machová E (2006). Antioxidant activity of medicinal plant polysaccharides. Fitoterapia, 77: 367-373.

Landry LG, Stapleton AE, Lim J, Hoffman P, Hays JB, Walbot V, Last RL (1997). An Arabidopsis photolyase mutant is hypersensitive to ultraviolet-B radiation. Proc. Natl. Acad. Sci. USA. 94: 328-332.

Levall MW, Bornman JF (1993). Selection in vitro for UV tolerant sugar beet (Beta vulgaris) somaclones. Physiol. Plant, 88: 37-43.

Murashige T, Skoog F (1962).A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Solomon S (1999). Stratospheric ozone depletion: A review of concepts and history. Rev. Geophys, 37: 275-316.

Stewart CN, Via LE (1993). A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques 14:748-749.

Tanaka A, Sakamoto A, Ishigaki Y, Nikaido O, Sun G, Hase Y, Shikazono N, Tano S, Watanabe H (2002). An ultraviolet-B-resistant mutant with enhanced DNA repair in Arabidopsis. Plant Physiol. 129: 64-71.

Turunen M, Latola K (2005). UV-B radiation and acclimation in timberline plants. Environ. Pollut. 137: 390-403.

Wang M (2004). Study on the elimination and fragmentation of exogenous chromosomes in the somatic hybrids of *Bupleurum scorzonerifolium*. Ph.D. thesis, Shandong University, Jinan.

Wang M, Xia G, Peng Z (2005). High UV-tolerance with introgression hybrid formation of Bupleurum scorzonerifolium Willd. Plant Sci. 168: 593-600.

Xia G, Chen H (1996). Plant regeneration from intergeneric somatic hybridization between *Triticum aestivum* L. and *Leymus chinensis* (Trin.) Tzvel. Plant Sci. 120: 197-203.