

BIOMASS AND FLAVONOIDS PRODUCTION IN CELL SUSPENSION CULTURES OF *Ficus deltoidea* ('MAS COTEK') IN RESPONSE TO ELICITATION AND PRECURSOR FEEDING

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ABSTRACT

The approaches of introducing elicitor and precursor into the cell culture were among some of the applications in optimizing the accumulation of secondary metabolites, without the need of compromising rapid recovery of the targeted compound. Three different elicitors (yeast extract, jasmonic acid and casein hydrolysate) and precursors of flavonoids (phenylalanine, cinnamic acid and chalcone) at different concentrations were introduced to *Ficus deltoidea* cell culture. The accumulation of three flavonoids: rutin, naringenin and quercetin were quantified and further verified using High Performance Liquid Chromatography (HPLC) and Liquid Chromatography – Mass Spectrometry (LC-MS). Among all the treatments tested, Murashige and Skoog (MS) medium supplemented with 3 mg/L casein hydrolysate recorded the highest biomass accumulation, in terms of dry weight, about 0.42 g higher than the control. Furthermore, the accumulation of rutin ($12.69 \pm 4.64 \mu\text{g/g DW}$) was found to be the highest in 1000 mg/L yeast extract treatment, quercetin ($4.75 \pm 0.37 \mu\text{g/g DW}$) in 0.001 mg/L chalcone treatment and naringenin ($11.22 \pm 2.15 \mu\text{g/g DW}$) in 0.005 mg/L chalcone treatment, respectively. Feeding of precursors into the medium resulted in suppressed production of rutin but a fairly good result in the accumulation of naringenin. Nevertheless, the accumulation of naringenin in all precursor treatments generally portrayed a higher production when compared to the control.

Keywords: *Ficus deltoidea*, cell suspension, flavonoid, elicitor

INTRODUCTION

Several strategies such as manipulating the nutrient, optimizing the culture conditions, feeding of precursor and elicitation can be applied in order to substantially increase the yields of secondary

metabolites in plant cell cultures [1]. As an example, feeding of 0.2 mmol/L of L-phenylalanine, one of the precursors in the phenylpropanoid pathway, to cell culture of *Cistanche deserticola* resulted in 75% higher production of phenylethanoid glycosides when compared to the cell culture without precursors [2]. Other than that, addition of 100 μ M of jasmonic acid into cell culture of *Erythrina americana* have successfully enhanced the production of alkaloid like erysodine by 434 % [3].

Ficus deltoidea is believed to be a good remedy for postpartum treatment and as health tonic by the local aborigines [4]. However, limited phytochemistry and pharmacology studies had been conducted on the antioxidant properties of *F. deltoidea* despite several major claims on its medicinal properties by the local aborigines. Likewise, no report has been documented so far on the enhancement of secondary metabolites, specifically the flavonoids *in vitro* culturing system. Hence, this study was carried out to enhance the production of flavonoid compounds in cell cultures of *F. deltoidea* by introducing elicitors and precursors.

EXPERIMENTAL DESIGN

The maintenance of the cell suspension cultures were done by regular sub-culturing of 2-week old culture in Murashige and Skoog's [5] media supplemented with 1 mg/L of picloram.

Five different concentrations of each precursor were studied, including phenylalanine, cinnamic acid and chalcone. Yeast extract (Bacto™, USA) was used as biotic elicitor and were added directly into the liquid MS medium prior to autoclaving. On the other hand, casein hydrolysate (Sigma-Aldrich, USA) and jasmonic acid (Sigma-Aldrich, USA) were used as the abiotic elicitors. Casein hydrolysate was directly added into the full strength MS liquid medium. As for jasmonic acid, it was dissolved in 99% ethanol (Merck, USA) and filter sterilized with 0.20 μ m syringe filter (Sartorius, Germany) before addition into the sterile MS liquid medium. Five different concentrations of jasmonic acid were studied. Medium without any addition of elicitors and precursors were used as a control.

In this present study, flavonoids quantification were done by High Performance Liquid Chromatography (HPLC) and further confirmed by Liquid Chromatography – Mass Spectrometry (LC-MS).

RESULTS AND DISCUSSION

Results showed that casein hydrolysate generally induced a higher biomass production when compared to the control (Figure 4.23). The highest biomass production was recorded in cell culture treated with 3 mg/L casein hydrolysate (0.68 ± 0.06 g DW), which was about 0.42 g higher than the control, followed by 1 mg/L casein hydrolysate treatment with a biomass production of 0.44 ± 0.10 g DW. This was in correlation with the addition of casein hydrolysate to the medium, which increased the growth rate of callus of *Cytisus aeolicus* Guss. Ex. Lindl. [6].

Figure 1. Biomass production in leaf-derived cell suspension of *F. deltoidea* treated with different concentrations of casein hydrolysate.

In Figure 2, yeast elicitation showed a nearly uniform production of rutin with supplementation of yeast higher than 1000 mg/L. The highest rutin content within the yeast treatment was recorded in 1000 mg/L with 12.69 ± 4.64 $\mu\text{g/g}$ DW, with almost 2.3 times higher than the rutin accumulation in the control. Similarly, yeast extract enhanced the production of sanguinarine in cell cultures of *Eschscholtzia californica* from 20 mg/L to 60 mg/L [7]. The positive effect of yeast extract is due to some cations, which could act as abiotic elicitors.

Figure 2. Flavonoid production in leaf-derived cell suspension of *F. deltoidea* treated with different concentrations of yeast.

Quercetin production was the highest at media supplemented with 0.001 mg/L chalcone where 4.75 ± 0.37 $\mu\text{g/g}$ DW of quercetin accumulation was achieved (Figure 3). Other than that, the highest accumulation of naringenin (11.22 ± 2.15 $\mu\text{g/g}$ DW) was recorded. This value was 280 % higher than the naringenin recorded in the control, in the treatment of 0.005 mg/L of chalcone. Chalcone was reported to be effective in producing demethylhomopterocarpin and maackiain in cell culture of *Trifolium pratense* [8].

Figure 3. Flavonoid production in leaf-derived cell suspension of *F. deltoidea* treated with different concentrations of chalcone.

CONCLUSION

The enhancement of three targeted flavonoid compounds were successfully optimized with greatest accumulation of rutin recorded in 1000 mg/L yeast supplemented in MS medium. Nevertheless, highest accumulation of quercetin and naringenin were recorded in 0.001 and 0.005 mg/L chalcone treatment, respectively.

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