

## LIST OF CONTENTS

	Page
ABSTRACT.....	ii
ABSTRAK.....	iii
ACKNOWLEDGEMENTS.....	iv
LIST OF CONTENTS.....	v
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
LIST OF SYMBOLS.....	xi
LIST OF ABBREVIATION.....	xi
CHAPTER 1 INTRODUCTION.....	1
1.1 Background.....	1
1.2 Importance of the Study.....	3
1.3 Research Objectives.....	4
CHAPTER 2 LITERATURE REVIEW.....	5
2.1 Oil Palm.....	5
2.1.1 Scientific Classification of Oil Palm.....	5
2.1.2 <i>Elaeis guineensis</i> .....	7
2.1.3 Flowers.....	7
2.1.4 Fruit.....	11
2.1.5 Economic Importance of Oil Palm.....	13
2.2 Introduction to microRNAs.....	15
2.2.1 Post-transcriptional Gene Regulation (PTGS).....	16

2.2.2	Biogenesis of miRNAs.....	16
2.2.3	miRNA-mediated Suppression Mechanism in Plants.....	20
2.2.4	miRNA Precursors (Pre-miRNA) .....	22
2.2.5	Origin and Evolution of Plant miRNA.....	22
2.2.6	Post-transcriptional Gene Regulation.....	24
2.2.6.1	siRNAs versus miRNAs.....	25
2.2.7	Regulation of miRNA Genes in Plants.....	29
2.2.8	Plant miRNA and Target mRNAs.....	29
2.2.8.1	miR172 and its APETALA2-like Targets.....	31
2.2.9	Other miRNAs Engaged in Flowering and Developmental Transition.....	34
 <b>CHAPTER 3 MATERIALS AND METHODS.....</b>		 35
3.1	Collection and Characterization of Oil Palm Tissue Samples.....	35
3.2	Plant Total RNA.....	38
3.3	Agarose Gel Electrophoresis.....	39
3.4	DNase Treatment.....	39
3.5	Gel Extraction of Small RNA.....	40
3.6	Primer Design.....	40
3.6.1	Potential Target RNA.....	40
3.6.2	miR172.....	41
3.7	One Step Reverse Transcription PCR.....	42
3.8	Gel Purification of PCR Products.....	43
3.9	Cloning of PCR Products.....	43
3.9.1	Ligation.....	43
3.9.2	<i>E.coli</i> Transformation.....	44

3.9.3	Colony PCR.....	45
3.9.4	Plasmid Isolation.....	46
3.10	DNA Sequencing.....	47
3.11	mFold and miRbase Analysis.....	48
3.12	Nested PCR of Six Putative Precursor egu-miR172 Paralogs.....	48
3.13	Quantitative Real time PCR Analysis.....	50
3.13.1	SYBR-Green.....	50
3.13.1.1	Selection of a Most Stable Endogenous Control.....	50
3.13.1.2	Reverse Transcription.....	52
3.13.1.3	PCR Amplification.....	52
3.13.2	Taqman Assay.....	54
3.13.2.1	MicroRNA Reverse Transcription.....	54
3.13.2.2	PCR Amplification.....	55
3.14	Computational Target Finding.....	55
3.14.1	Potential Target for miR172 in EST of Oil Palm.....	55
3.14.2	BLAST Analysis.....	56
3.15	Rapid Amplification of cDNA Ends (RACE).....	56
3.15.1	Ligating the RNA Oligo to Truncated mRNA.....	57
3.15.2	RNA Precipitation.....	57
3.15.3	Reverse Transcribing mRNA.....	58
3.15.4	Amplifying cDNA Ends.....	59

<b>CHAPTER 4 RESULTS AND DISCUSSION.....</b>	<b>61</b>
4.1 Confirmation of Putative miR172 Gene Expression in Oil Palm Tissues	61
4.2 Potential egu-miR172 Paralogs.....	64
4.3 Expression Analysis of miR172 in Oil Palm using Nested PCR.....	76
4.4 Quantitative Reverse Transcription PCR Analysis of miR172 Precursors	78
4.4.1 Selection of Internal Control.....	78
4.4.2 Expression of miR172 Precursors in Oil Palm.....	79
4.5 miR172 Target Prediction.....	83
4.6 Experimental Validation of Predicted AP-like Target.....	91
4.7 Expression Profiles of miR172 and its Potential Target Gene.....	93
<b>CHAPTER 5 CONCLUSION AND FURTHER STUDIES.....</b>	<b>105</b>
<b>REFERENCE.....</b>	<b>107</b>
<b>APPENDIX.....</b>	<b>131</b>

## LIST OF TABLES

Table No.	Page
3.1 Developmental Stages of Oil Palm Flowers.....	36
3.2 Reaction Components for One Step RT-PCR.....	42
3.3 Ligation of DNA in the pGEM®-T Easy Vector.....	44
3.4 Colony PCR using GoTaq® Flexi DNA Polymerase.....	45
3.5 Six Primer Sets for Nested PCR.....	49
3.6 Reaction Components for Nested PCR.....	49
3.7 Endogenous Controls Forward and Reverse Primers.....	51
3.8 Reaction Components for Reverse Transcription.....	52
3.9 Reaction Components for Quantitative Reverse Transcription PCR.....	53
3.10 Reaction Components for Reverse Transcription.....	54
3.11 Reaction Components for Real-Time PCR.....	55
3.12 Reaction Components for Amplifying cDNA Ends.....	59
3.13 Thermal Cycling Profile for Amplifying cDNA Ends.....	60
4.1 Predicted Secondary Structures of egu-miR172 Paralogs.....	65
4.2 Average Expression Stability Values ( <i>M</i> ) of Four Control Genes.....	79
4.3 Predicted miR172: mRNA Target hybrids.....	84

## LIST OF FIGUERS

Figure No.	Page
2.1 Oil Palm Plant.....	6
2.2 Features of Reproductive Development in Oil Palm.....	8
2.3 Macroscopic View of Mature Female and Male Inflorescences of Oil Palm.....	10
2.4 Oil Palm Fruit.....	12
2.5 Uses of Oil Palm.....	14
2.6 miRNA Biogenesis in Plants and Animals.....	19
2.7 The Actions of miRNAs in Target Regulation.....	21
2.8 Biogenesis of miRNAs and siRNAs.....	27
2.9 A Model for the MIR Genes of Dual Function.....	28
3.1 Reproductive Development in Oil Palm.....	37
3.2 Multiple Alignment for miR172 Primer Design.....	41
4.1 RNA Extraction using CTAB Modified Method.....	61
4.2 Agarose Gel of RT-PCR Products.....	63
4.3 Multiple Sequence Alignment of egu-miR172 Paralogs with Their Orthologs from other Plant Species.....	73
4.4 Nested PCR Amplification of egu-pre-miR172a.....	77
4.5 Quantitative Reverse Transcription PCR Expression Profiles of egu- miR172 Paralogs.....	80
4.6 Candidate miR172 Target from Oil Palm.....	88
4.7 The Predicted miR172: AP2-like Targets Hybrid.....	89
4.8 Agarose Gel (1%) of RT-PCR Products.....	91
4.9 Sequence Alignment of 571bp RT-PCR Product (Query) with EST EL692343.1.....	92

4.10	Quantitative Reverse Transcription PCR of Mature miR172 and AP2-like Target from Oil Palm.....	95
4.11	Expression Comparison of egu-miR172a Paralogs and Isoforms.....	97
4.12	5' Race.....	99

## **LIST OF SYMBOLS**

bp	Base pairs
°C	Degree Celsius
%	Percentage
µl	Microliter
µg	Microgram
g	Gravity
M	Molar
ml	Mililiter
mM	Milimolar
V	Voltage

## **LIST OF ABBREVIATION**

3' UTR 3'	Untranslated Region
BLAST	Basic local alignment search tool
cDNA	Complementary Deoxyribonucleic Acid
CTAB	Cetyltrimethylammonium Bromide
DNA	Deoxyribonucleic Acid

dNTPs	Deoxyribonucleoside Triphosphates
EtBr	Ethidium Bromide
Egu-miR172	<i>Elaeis guineensis</i> MicroRNA
IPTG	Isopropyl- $\beta$ -D-thiogalactopyranoside
MgCl <sub>2</sub>	Magnesium Chloride
MgSO <sub>4</sub>	Magnesium Sulphate
miRNA	MicroRNA
mRNA	Messenger RNA
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCBI	National Centre for Biotechnology Information
OD	Optical Density
PCR	Polymerase Chain Reaction
pH	Potenz Hydrogen
PVP	Polyvinylpyrrolidone
RISC	RNA Induced Silencing Complex
RNA	Ribonucleic Acid
RNAi	RNA Interference
rRNA	Ribosomal Ribonucleic Acid
siRNA	Short Interference RNA
TBE	Tris-Borate-EDTA