

Elicitor-induced production of natural products from the suspension culture of
Scutellaria baicalensis and *Panax ginseng*

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It is well known that if a plant is exposed to a pathogenic microorganism, it often produces a variety of chemicals called phytoalexins. These phytoalexins may be used as agrochemicals in nature and sometimes they have a significant biological activities for human. The signaling substances that stimulate the induction of these chemicals are termed elicitors. When an elicitor is recognized by a plant cell, the signal is transduced and, in turn, biosynthetic enzymes which involved in the production of antibiotic chemicals are activated. Among the best studied endogenous signalling molecules are ethylene, salicylic acid and jasmonic acid (JA). Increasing evidence suggests that JA may function in the signal systems for the production of secondary metabolites.

The root of *Scutellaria baicalensis* Georgi (Labiatae) is known to contain a number of flavone derivatives and it is often used as an active principle in Oriental herbal medicines. The cell suspension cultures of the plant has been established and the production of flavonoids was reported. Recently, Morimoto et al identified a rapid and novel system to effectively metabolize a large amount of hydrogen peroxide in the suspension cells of *S. baicalensis*. A wide variety of elicitors have been employed to alter cell metabolism in plant cell cultures. In a study searching for a possible agent to manipulate the second metabolism of the *S. baicalensis* cells, we discovered that triterpenoids were induced by the treatment of yeast elicitor preparation. The present results show that the production of triterpenoids from the *S. baicalensis* suspension culture is induced by yeast elicitor and the induced compounds are mainly accumulated in the culture media. The results also shows that the elicitation may be mediated by a second messenger jasmonic acid, linked by the preceding PLA₂ activation. When growth-phase cell suspension cultures of *Scutellaria baicalensis* were treated with 50 µg/ml of yeast elicitor preparation, the levels of both oleanolic acid and ursolic acid were transiently increased in the culture medium rather than in the cells. The maximal triterpenoid concentration was found in approximately 35 hours after yeast elicitor treatment, whereas the time for reaching a maximum level was about 20 hours after exogenous methyl

jasmonate treatment. Elicitor treatment also increased PLA₂ activity by two folds and the simultaneous treatment of aristolochic acid, a PLA₂ inhibitor, inhibited triterpenoids accumulation as well as PLA₂ activity in the culture. These results suggest that the biosynthesis of cytotoxic triterpenoids may well be induced by the treatment of elicitors and that the elicitation may be mediated by so called octadecanoid pathway.

In an independent research with *Panax ginseng* suspension culture, a self defense chemical produced by elicitation was separated and its structure was identified. The result offers the possible development of useful biopesticide in ginseng cultivation. When we treated suspension cultures of *Panax ginseng* with yeast extract, a universal elicitor, a distinct compound was released into the cell cultured medium. Ethylacetate fraction of the yeast-elicitor treated cell culture medium was separated by silica gel column and subsequent Sephadex LH-20 column chromatography. Compound 1 was isolated and its structure was identified as 2,5-dimethoxy-1,4-benzoquinone by MS, ¹H-NMR, ¹³C-NMR. Compound 1 was also increased even after *Botrytis cinerea* 9802, a well known pathogenic fungus of crop plants and fruit trees, culture broth treatment. The production of 2,5-dimethoxy-1,4-benzoquinone was increased slowly and reached maximum level ($54.5 \pm 5.16 \mu\text{g/g}$ fresh weight) after 12hr of addition of $50 \mu\text{g/ml}$ yeast elicitor preparation (MW<10,000) while the production of the compound reached maximum level ($39.2 \pm 10.8 \mu\text{g/g}$ fresh weight) after 24hr of addition of $100 \mu\text{g/ml}$ *Botrytis cinerea* 9802 culture broth. Since the compound has never been known to be produced in *Panax ginseng* and it has been known to be antimicrobial, we tentatively conclude that the compound is a new type of phytoalexin which is induced by elicitation.

The results presented above show that elicitation is a useful tool to increase the yield of a certain secondary metabolite and possibly increase the activity of the enzymes which is involved in the biosynthesis of a certain secondary metabolite. For the biotechnological approach to manipulate a secondary metabolism of a plant, more thorough research towards plant-microbe interaction and/or subsequent signal transduction pathway should be essential.