UNIVERSITI TEKNOLOGI MARA

OPTIMIZATION OF CULTURE CONDITIONS FOR BROMELAIN PRODUCTION IN CALLUS OF *Ananas comosus* VAR. MD2

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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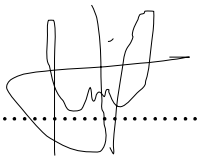
Signature of Student	:	 ……..
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ABSTRACT

MD2 pineapple is currently the most preferred variety on the international market due to the pleasant aroma, high brix acidity ratio and as source of bromelain. Bromelain enzyme is proteases (natural proteolytic enzymatic complex) presence in stems, fruits and tissues of the pineapple (*Ananas comosus*) possessing notable therapeutic properties including antitumor agent, bronchitis and sinusitis reliever. The use of callus culture with optimized medium composition and culture conditions as a biotechnological method to harvest bromelain and accumulation of biomass has led to significant interest in *in vitro* plant study. This study aims to initiate callus culture of MD2 pineapple with exogenous PGRs and enhance bromelain content with manipulation of sucrose, thiamine and MS strength. Callus culture of pineapple var. MD2 was established by using crown explant and inoculated on MS medium fortified with varying concentrations of 2,4-D (0.5, 1.0, 2.0, 4.0, 6.0 mg/l) alone and in combination with BAP (1.0, 2.0, 3.0 mg/l). All 21 treatments showed explant callusing excluding control. Combination of 2.0 mg/l 2,4-D + 2.0 mg/l BAP had significantly ($p<0.05$) induced 91.67 ± 8.33 % callus after 23 days of culture with 0.25 ± 0.07 g callus fresh weight (FW). However, there was moderate correlation between the earliness of callus formation and frequency of callus formation ($p>0.5$). MS medium fortified with different 2,4-D concentrations (0.5, 1.0, 2.0, 4.0, 6.0, 8.0 mg/l) were combined with constant 2.0 mg/l TDZ for callus proliferation. Callus FW, 1.52 ± 0.03 g recorded in MS media supplemented with 6.0 mg/l 2,4-D + 2.0 mg/l TDZ was highly significant ($p<0.05$) and optimal for callus proliferation. Callus exhibits totipotentiality as shoot and root formation were documented. Analysis of the high-performance liquid chromatography (HPLC) shows the presence of crude bromelain in callus culture (R^2= 0.9913). Bromelain augmentation strategies were investigated in MS medium supplemented with optimum PGRs and different sucrose concentrations (0, 10, 20, 30, 40 g/l), thiamine (0.0, 0.4, 0.8, 1.2, 1.6 µM) and MS strength (Without MS, a quarter, half, three quarter, full). Optimum medium for enhanced bromelain content in full strength of MS medium (1.22 ± 0.04 mg/ml), 40 g/l sucrose (1.38 ± 0.02 mg/ml) and 1.2 µM thiamine (1.36 ± 0.40 mg/ml) were statistically significant ($p<0.05$). Callus growth defined from FW in full strength MS medium (0.99 ± 0.18 g) and 30 g/l sucrose (2.24 ± 0.02 g) were statistically significant ($p<0.05$). However, addition of thiamine showed no significant difference for growth of callus. PGRs play pivotal role in callus induction, proliferation and morphology of callus. Manipulated culture conditions successfully enhanced bromelain content in callus culture. Culture medium optimized for bromelain production are not necessarily optimized for callus growth. Callus culture has a great potential to be an alternative source of bromelain and planting materials.

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