## **UNIVERSITI TEKNOLOGI MARA**

# THE EFFECTS OF NAA AND PRECURSORS ON GROWTH AND THE PHYTOCHEMICAL CONSTITUENTS OF Elaeis guineensis Jacq. EMBRYOID CULTURES

### ZATI HANANI BINTI TERMIZI

Thesis submitted in fulfillment of the requirements for the degree of **Master of Science** 

**Faculty of Applied Sciences** 

February 2017

#### ABSTRACT

*Elaeis guineensis* Jacq. (oil palm) is a very important commercial crop in Malaysia. This plant is a valuable source of oil and phytochemical compounds used in food, cosmetics, pharmaceuticals and biofuels. Conventional propagation of oil palm cannot be done vegetatively since it has a single growing apex. Hence, propagation in vitro has great advantages in the case of oil palm where vegetative propagation is possible only via tissue culture. Somatic embryogenesis is an important pathway for the oil palm in vitro propagation in order to increase the number of new regenerated plantlets. The evolving commercial importance of the phytochemicals has a great interest particularly in the production of secondary metabolites by means of cell culture technology. The presence and accumulation of phytochemical compounds can be an added value to the oil palm tissue culture. This study aimed to determine the effects of Naphthaleneacetic acid (NAA) and precursors (glutamine and phenylalanine) on fresh weight, number of shoots and the phytochemical constituents of oil palm embryoid culture. Oil palm embryoids from clone PL 213, PL 209 and PL 220 were cultured on Murashige & Skoog (MS) media supplemented with 0.0, 0.5, 1.0 and 2.0 mg/L NAA. Then, the best clone was selected to be cultured on MS media supplemented with precursors which were glutamine (0, 250, 500 mg/L) and phenylalanine (0, 75, 100 mg/L). The phytochemical screening of embryoids and shoots methanolic extract was done using Gas Chromatography-Mass Spectrometry (GC-MS). The histological study was carried out for the precursor treatment to observe the structure and the development of embryoids. Results from NAA experiment showed that clone PL 213 produced the highest mean of fresh weight (55.30  $\pm$  3.40 g) and number of shoots (123.00  $\pm$  4.00) in MS0 (control) after 16 weeks of culture. Clone PL 213 also showed the highest number of phytochemical constituents in embryoids and was selected as the best clone to be used in the precursor experiment. Findings from precursor experiment using clone PL 213 indicated that the highest mean of fresh weight ( $6.39 \pm 2.14$  g) was found in MS+500 mg/L glutamine while the highest mean of number of shoots (20.00  $\pm$  7.87) was produced in MS+250 mg/L glutamine after 28 days of culture. However, MS0 gave the highest mean of fresh weight  $(6.33 \pm 1.30 \text{ g})$  and MS+100 mg/L phenylalanine produced the highest mean of number of shoots  $(31.00 \pm 7.00)$  after 28 days of culture. The number of phytochemical constituents was higher in embryoids and shoots treated with glutamine and phenylalanine compared to MS0. These precursors influenced growth and secondary metabolism of oil palm embryoid culture as it could serve as an alternative nitrogen source and constituents of proteins. Generally, histological study of oil palm embryoid showed that adjacent numerous centers of meristematic cells with dense cytoplasm. Also, differentiated cells containing high storage lipid content were obtained within the cytoplasm. As a conclusion, NAA did not enhance the growth meanwhile glutamine and phenylalanine gave a positive effect on the growth of oil palm embryoid cultures. However, NAA and these precursors can promote the accumulation of phytochemical constituents. Therefore, it was suggested that oil palm embryoid culture can be further exploited and manipulated with different concentration of precursor to obtain more valuable phytochemical compounds.

### ACKNOWLEDGEMENT

First of all, I wish to thank God for giving me the opportunity and health so that I can complete my research and this thesis successfully.

My gratitude and thanks go to my beloved supervisor, Associate Professor Dr. Norrizah Jaafar Sidik for the guidance, support and patience during conducting me for few years of my study. I also would like to thanks my co-supervisors Dr. Ahmad Tarmizi Hashim and Associate Professor Dr. Norizan Ahmat for their support.

My appreciation also goes to the staff of Malaysian Palm Oil Board and Faculty of Applied Sciences, Universiti Teknologi MARA for providing the facilities, knowledge and assistance. Thanks also to RMI, Universiti Teknologi MARA and The Ministry of Higher Education for the grant FRGS/2/2010/SG/UiTM/03/22.

Thanks to my father, Hj. Termizi Yahya who always support me during my study and also giving the financial support in order to help me to complete my study. Thanks also to my mother, **Example 1** and my siblings for their patience, motivation and encouragements that always give me the excitement to further study.

Finally, special thanks to my husband, Mr. Mohd Harris Md Alimuddin and also to my friends who always helped me in this research and gave the moral support until I completed my study successfully. Alhamdulillah.

## TABLE OF CONTENTS

Page

CONFIRMATION BY PANEL OF EXAMINERS	ii				
AUTHOR'S DECLARATION					
ABSTRACT					
ACKNOWLEDGEMENT	v				
TABLE OF CONTENTS LIST OF TABLES LIST OF FIGURES LIST OF PLATES					
				LIST OF SYMBOLS	xiii
				LIST OF ABBREVIATIONS	xiv
				CHAPTER ONE: INTRODUCTION	
1.1 Background of Study	1				
1.2 Problem Statement	3				
1.3 Significance of Study	4				
1.4 Objectives of Study	5				
1.5 Scope and Limitation of Study	5				
CHAPTER TWO: LITERATURE REVIEW					
2.1 Oil Palm	7				
2.2 Oil Palm Tissue Culture	9				
2.3 Oil Palm Embryoid	10				
2.4 Plant Growth Regulator	11				
2.5 Precursor	13				
2.5.1 Glutamine	15				
2.5.2 Phenylalanine	16				
2.6 Production of Phytochemicals by Plant Tissue Culture	17				
2.6.1 Uses of Phytochemicals	18				
2.6.2 Factors Influencing Phytochemicals Accumulation	20				
2.7 Gas Chromatography-Mass Spectrometry (GC-MS)	21				

vi

### CHAPTER THREE: EFFECTS OF NAA ON GROWTH OF OIL PALM EMBRYOID CULTURES AND ITS PHYTOCHEMICAL CONSTITUENTS

3.1	Introduc	tion	24
3.2	Material	s and Methods	25
	3.2.1	Plant Materials	25
	3.2.2	NAA and Media Preparation	25
	3.2.3	Embryoid Multiplication	26
	3.2.4	Embryoid Culture and Maintenance	26
	3.2.5	Fresh Weight and Number of Shoots	27
	3.2.6	Morphological Study of Oil Palm Embryoid Culture	27
	3.2.7	Extraction Process	27
	3.2.8	Phytochemical Screening of Oil Palm Embryoid Cultures	27
		using Gas Chromatography-Mass Spectrometry (GC-MS)	
	3.2.9	Statistical Analysis	28
3.3	Results a	and Discussion	28
	3.3.1	Effects of Different Concentrations of NAA on Fresh Weight	28
		and Number of Shoots	
	3.3.2	Morphological Study of Oil Palm Embryoid Culture	32
	3.3.3	Phytochemical Screening of Oil Palm Embryoid Cultures	35
		using Gas Chromatography-Mass Spectrometry (GC-MS)	
3.4 Conclusion			40

## CHAPTER FOUR: EFFECTS OF GLUTAMINE ON GROWTH OF OIL PALM EMBRYOID CULTURES AND ITS

#### PHYTOCHEMICAL CONSTITUENTS

4.1	Introduction	41	
4.2	4.2 Materials and Methods		
	4.2.1 Plant Materials	42	
	4.2.2 Glutamine and Media Preparation	42	
	4.2.3 Embryoid Multiplication	43	
	4.2.4 Embryoid Culture and Maintenance	43	

22