

Full Length Research Paper

High temperature stress decreases root iridoid glycosides biosynthesis of *Scrophularia ningpoensis* during florescence

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Radix Scrophulariaceae is an herbal medicine used as antipyretic and antibacterial. The objective of this study was to investigate the effect of high temperature stress on the secondary metabolites of *Scrophularia ningpoensis* Hemsl (*S. ningpoensis*). Potted *S. ningpoensis* plants of three ecotypes in florescence were subjected to high temperature stress for 15 days. Results showed that under high temperature stress, contents of iridoid glycosides and cinnamic acid in *S. ningpoensis* roots were significantly lower than the control in all ecotypes. The present study indicates that high temperature stress has an obvious inhibitory effect on the production of iridoid glycosides and cinnamic acid.

Key words: *Scrophularia ningpoensis*, secondary metabolites, cinnamic acid, iridoid glycosides.

INTRODUCTION

Radix Scrophulariae dried roots from some species of the *Scrophularia* genus, present curing capabilities such as trypanocidal, antipyretic, leishmanicidal, antiprotozoal, antimycobacterial, antimalarial and plasmodia Fabi enzyme inhibiting properties (Nguyen et al., 2005; Tasdemir et al., 2005, 2008; Bas et al., 2007). Since ancient times, the dried roots of *Scrophularia ningpoensis* Hemsl (*S. ningpoensis*) have 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. Roots of the Scrophulariaceae family are characterized by containing many types of iridoid glycosides such as harpagoside, aucubin, catalpol and harpagide, which are defined as secondary metabolites (Bowers,

1991; Qian et al., 1992; Nass and Rimpler, 1996; Li et al., 1999).

Understanding the physiological mechanisms of iridoid glycosides accumulation in response to environmental stresses is critical for the scale-up production of iridoid glycosides. Temperature is one of the major environmental factors affecting plant growth and development. It probably alters the secondary metabolite production in plants. It is the hottest period in China from August to September, when the *S. ningpoensis* is just right in its florescence phase. Hence, *S. ningpoensis* plants during florescence are usually exposed to high temperature stress (higher than 35°C), especially in recent years due to the global climate change. However, there has been

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Table 1. The root contents of catalpol, harpagoside, aucubin, harpagide and cinnamic acid in *Scrophularia ningpoensis* Hemsl of three ecotypes after 15 days of high temperature stress during florescence.

Treatment	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 CK	1.31±0.01 ^e	9.38±0.05 ^b	12.54±0.14 ^b	0.76±0.01 ^b	4.50±0.06 ^c
AG HS	0.48±0.01 ^f	4.19±0.04 ^d	9.84±0.10 ^c	0.35±0.01 ^d	1.22±0.03 ^e
NC CK	2.02±0.04 ^c	4.60±0.03 ^c	7.25±0.07 ^d	1.21±0.02 ^a	4.43±0.05 ^c
NC HS	1.78±0.01 ^d	4.34±0.03 ^{cd}	5.79±0.06 ^e	1.15±0.03 ^a	2.70±0.02 ^d
DY CK	3.90±0.04 ^a	10.07±0.06 ^a	18.70±0.25 ^a	0.71±0.01 ^b	8.89±0.12 ^a
DY HS	2.41±0.01 ^b	9.77±0.05 ^{ab}	13.59±0.16 ^b	0.62±0.01 ^c	5.21±0.06 ^b

AG, NC, DY represented roots of *Scrophularia ningpoensis* Hemsl plantings from Anguo (Hebei province) ecotype, Nanchuan (Chongqing city) ecotype, and Dongyang (Zhejiang province) ecotype, respectively. CK and HS represented the *Scrophularia ningpoensis* Hemsl plants under temperature regimes of 28/23 and 45/38°C, respectively. Means ± standard deviation (SD) (n = 3) were shown. The different letters in the same column indicate significant differences among treatments.

there has been little research regarding the production of secondary metabolites in *S. ningpoensis* under high temperature stress. Therefore, the objective of this study was to investigate the effect of high temperature on root contents of secondary metabolites in *S. ningpoensis* during florescence.

MATERIALS AND METHODS

The plant materials of three ecotypes were the same as that in Wang et al. (2010). On 5th March 2008, well-preserved buds of *S. ningpoensis* from Anguo (AG) in Hebei Province, Nanchuan (NC) in Chongqing City, and Dongyang (DY) in Zhejiang Province were planted into pots filled with acid-washed sand in a greenhouse. Sixty milliliter 1/4 Hoagland nutrition was applied to each pot at three-day intervals. On 5th May, plants with uniform height and growth status were selected and transferred into plastic pots (30 cm diameter, 20 cm depth) filled with 2.1 kg vermiculite. Each pot was planted with 4 seedlings and filled with 0.9 L 1/4 strength Hoagland and Arnon solution. The pots were transported to a controlled growth chamber (light/dark regime of 13/11 h at 28/23°C, relative humidity of 50 to 55%, photosynthetic photon flux density of 150 to 200 μmol m⁻² s⁻¹). Hoagland and Arnon solution (1/4 strength) was added at two-day intervals to provide the nutritional and water requirements for seedlings.

For each ecotype, 12 pots were chosen and divided into two equal groups randomly. Almost all young plants blossomed out on 3rd August. After one week, high temperature stress treatment was applied to the 120-day-old seedlings. For different temperature treatments, two groups of each ecotype were equally separated and transferred into two growth chambers. One chamber was set on a light:dark regime of 13:11 h and a coinciding thermoperiod of 45:38°C (high temperature stress), while the other was set on a light:dark regime of 13:11 h and coinciding thermoperiod of 28:23°C (control). After 15 days, the roots of all treatments were gathered, washed carefully and dried at 50°C for 96 h for determination of catalpol, harpagoside, aucubin, harpagide and cinnamic acid. These metabolite contents were determined according to the method of Wang et al. (2010) through high-performance liquid chromatography (HPLC). The results presented were the mean of three replicates. Means were compared by one way analysis of variance Duncan's multiple range test at the 5% level of significance.

RESULTS AND DISCUSSION

As stated in the introduction, *S. ningpoensis* accumulate

lots of iridoid glycosides such as catalpol, aucubin, harpagide and harpagoside (Jessen et al., 1993; Li et al., 2000; Tasdemir et al., 2008; Jeong et al., 2008). A survey of the contents of the secondary metabolites in the dry roots of three *S. ningpoensis* ecotypes showed that the high temperature stress tended to decrease the secondary metabolite (that is, catalpol, harpagoside, aucubin, harpagide, and cinnamic acid) contents in dry roots of *S. ningpoensis* during florescence. The root contents of those five secondary metabolites in *S. ningpoensis* plants under control condition were higher than those under 45/38°C treatment in all ecotypes (Table 1). Compared with the controls, the high temperature stress decreased the contents of catalpol, harpagide, aucubin, cinnamic acid and harpagoside by 63.30 ± 2.35, 55.36 ± 1.89, 21.55 ± 0.69, 50.85 ± 3.04 and 72.89 ± 2.93%, respectively, in the roots of AG ecotype, by 11.86 ± 0.99, 5.74 ± 0.67, 20.13 ± 1.03, 4.65 ± 0.57 and 39.20 ± 1.37 %, respectively, in NC ecotype, and by 38.23 ± 1.99, 3.02 ± 0.16, 27.33 ± 0.91, 12.68 ± 2.04 and 41.34 ± 2.67%, respectively, in DY ecotype. The highest contents of catalpol, harpagide, aucubin and harpagoside were found in the control plants of DY ecotype, indicating the best quality of DY ecotype. In contrast with our results, Zobayed et al. (2005) reported that high temperature (35°C) treatment increased the hypericin, pseudohypericin and hyperforin concentrations in the shoot tissues of St. John's wort. This difference might be explained by the fact that there are different secondary metabolite pathways in the two species tested. The highest cinnamic acid content was observed in the control plants of NC ecotype, suggesting that the NC ecotype is more suitable for the cinnamic acid production. Among the five secondary metabolites, the harpagoside contents showed the biggest reductions in all ecotypes, implying that the harpagoside metabolism is the most sensitive to high temperature stress.

The secondary metabolites of *S. ningpoensis* were sensitive to high temperature during florescence. The high

temperature has an inhibitory effect on accumulation of secondary metabolites in *S. ningpoensis* at florescence stage. Therefore, effective cooling measures should be taken to improve Radix Scrophulariaceae quality during florescence. Further research elucidating the iridoids glycoside biosynthesis pathway would provide a better understanding regarding the effect of temperature on iridoids glycoside production.

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