Response of Scutellarin Content to Heavy Metals in *Erigeron Breviscapus*

Huabin Xiong, Changqun Duan, Xinxiang A, and Minghong Chen

Abstract—Part of hypotheses point out the quantity of plant secondary metabolites will increase in order to adapt environmental stress. Secondary metabolites accumulation of scutellarin in *Erigeron breviscapus* was investigated under different heavy metal stress. The results showed that exposure to cadmium, chromium and mercury stress scutellarin content of stem was higher sometimes, but its change from stress of copper and arsenic were opposite. In leaf and flower, scutellarin concentration was also decrease rapidly. So we inferred that after toxic substances entry into plant body, level of some secondary metabolites will exhibit decrease trend in some extent, and not only enhanced any time. Therefore, the change of secondary metabolites in medicine plant should attract more attention under stress environment in future.

Index Terms—Inhibit, scutellarin, secondary metabolites, stress.

I. INTRODUCTION

The secondary metabolites are the products from plant itself that adapt to surrounding environment during the long-term course of evolution. Meantime, plant could accommodate oneself to changes for environment and enhance survival capabilities that are its essential ecological function in changed natural environment, especially environmental stress. Among them, the product of secondary metabolites which can protect plant from surrounding environment is rather important. That is to say, secondary metabolites play a crucial function in the adaptation of plants to the alternative environmental, and can synthesis quantities of secondary metabolites with limitless ability. Some biotic and abiotic stress factors, such as low temperature, strongly light intensity, poor nutrition elements, water deficit and microbial attack, will reduce plant defence mechanisms and motivate many complex biochemical process [1]. Changes have been reported at genetic or protein level that is brought about by stress conditions and are reflected in a profound alteration of the metabolite pool of the affected plants [2]-[5].

Therefore, some hypotheses of generation mechanism of secondary metabolites are presented, like growth/differentiation balance (GDB) [6], carbon/nutrient balance

(CNB) [7], optimum defense (OD) [8] and resource availability (RA) [9]. What's more important, these four hypotheses have confirmed that the quantities of plant secondary metabolites exposure to environmental stress will enhance. Moreover, Holopainen [1] and Szathmary [4] also point out that despite the complex nature and inconclusive reports on the regulatory mechanisms and biosynthesis of different secondary metabolites in plants, a significant body of research evidence indicates that their biosynthesis and accumulation remain remarkably under the control of the environment.

In plants for medical excessive heavy metals will effect its normal growth and development, and accumulate secondary metabolites. It is found that through the correlation analysis of content of secondary metabolites and trace elements content of aucubin is closely correlation with arsenic in different *Scrophularian ningpoensis*, it suggested that arsenic is link with synthesis of aucubin [10]. The content of lead and mercury in all geoh herbs, like *Coptis chinensis*, *Coptis deltoidea*, *Ligusticum chuanxiong*, *Fritillaria cirrhosa*, *Gastrodia elata*, *Curcuma aromatica*, *Citrus aurantium*, *Ophiopogon japonicus*, *Aconitum carmichaeli*, *Angelica dahurica* var. *formosana* and *Codonopsis* var. *modesta*, which are higher than other non-geoh herbs from Sichuan province, China [11]. The synthesis and accumulation of artemisinin are obvious enhanced when the concentration of cadmium arrive to 0.15 mg kg^{-1} [12]. Hence, the research of the changes of secondary metabolites for medical plant from heavy metal is rather essential.

Erigeron breviscapus is a common medicine plant in traditional Chinese medicine that mainly grown in Yunnan province of Southwest China [13]. It has a long time used in remedy brain thrombosis and associated paralysis, coronary arteriosclerosis and myocardial ischemia through enhancing blood circulation, preventing blood coagulation and dilating brain blood vessels, especially as a long-range therapeutic drug without evident side effects [14]-[15]. The previous research indicated that scutellarin is one of the most important active constituents in *Erigeron breviscapus* [16].

In order to analysis influence of secondary metabolites accumulation from stress environment the content change of scutellarin in *Erigeron breviscapus* was investigated under different heavy metal stress, and which will also offer some benefit introduction for its cultivar how to select appropriate soil environment.

II. MATERIALS AND METHODS

A. Pot Experiment

Pot-test experiments were conducted in Yuhua campus

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(24°50'39" North latitude and 102°51'09" East longitude) of Yunnan University of Nationalities between November, 2011 and July, 2012. The soil used was collected from an orchard in suburban Kunming, China. And it was collected from surface horizon (0-15 cm), air-dried, crushed, passed through a 5 mm sieve and mixed well with heavy metals. There were four treatments with three replicates for each heavy metal (Table I). Meantime, each plastic pot filled with 35 kg prepared soil incubated for 30 days after adding heavy metal was watered with tap water daily to maintain 3 to 4 cm submergence throughout experiment period. When there were 2-3 leaves, the prepared seedlings of *Erigeron breviscapus* were transplanted to soil. The pots were laid out in the greenhouse and watered twice or thrice for one week to maintenance moderate soil moisture. The sample was harvested until flower dead (Fig. 1).

TABLE I: ORIGIN AND CONCENTRATION OF HEAVY METALS USED IN THE TESTED SOIL

Treatment	Treatment element / molecular formula of origin substances(mg kg ⁻¹)		
	Cr/K ₂ Cr ₂ O ₇	Cd/CdCl ₂ ·2.5H ₂ O	Cu/CuSO ₄ ·5H ₂ O
T ₀	0	0	0
T ₁	10	0.5	10
T ₂	50	2	50
T ₃	100	5	100

Continue

Treatment	Treatment element / molecular formula of origin substances(mg kg ⁻¹)	
	Pb/Pb(NO ₃) ₂	Hg/HgCl ₂
T ₀	0	0
T ₁	50	0.5
T ₂	100	2
T ₃	200	5

Fig. 1. Pot experiment of different heavy metal stress

B. Extraction of Scutellarin

The stem, leaf and flower tissues of *Erigeron breviscapus* were dried at room temperature and then ground to 80 mesh powders. The sample (50mg) was immersed with 60 ml 80% methanol for 30 minutes, then, extracted with the supernatants 30 minutes (60 °C and 59 KHz). After the procedure was complete the extract volume was increased to 100 ml with adding 80% methanol. The extract was filtered with filter paper, and 3ml liquid was transferred into

colorimetric ware (1cm diameter). Scutellarin concentration was measured with ultraviolet spectrophotometer in 335 nm wavelength. The following standard working solutions with concentrations in the range of 0-0.015mg mL⁻¹ for scutellarin were obtained. In this study, the yield of scutellarin [17] was defined:

$$\text{Yield of scutellarin (mg. kg}^{-1}\text{)} = \frac{\text{mass of scutellarin extracted}}{\text{mass of material}}$$

C. Data Analysis

In order to analysis the content of scutellarin from heavy metal stress and uncontaminated soil, relative difference (RD) was used.

$$RD(\%) = \frac{(T - T_0) \times 100}{T_0}$$

where: T was scutellarin concentration of every treatment under five heavy metals stress respectively and T₀ was scutellarin concentration in non-population soil.

III. RESULTS AND DISCUSSION

As increasing demand of phytomedicines in the all world, quality of medicine plant is attracting more interesting for its excellent therapy with no side effect. But, it is clear that quality in medicine plant include two important parts, the first one is good food safety of medicine plant, that is, all toxic substances in these plants are satisfied to international food standard and not bring any damage to human health. And the second one is good medicine quality, which will guarantee its therapy to disease and not delay to improve human health.

However, with the rapid progress of all global economy, numerous of toxic substances, such as heavy metals and environmental hormones, have been detected in water, soil and food. Therefore, many research focusing on absorption, accumulation and transformation from soil and water to plant have been conducted from the begin of last century. But, to medicine plant, can stress from toxic substances influence accumulation of secondary metabolites, or even change major medicine substances content? In order to illustrate the phenomenon, scutellarin content change was investigated exposure to five differential heavy metals.

A. Scutellarin Content Change of Stem

TABLE II: SCUTELLARIN CONTENT CHANGE OF STEM UNDER HEAVY METALS STRESS

Treatment	Cd	Cr	Hg	Cu
T1	1.5666	-24.2820	27.1540	-12.2715
T2	11.4883	34.9869	-46.7363	-1.8277
T3	-29.5039	-11.2272	12.5326	-9.9217

Continue

Treatment	Pb
T1	-32.3760
T2	-36.8146
T3	-31.0705

When the cadmium concentration is low than 2mg. kg⁻¹ in soil, change of scutellarin content in stem is not very obvious, but with increase of cadmium content the decreasing degree of scutellarin is rather distinguish and excess one quarter of

control. Similarly, under chromium and mercury stress scutellarin content in stem is almost all reduced and even arrives to half of control in some concentration. Besides, as T_2 and T_1 in Table II show, copper and lead added artificially to soil respectively, scutellarin content in stem exhibited described decrease trend in spite of any metal concentration.

B. Scutellarin Content Change of Leaf

TABLE III: SCUTELLARIN CONTENT CHANGE OF LEAF UNDER HEAVY METALS STRESS

Treatment	Cd	Cr	Hg	Cu
T_1	-7.2597	-21.9836	-14.5194	-9.1002
T_2	1.9427	-47.1370	-21.8814	-3.2720
T_3	-50.3067	-19.3252	3.0675	0.0000

Continue

Treatment	Pb
T_1	-7.6687
T_2	-0.5112
T_3	-3.3742

In leaf of *Erigeron breviscapus*, scutellarin content is only minor increase when cadmium and mercury concentration are 2 mg kg^{-1} and 5 mg kg^{-1} according to Table III, respectively. Oppositely, on the condition of other concentration and metal elements scutellarin content is a negative growth, it is inferred that scutellarin synthesis is inhibited seriously.

C. Scutellarin Content Change of Flower

TABLE IV: SCUTELLARIN CONTENT CHANGE OF FLOWER UNDER HEAVY METALS STRESS

Treatment	Cd	Cr	Hg	Cu
T_1	-29.3383	-33.9576	-13.6080	-38.9513
T_2	-44.9438	-47.8152	-65.9176	-26.5918
T_3	-75.4057	-30.7116	-32.8340	-42.5718

Continue

Treatment	Pb
T_1	-32.8340
T_2	-58.4270
T_3	-27.5905

Under every concentration of all metal elements stress scutellarin content were reduce severely in flower. The decreasing degree of scutellarin is higher than in stem and leaf and the maximum value arrive to 65 percentages. The result is shown clearly in Table IV.

D. The Further Discussion

The result of this study show *Erigeron breviscapus* can cause some change in some extent within different heavy metals stress scutellarin content of different tissues. And the total trend is reduced, especially in leaf and flower tissues. Concurrently, it is inferred that although some toxic substances in condition of low dose can promote plants growth and secondary metabolites accumulate in mentioned extent [18]-[19], the increasing level of secondary metabolites is very limited. Such as the concentrations of five flavone components in *S. baicalensis* Georgi were not significantly affected by spiking low concentrations of As

($\leq 200 \text{ mg kg}^{-1}$) into soils, but high levels of As inhibited the generation of baicalin and wogonin [20]. Therefore, the increase of secondary metabolites by addition of toxic substances is very difficult, and its effect is mainly brought some poison to plants.

In synthesis of optimum defense the generation of secondary metabolites is reduce chance of plant growth pay a price. Plant growth is very slow under environment stress, and has a low cost for generation of secondary metabolites. Compensatory ability for damaged in plant is very low, defense benefit of secondary metabolites is promoted. So, plant would produce more secondary metabolites in stress condition. In addition, according to the results of study we speculate that this phenomenon is depend on that plant growth is affected slightly. If damage and time of stress have been become more seriously, all defense system would deplete extremely energy to maintain longer life, and the increasing level of secondary metabolites will very difficult. According to resource distribution strategy, every plant is three important functions, which include growth, maintain survival and propagation. But if plant encounter environmental stress in the course of growth and development, resource distribution strategy will change to adapt to alternative environment, function of maintain survival also will become major aim. Therefore, with the purpose of adapt to environmental change and survival in further, plant will activate secondary metabolites defense system and synthesis a great deal of secondary metabolites to defense disadvantage and extreme environment. Thus, in order to guarantee excellent medicine quality of plant for medicine in contaminated soil will not adopt to cultivar medicine plants.

On the other hand, some trace metal ion, like magnesium, zinc and iron, they are essential elements of enzyme synthesis, and these material in plant can affect secondary metabolites' change. When they are higher than a normal condition, plant would not toxic severely. Instead, some secondary metabolites, especially key medicinal components, will massive synthesis [21]-[23]. Therefore, the research how to influent synthesis of secondary metabolites in high concentration condition of trace elements will very hot focus in future.

In the research secondary metabolites of *Erigeron breviscapus* only analysis change of scutellarin content, but from the secondary metabolites prospective, secondary metabolites category that have been changed under heavy metal stress should very rich. Hence, in order to research change rule of secondary metabolites after exposure to toxic substances, research objects of secondary metabolites will not only focus on known and interesting metabolites substances, but also investigate deeply category and quantities of all changed metabolites, so all these work will help to master plant response mechanism for stress environment.

For example, using a combination of HPLC-ESI-MS techniques, during cold acclimation a total of 40 phenolic and flavonoid compounds in wheat (*Triticum aestivum* L.) leaves were identified. Moreover, the trimethylated flavone, 3', 4', 5'-trimethyltricetin was identified for the first time in the native extracts of both control and cold-acclimated wheat leaves [24]. Yukino Yakabe reported first that some

compounds for formation of furanocoumarin phytoalexins could be induced in *Glehnia littoralis* root cultures by treatment with ascorbic acid (AsA), and the modulation of the phytoalexin biosynthetic pathway in *G. Littoralis* be induced also by iron deficiency [25].

In short, the formation of medicine plant quality is link closely with plant living surrounding, in which medicine plant have been evolve and adapt to the environment for hundreds of years, even thousands of years. Therefore, in order to explain formation mechanism of quality, growth surrounding of medicine plant should pay the more attention, especially on the stress environment condition.

For the discovery of novel and innovative drugs, the wide chemical diversity of secondary metabolites represents an extremely rich biogenic resource throughout the plant kingdom [5]. Therefore, several schools of thought, substantiated with numerous scientific evidence, implicate the highly ordered interactions between plants and their biotic and abiotic environments as being a major driving force behind the emergence of specific secondary metabolites [26].

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