P 68 Effect of Fungal Elicitor Exposure Time on Biosynthesis of Sanguinarine by *Papaver somniferum* Cell Cultures

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Objectives

This study was conducted to optimize elicitor exposure time on sanguinarine production of Papaver somniferum cell cultures. Determination of the secondary metabolite profiles in response to fungal elicitation is essential for maximization of yield by optimization of the exposure time. Here we report the exposure time effect of two fungal elicitors, *Verticillium dahliae* and *Botrytis cinerea* and methyl jasmonate on sanguinarien production.

Materials and Methods

1. *Elicitor Treatment*: Fungal elicitor was prepared from *Botrytis* and *Verticillium*. A section (1 cm^2) of mycelia cultured on potato dextrose agar medium was frown in 50ml B5 medium, including supplements but lacking phytohormones, on a gyratory shaker (120 rpm) at 24℃ in the dark for 6 days. Mycelia and remaining medium were homogenized at maximum speed for 10 min, autoclaved (121℃) for 20 min, and subsequently centrifuged under sterile conditions with the supernatant serving as elicitor.

2. *Quantification of sanguinarine*: Sanguinarine was extracted from dried cells with methanol (pH2.0). Analysis of sanguinarine was performed by using HPLC (Waters, Massachusetts, USA) with a reversed phase C18 column (μbondapack, Waters) and MeOH/H_2O eluant system (55:45). The chromatogram was monitored between 200 and 600 nm using a photodiode array detector. Sanguinarine contents were estimated from a calibration curve prepared with authentic standards purchased from Sigma.

Results and Discussion

Elicitor screening and transient studies with *Papaver* cells were conducted to demonstrate the importance of both elicitor specificity and exposure times on sanguinarine production. We saw an enhancement in the levels of sanguinarine upon addition of *Botrytis* to our cell culture system. Botrytis seems to be an excellent choice as an elicitor for enhancement in sanguinarine yields. Elicitor exposure time also plays an significant role in transient induction of genes, activation of enzymes and accumulation of sanguinarine. The production of sanguinarine increased over 10-fold on day 3 after addition of *Botrytis*. The methods described in this report could be generally be used in devising strategies for enhancement in productivity of secondary metabolites.

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