

Elicitor-stimulation of Benzophenanthridine Alkaloid Formation in Suspension Cultures of *Toddalia asiatica*

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Abstract

Secondary metabolites production in plants represents the adaptation of plants to environmental stress, or defensive/ protective/ offensive chemicals against pathogens. Plant cells *in vitro* show physiological and morphological responses to microbial, physical, or chemical factors, which are known as elicitors. Hence the biosynthetic potential of the suspension cultures of *Toddalia asiatica* Linn.(Rutaceae) to stimulate the formation of benzophenanthridine alkaloid nitidine by the addition of proper stimulants to the culture medium was investigated. For the elicitor studies, 5 ml of cell suspension cultures cultured in MS supplemented with 2,4-D(1 mg/l) + BA(0.5 mg/l) were transferred into each well of pre-sterilized six-well plate separately under laminar flow with 50% v/v as inoculum. Dose dependent induction experiments were carried out by feeding abiotic elicitors, quercetin (50 µM, 100 µM, 500 µM) and benzoic acid (10 µM, 50 µM, 100 µM) separately into each well of six-well plates by adding the elicitors on day 1, 9 and 12 in order to optimize the concentration and the day of addition. The six well plates were incubated at 25±2 °C and 120 rpm in refrigerator shaker incubator, 16h photoperiod. The elicitor treated cultures were harvested after completion of 12 days of incubation period. Whenever the elicitors were added, each concentration in triplicate was added and with a suitable control. Among the different concentrations of quercetin (50 µM, 100 µM, 500 µM) and benzoic acid (10 µM, 50 µM, 100 µM), quercetin 50 µM and 100 µM and benzoic acid 10 µM and 50 µM were found to increase alkaloids, nitidine accumulation. Not only the time needed to reach a high yield of secondary metabolites is decreased by elicitation, but it is also a process strategy that can be easily integrated with other yield enhancement strategies.