Flavonoids and Antioxidative Enzymes in Temperature-Challenged Roots of *Scutellaria baicalensis* Georgi

Yuan Yuan, Lingfei Shuai, Shunqin Chen, Luqi Huang*, Shuangshuang Qin, and Zhaochun Yang

Institute of Chinese Materia Medica, Academy of Chinese Medical Sciences, Beijing 100700, China. E-mail: huangluqi@263.net

* Author for correspondence and reprint requests

Z. Naturforsch. **67c**, 77–85 (2012); received March 21/October 27, 2011

The active compounds in the roots of *Scutellaria baicalensis* Georgi, a traditional Chinese medicinal plant, are mainly flavonoids which have anti-inflammatory, antitumour, and anti-HIV activity, respectively. The increasing annual average temperature has rendered the *S. baicalensis* plants grown in some ancient producing regions no longer suitable for their medicinal usage. Hydrogen peroxide plays an important role in root responses to abnormal temperature in *S. baicalensis*. Baicalin and baicalein and antioxidative enzymes were anticipated to detoxify H_2O_2 in *S. baicalensis*. Here, we show that abnormal temperatures (10 and 40 °C) decreased the content of flavonoids as compared with the normal temperature (30 °C), and the transcripts of UDP-glucuronate:baicalein 7-*O*-glucuronosyltransferase and *β*-glucuronidase involved in the interconversion between baicalin and baicalein were affected by the 40-°C treatment. High temperature also increased the activities of catalase and peroxidase. Reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that the transcript levels of peroxidase 2, peroxidase 3, monodehydroascorbate reductase 2, and dehydroascorbate reductase were significantly increased under high-temperature conditions. The respective genes would be candidates for improvement of the adaptation of *S. baicalensis* plants to abnormal temperatures and for regulation of the contents of the active compounds.

Key words: Scutellaria baicalensis Georgi, Abnormal Temperature, Active Compounds

Introduction

Scutellaria baicalensis Georgi is a traditional Chinese medicinal plant whose bioactive compounds include the flavones, or flavone glycosides, respectively, baicalin, baicalein (Fig. 1), wogonoside, wogonin, neobaicalein, visidulin I, and oroxylin A. These compounds possess anti-inflammatory, antitumour, and anti-HIV activities (Blach-Olszewska *et al.*, 2008). Most *S. baicalensis* plants grow naturally in northern China, where the average temperature is about 10 °C in spring and autumn and around 30 °C in summer (http://cdc.cma.gov.cn/). Temperature is an important environmental factor that may affect the medicinal quality of *S. baicalensis* (Li *et al.*, 2008). The baicalin content of *S. baicalensis* was previously shown to be correlated with the mean temperature in June and July (Yuan *et al.*, 2010). The Intergovernmental Panel on Climate Change Working Group II reported that many natural systems are being affected by regional

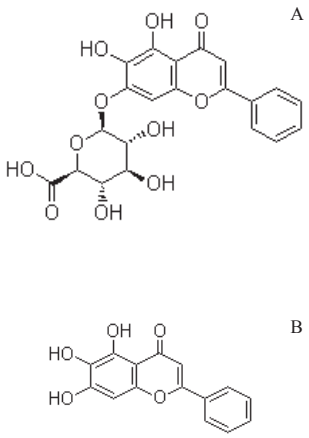

Fig. 1. Chemical structures of the flavonoids (A) baicalin and (B) baicalein.

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DHAR, dehydroascorbate reductase; GPX, glutathione peroxidase; GUS, *β*-glucuronidase; MDAR, monodehydroascorbate reductase; POD, peroxidase; SOD, superoxide dismutase.

climate change, particularly temperature increase (Rosenzweig *et al.*, 2008). The increasing annual average temperature in Hubei Province, an ancient *S. baicalensis*-producing region, has made the *S. baicalensis* plants from this area unsuitable for medicinal use.

High temperature is an important abiotic factor that can affect plant growth and productivity by inhibiting photosynthesis, damaging cell membranes, and inducing senescence and cell death (Xu *et al.*, 2006). One mechanism of injury at high temperature involves the overproduction of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) (Mittler, 2002). To control the ROS levels in their cells, plants produce several ROS-scavenging enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), and glutathione peroxidase (GPX). The activity levels of SOD and CAT in lotus seeds were shown to increase during the early phase of treatment at 100 °C and then decrease (Ding *et al*., 2008), while Cu/Zn SOD and APX expression in potato was upregulated by oxidative stress and high temperatures (Tang *et al*., 2006).

Low temperature also affects the ROS and detoxifying enzyme activity levels in plants. A temperature of 5 °C increased the $O_2^{\bullet-}$ content and activities of SOD and APX in tea (Vyas and Kumar, 2005). Pukacka and Ratajczak (2005) reported increased $O_2^{\bullet-}$ and H_2O_2 levels and changes in low-molecular weight antioxidants and enzymatic scavengers such as APX, DHAR, glutathione reductase (GR), CAT, SOD, and guaiacol peroxidase in *Fagus sylvatica* seeds following storage at 4, 20, and 30 °C. High and low temperatures lowered CAT activity and boosted SOD activity in tomato and watermelon plants (Rivero *et al.*, 2003).

Flavonoids are major secondary plant metabolites that function as antioxidants to detoxify ROS (Morimoto *et al.*, 1998). Flavonoid accumulation protects plants against various stressful conditions, including cold treatment (Lillo *et al.*, 2008) and freezing (Hannah *et al.*, 2006). In *S. baicalensis*, baicalein is involved in scavenging of H_2O_2, a peroxidase substrate (Morimoto *et al.*, 1998). Baicalin and baicalein are synthesized via the phenylpropanoid pathway which is initiated by phenylalanine ammonia-lyase (PAL). Baicalin is hydrolyzed to baicalein by β-glucuronidase (GUS), and is formed from baicalein by UDP-glucuronate:baicalein 7-*O*-glucuronosyltransferase (UBGAT). PAL expression and the anthocyanin content in tomato plants were shown to be increased in response to lower temperatures (Lovdal *et al.*, 2009). In comparison, high temperatures decreased the concentration of phenolic compounds in apple (Devic *et al.*, 1995), while Olsen *et al.* (2009) found that the initial anthocyanin flux and flavonol degradation were temperature-independent.

In this study, we analysed how high and low temperatures affect flavonoid accumulation and the transcription and activity of antioxidative enzymes in *S. baicalensis*. We also investigated the function of the flavonoids in *S. baicalensis* at 10 and 40 °C.

Material and Methods

Plant materials and experimental conditions

Seeds of *S. baicalensis* obtained from the Institute of Chinese Materia Medica, Academy of Chinese Medical Sciences (Beijing, China) were grown in pots containing 500 g of a mixture of peat and sand (2:1) in a growth chamber maintained at 30 °C under long-day conditions (light intensity, 600 μM m^{-2} s^{-1}). The plants were irrigated every third day with 80 g of distilled water. Three-month-old *S. baicalensis* plants were exposed to temperatures of 10, 30, and 40 °C, respectively. The roots were sampled 30, 40, and 50 d after treatment.

Flavonoid content

Powdered root material (100 mg) was extracted in 1 mL ethanol for 1 h at room temperature. The solution was filtered through a membrane filter (0.2 μm), and the concentrations of the flavonoids were determined by HPLC using a Diamonsil C_{18} column (4.6 × 250 mm, 5 μm) at a flow rate of 1.0 mL min^{-1}. The detection wavelength was set at 280 nm, and the column temperature was maintained at 30 °C. The mobile phase consisted of (A) acetonitrile/deionized water/methanoic acid (21:78:1, v/v/v) and (B) acetonitrile/deionized water/methanoic acid (80:19:1, v/v/v). HPLC-grade acetonitrile (Merck, Darmstadt, Germany) was used. The conditions were A:B (100:0, v/v) for 15 min, followed by linear changes to A:B (87:13, v/v) within 25 min, A:B (52:48, v/v) for 40 min, and A:B (0:100, v/v) for 60 min. Peaks were identified based on the retention time of standards

(0.208 mg mL^{-1} baicalin and 0.602 mg mL^{-1} baicalein) obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The injection volume was 20 μL for the sample solution, and the experiment was repeated six times. The amounts of baicalin and baicalein were calculated according to the method of Li *et al.* (2009).

Quantitative analysis of the total flavonoid content was performed on a sample using the aluminum chloride colorimetric assay method (Marinova *et al.*, 2005). The total flavonoid content was calculated as baicalin equivalents. The experiment was repeated six times.

H_2O_2 concentration

Fresh root tissue (0.1 g) was ground in liquid nitrogen and extracted with 50 mM K_3PO_4 buffer (pH 7.8), and the H_2O_2 concentration was measured by monitoring the titanium peroxide complex absorbance at 415 nm, as described by Patterson *et al.* (1984).

Assay of enzymatic activities

Fresh root tissue (100 mg) was ground in liquid nitrogen and extracted with 50 mM Na_3PO_4 buffer [pH 7.0 for CAT and pH 7.8 for SOD and POD (peroxidase)] containing 1% (w/v) polyvinyl pyrrolidone and 0.1 mM Na_2EDTA. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000 x *g* for 20 min. The supernatant was used to determine the enzyme activities and protein concentration. The extractions and enzyme activity assays were done at 4 and 25 °C, respectively.

The SOD activity was measured spectrophotometrically as described by Beyer and Fridovich (1987) with 1 unit of SOD being defined as the amount of SOD required to inhibit the photoreduction of nitroblue tetrazolium by 50%. The CAT activity was assayed according to Clairborne (1985) with the decomposition of H_2O_2 followed by the decline in absorbance at 240 nm for 2 min. One unit of CAT converts 1 mmol of H_2O_2 min^{-1}. The POD activity was determined as described by Chance and Maehly (1955) using guaiacol as an electron donor.

The PAL activity was determined as described by Ke and Saltveit (1986). The sample was ground in liquid nitrogen and extracted with 0.1 M $Na_2B_4O_7$ (pH 8.8). The change in absorbance at 290 nm was monitored in 1-cm light path cells at 10- to 15-min intervals for 30 min at 30 °C. Under these conditions, a change in absorbance of 0.01 was found to be equivalent to the production of 1 μg mL^{-1} cinnamic acid.

A quantitative analysis of the GUS activity was done by the MUG assay in root extracts (Wang *et al.*, 2002). The production of 4-methylumbelliferone (4-MU) was measured using a fluorometer (CytoFluor; Applied Biosystems, Foster City, CA, USA). The amount of 4-MU was determined from a standard curve. The protein concentration in the extracts was measured by the method of Bradford (1976).

Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA), quantified by spectrophotometry, and reverse-transcribed using a PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Dalian, China) following the manufacturer's instructions (the concentration of RNA in the reaction tube was 100 μg mL^{-1}). Gene expression was investigated using the primers listed in Table I. The RT-PCR conditions were 94 °C for 3 min, followed by 31 cycles at 94 °C for 30 s, annealing temperature according to Table I for 40 s, and 72 °C for 40 s, with a final hold at 72 °C for 10 min. The amplified fragments were separated on 1.5% agarose gels. Expression of 18S rRNA was used as an internal control to normalize for sample-to-sample variation in the amount of total RNA. The experiments were repeated at least three times using independently isolated RNA samples.

Statistical analyses

The effects of high- and low-temperature treatment were analysed with two-way analysis of variance (ANOVA). Asterisks shown above a column in the figures indicate significant differences between high- and low-temperature-treated and control (30 °C) roots, respectively. In addition, significant differences ($P < 0.05$) among the different temperatures were calculated with Tukey's multiple comparison test.

Table I. Primers used in this study for RT-PCR amplification.

Gene (GenBank No.)	Primer	Annealing temperature [°C]
PAL (EF501766)	5'-ATTCGGAAAAGGGACGGAG-3' 5'-GGGCAAGCATGGAGTGATG-3'	65
UBGAT (EF512580)	5'-AGCCAAGGAAGCCATAGTCAAC-3' 5'-CCGAAACAAAGGAAGACGACA-3'	60
GUS (AB040072)	5'-AGAGCAGTGTGAAGATAAGC-3' 5'-CATAGTAGGTCCAGGCAAG-3'	58
POD1 (AB024437)	5'-TGTCAAACCTACCACCACCT-3' 5'-GCGTATGCCAAATCCAGAGT-3'	52
POD2 (AB024438)	5'-CCCAGTGTGGCAACTTTCGT-3' 5'-ACAATGGCGTCTGTTTCTCCTC-3'	54
POD3 (AB024439)	5'-ACGATAGGACAGGCGAGGTG-3' 5'-TTGAAGAAAGCAGCCGAGTT-3'	53
SOD1 (HQ395746)	5'-AGTCCTCCCTTTCGTTCC-3' 5'-ACCGTTCTGGGTTTGTTG-3'	55
SOD2 (HQ395747)	5'-GGTGACCTGGGAAACATAG-3' 5'-AAAGAGGAGCAACCTTAGAG-3'	55
DHAR (HQ395748)	5'-ATTGATGCGGCTCTTCCC-3' 5'-CTGCGATAACATACTCTTCTGC-3'	46
MDHAR1 (HQ395749)	5'-TGGTTCTGATGGTCGTGT-3' 5'-GCAGTTAGCAGGGATTTA-3'	40
MDHAR2 (HQ395750)	5'-TGGTGGATACATAGGTCTG-3' 5'-TCGCATAATAGCCTTCAT-3'	40
MDHAR3 (HQ395751)	5'-TCATCAAGGGCACAGTAG-3' 5'-TTCAGCAGATTTGCGAGA-3'	40
APX (HQ395752)	5'-TACGCCAAGAGGATAGCA-3' 5'-GGTAAATCGTCTGGGAAG-3'	40
18S (FJ527609)	5'-CGTTGACTACGTCCCTGCCCTT-3' 5'-GTTCACCTACGGAAACCTTGTTACGAC-3'	60

Results

Effects of high and low temperatures on flavonoid content

The total flavonoid content and levels of baicalin and baicalein did not change over time at 30 °C. In comparison, exposure to 10 °C decreased the total flavonoid content and baicalin levels after 40 days. At 40 °C, the baicalein content decreased after 30 days and the plants were dead after 50 days at 40 °C (Fig. 2).

To better understand how temperature changes affect flavonoid accumulation, we analysed the transcript levels of three flavonoid biosynthetic genes, *i.e. PAL*, *GUS*, and *UBGAT*, under various conditions. We found that high temperature reduced the level of *GUS* and increased that of *UBGAT* transcript, respectively, whereas low temperature did not affect the levels of these transcripts compared with their levels at 30 °C (Fig. 3). At 40 °C, the expression of *UBGAT* after 40-days exposure was significantly higher than that after 30 days. The expression of all genes did not change during the exposure time under 30 °C.

Effect of temperature on antioxidative enzyme activities

Because baicalein is an important antioxidant that functions to eliminate H_2O_2 in *S. baicalensis* (Morimoto *et al.*, 1998), we analysed the H_2O_2 content at different temperatures (Fig. 4). The H_2O_2 content was unaffected by a temperature change.

We further analysed the activities of four enzymes involved in ROS elimination. At high temperatures, the POD activity was increased after 40 days and the CAT activity after 30 and 40 days. Low temperature did not affect the antioxidative enzyme activities.

Effect of temperature on the expression of genes encoding antioxidative enzymes

Two full-length clones encoding SOD were found from a full-length cDNA library constructed in our laboratory, and the levels of the respec-

tive transcripts at different temperatures were analysed by RT-PCR. Our results indicate that the expression of both *SOD* genes was unaffected by a change in temperature.

A BLAST analysis using the sequences of known *Arabidopsis POD* genes against those in GenBank identified three *POD* genes in *S. baicalensis*. We also identified *S. baicalensis APX*, *DHAR*, and *MDAR* genes. We further analysed the expression levels of these genes at different temperatures by RT-PCR. Our results indicate that the expression levels of *POD2* and *POD3* increased at high temperatures compared with the levels at 30 °C. The expression of *POD1*, *MDHAR1, MDHAR3*, and *APX* was not affected by high or low temperatures (Fig. 5). *DHAR* expression was increased at high and low temperatures after 40 days of exposure compared with the level at 30 °C. Only *MDHAR2* expression was increased at high temperatures after 40 days (Fig. 5).

Discussion

It is generally believed that temperature affects flavonoid production in plants. For example, high temperatures led to a lower level of the flavonoid kaempferol in broccoli (Mrkic *et al*., 2006). Pan *et al*. (2004) reported a progressive reduction in the anthocyanin content in strawberry fruits at

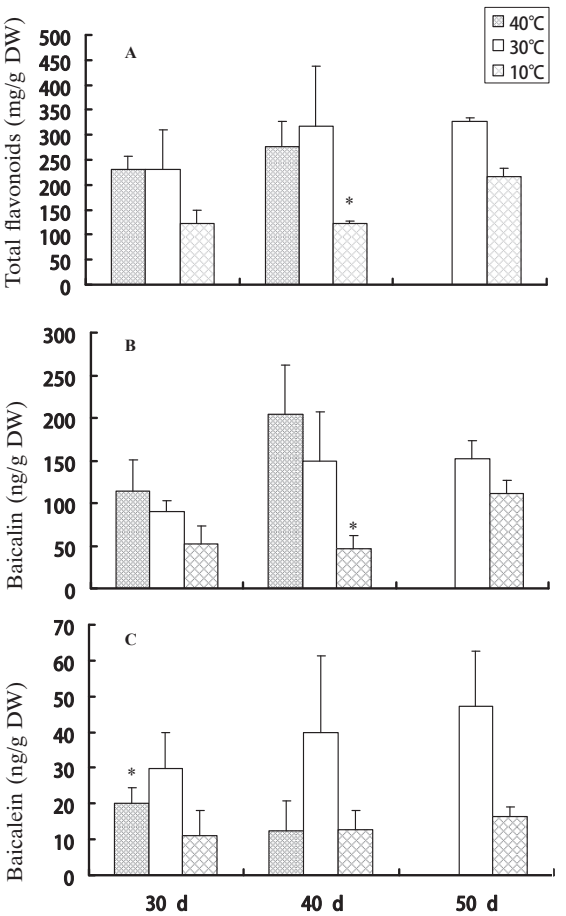

Fig. 2. Temperature effects on the content of flavonoid compounds. The asterisks above the columns indicate significant differences between control and high- and low-temperature-treated roots according to Tukey's multiple test ($P < 0.05$). (A) Total flavonoid content; (B) baicalin content; (C) baicalein content.

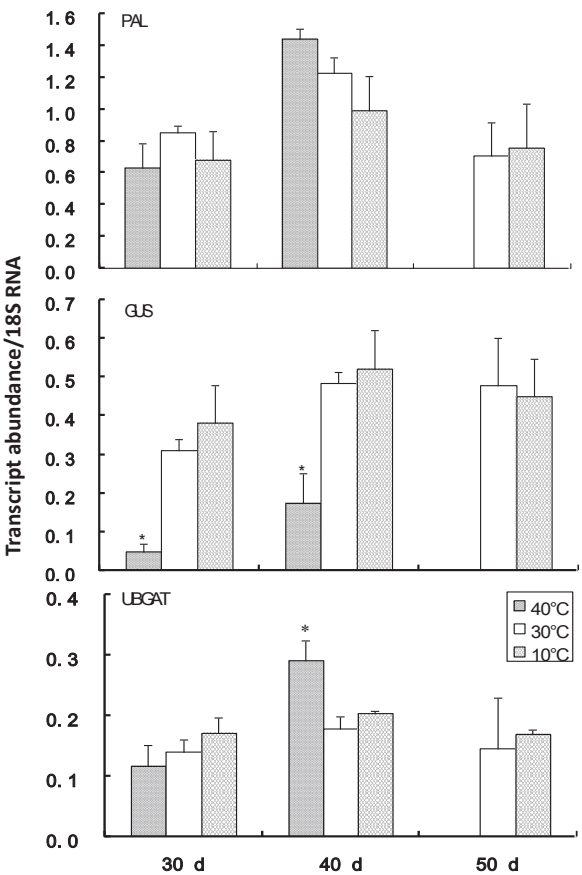

Fig. 3. Temperature effects on the expression level (transcript abundance/18S RNA) of flavonoid biosynthetic genes. The asterisks above the columns indicate significant differences between control and high- and low-temperature-treated roots according to Tukey's multiple test ($P < 0.05$).

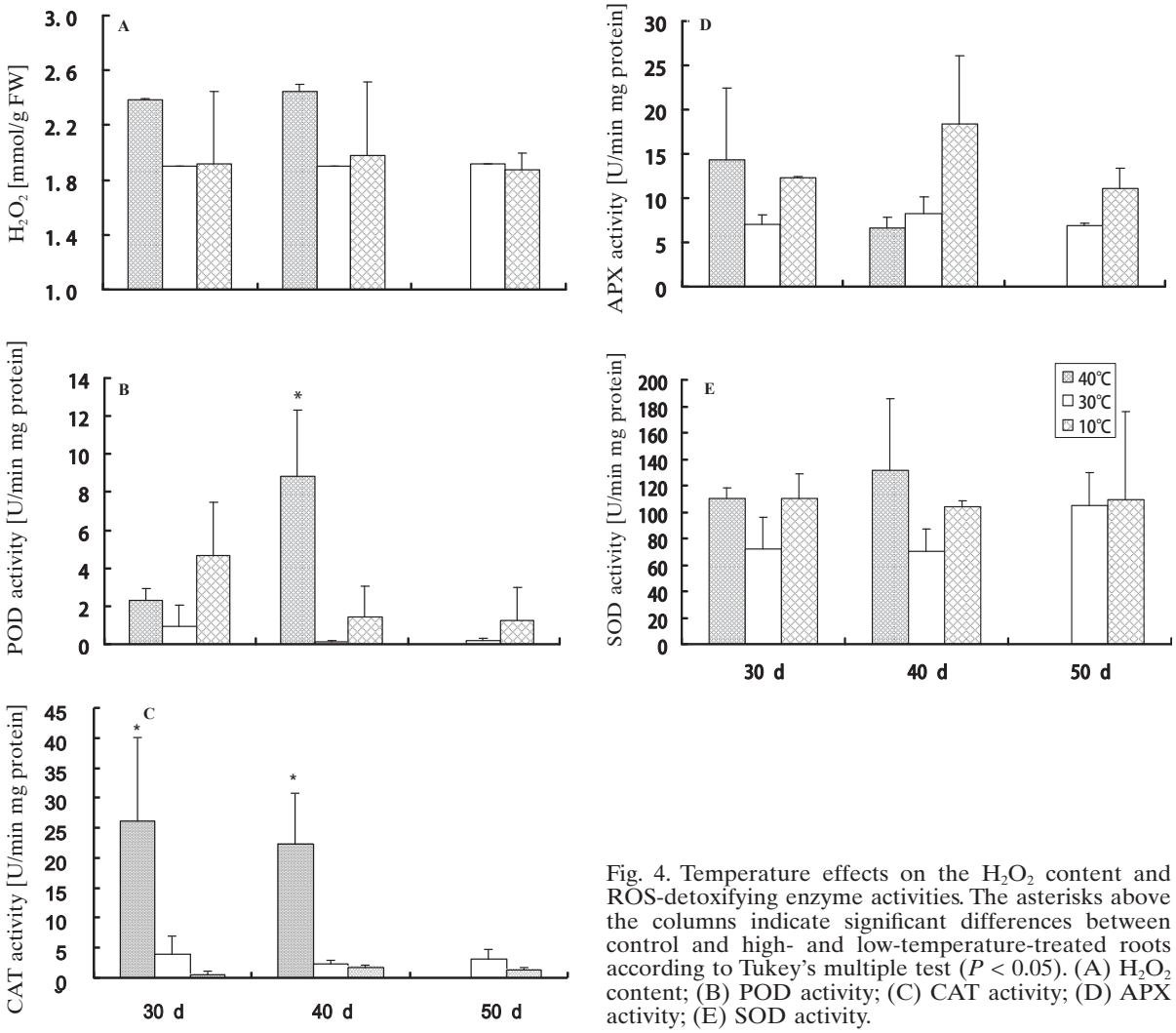

Fig. 4. Temperature effects on the H_2O_2 content and ROS-detoxifying enzyme activities. The asterisks above the columns indicate significant differences between control and high- and low-temperature-treated roots according to Tukey's multiple test ($P < 0.05$). (A) H_2O_2 content; (B) POD activity; (C) CAT activity; (D) APX activity; (E) SOD activity.

temperatures ranging from 30 to 45 °C, and they suggested that this reduction is responsible for the onset of oxidative damage. Low temperatures also affect the flavonoid content in plants. For example, Akhtar *et al.* (2010) reported flavone accumulation in *Lemna gibba* upon exposure to low temperatures. Anthocyanins also show markedly increased levels in tomato plants when the growth temperature is lowered from 24 to 18 or 12 °C. The medicinal quality of the roots of *S. baicalensis* is closely related to the flavonoid content. We found that a low temperature decreased the total flavonoid content and the level of the active compound baicalin, while high temperature decreased the baicalein content.

Further work was done to determine whether the change in the rate of flavonoid biosynthesis caused the decrease in these active compounds. *PAL* expression was increased in response to lower temperatures, which is in agreement with a corresponding increase in the flavonoid content in the leaves of tomato (Lovdal *et al.*, 2009). The transcription of the chalcone synthase (*CHS*) gene and other biosynthesis genes in the isoflavone calycosin-7-*O*-beta-D-glucoside pathway was up-regulated in *Astragalus* transferred from 2 to

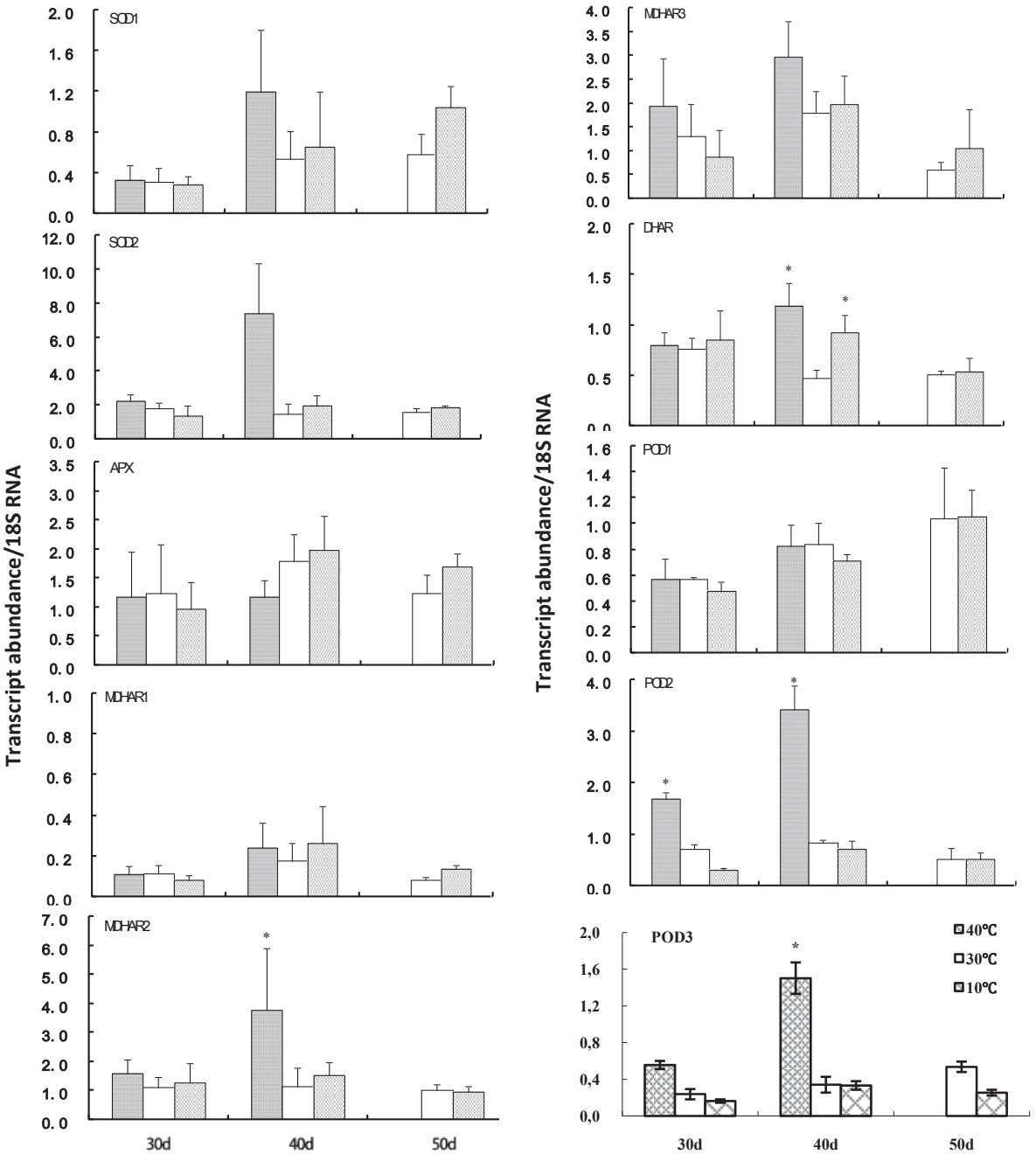

Fig. 5. Temperature effects on the expression level(transcript abundance/18S RNA) of genes encoding the antioxidative enzyme. The asterisks above the columns indicate significant differences between control and high- and low-temperature-treated roots according to Tukey's multiple test ($P < 0.05$).

25 °C (Pan *et al.*, 2008). In the roots of *S. baicalensis*, the transcript levels of *PAL* did not change after high- and low-temperature treatment, indicating that the changes of active compounds were not due to the alterations in the rate of flavonoid biosynthesis. Nevertheless, higher expres-

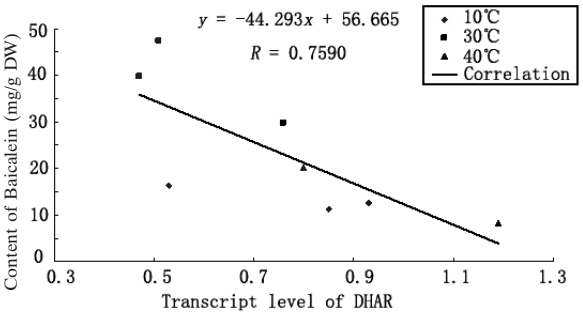

Fig. 6. Relationship between DHAR transcript level (transcript abundance/18S RNA) and baicalein content at all time points and temperatures. Correlation analysis using Excel software with linear regression formulae.

sion of *UBGAT* and lower expression of *GUS* in response to high-temperature treatment were observed, indicating that high temperature promotes the synthesis of baicalin from baicalein.

Both low and high temperature decreased the baicalein content, however, no change in flavonoid biosynthetic gene expression was found, indicating that the change of flavonoid levels might be related to the degradation pathway. Flavonoids protect plants against various biotic and abiotic stresses, and many of the biological roles of flavonoids are attributed to their potential cytotoxicity and antioxidant abilities (Pourcel *et al.*, 2007).

Flavonoids are important antioxidants that function to eliminate ROS after abnormal temperature exposure. A similar change in flavonoid content and flavonoid scavenging of $O_2^{\bullet-}$ and $OH^{\bullet}$ observed in soybean seedlings suggests that the flavonoids enhance the degradation of ROS (Peng and Zhou, 2009). In the roots of *S. baicalensis*, high temperatures did not affect the H_2O_2 content, but they increased the activity levels of POD and CAT. Low temperature did not affect the H_2O_2 content and the activities of APX, CAT, SOD, and POD.

Transcript levels of ROS-eliminating enzymes varied at different temperatures. To determine the transcript of which gene was related to the flavonoid content, the transcript levels of two *SOD*, one *APX*, one *DHAR*, three *MDHAR*, and three *POD* genes at 10, 30, and 40 °C were analysed. Among these genes, *POD2*, *POD3*, and *MDHAR2* responded strongly to high temperature only, whereas *DHAR* responded strongly to both low and high temperatures. The increase in the transcript level of *S. baicalensis DHAR* was significantly correlated with the content of baicalein (Fig. 6), indicating that high *DHAR* expression might lead to a high level of antioxidant recycling in the ascorbate-glutathione cycle, then promoting the conversion from baicalin to baicalein. Kim *et al.* (2010) reported that peroxidase contributes to ROS production during *Arabidopsis* root response to potassium deficiency. Cotton POD may be responsible for the high level of ROS production that occurs during cotton fiber elongation (Mei and Qin, 2009). These genes are good candidates for the adaptation of *S. baicalensis* to high and low temperatures and in the regulation of the flavonoid content in *S. baicalensis*.

Acknowledgements

This work was supported by China Postdoctoral Funding (201003228), Beijing Nova Program (2008B82), and the Self-selection Program of China Academy of Traditional Chinese Medicine (ZZ2008062).

Akhtar T. A., Lees H. A., Lampi M. A., Enstone D., Brain R. A., and Greenberg B. M. (2010), Photosynthetic redox imbalance influences flavonoid biosynthesis in *Lemna gibba*. Plant Cell Environ. **33**, 1205–1219.

Beyer W. F. and Fridovich I. (1987), Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. **161**, 559–566.

Blach-Olszewska Z., Jatczak B., Rak A., Lorenc M., Gulanowski B., Drobna A., and Lamer-Zarawska E. (2008), Production of cytokines and stimulation of resistance to viral infection in human leukocytes by *Scutellaria baicalensis* flavones. J. Interferon Cytokine Res. **28**, 571–581.

Bradford M. M. (1976), A rapid and sensitive technique to determine protein concentrations. Anal. Biochem. **72**, 248–254.

Chance B. and Maehly A. C. (1955), Assay of catalase and peroxidase. Methods Enzymol. **2**, 764–775.

Clairborne A. (1985), Catalase activity. In: Handbook of Methods for Oxygen Radical Research (Greenwald R. A., ed.). CRC Press, Boca Raton, FL, pp. 283–284.

Devic E., Guyot S., Daudin J. D., and Bonazzi C. (1995), Effect of temperature and cultivar on polyphenol re-

tention and mass transfer during osmotic dehydration of apples. J. Agric. Food Chem. **58**, 606–614.
Ding Y., Cheng H., and Song S. (2008), Changes in extreme high-temperature tolerance and activities of antioxidant enzymes of sacred lotus seeds. Sci. China Life Sci. C **51**, 842–853.
Hannah M., Wiese D., Freund S., Fiehn O., Heyer A., and Hincha D. (2006), Natural genetic variation of freezing tolerance in *Arabidopsis*. Plant Physiol. **142**, 98–112.
Ke D. and Saltveit M. E. (1986), Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. HortScience **21**, 1169–1171.
Kim M. J., Ciani S., and Schachtman D. P. (2010), A peroxidase contributes to ROS production during *Arabidopsis* root response to potassium deficiency. Mol. Plant. **3**, 420–427.
Li H., Huang L. Q., Yang B., Feng X. F., Li W., and Tang N. (2008), Content change of active compounds in different growth and development periods of *Scutellaria baicalensis*. China Trad. Herb Drugs **39**, 604–607.
Li S. M., Luo Y. J., Yuan Y., and Huang L. Q. (2009), HPLC determination of baicalin and baicalein in the callus of *Scutellaria baicalensis* Georgi. Chin. J. Exp. Trad. Med. Form. **8**, 1–3.
Lillo C., Lea U., and Ruoff P. (2008), Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. Plant Cell Environ. **31**, 587–601.
Lovdal T., Olsen K. M., Slimestad R., Verheul M., and Lillo C. (2009), Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato. Phytochemistry **71**, 605–613.
Marinova D., Ribarova F., and Atanassova M. (2005), Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J. Univ. Chem. Technol. Metall. **40**, 255–260.
Mei W. and Qin Y. (2009), Cotton GhPOX1 encoding plant class III peroxidase may be responsible for the high level of reactive oxygen species production that is related to cotton fiber elongation. J. Genet. Genom **36**, 141–50.
Mittler R. (2002), Oxidative stress, antioxidant and stress tolerance. Trends Plant Sci. **7**, 405–410.
Morimoto S., Tateishi N., Matsuda T., Tanaka H., Taura F., Furuya N., Matsuyama N., and Shoyama Y. (1998), Novel hydrogen peroxide metabolism in suspension cells of *Scutellaria baicalensis* Georgi. J. Biol. Chem. **273**, 12606–12611.
Mrkic V., Cocci E., Rosa M. D., and Sacchetti G. (2006), Effect of drying conditions on bioactive compounds and antioxidant activity of broccoli (*Brassica oleracea* L.). J. Sci. Food Agric. **86**, 1559–1566.
Olsen K. M., Slimestad R., Lea U. S., Brede C., Lovdal T., Ruoff P., Verheul M., and Lillo C. (2009), Temperature and nitrogen effects on regulators and products of the flavonoid pathway: experimental and kinetic model studies. Plant Cell Environ. **32**, 286–299.
Pan J., Vicente A. R., Martinez G. A., Chaves A. R., and Civello P. M. (2004), Combined use of UV-C irradiation and heat treatment to improve postharvest life of strawberry fruit. J. Sci. Food Agric. **84**, 1831–1838.
Pan H., Wang Y., Zhang Y., Zhou T., Fang C., Nan P., Wang X., Li X., Wei Y., and Chen J. (2008), Phenylalanine ammonia lyase functions as a switch directly controlling the accumulation of calycosin and calycosin-7-*O*-beta-D-glucoside in *Astragalus membranaceus* var. *mongholicus* plants. J. Exp Bot. **59**, 3027–3037.
Patterson B. D., Macrae E. A., and Ferguson I. B. (1984), Estimation of hydrogen peroxide in plant extracts using titanium(IV). Anal. Biochem. **134**, 487–492.
Peng Q. and Zhou Q. (2009), Antioxidant capacity of flavonoid in soybean seedlings under the joint actions of rare earth element La(III) and ultraviolet-B stress. Biol. Trace Elem. Res. **127**, 69–80.
Pourcel L., Routaboul J., Cheynier V., Lepiniec L., and Debeaujon I. (2007), Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends Plant Sci. **12**, 29–35.
Pukacka S. and Ratajczak E. (2005), Production and scavenging of reactive oxygen species in *Fagus sylvatica* seeds during storage at varied temperature and humidity. J. Plant Physiol. **162**, 873–885.
Rivero R. M., Sanchez E., Ruiz J. M., and Romero L. (2003), Influence of temperature on biomass, iron metabolism and some related bioindicators in tomato and watermelon plants. J. Plant Physiol. **160**, 1065–1071.
Rosenzweig C., Karoly D., Vicarelli M., Neofotis P., Wu Q., Casassa G., and Menzel A. (2008), Attributing physical and biological impacts to anthropogenic climate change. Nature **453**, 353–357.
Tang L., Kwon S. Y., Kim S. H., Kim J. S., Choi J. S., Cho K. Y., Sung C. K., Kwak S. S., and Lee H. S. (2006), Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature. Plant Cell Rep. **25**, 1380–1386.
Vyas D. and Kumar S. (2005), Tea (*Camellia sinensis* (L.) O. Kuntze) clone with lower period of winter dormancy exhibits lesser cellular damage in response to low temperature. Plant Physiol. Biochem. **43**, 383–388.
Wang E., Gan S., and Wagner G. (2002), Isolation and characterization of the CYP71D16 trichome-specific promoter from *Nicotiana tabacum* L. J. Exp. Bot. **53**, 1891–1897.
Xu S., Li J. L., Zhang X. Q., Wei H., and Cui L. J. (2006), Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplast in two cool-season turfgrass species under heat stress. Environ. Exp. Bot. **56**, 274–285.
Yuan Y., Hao J. D., Yang B., Li H., Li Z., Huang L. Q., Feng X. F., Chen S. Q., Luo Y. J., and Zhang Z. J. (2010), Climate change affected the best producing area of Chinese herbal medicine *Scutellaria baicalensis* Georgi. J. Trad. Med. (Russia) **3s**, 241–248.