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Full Length Research Paper

Drought stress increases iridoid glycosides biosynthesis in the roots of *Scrophularia ningpoensis* seedlings

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In China, *Scrophularia ningpoensis* Hemsl (Scrophulariaceae) has been cultivated for many years; however, the effects of drought on the content of secondary metabolites in Radix Scrophulariaceae are still unknown. The study investigated the medicinal components of *S. ningpoensis* including harpagoside, aucubin, catalpol, harpagide, and cinnamic acid in relation to drought stress. Three ecotypes of *S. ningpoensis* at seedling stage were subjected to progressively three levels of osmotic stress for 10 days and then the superoxide anion content of leaves at different stages was determined, as well as the content of the components in the roots of *S. ningpoensis* detected by High-Performance Liquid Chromatography (HPLC). The contents of four iridoids glycosides in the roots along with the superoxide anion content in leaves under osmotic stress were higher than those with no osmotic stress in every ecotype, whereas cinnamic acid decreased. Drought stress could increase the iridoid glycosides and decrease the cinnamic acid content of roots in *S. ningpoensis* seedlings.

Key words: *Scrophularia ningpoensis* Hemsl, cinnamic acid, iridoid glycosides, osmotic stress.

INTRODUCTION

Radix Scrophulariaceae present curing capabilities such as trypanocidal, leishmanicidal, antiprotozoal, antimycobacterial, antimalarial, and plasmodia FabI enzyme inhibiting properties (Tasdemir et al., 2005, 2008; Bas et al., 2007a,b). *Scrophularia deserti* Del. (Scrophulariaceae) is used in traditional medicine as an antipyretic and a remedy for kidney diseases, tumors, and lung cancer in the Mideast (Stavri et al., 2006). Since ancient times, dried root of *Scrophularia ningpoensis* Hemsl (Scrophulariaceae) has been used medicinally in East Asia (Wang et al., 2004; Xu et al., 2004), and it has been cultivated in many provinces of China for hundreds of years.

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It is believed that some secondary metabolites are partly responsible for the medicinal properties of Scrophulariaceae roots (Stavri et al., 2006). Roots of the Scrophulariaceae family are characterized by many types of iridoid glycosides contained, which are defined as secondary metabolites (Qian et al., 1992; Nass and Rimpler, 1996; Li et al., 1999). A number of secondary metabolites in *S. ningpoensis* roots, such as harpagoside, aucubin, catalpol, harpagide, and cinnamic acid have been reported to possess the main medicinal activities of *S. ningpoensis* (Li et al., 2000; Tasdemir et al., 2008; Jeong et al., 2008).

It is significant to note that differences have been found in the medical and chemical contents of *S. ningpoensis* roots grown in different conditions (Gong et al., 2008). Different ecotypes of *S. ningpoensis* have been formed due to adaptation, and corresponding responses to different environmental conditions (Zhao, 2008).

During the past decades many researches have been

conducted on crop plants under drought stress, but no medicinal plants (Tan et al., 2006; Jaleel et al., 2007a). Medical chemicals are synthesized and accumulated based on some gene induced by environmental conditions, which is found to have an important effect on the medical plants' defense against adversities. For example, Munné-Bosch et al. (2001) postulated that the antioxidant activity of carnosic acid, a membrane-associated antioxidant found in chloroplasts, provides protection for drought-stressed *Salvia officinalis* L. (subs. officinalis) leaves. In addition, Jaleel et al. (2007a, b) found that the ajmalicine or total indole alkaloid accumulation in the roots of *Catharanthus roseus* (L.) G. Don. (Apocynaceae) increased significantly under drought stress conditions. The authors suggested that plants under drought stress were highly affected by components of the anti-oxidative system and secondary metabolite contents.

It is critical to understand the physiological mechanism of secondary compounds accumulation of those medicinal plants cultivated in good agricultural practice (GAP) in response to different water situations (Tan et al., 2006). Osmotic solutions are used to impose water stress reproducibly under *in vitro* conditions. PEG 6000 has frequently been used to induce water stress and maintain uniform water potential throughout the experimental period for their characters of inert, non-ionic and virtually impermeable chains (Van den Berg and Zeng, 2006). However, the effects of drought stress on the content of secondary metabolites in *S. ningpoensis* roots are still unknown. Therefore, the aim of this study was to investigate the content of the five compounds in response to drought stress. For this purpose, some secondary metabolites of the three ecotypes of *S. ningpoensis* were measured under different levels of osmotic stress at the seedling stage.

MATERIALS AND METHODS

Plant material

The buds of *S. ningpoensis* were collected at November, 2006 from five main regions in China: Anguo (AG) in Hebei Province, Zhenping in Shaanxi Province, Badong in Hubei Province, Nanchuan (NC) in Chongqing City, and Dongyang (DY) in Zhejiang Province. Voucher specimens (L1106AG, L1106ZP, L1106BD, L1106NC and L1106DY) were deposited at College of Life Sciences, Northwest A and F University, Yangling, Shaanxi Province, P. R. China. These buds were classified into three species by collating statistical data in the next year and by variance analysis based on morphologic characteristics when cultivating them in the same condition (Zhao, 2008). The buds used in the experiment were collected from AG, NC, and DY after careful selection.

Treatment

The selected buds from AG, NC and DY were placed into pots filled with acid-washed sand on 5 March, 2008. The pots were transferred into a greenhouse with the temperature and the Relative Humidity (RH) maintained at 20-25°C and 40-50%, respectively, under normal daylight and with well-watered conditions from 25 March to 5 May. Hoagland nutrition (15 ml) solution and water (45 ml) were applied at three-day intervals. On 5 May, the young seedlings were transferred to hydroponic systems and transported to a controlled growth chamber (light/dark regime of 13/11 h at 28/23°C, relative humidity of 50-55%, photosynthetic photon flux density of 150-200 $\mu mol \cdot m^{-2} s^{-1)}$. Every aquarium was planted with two seedlings and filled with 700 ml Hoagland nutrition that was replenished every 72 h. An air pump was employed to ventilate nutrition four times per day at 60 min per ventilation.

For every ecotype, 18 aquariums with two seedlings each were chosen and divided into three groups with three replications. Osmotic stress treatment was applied to 54-day-old seedlings by adding nothing (level 0, control treatment), 100 gL^{-1} PEG 6000 (level 1, water potential is -0.2 MPa), and 170 g L^{-1} PEG 6000 (level 2, water potential is -0.4 MPa) into Hoagland nutrition. On the 0th, 1st, 3rd, 5, 7, and 10th day of osmotic stress treatment, the matured leaves were randomly selected, frozen in liquid nitrogen, and then stored at -78°C until use. On the 10th day, all the roots of every treatment were gathered and dried in 50°C for 96 h.

Superoxide anion determination

Superoxide anion (O_2^-) content was assayed following the method of Zhu et al. (2009).

Determination the quantity of secondary metabolites in *S. ningpoensis* roots

Chromatography and reagent

A waters HPLC system (Milford, MA, USA) equipped with a 2487 binary pump, a manual sample injector, and a waters 2996 photodiode array detector was used to perform HPLC analysis. The HPLC fingerprint was carried out on a C18 column (Waters, Sun Fire C18, 4.6 × 250 mm, 5 µm) at 30°C with a sample injection volume of 20 µl. Empower2 software was used for data acquisition at a flow rate of 1.0 ml min-1. A gradient elution of A (methanol) and B (ultrapure water) was used as follows: 0-10 min, 5-35% A; 10-15 min, 35-45% A; 15-20 min, 45-51% A; 20-40 min, 51-61% A; 40-45 min, 61-80% A; 45-60 min, 80-80% A; on-line UV spectra were recorded with a diode array detector as 200 and 278 nm.

HPLC-grade methanol was bought from Tedia Co., Inc. (Fairfeld, OH, USA), and analytical grade methanol was obtained from the Xi'an Chemical Reagent Plant (Xi'an, China). Harpagide, harpagoside, aucubin, catalpol, and cinnamic acid were purchased from the National Institute for the Control of Biological and Pharmaceutical Products (Beijing, China). Ultrapure water was generated with an Ultrapure Water System (UPW, Shanghai Ultrapure Technology, Shanghai, China).

Sample preparation

Five secondary metabolites content was assayed by HPLC following the method of Yu (2009). All the dried roots from every treatment were ground to pass through a 0.5 mm sieve. Powdered root samples weighing 0.1 g were extracted by 10 ml distilled water through 40 min ultrasonic treatment at 30°C and centrifuged at 4,000 rpm for 15 min. The resulting liquid was solution A. Solution B was acquired by replacing distilled water with 50% methanol and then following the same procedure used with solution A. The solution for HPLC was gained after 12 h by mixing 5 ml of A, 5 ml of B and 0.1% chitosan for protecting C18 column from amylose. The

Table 1. Calibration curves of five chemicals.

Standard chemicals	Solution concentration (mg mL^{-1})	Standard curve	R^2	Test range (mg mL^{-1})
Catalpol	0.2999	Y = 15734.9x + 23709	0.9975	0.1500-2.9990
Aucubin	0.2399	Y = 14401.9x + 46362	0.9995	0.1200-2.3990
Harpagide	0.2728	Y = 13072.8x + 6576.5	0.9996	0.0136-0.2728
Harpagoside	0.1720	Y = 1520.35x + 592.40	0.998	0.0860-1.7200
Cinnamic acid	0.0102	Y = 3659.85x + 2033.95	0.9917	0.0051-0.1020

Y: peak area; x: concentration of chemicals (μg mL^{-1}).

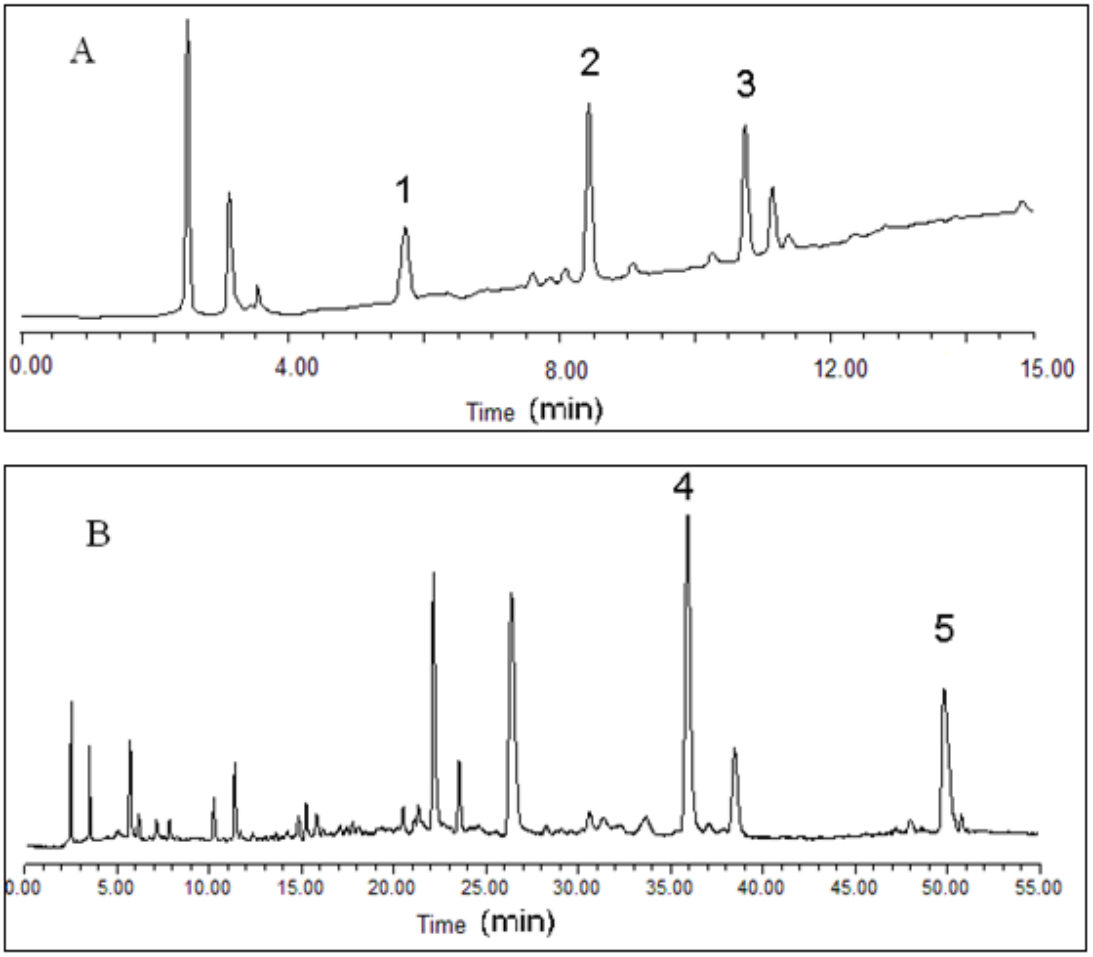

Figure 1. Representative HPLC chromatograms in two determined wavelengths (A: 200 nm, B: 278 nm) of *S. ningpoensis*. 1, catalpol; 2, aucubin; 3, harpagide; 4, harpagoside; 5, cinnamic acid.

solution was filtered through a 0.45 μm organic membrane filter before injecting it to the HPLC system.

Separate standard solutions (Table 1) were prepared by dissolving the chemicals in methanol. Injection volumes of 0, 2, 5, 10, 15, and 20 μl were used to calibrate the HPLC system with the catalpol, aucubin, harpagide, harpagoside, and cinnamic acid standards (Table 1). The five compounds in the samples were established through consistency of retention time (Figure 1) and spectra in comparison with the standards (Figure 2). The spectra of these five kinds secondary metabolites are different each other. The optical absorption peak of catalpol at 195.7 nm, and that of aucubin is at 194.5 nm, harpagide is at 196.8 nm. But harpagoside has two strong optical absorption peaks at 202.7 and 280.6 nm, so does cinnamic acid at 198.0 and 273.5 nm (Figure 2).

Method validation

Method precision and repeatability were evaluated using successive analyses of five replicates of the same powder sample.

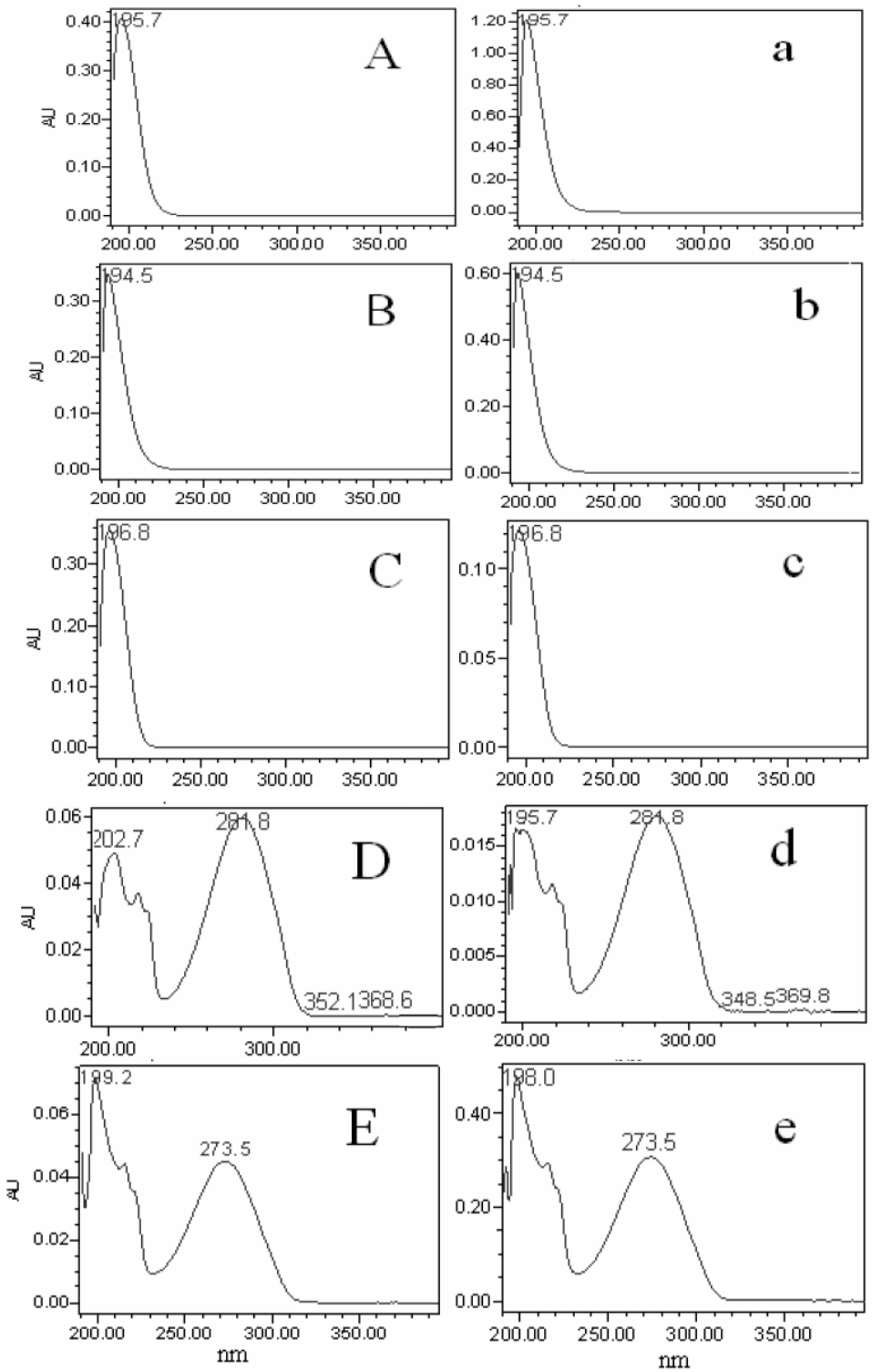

Figure 2. The spectra of catalpol (A, a), aucubin (B, b), harpagide (C, c), harpagoside (D, d) and cinnamic acid (E, e). A, B, C, D and E were the spectrum of the standard of secondary metabolites; a, b, c, d and e were the spectrum of secondary metabolites in *S. ningpoensis* roots.

The Relative Standard Deviations (R.S.D.) were ≤2.3% for retention time (tR) and ≤2.1% for the Peak Area (PA) of characteristic peaks. Stability of the sample solutions was determined by analyzing samples at 0, 2, 4, 8, 16, and 24 h after their preparation. The recovery test was carried as followings: three different quantities (low, medium and high) of authentic standards were added into

Table 2. Analytical results of recoveries (n=3).

Chemicals added into samples solution (μg mL^{-1})		Found (μg mL^{-1})	R.S.D. (%)	Recovery (%)
Catalpol	2.999	2.878 ± 0.012	0.400	95.97
	15.000	14.796 ± 0.181	1.207	98.64
	45.000	44.238 ± 0.583	1.296	98.30
Aucubin	2.399	2.365 ± 0.034	1.417	98.58
	12.000	11.682 ± 0.126	1.050	97.35
	36.000	36.209 ± 0.389	1.081	100.58
Harpagide	2.728	2.835 ± 0.041	1.503	103.92
	13.514	13.509 ± 0.257	1.902	99.96
	40.542	39.308 ± 1.306	3.222	96.96
Harpagoside	1.720	1.685 ± 0.019	1.105	97.96
	8.600	8.577 ± 0.154	1.791	99.73
	25.800	25.812 ± 0.806	3.124	100.05
Cinnamic acid	0.102	0.997 ± 0.002	1.960	97.74
	0.610	0.603 ± 0.013	2.131	98.85
	1.830	1.844 ± 0.076	4.153	100.76

R.S.D. (%) = (S.D./mean) × 100%, Recovery (%) = (found concentration /concentration added into samples solution) × 100%.

samples. The resultant samples were extracted and analyzed as described in Table 2. The quantity of each chemical was subsequently obtained from the corresponding calibration curve. The recovery of the five standards ranged from 95.97 to 103.92% (Table 2). From the results of precision test and recovery test, it was known that the method manifested good precision and accuracy.

Experimental design and statistical analysis

All treatments were arranged in a completely randomized block design with three replicates. Statistical analysis was performed using one way analysis of variance (ANOVA) and followed by Duncan's multiple range tests (DWRT) with SAS for Windows 8e (SAS Institute Inc., Cary, NC,USA). And SAS for Windows 8e was used to determine correlation coefficients.

RESULTS

O_2^- content in *S. ningpoensis* leaves among three ecotypes under osmotic stress O_2^- content in the leaves of *S. ningpoensis* increased gradually under osmotic stress at early stage (Figure 3). At the later stage, the rapid rise was observed in the DY species under osmotic stress. The O_2^- content of the NC species accumulated fastest at the beginning and then fluctuated on the seventh day. The peak of the O_2^- content was seen at the end of both osmotic treatment levels for NC and DY, while that of the AG species was seen at the middle stage. In the later treatment stage, it was observed that the O_2^- contents of the DY species in the two osmotic levels were higher than those of AG and NC, wherein no significant difference was displayed. At the end of the experiment, the O^{2-} content of AG, NC, and DY in high osmotic stress increased by 61.6, 184.1, and 218.7% compared with those in the control, respectively.

Secondary metabolites of *S. ningpoensis* roots among three ecotypes under osmotic stress

Secondary metabolite (that is, catalpol, harpagoside, aucubin, harpagide, and cinnamic acid) contents in dry roots changed within 10 days of different osmotic stress intensities (Table 3). The contents of catalpol, harpagide, aucubin, and harpagoside in the roots of 0 gL^{-1} PEG6000 (control) were lowest among the three treatments in all ecotypes (Table 3). Cinnamic acid content in the roots of 0 gL^{-1} PEG6000 (control), however, was the highest of the three treatments in all ecotypes. The highest catalpol content was found in 100 gL^{-1} PEG6000 treatment in the DY ecotype, while the highest harpagide and harpagoside contents were found in 100 g L^{-1} PEG6000 treatment on the AG ecotype. The highest aucubin and cinnamic acid contents were found in 170 and 0 gL^{-1} PEG6000 treatments, respectively, on the NC ecotype.

Table 4 shows that significantly higher catalpol and aucubin contents under level 2 as compared with the other two water conditions. Furthermore, the contents of harpagide and harpagoside in the roots of level 1 were highest among the three levels while those of level 2

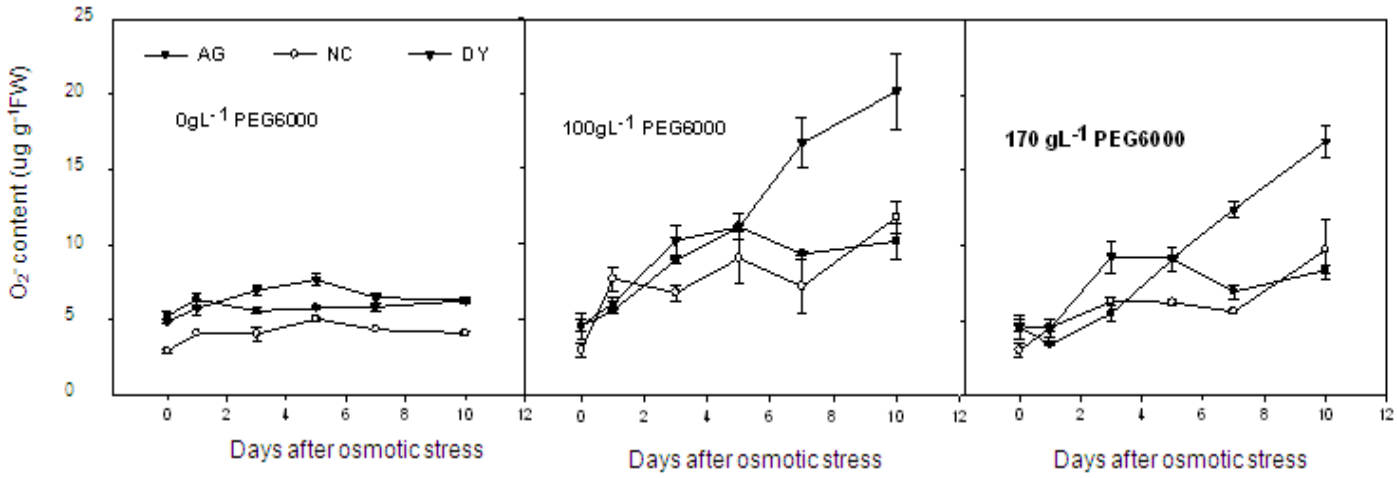

Figure 3. O_2^- content in *S. ningpoensis* leaves among three ecotypes under osmotic stress. AG, NC, DY represented roots of *S. ningpoensis* seedlings from Anguo (Hebei Province) ecotype, Nanchuan (Chongqing City) ecotype, and Dongyang (Zhejiang Province) ecotype, respectively; Vertical bars represent standard errors (n=3).

Table 3. Catalpol, harpagoside, aucubin, harpagide, and cinnamic acid contents in dry roots of *S. ningpoensis* among three ecotypes 10 days of osmotic stress.

Treatments	Catalpol (mg g^{-1}DW)	Harpagide (mg g^{-1}DW)	Aucubin (mg g^{-1}DW)	Cinnamic acid (mg g^{-1}DW)	Harpagoside (mg g^{-1}DW)
AG 0	1.029 ± 0.014C	1.801 ± 0.045C	8.984 ± 0.104C	0.941 ± 0.020A	3.292 ± 0.039C
AG 1	1.218 ± 0.014B	3.828 ± 0.041A	11.257 ± 0.146A	0.901 ± 0.019B	7.668 ± 0.124A
AG 2	1.430 ± 0.015A	1.972 ± 0.044B	9.408 ± 0.111B	0.650 ± 0.013C	7.239 ± 0.115B
NC 0	1.324 ± 0.014C	1.400 ± 0.050C	7.513 ± 0.125C	1.176 ± 0.027A	3.512 ± 0.043B
NC 1	1.455 ± 0.015B	2.201 ± 0.043B	9.286 ± 0.109B	0.923 ± 0.020B	5.289 ± 0.078A
NC 2	1.648 ± 0.015A	2.543 ± 0.043A	18.793 ± 0.309A	0.774 ± 0.016C	3.642 ± 0.046B
DY 0	1.211 ± 0.014C	2.413 ± 0.043C	9.417 ± 0.111C	0.874 ± 0.018A	3.160 ± 0.036C
DY 1	1.966 ± 0.054A	2.886 ± 0.042A	11.269 ± 0.146A	0.678 ± 0.013B	3.457 ± 0.042B
DY 2	1.672 ± 0.015B	2.727 ± 0.042B	10.457 ± 0.130B	0.695 ± 0.014B	4.419 ± 0.061A

AG, NC, DY represented roots of *S. ningpoensis* seedlings from Anguo (Hebei Province) ecotype, Nanchuan (Chongqing City) ecotype, and Dongyang (Zhejiang Province) ecotype, respectively. 0, 1, 2 represented Hoagland nutrition with 0, 100, 170 g/L PEG 6000, respectively. Different letters (A, B and C) indicated significant difference at p=0.01. Means ± standard deviation (S.D.) (n=3) were shown.

Table 4. Results of analysis of variance (ANOVA) of catalpol, harpagoside, aucubin, harpagide and cinnamic acid contents in dry roots of *S. ningpoensis* in 10 days different levels the osmotic stress.

	Catalpol (mg g^{-1}DW)	Harpagide (mg g^{-1}DW)	Aucubin (mg g^{-1}DW)	Cinnamic acid (mg g^{-1}DW)	Harpagoside (mg g^{-1}DW)
Level 0	1.295±0.062C	1.595±0.460C	8.984±0.646C	0.984±0.075A	3.365±0.113C
Level 1	1.440±0.209 B	2.972±0.356A	10.604±0.508B	0.847±0.066B	5.471±0.917A
Level 2	1.583±0.059 A	2.357±0.216B	12.539±2.352A	0.706±0.030C	5.057±0.844B

0, 1, 2 represented Hoagland nutrition with 0, 100, 170 g/L PEG 6000, respectively. Different letters (A, B and C) indicated significant difference at p=0.01. Means ± standard deviation (S.D.) (n=3) were shown.

were higher than that in level 0 (control). The cinnamic acid content significantly decreased with drought densities.

Table 5 shows the differences between the secondary metabolite contents among the three ecotypes of *S. ningpoensis* (that is, Anguo, Nanchuan, and Dongyang).

Table 5. Results of analysis of variance (ANOVA) of catalpol, harpagoside, aucubin, harpagide and cinnamic acid contents in dry roots of different ecotypes *S. ningpoensis* in 10 days the osmotic stress.

	Catalpol (mg g^{-1}DW)	Harpagide (mg g^{-1}DW)	Aucubin (mg g^{-1}DW)	Cinnamic acid (mg g^{-1}DW)	Harpagoside (mg g^{-1}DW)
AG	1.226±0.088C	2.533±0.488 B	9.883±0.534C	0.830±0.070 B	6.067±1.048 A
NC	1.446±0.072 B	1.715±0.500 C	11.864±2.633 A	0.958±0.090 A	4.148±0.432 B
DY	1.616±0.167 A	2.676±0.111 A	10.381±0.418 B	0.749±0.049C	3.679±0.288 C

AG, NC, DY represented roots of *S. ningpoensis* seedlings from Anguo (Hebei Province) ecotype, Nanchuan (Chongqing City) ecotype, and Dongyang (Zhejiang Province) ecotype, respectively. Different letters (A, B and C) indicated significant difference at p=0.01. Means ± standard deviation (S.D.) (n=3) were shown.

Table 6. Correlation analysis of catalpol, harpagoside, aucubin, harpagide, and cinnamic acid contents in dry roots and O_2^- content in the leaves of *S. ningpoensis* after 10 days of osmotic stress.

	O_2^-	Catalpol	Aucubin	Harpagide	Harpagoside	Cinnamic acid
O_2^-	1					
Catalpol	0.828**	1				
Aucubin	0.364	0.426*	1			
Harpagide	0.460	0.257	0.395*	1		
Harpagoside	0.086	- 0.129	- 0.072	0.433*	1	
Cinnamic acid	- 0.779**	- 0.590*	-0.395*	- 0.411*	- 0.243	1

* The level of significance was indicated as follows: 0.05 > p >0.01.** The level of significance was indicated as follows: 0.01 > p.

Among the three, the Anguo ecotype had highest harpagoside content, lowest catalpol and aucubin; the Nanchuan ecotype had highest aucubin and cinnamic acid, lowest harpagide content; while the Dongyang ecotype had highest catalpol and harpagide, average aucubin, and lowest cinnamic acid and harpagoside content.

Correlation analysis

There were significant positive correlations between superoxide anion (O_2^-) content in *S. ningpoensis* leaves on 10th d osmotic stress with catalpol or harpagide content (Table 6). The content of cinnamic acid was significant negatively related to O_2^- content in the leaves and catalpol, aucubin, and harpagide contents in the roots. Those results can be interpreted by O_2^- content in the leaves have strong effect on catalpol, harpagide and cinnamic acid content in the roots of *S. ningpoensis* seedlings.

DISCUSSION

The dried roots of *S. ningpoensis*, has been used as traditional medicine since ancient times. One significant characteristic of the genus Scrophulariaceae is the accumulation of iridoid glycosides such as catalpol, aucubin, harpagide, and harpagoside (Li et al., 2000; Tasdemir et al., 2008; Jeong et al., 2008). It features properties as varied as anti-inflammatory, antiviral, and antimicrobial effects. The concentrations of iridoid glycosides result in significant differences in the roots of *S. ningpoensis* grown under different conditions (Gong et al., 2008; Yu, 2009). We surveyed superoxide anion content in the leaves and several secondary metabolites content in the dry roots of three *S. ningpoensis* ecotypes under different osmotic stress levels. The results showed that iridoid glycoside contents in the roots were increased by osmotic stress. The cinnamic acid content, however, did not undergo such as increase.

Oxidative damage is generated under osmotic stress through the formation of ROS in plants (Zhao et al., 2008; Costa et al., 2007). Stress conditions commonly induce the accumulation of ROS. The O_2^-, an ROS regularly synthesized in the chloroplast and mitochondrion and in some micro bodies, is the most important one. In the present study, the O_2^- content transformation process could be separated into three stages: rising, stable, and fast rising. The O_2^- contents of the NC and DY species showed the three stages clearly, but that of the AG species only showed the rising and stable stages. In this study, we also found that catalpol, harpagoside, aucubin, and harpagide contents in *S. ningpoensis* roots were increased by osmotic stress, but cinnamic acid content was decreased (Tables 3 and 5). Active oxygen species like superoxide anion are the key components

contributing to cellular redox poise (Shao et al., 2005). They participate in all processes controlled by redox reactions, and their content is regarded as an “alarm” signal that initiates pre-emptive defense responses or cell death. Oxidative stress is caused by an imbalance between ROS production and a biological system’s ability to detoxify these reactive intermediates readily or repair the resulting damage easily (Mena et al., 2009). It is important to note that they provide essential information on cellular redox state and regulate gene expression associated with biotic and abiotic stress responses to optimize defense (Shao et al., 2008; Bartosz, 2009). The significant positive correlations between O^{2-} content in *S. ningpoensis* leaves on 10th day osmotic stress with catalpol or harpagide content (Table 6) confirmed this frame of reference.

Several investigations have shown catalpol and aucubin with protecting activities against oxidative stress on animal experiments (Mao et al., 2007; Zhang et al., 2007; Jin et al., 2008). Jeong et al. (2008) reported that iridoid glycosides from *S. buergeriana* had both cognitive-enhancing and antioxidant activities. The biochemistry of oxygen activation and detoxification analyzed in the past and the identification of similarities between plants and animals opened a new field of research (Grassmann et al., 2002). Iridoids glycosides such as catalpol, harpagide, aucubin and harpagoside maybe helpful *S. ningpoensis* defense oxidative stress. Further studies are required to confirm these functions such as scavenging and/or detoxifying free radicals, blocking production, and enhancing the activities of antioxidant enzymes.

Despite a few decades of research, the enzymes, genes, and biochemical pathways involved in iridoids glycosides biosynthesis are the largely uncharacterized. The results of current investigation suggest the potential of using suitable water stress as strategy for increasing the content of iridoids glycoside. To meet the strong market demand, improve iridoids glycosides accumulation in roots by change water conditions at seedling stage may be feasible in *S. ningpoensis* cultivation.

Based on our study, drought stress can increase iridoid glycoside (that is, catalpol, aucubin, harpagide, and harpagoside) accumulation and decrease cinnamic acid content in the roots of *S. ningpoensis* seedlings. The anti-drought characteristic of medical herbs is a comprehensive activity of many components which presents a complex problem with various physiological and chemical changes.

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