Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental and Experimental Botany 56 (2006) 44–53
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Comparison of resveratrol, SOD activity, phenolic compounds and free amino acids in *Rehmannia glutinosa* under temperature and water stress

Ill Min Chung [a,*,1], Jong Jin Kim [b,1], Jung Dae Lim [c], Chang Yeon Yu [c], Seung Hyun Kim [d], Sang Joon Hahn [a]

[a] *Department of Applied Life Science, College of Life and Environment Science, Konkuk University, GwangJinKu, HwaYangDong, Seoul 143-701, Korea*
[b] *Department of Environmental Science, College of Life and Environment Science, Konkuk University, GwangJinKu, HwaYangDong, Seoul 143-701, Korea*
[c] *College of Agriculture and Life Science, Kangwon National University, Chunchon 200-701, Korea*
[d] *Department of Agricultural Environmental Chemistry, University of California, Davis, CA 95616, USA*

Received 30 September 2004; accepted 4 January 2005

Abstract

The objective of this study was to determine the contents of secondary metabolic substances such as resveratrol, SOD, phenolic compounds and free amino acids in *Rehmannia glutinosa* under environmental stress. The content of resveratrol varied from 2.96 to 38.87 $\mu g\,g^{-1}$, while SOD activity varied from 9.40 to 28.43%. Of the 16 individual phenolic compounds, myricetin showed the highest concentrations (34.30, 60.96, 35.70 $\mu g\,g^{-1}$) in the water deficiency (−1.18 MPa) and control (−1.04 MPa) and low temperature (15 °C) treatment. The 21 free amino acids in *R. glutinosa* were only detected in small amounts and varied from 0.82 to 5.69 $\mu g\,g^{-1}$. Overall, these substances decreased at high temperature and with a water deficiency. Other than for SOD activity, these substances were negatively correlated ($r^2 = -0.99^{**}$) with temperature and positively correlated with water stress ($r^2 = 0.99^{***}$). Also, other than for SOD activity under the water deficiency treatment, resveratrol, SOD activity, phenolic compounds and free amino acids had over correlation coefficients ($r^2 = 0.99^{**}$) in all treatments. Our study suggests that it might be feasible to improve or develop *R. glutinosa* cultivation methods with high functional substances such as resveratrol, even under poor environmental conditions for cultivation.

Keywords: *Rehmannia glutinosa*; Resveratrol; SOD activity; Phenolic compounds; Free amino acids; Temperature stress; Water stress

1. Introduction

Rehmannia glutinosa (Gaertn.) Libosch. is a perennial medicinal plant that originates from China and belongs to the family Scrophulariaceae. For centuries, many oriental medical books in countries such as China, Japan and Korea have listed this plant's roots because of its medicinal purposes (Zhu et al., 2003). Although there are seven species in the genus *Rehmannia*, only three are used for medicinal purposes: *R. glutinosa* Libosch. in Korea, *R. glutinosa* var. *purpurea* Makino in Japan and *R. glutinosa* var. *hueichingensis* (Chao and Schih) Hsiao in China. *R. glutinosa* is generally cultivated in the southern provinces of Korea due to its mild climate, where it is used in three ways: fresh, dried and steamed. Each is known to have different medicinal effects (Hasegawa et al., 1982). The cultivated areas of *R. glutinosa* in Korea has progressively decreased over the last 20 years. In 2002, only 90 ha were planted, which was just 6% of the total cultivated area of medicinal plants. This trend is due to increasing imports of cheaper medicinal plants from China, where over 2100 M/T are imported every year (Ministry of Agriculture and Forestry, 2003).

* Corresponding author. Tel.: +82 2 450 3730; fax: +82 2 446 7856.
E-mail address: imcim@konkuk.ac.kr (I.M. Chung).
[1] These two authors contributed equally to this work.

0098-8472/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2005.01.001

R. glutinosa contains rehmaglutin A, B, C and D as an iridoid and catalpol, aucubin, leonuride, melittoside, rehmanniosides, glucosides, amino acids and β-stigmasterol as an iridoid with glucosides, and has been reported to have various medical effects such as reducing blood pressure in rabbits, diuretic properties in mice with glycosuria and antibiotic effects against bacteria. *R. glutinosa* also contains resveratrol, phenolic compounds, free amino acids and SOD, all of which have been reported to have physiologically active substances that have anti-oxidative properties, aid the defence mechanism, or possess chemicals related to resistance to environmental stresses in human beings or plants (Kubo et al., 1994; Kim et al., 2000).

Resveratrol (*trans*-3,5,4′-trihydroxystilbene), a type of stilbene, is found in peanuts, mulberries and grapes and accumulates in plants after exposure to UV light, aluminium chloride or infection by *Plasmospora viticola* or *Botrytis cinerea* (Langcake, 1981; Sobolev and Cole, 1999). It is known to have advantageous effects for diabetes, constipation, allergies and headaches. In addition, it can trigger apoptosis, has antibacterial properties and can reduce inflammation (Kubo et al., 1994). It also contains superoxide dismutase (SOD; EC 1.15.1.1), which is present in most organisms, and was first found by McCord and Fridovich (1969). There are three types of SOD, Cu/Zn-SOD, Mn-SOD and Fe-SOD, all of which have the following removal system for several in vivo reactive oxygen species (ROS):

$$2O_2{}^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$$

The Cu/Zn-SOD type has generally been reported to occur in the cytosol of most higher plants. Recently, there has been an increase in interest in SOD as several research reports related to human health have noted resistance mechanisms against environmental stresses (Bryan et al., 2000). Free amino acids and phenolic compounds composed of phenolic acid (C_6–C_1), coumarins (C_6–C_3), flavonoids (C_6–C_3–C_6) and condensed tannins are known to possess chemicals with anticancer and anti-oxidative properties and can control physiological metabolites in humans and plants. The contents of such chemicals vary with species, growth stage and cultivation method (Hong et al., 1989).

Since the industrial revolution in the 19th century, the climate of the Earth has changed as a result of developing industry and science. This has led to abnormal climatic conditions around the world. However, little is known about variations in the aforementioned advantageous substances in *R. glutinosa* to changing environmental conditions, such as severe drought or elevated air temperatures. As a result, a major focus has been placed on the analysis and evaluation of medicinal chemicals in *R. glutinosa* and their effects against several diseases.

The main purposes of this study were: (1) to examine the content of resveratrol, phenolic compounds, free amino acids and SOD activity in *R. glutinosa* cultivated under water and temperature stress and; (2) to determine correlations between changes of these substances and environmental factors. Such information in this study could help to both develop more adaptable methods for cultivating *R. glutinosa* and in producing new cultivars with highly functional substances.

2. Materials and methods

2.1. Plant material and growth conditions

R. glutinosa seedling which was grown in the seedling culture box on 25 July 2003 and transplanted into plastic pots (27 cm in diameter and 30 cm in height) filled with a sandy loam soil on 11 September 2003. They were then cultivated in a greenhouse at Konkuk University. Fifty days after transplanting (11 September), they were exposed to two temperature and water conditions: low temperature (day: 20 °C, night: 15 °C, light intensity: 2050 μmol m^{-2} s^{-1}), high temperature (day: 35 °C, night: 30 °C, light intensity: 2050 μmol m^{-2} s^{-1}), water deficiency (water potential in the soil: -1.18 ± 0.04 MPa), control (water potential in the soil: -1.04 ± 0.07 MPa). Plants were harvested from each replicate and stored at low temperature (below −30 °C) until analysis. The experiment consisted of a completely randomized design with three replicates.

2.2. Measurement of superoxide dismutase (SOD) activity

2.2.1. Enzyme extraction

For each sample, 0.2 g of dried root *R. glutinosa* was ground, mixed with 0.4 g polyvinylpolypyrrol-idome (PVP) and 2 mL extraction buffer with pH 7.0, 100 mM potassium phosphate, 10 mM sodium ascorbate and 5 mM EDTA. After the homogenate was centrifuged at 15,000 rpm for 20 min, the upper solution was extracted using a PD-10 column of Sephadex G-25 and was used to estimate SOD activity.

2.2.2. SOD activity test by the nitro blue tetrazolium (NBT) reduction method

SOD activity of root *R. glutinosa* was measured by the nitro blue tetrazolium (NBT) reduction method (Beyer and Fridovich, 1987). Test tubes containing reaction solution with 3 mL of assay buffer, 60 μL of crude enzyme and 30 μL of riboflavin were illuminated for 7 min in an aluminum foil lined box containing two 20-W Slyvania Groiux Fluorescent lamps at 25 °C. After reaction, the absorbance of the blank solution and reaction solution was measured with a spectrophotometer (Hitachi Ltd., Tokyo, Japan) at 560 nm. SOD activities were calculated as a following equation:

$$\text{SOD activity}\,(\%) = (1 - A/B) \times 100$$

A: absorbance of sample; B: absorbance of blank.