The effects of various biotic elicitors on the accumulation of scopolamine and hyoscyamine by adventitious hairy root cultures of *Scopolia parviflora*

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Abstract

Hyoscyamine and scopolamine are the pharmaceutically valuable anticholinergic drugs. The aim of our study is to increase the contents of both hyoscyamine and scopolamine by means of elicitation. Various biotic elicitors derived from 3 fungi and 1 yeast were inoculated to the hairy root cultures. When homogenate and supernatant of elicitors treated with hairy root cultures, that of yeast elicitor was only increased scopolamine production. However, all of the fungal elicitors did not influenced the productions of scopolamine and hyoscyamine. Our results will contribute to mass production of tropane alkaloids by hairy root cultures of *Scopolia parviflora*.

Introduction

The low yield of useful secondary metabolites in vitro culture is one of the major limitations, and many strategies have been tried to improve the production. The employment of fungal preparations or chemicals, termed as elicitors, becomes one of the most important strategies. Although several researchers have reported the strong and rapidly stimulating effect of various elicitors, the contradictory results were sometimes observed in other cases. Therefore, we investigated the effects of biotic elicitors originated different fungus strains and yeast strain in order to the selection of optimal biotic elicitor in *S. parviflora* hairy root culture.

Materials and methods

1. Establishment and culture of adventitious hairy roots

The adventitious hairy roots of *S. parviflora* were induced from the rhizome of a mature plant and maintained on B5 medium supplemented 5% sucrose and 0.1 mg/l IBA.

2. Elicitor prepation and elicitation

Three fungus strains (*Alternaria alterinata*, *Botrytis cinerea*, and *Fusarium solani*) and one yeast (*Candida albicans*) were used as biotic elicitors. Fungal homoganates were prepared as previously described[1)] and fungal supernatants were obtained using the method of Eilert et al.[2)]. Yeast homogenate was prepared by autoclaving the entire culture and yeast supernatant by centrifuging. Each homogenate elicitors was added 3 ml/flask and each supernatant elicitors was 2 ml/flask.

3. Analysis of hyoscyamine and scopolamine

Hairy roots were harvested, weighed, and analyzed using by HPLC (MeCN : 50 mM K_2HPO_4 = 22 : 78, 4.6 × 25 cm TSK gel ODS column, and UV 215 nm). Analysis of hyoscyamine and scopolamine was investigated on 0, 12, 24, and 48 (72 hour in case of homoganate) hour after treatments of elicitors.

Results and discussion

Both of homogenate and supernatant yeast elicitors stimulated the specific production, which increased the scopolamine production, but not hyoscyamine production (Fig. 1). Especially, yeast homogenate continuously augmented the scopolamine production through the exposure time, whereas yeast supernatant showed the highest scopolamine production (2.2 fold) at 24 exposure time and then declined rapidly (data not shown). However, fungal homogenate and supernatant elicitors did not increase neither hyoscyamine nor scopolamine production. In case of *A. alterinata* homogenate, the hyoscyamine and scopolamine production were decreased over the exposure time.

Our results suggest that tropane alkaloid pathway could not be activated in response to fungal elicitors. Moreover, the fact that *C. albicans* was proved the optimal biotic elicitor indicates the requirement of more expaned study to other yeast strain in terms of elicitation.

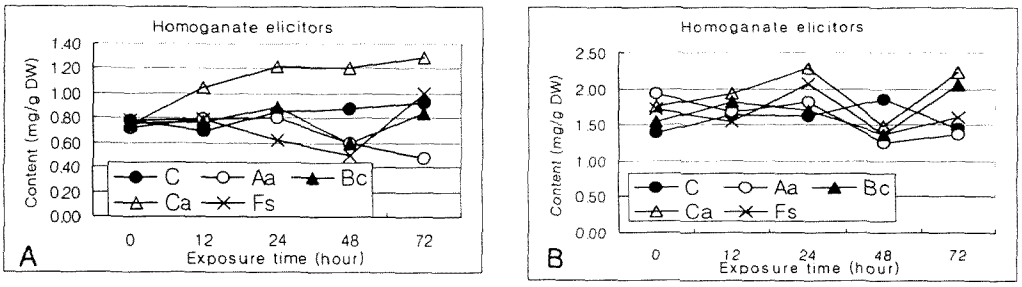

Fig. 1. The effect of homogenate elicitors on scopolamine (A) and hyoscyamine (B) production. (C: control, Aa: *A. alterinata*, Bc: *B. cinerea*, Fs: *F. solani*, and Ca: *C. albicans*)

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References

1. C. Wang, J. Wu, X. Mei, "Enhancement of Taxol production and excretion in *Taxus chinensis* cell culture by fungal elicitation and medium renewal" (2001), Appl Microbiol Biotechnol, 55, 404-410
2. U. Eilert, W.G.W. Kurz and F. Constabel, "Stimulation of Sanguarine accumulation in *Papaver somniferum* cell cultures by fungal elicitors" (1985), J. Plant Physiol. 119, 65-76.