Industrial Crops & Products 122 (2018) 473–482

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

# Changes in the physiological characteristics and baicalin biosynthesis metabolism of *Scutellaria baicalensis* Georgi under drought stress

Lin Cheng, Mei Han*, Li-min Yang*, Li Yang, Zhuo Sun, Tao Zhang

*Cultivation Base of State Key Laboratory for Ecological Restoration and Ecosystem Management of Jilin Province and Ministry of Science and Technology, College of Chinese Medicinal Materials, Jilin Agricultural University, 130118, Changchun, Jilin, PR China*

ARTICLE INFO

*Keywords:*
*Scutellaria baicalensis* Georgi
drought stress
physiological characteristics
baicalin
secondary metabolism

ABSTRACT

Drought stress is an important ecologically limiting factor that strongly affects the physiological and biochemical reactions of medicinal plants and changes the secondary metabolic process. *Scutellaria baicalensis* Georgi (SBG), belonging to the Lamiaceae family, is a traditional medicinal herb that is well known for its high flavonoid content. Baicalin is the most important bioactive constituent of these flavonoids. In the current study, the effects of progressive drought stress on plant physiological characteristics and the secondary metabolism of baicalin were investigated during the vegetative period in two-year-old SBG. The results showed that the relative water content of leaves was reduced, fresh and dry root weight decreased significantly, the contents of malondialdehyde (MDA), proline (Pro), soluble sugar (SS) and soluble protein (SP) increased, and the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) increased during mild and moderate drought but were inhibited in severe drought. The content of baicalin increased significantly under mild drought stress but decreased under severe stress. Meanwhile, the expression patterns and activities of key enzymes upstream of baicalin biosynthesis including Phenylalanine ammonialyase (*PAL*), Cinnamate-4-Hydroxylase (*C4H*), 4-Coumarate:Coenzyme A Ligase (*4CL*) and Chalcone Synthase (*CHS*), which were in the secondary metabolic pathways, were consistent with the accumulation of baicalin. These results demonstrate that an appropriate degree of drought stress may promote baicalin accumulation by stimulating the expression and activities of the key enzymes involved in baicalin biosynthesis; in this process, antioxidant enzyme was closely related to the key enzyme of secondary metabolic pathway, which plays an important role in regulating the active ingredient accumulation which plays an important role in regulating baicalin accumulation. During agricultural production, SBG should not be harvested after prolonged drought but instead should be harvested several days after rain or irrigating to ensure high baicalin content.

## 1. Introduction

Drought stress, one of the most serious abiotic stresses, causes stomata to close and limits photosynthesis, thus impacting the physiological processes and productivity of plants, especially the synthesis and accumulation of secondary metabolites (Boyer, 1970; Selmar and Kleinwächter, 2013). Drought stress is a deficiency in water supply, detected as a reduction in soil water content or from the physiological responses of the plant to water deficit (Ihuoma and Madramootoo, 2017). Under limiting water content of soil, chemical signal is transmitted to the leaf through xylem pathways, which leads to physiological responses such as stomatal closure, reductions in photosynthesis rate, affecting in photosynthetic proton and electron transport and changing in photosynthetic carbon reduction and carbon oxidation cycles (Cornic and Fresneau, 2002; Zivcak et al., 2014).

The main active compounds in medicinal plants are secondary metabolites. Therefore drought stress has a greater impact on medicinal plants. In recent years, several medicinal plants including *Bupleurum chinense* DC. (Zhu et al., 2009), *Salvia officinalis* L. (Nowak et al., 2010) and *Salvia miltiorrhiza* Bunge. (Liu et al., 2011b) were intentionally planted under drought conditions to influence the content of secondary metabolites. *Scutellaria baicalensis* Georgi. is a perennial herb in the Lamiaceae family, and its roots are used to treat heat and dampness (in traditional Chinese medicine) for detoxification, to promote hemostasis,

*Abbreviations:* SBG, *Scutellaria baicalensis* Georgi; MDA, malondialdehyde; Pro, proline; SS, soluble sugar; SP, soluble protein; SO, Dsuperoxide dismutase; PO, Dperoxidase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; *PAL*, Phenylalanine ammonialyase; *C4H*, Cinnamate-4-Hydroxylase; *4CL*, 4-Coumarate:Coenzyme A Ligase; *CHS*, Chalcone Synthase

* Corresponding authors.
*E-mail addresses:* 429068429@qq.com (L. Cheng), idream9999@126.com (M. Han), 3465637993@qq.com (L.-m. Yang), 184677837@qq.com (Y. Li), 329575068@163.com (Z. Sun), 251073371@qq.com (T. Zhang).

https://doi.org/10.1016/j.indcrop.2018.06.030
Received 12 January 2018; Received in revised form 28 April 2018; Accepted 8 June 2018

and as a tocolytic (Zhao et al., 2016). It is widely distributed in the arid and semiarid areas of China, Russia, Mongolia, Europe and Japan (Shang et al., 2010). Increasing market demand has nearly depleted the natural populations of the plant, but *S. baicalensis* is now widely cultivated in most areas of the world (Bochořáková et al., 2003; Liu et al., 2017). The physiological mechanisms that contribute to SBG's adaptation to drought conditions have not been clearly elucidated.

Plant physiological characteristics change under stressful environmental conditions such as during drought, floods, and frost. Malondialdehyde (MDA)is regarded as an indicator of abiotic stress and is the end product of membrane-lipid peroxidation following oxidative damage. Under drought stress, the content of MDA increased with the increasing of membrane-lipid peroxidation (Rahimi et al., 2018). Plants mainly reduce the damage of these oxides by controlling the antioxidant system and regulating the content of osmoregulating compounds (Blokhina et al., 2003; Morgan, 1984). The antioxidant system consists of both enzymatic antioxidant enzymes including SOD (superoxide dismutase), POD (peroxidase) and CAT (catalase) and non-enzymatic antioxidant enzymes such as APX (ascorbate peroxidase) and GR (glutathione reductase). The non-enzymatic antioxidant system also includes phenols and flavonoids, which have higher pharmacological activity (Bozin et al., 2008). The main osmoregulating substances used by plants are mainly composed of Pro (proline), SS (soluble sugar) and SP (soluble protein) (Liu et al., 2011b). In the early stage of drought stress, the activities of antioxidant enzymes enhanced and osmoregulation substances synthesis increased. However, the activities of antioxidant enzymes inhibited and the content of osmotic regulation decreased duration of drought stress (Anjum et al., 2011; Aziz et al., 2018).

The formation of secondary metabolites in medicinal plants is closely related to the growth environment. Baicalin, the main active ingredient of *S. baicalensis*, is an important flavonoid compound (Makino et al., 2008). Baicalin is mainly distributed in the root and is synthesized by the flavonoid pathway, which is part of phenylpropanoid metabolism (Noel et al., 2005). Naringin is the intermediate of flavonoid biosynthesis. The general metabolic pathway for the biosynthesis of flavonoids involves phenylalanine ammonialyase (*PAL*), cinnamoyl 4-hydroxylase (*C4H*) and 4-Coumarate: Coenzyme A Ligase (*4CL*), followed by chalcone synthase (*CHS*). Baicalin is formed by other downstream enzymes (Fig. 1). The previous studies showed that environmental factors could promote the expression of key enzyme gene in secondary metabolic pathway and increase the content of baicalin (Chen et al., 2010; Xu et al., 2010; Yang et al., 2011). The expression of these key enzymes in the flavonoid metabolic pathway plays an important role in the synthesis of baicalin (Zhao et al., 2016).

Water is an essential ecological component of plant growth and development, and the plant root system is the major moisture absorbing organ. Therefore, soil water content is crucial to the production and quality of root metabolites (Kliebenstein and Osbourn, 2012; Ncube et al., 2012). Generally, drought stress severely impacts crop production and is unfavorable to agriculture. However, medicinal plants growing in semiarid areas have been shown to have a relatively high content of medicinal compounds (Kleinwächter and Selmar, 2015). Some studies have shown that an appropriate degree of drought stress induces secondary metabolic processes and increases secondary metabolites (Zhu et al., 2009; Bettaieb et al., 2009; Nowak et al., 2010; Bettaieb et al., 2011; Liu et al., 2011a). Nevertheless, economic efficiency may be constrained by normal plant growth conditions in these cases. Finding the perfect balance between medicinal plant production and quality by controlling soil water content is important for increasing both the quality and yield of medicine.

In the present study, physiological drought indicators including leaf water status, antioxidant enzymes, osmoregulating molecules and secondary metabolism (key enzyme activities, gene expression, and secondary metabolites) were analyzed. This study attempts to illuminate the mechanisms underlying the physiological and biochemical responses of SBG to drought stress and provide guidelines for the cultivation of SBG with high baicalin content.

## 2. Materials and methods

### 2.1. Plant materials

The plant material used was biennial SBG. The seeds of SBG were purchased from Hebei Province and potted in early April 2014. The dimensions of the pots were 30.50 cm deep, with a rim diameter of 31.20 cm, a base diameter of 20.00 cm, and a soil depth of 28.00 cm. Each pot was buried entirely in the soil and kept at the same temperature as the soil. The soil used in the experiment was sandy loam, with 25.74 g/kg of organic matter, 99.47 mg/kg of alkali nitrogen, 27.24 mg/kg of available phosphorus, and an available potassium content of 136.78 mg/kg. The saturated water content of the soil was 33.86%. Plant emergence occurred in mid-May, and three shoots of approximately the same height were kept in each pot. The plants were provided with a normal water supply for the first year.

### 2.2. Experimental design

Progressive drought stress was applied during the vegetative period with two-year-old SBG. The experiment was conducted from June to July 2015 at the medical botanical garden of Jilin Agricultural University, Jilin Province, China. The treat group was irrigated with enough water to reach saturation, i.e., the soil water content was approximately 33.86%; the soil water content of the control group was maintained at a normal level of 26.00% based on the average rainfall over 10 years in the Changchun area. The soil moisture was monitored using an HH2 soil moisture tester (Delta-t Devices Ltd, UK) every day until the SBG plants wilted. Samples were taken in triplicate every 2 days. The samples were collected at 10:00 am and brought back to the lab in ice chests. The roots were immediately washed clean, frozen with liquid nitrogen, and stored at -80 °C. A rain shelter was set up during the experiment; the plastic film was rolled up on sunny days and replaced at night and on rainy days.

### 2.3. Determination of fresh and dry root weight

The roots of *S. baicalensis* were washed by tap water and rinsed three times with distilled water, then the surface moisture was wiped

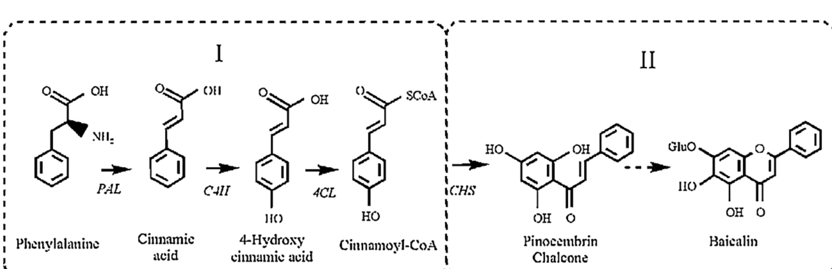


**Fig. 1.** Baicalin metabolic pathway in *Scutellaria baicalensis* Georgi. (I) Synthesis pathway upstream of baicalin (phenylpropanoid metabolism). (II) Baicalin synthesis from the flavonoid metabolic pathway. Enzyme abbreviations: *PAL*, phenylalanine ammonialyase; *C4H*, cinnamate 4-hydroxylase; *4CL*, 4-coumarate-CoA ligase; *CHS*, chalcone synthase.