

Enhanced Secondary Metabolite Biosynthesis by Abiotic Elicitor in Transformed Plant Root System

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Abstract

Plants generally produce secondary metabolites in nature as a defense mechanism against pathogenic and insect attack. In this study, we applied several abiotic elicitors in order to enhance growth and ginseng saponin biosynthesis in the hairy roots of *P. ginseng*. Generally, elicitor treatments were found to inhibit the growth of the hairy roots, although simultaneously enhancing ginseng saponin biosynthesis. The addition of selenium at inoculum time did not significantly affect ginseng saponin biosynthesis. However, when 0.5 mM selenium was added as an elicitor after 21 days of culture, ginseng saponin content and productivity increased to about 1.31 and 1.33 times control levels, respectively. These results suggest that processing time for the generation of ginseng saponin in a hairy root culture can be reduced via the application of an elicitor.

Introduction

In general, plants produce secondary metabolites in nature as a defense mechanism against pathogenic and insect attack. In recent research into in vitro culture systems, a wide variety of elicitors have been employed in order to modify cell metabolism. These modifications are designed to enhance the productivity of useful metabolites in the cultures of the plant cells/tissues. The cultivation period, in particular, can be reduced by the application of elicitors, while maintaining high concentrations of product. Elicitation strategies are compounds or treatments which

cause plants to synthesize elevated levels of phytoalexins. The active mechanisms employed by elicitors are complex and distinctive. As little is known regarding the biosynthetic pathways of most secondary plant metabolites, the effects of elicitation on a plant cell/tissue culture are difficult to predict. Therefore, elicitation approaches tend to be empirical steps. The effects of elicitors rely on a host of factors, including the concentration of the elicitor, the growth stage of the culture at the time of elicitation, and the contact duration of elicitation. Both biotic and abiotic elicitors can be used to stimulate secondary metabolite biosynthesis in plant cell/tissue cultures, thereby reducing the processing time necessary for high product yields. In this study, *P. ginseng* C.A. Meyer hairy root cultures, generated by infection with *Rhizobium rhizogenes* KCTC 2744, were employed in order to enhance the biosynthesis of a secondary metabolite, via the application of abiotic elicitors.

Materials & Methods

The hairy roots of *P. ginseng* C.A. Meyer were induced and maintained, as previously described. In all of the experiments in this study, the hairy roots were cultivated in liquid hormone-free 1/2 MS medium that contained 30 g/L sucrose. The cultures were then incubated at 23±1°C in darkness in a 250 mL Erlenmeyer flask, on a rotary shaking incubator at 80 rpm. In order to determine the effects of several elicitors on hairy root growth and ginseng saponin biosynthesis in *P. ginseng* hairy roots in 250 mL flask cultures, selenium (as selenite; 0 to 10 mg/L) was applied. 0.2 mL of elicitors were added to each sample flasks at inoculum time. The pH of the prepared elicitor solutions was adjusted to 5.8, using either 1 N NaOH or 1 N HCl. At each experiment, the control was incubated to same culture condition for same period. For selenium elicitation experiments, after 21 days of culture which did not added any elicitor (1st culture), different concentrations of the prepared selenium elicitors (as selenite; 0 to 0.5 mM) were added to culture media. On day 3 of elicitation (2nd culture), the hairy roots were harvested, and the biomass and ginseng saponin content were measured. To compare with the elicitation effect of elicitor, the control was incubated to same culture condition for 24 days. All experiments are performed at least two

independent experiments. In order to determine the weight of the biomass, dry weight was measured gravimetrically after drying the roots for 24 hours at 60°C. Results are expressed as the mean value of at least two independent measurements. Ginseng saponin content was measured via Vanillin-H₂SO₄ colorimetry.

Results & Discussion

In order to investigate the effect of selenium on *P. ginseng* biomass and ginseng saponin biosynthesis, prepared selenium was added to medium at inoculum time. Selenium was found to strongly inhibit hairy root growth, but caused a slight increase in ginseng saponin content, as is shown in Figure 1. The addition of 0.1 mM selenium resulted in a ginseng saponin content of 80.5±4.4 mg/g (1.07 times control level), and a productivity of 402.3 mg/L (1.04 times control level). These results indicate that the addition of selenium at inoculum time do not greatly improve the productivity of ginseng saponin in *P. ginseng* hairy root cultures. Carvalho *et al.* reported similar results that the effects of selenium supplementation on germination and plant growth and at sufficiently high concentrations, it can inhibit both the growth and germination of seeds. As shown in Figure 2, elicitation via the addition of 0.1 mM Se resulted in inhibited biomass growth. However, when 0.5 mM Se was added, biomass was slightly higher than that observed in the control sample. The ginseng saponin content of hairy roots increased directly with increases in the added amount. As a result of the addition of 0.5 mM Se, ginseng saponin content was 74.7±7.3 mg/g (1.31 times control value), and the productivity was 594 mg/L (1.33 times control value). Treatment with selenium as an elicitor is associated with several advantages in terms of both economy and operation compared with other treatments such as yeast elicitor treatments. Chief among these advantages are the low preparation cost and the ease of the preparation process. Preparation of a selenium elicitor is fairly simple, since the elicitor is applied as a solution, whereas the preparation of a yeast elicitor requires several processing steps, including repeated ethanol precipitation and purification steps. Also, the time required for preparation of a selenium elicitor is much less than that required for the preparation of yeast elicitor; the preparation of a yeast elicitor takes approximately 1 week. Therefore, application

of selenium as an elicitor may dramatically reduce the costs associated with the large-scale production of ginseng saponin (ginsenosides).

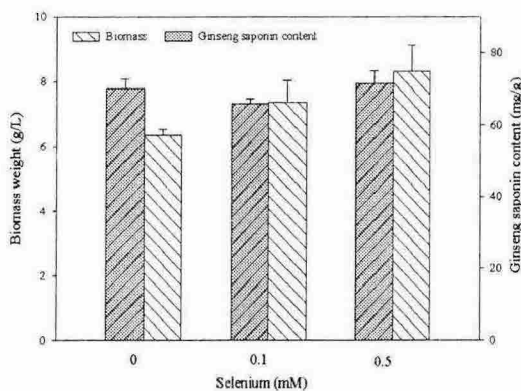


Figure 1. Effect of selenium on growth of hairy roots.

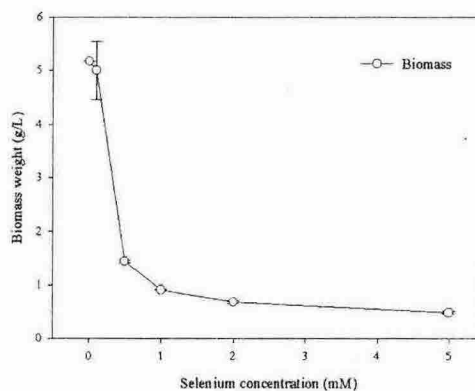


Figure 2. Effect of selenium elicitor on growth and secondary metabolite accumulation of hairy roots.

References

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