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# EFFECTS OF PRASEODYMIUM ON FLAVONOIDS PRODUCTION AND ITS BIOCHEMICAL MECHANISM OF *SCUTELLARIA VISCIDULA* HAIRY ROOTS *IN VITRO*

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**Abstract**

In the present study the effect of rare earth element Praseodymium (Pr) on flavonoids production and its biosynthesis key enzymes including Peroxidase (POD; EC 1.11.1.7), Polyphenol oxidase (PPO; EC 1.10.3.1) and Phenylanlanine ammonialyase (PAL; EC 4.3.1.5) in hairy roots of *Scutellaria viscidula* was investigated. After 7 days of suspension culture, activity of POD, PPO and PAL and total flavonoid production showed the same response tendency, which was ascending first and then descending with the $Pr^{3+}$ elicitor increasing, and the $Pr(NO3)_3$ concentration reaching the knee point was 15, 25, 25, 25, 25, ordinarily. Subsequently mechanism of flavonoids accumulation by Pr elicitation at the biochemical level is very significant for application of rare earth element to large scale production of plant nature drugs.

## Introduction

Flavonoids, a widespread plant secondary metabolites, are of immense economic functions such as potential drugs, food nutraceuticals and industrial materials, while their importance was also manifested by the larger and larger demands. However, their low yields in natural plant and the remarkable complexity for chemical synthesis so that supply falls short of demand, so an alternative and effective strategy is focused on both of hairy roots culture and elicitation treatment, which furthermore is considered as a useful experimental system and a promising production mode for the active constituents from medicinal plants (Giri & Narasu, 2000).

Recently, the advantages of hairy roots culture technology make people pay more and more attention on the study and application to enhance nature products, due to fast and all-weather growth as well as elevated and stable productivity rather than traditional cell cultures or directly isolating from natural plant (Savitha *et al*., 2006). *Scutellaria viscidula* Bunge is a perennial herbs subordinating to the family *Labiate*. Its roots are widely used in treating inflammatory and bacterial diseases as the potent traditional Chinese medicine with long officinal history in the Orient countries, owing to the chief bioactive ingredients, including baicalin, baicalein, wogonoside, wogonin and other flavonoids compounds (Yamoto, 1991). And especially in recent studies remarkable efficacy was found in resisting human immune-deficiency virus (HIV-1) and T-cell leukemia virus (HTLV-1), inhibiting some kinds of tumor cell dissemination, and scavenging effectively free-radical, etc. (Yano *et al*., 1994; Motoo & Sawabu, 1994; So *et al*., 1997; Ikemoto *et al*., 2000; Ye *et al*., 2002). Further more, hairy roots culture system of *S. viscidula* has been established as a potential method of producing flavonoids (Wang *et al*., 2008).

A variety of elicitors have successfully been applied to enhance secondary metabolites, and most of the studies were performed by shake flask culture (Bais *et al*., 2000). Elicitors are a group of biotic and abiotic compounds, which can trigger the increased production of flavonoids and other resistance-related compounds by stimulating plant cells (Savitha *et al*., 2006). Praseodymium (Pr), one of the more abundant of rare-earth elements, have already been studied on its biological effects, but was only reported in synthesis regulation of polysaccharide in *Russula* sp., (Li, 2007). Moreover, so far little information is available about its roles on plant secondary metabolism.

In the present study, the production of total flavonoids induced by $Pr^{3+}$ elicitor in hairy roots from *S. viscidula*, and elucidated the effect of elicitation on three enzymes Peroxidase (POD; EC 1.11.1.7), Polyphenol oxidase (PPO; EC 1.10.3.1) and Phenylanlanine ammonialyase (PAL; EC 5.3.1.5), which have a great influence on plant development and phenols biosynthesis including flavonoids (Zhang *et al*., 2005). Therefore, flavonoids regulation mechanism based on hairy roots and elicitation treatment was discussed at the biochemical level. The result will preliminarily pay the way to enhance flavonoids accumulation through rare earth elements application technology in medicinal plant production.

## Materials and Methods

**Plant materials:** *S.viscidula* was cultivated in the botanical garden of Southwest University in China. The hairy root cultures were implemented (Fig. 1) and the technical process was reported previously reported (Wang *et al*., 2008).

**Elicitor preparation and elicitation treatment:** About 100 mg of fresh hairy roots (3 cm in length) were inoculated into 250 ml conical flasks containing 150 ml of liquid $1/2MS_0$. In form of $Pr(NO_3)_3$ solution at the concentration of 1 mmol/L and pH 5.5, $Pr^{3+}$ was used at the levels of 0、5、10、15、20、25 and 30 mmol/L in the $1/2MS_0$ medium, respectively. The hairy roots from *S. viscidula* were cultured in this liquid complex medium on rotary shaker (110 rpm) at 25±1°C for 7 days.

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Fig. 1. Suspension culture of *S.viscidula* hairy root.

**Collection and measurement of experimental parameters:** The POD, PPO and PAL were extracted and their activities were measured through the guaiacol oxidation method (Li, 2000), the pyrocatechol colorimetric method (Wang & Shang, 2000) and the spectrophotometry method (Zou, 2000), respectively. The activity of all enzymes is expressed in U/min·gFw. The extraction and determination of total flavonoids by colorimetric method, and the content of baicalin was detected by the HPLC method. Both above mentioned processes were described previously (Wang *et al*., 2008). All treatments were performed in triplicate. The results are represented by the figures and the mean ± standard error (S.E.) (Çag, *et al*., 2009).

## Results

**Effect of elicitation on activity of key enzymes POD, PPO and PAL:** Increased with $Pr^{3+}$ concentration, the POD activity of hairy roots raising and reached the maximum in 15 mmol/L $Pr(NO_3)_3$ and then went down to some extent in liquid medium 20, 25 and 30 mmol/L $Pr^{3+}$(Fig. 1). All of samples treated with $Pr^{3+}$ were higher than the control, and the POD activity in 30 mmol/L $Pr(NO_3)_3$ solution was only a little higher than the control, speculating that the activity was probably less than the control in $Pr^{3+}$ solution of higher concentration (Fig. 2).

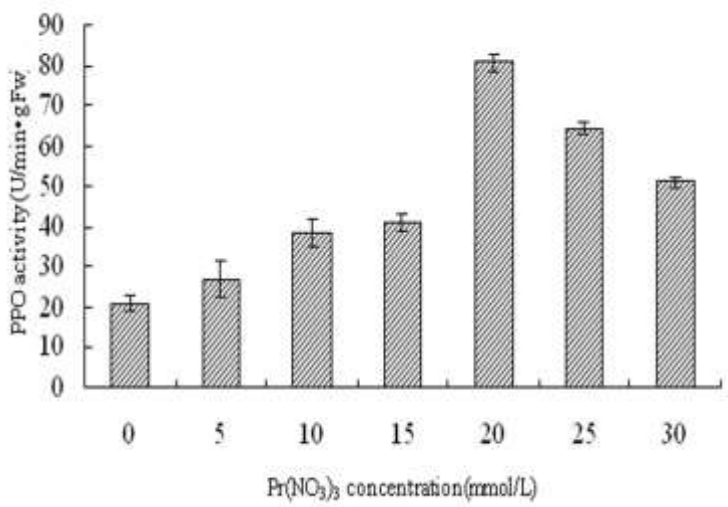


Fig. 2. Effect of $Pr^{3+}$ elicitor on POD activity in hairy roots of *S. viscidula*. (Data presented as means ± S.E., n=3).

PPO activity in hairy roots was significantly different from each other among 6 elicitation treatment and the control group. After treatment by $Pr(NO_3)_3$, PPO activity was induced quickly and reached the peak value in 20 mmol/L $Pr^{3+}$ elicitor under suspension culture condition and then declined in $Pr^{3+}$ solution of higher concentration. However, PPO activity in all treatments were higher than the control group (Fig. 3).

The activity of PAL in hairy roots treated by $Pr(NO_3)_3$ were much higher than the control (Fig. 3). PAL activity enhanced gradually to the peak in $Pr(NO_3)_3$ solution of no higher than 25 mmol/L, and reduced subsequently. However the minimum did still elevate obviously by 3.54 fold compared with the control group (Fig. 4).

**Effect of $Pr^{3+}$ elicitor on the content of total flavonoids:** After treatment with $Pr^{3+}$ elicitation, the change of total flavonoids production in hairy root culture is shown in Fig. 3. The response to $Pr^{3+}$ elicitor was to ascend and then descend evidently, but flavonoids content in all treatments were still obviously higher than that in the control. The total flavonoids production in suspension medium containing gradient concentration $Pr^{3+}$ increased by 1.206, 2.259, 2.712, 3.415, 4.502 and 4.088 folds respectively compared with the control (Fig. 5).

## Discussion

POD, PPO and PAL were three crucial enzymes involved in phenolic compound including flavonoids biosynthesis pathway, and meanwhile they were important protective enzymes, which defended against various environmental stresses such as hypoxia stress in suspension culture condition and toxicity stress from excessive heavy-metal Pr in this experiment. So these enzymes activities were not only induced by elicitation and mild water factor, but also inhibited by the severe water stress. Flavonoids had analogous physiological function and response mechanism too. This experiment showed that all of the POD, PPO and PAL activities and flavonoids production increased firstly and decreased afterwards, corresponding to the gradient variation of $Pr^{3+}$ concentration. The similar conclusion has also been demonstrated by the study on biological effects of other abiotic stress such as $La^{3+}$ (Mu *et al*., 2006) and sound wave (Wang *et al*., 2003).

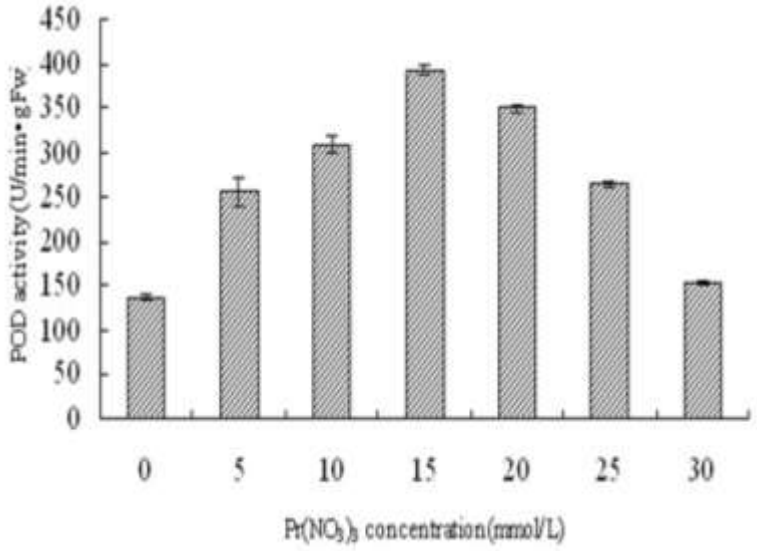


Fig. 3. Effect of $Pr^{3+}$ elicitor on PPO activity in hairy roots of *S. viscidula*. (Data presented as means ± S.E., n=3)

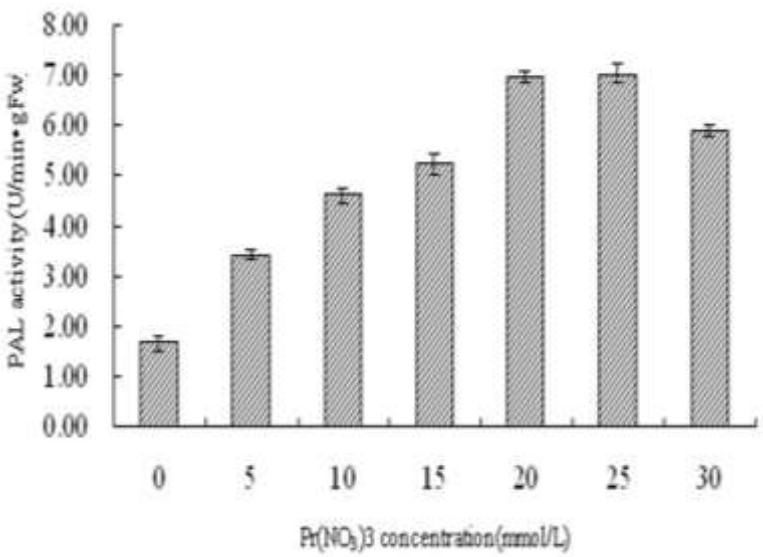


Fig. 4. Effect of $Pr^{3+}$ elicitor on PAL activity in hairy roots of *S. viscidula*. (Data presented as means ± S.E., n=3)

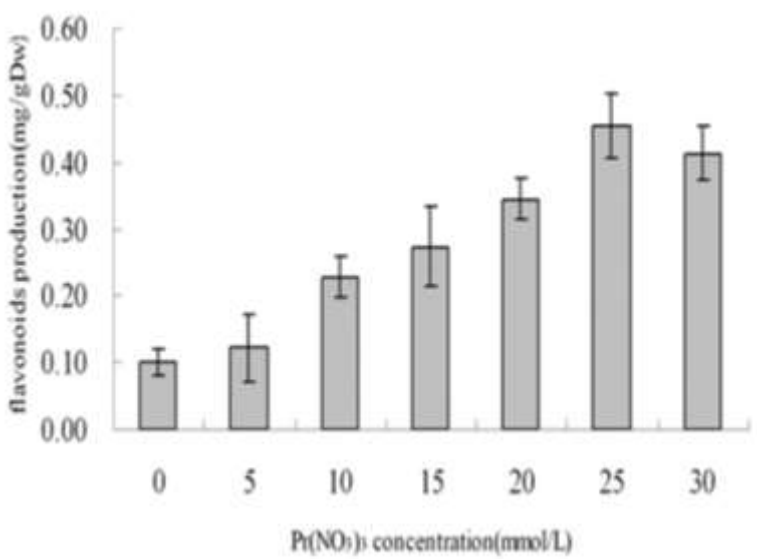


Fig. 5. Effect of $Pr^{3+}$ elicitor on total flavonoids production in hairy roots of *S. viscidula*. (Data presented as means ± S.E., n=3)

POD exists in various plant cells, and is considered to participate in ethylene and flavonoids biosynthesis (Wang *et al*., 2001). After treatment with $Pr^{3+}$ elicitor, POD activity rose to the threshold value in medium with 15 mmol/L $Pr(NO_3)_3$. PPO is a copper-containing metalloprotein with two conserved Cu-binding domains in the active center, and catalyzes polyhydric phenol hydroxylation of xenol and dehydrogenation from hydroxyl-phenol to quinine (Liu *et al*., 1989; Han and Lu, 1994). Positive ion like $Cu^{2+}$, $Pr^{3+}$ had probably similar mechanism of enzyme activation. Peak value of PPO activity appeared when $Pr(NO_3)_3$ is 25 mmol/L, which was the most appropriate activating concentration of PPO enzyme.

PAL is the first committed enzyme involved in the plant phenylpropanoid pathway (Amrita & Brian, 2001), and is the hotspot in secondary metabolism field, too. PAL activity reached the highest value in medium with 25 mmol/L $Pr(NO_3)_3$, when this enzyme could drive mostly the flavonoids biosynthesis. The result revealed that there was a non-linear relationship between biological effect and chemical elicitation. Appropriate content of $Pr^{3+}$ elicitor can stimulate the flavonoids accumulation, covering in Yuan's research report (Yuan *et al*., 2002). When elicitation degree exceeded the threshold value, protein synthesis would be inhibited by the excessive rare earth element, and hence the enzyme activity fell afterwards.

Taking a wide view of the four figures, the $Pr(NO_3)_3$ concentration reaching the threshold value of POD activity was lower than others. Because as a representative biomarker for oxidative stress, POD is more sensitive to hypoxia environment of suspension culture. Besides, it is necessary to mention that the same response trend, ascending first and then descending with the $Pr^{3+}$ elicitor increasing, was found in different metabolism enzymes activity and total flavonoid production. The phenomena demonstrated mechanism of flavonoids accumulation from the enzymological point of view, and furthermore, coinciding well with the flavonoids regulation pattern by some biotic and abiotic elicitor such as chemical (Belhadj *et al*., 2008), physical (Antognoni *et al*., 2007), and pathogen (Krolicka *et al*., 2008) and so on.

A diverse process of Pr elicitation was revealed through the change of the production of total flavonoids and expression activity of key enzymes in flavonoids metabolism in hairy roots of *S. viscidula*. Futher more, secondary metabolism was effectively up regulated only when the most proper $Pr^{3+}$ elicitor worked, and thus it is feasible for the appropriate and efficient $Pr^{3+}$ to be used as a physiologically acceptable elicitor.

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