

TABLE OF CONTENTS

	Page
TABLE OF CONTENTS.....	i
LIST OF TABLES.....	ii
LIST OF FIGURES.....	iii
INTRODUCTION.....	1
LITERATURE REVIEWS.....	3
Botany of Orchid.....	3
Flavonoids.....	5
Anthocyanin Biosynthesis.....	6
Molecular Genetics of Anthocyanin Biosynthesis.....	8
Metabolic Engineering Regulatory Genes Involved in Anthocyanin Biosynthesis.....	12
Genetic Engineering in Orchid.....	20
Constitutive Promoter to Control Gene Expression.....	24
MATERIALS AND METHOD.....	27
Materials.....	27
Methods.....	32
RESULTS AND DISCUSSION.....	48
Study on Temporal Expression of Structural and Regulatory Genes Involved in Anthocyanin Biosynthesis of Orchid Flowers.....	48
Analysis Optimum Parameters for <i>Dendrobium</i> Bombardment Using Transient GUS Expression.....	56
Maize Regulatory Genes Transformation.....	62
CONCLUSION.....	79
LITERATURE CITED.....	81
APPENDIX.....	97

LIST OF TABLES

Table		Page
1	Transcription factors functionally associated with the control of flavonoid pathways in plants.....	18
2	Various plasmids utilized to be the vectors for orchid transformation.....	28
3	Oligonucleotide primers utilized for PCR detection.....	29
4	Oligonucleotide primers utilized for RT-PCR analysis.....	30
5	<i>Gus</i> gene expression and survival percentage of orchid protocorms 3d after bombardment with different parameters.....	59
Appendix Table		
1	The medium used for tissue culture and selection.....	98
2	Chemical of MS medium and VW medium	99

LIST OF FIGURES

Figure	Page
1 Basic structure of flavonoid molecule.....	6
2 Schematic anthocyanin pathways.....	7
3 The structural genes and their transcription factors involved in phenylpropanoid and anthocyanin biosynthesis.....	12
4 Dot blot apparatus.....	33
5 The pBpSA13 construction.....	37
6 The pC1SA12 construction.....	38
7 The total RNA extracted from the orchid flower.....	49
8 The samples of orchid flower used in the study of temporal expression of the regulatory and structural genes involved in anthocyanin biosynthesis.....	50
9 RNA dot blot analysis showing expression of the structural and regulatory genes involved in anthocyanin biosynthesis at various stages of development.....	53
10 Comparison of affecting parameters for bombardment in orchid protocorm.....	59
11 Transient GUS activity in sepal of <i>Dendrobium</i> orchid with different promoter.....	60
12 The anthocyanin pigments spots in the sepal cells.....	63
13 Characters of GFP-expressing protocorm during hygromycin selection.....	65
14 PCR and PCR southern blot hybridization of <i>hph</i> and <i>gfp</i> genes	67
15 Genomic southern blot hybridization of <i>gfp</i> gene.....	68
16 The position of the specific primer in transcription factor gene and Nos terminator.....	69

LIST OF FIGURES (Continued)

Figure		Page
17	PCR analysis of hygromycin-resistance plantlet expressing GFP using two pair of specific primers to the transcription factor gene and Nos terminator.....	70
18	Genomic southern blot hybridization of putative transgenic independent lines.....	71
19	Phenotype resulting from the transcription factor gene expression in putative transgenic orchid.....	72
20	Northern analysis of <i>Myc</i> transcription factor, maize <i>B-peru</i> , gene involved in anthocyanin biosynthesis.....	74
21	RT-PCR analysis of putative transgenic orchid using the pair of regulatory and structural gene primer.....	76
 Appendix Figure		
1	Schematic of the vectors used for genetic transformation....	100
2	Schematic of the plasmids containing the <i>B-peru</i> and <i>C1</i> inserted in p35SBpCDNA and p35SC1.....	103