

Induction of Callus and Expression of Compounds in *Capsicum frutescens* Supplemented with 2, 4-D

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Abstract—“Cili padi” or *Capsicum frutescens* is one of many capsicum species from the nightshade family; Solanaceae. It is famous in Malaysia and is widely used as a food ingredient. The bright colours of Capsicum varieties are due to a combination of esters of capsanthin, capsorubin, zeaxanthine, cryptoxanthine and other carotenoids. These extractable colours of chilli pepper have been broadly used in the food processing business to produce a wide range of products like sausages and meat products, as well as for cheeses, butters, salad dressings, condiment mixtures, gelatine desserts and processed foods. *Capsicum frutescens* also possess vast medicinal properties, for example the compound ‘capsaicin’ has been reported to provide temporary relief for post-surgical pain and mouth sores caused by radiation therapy and chemotherapy. The objectives of this study are to determine the most optimum 2,4-Dichlorophenoxyacetic acid (2,4-D) hormone concentration for callus induction from stem explants *C. frutescens* and the effects of different 2,4-D concentrations on expression of compounds from *C. frutescens*. Seeds of *C. frutescens* were germinated on MS media without hormone (MS basal media) to produce aseptic seedlings of this species, which were then used to supply explants for subsequent tissue culture experiments. Stem explants were excised from aseptic seedlings and cultured on MS media supplemented with various concentrations of 2,4-D (0.1, 0.3 and 0.5 mg/L) to induce formation of callus. Fresh weight, dry weight and callus growth percentage in all samples were recorded. The highest mean of dry weight was observed in MS media supplemented with 0.5 mg/L 2,4-D, where 0.4499 ± 0.106 g of callus was produced. The highest percentage of callus growth (16.4%) was also observed in cultures supplemented with 0.5 mg/L 2,4-D. The callus samples were then subjected to HPLC-MS to evaluate the effect of hormone concentration on expression of bioactive compounds in the different samples. Results showed that caffeoylferuloylquinic acids were present in all samples, but was most abundant in callus cells supplemented with 0.3 & 0.5 mg/L 2,4-D. Interestingly, there was an unknown compound observed to be highly expressed in callus cells supplemented with 0.1 mg/L 2,4-D, but its presence was less significant in callus cells supplemented with 0.3 and 0.5 mg/L 2,4-D. Furthermore, there was also a compound identified as octadecadienoic acid, which was uniquely expressed in callus grown with 0.5 mg/L 2,4-D, but absent in callus cells grown with 0.1 and 0.3 mg/L 2,4-D. The results obtained in this study indicated that plant growth regulators played a role in expression of secondary metabolites in plants. The increase or decrease of these growth regulators may have triggered a change in the secondary metabolite biosynthesis pathways, thus causing differential expression of compounds in this plant.

Keywords—callus, *in vitro*, secondary metabolite, 2,4-dichlorophenoxyacetic acid.

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