

## TABLE OF CONTENTS

|                                                                                                                      | Page     |
|----------------------------------------------------------------------------------------------------------------------|----------|
| ABSTRACT (THAI) .....                                                                                                | I        |
| ABSTRACT (ENGLISH).....                                                                                              | II       |
| ACKNOWLEDGEMENTS .....                                                                                               | III      |
| TABLE OF CONTENTS .....                                                                                              | IV       |
| LIST OF TABLES.....                                                                                                  | VII      |
| LIST OF FIGURES.....                                                                                                 | VIII     |
| LIST OF ABBREVIATIONS .....                                                                                          | XI       |
| <b>CHAPTER</b>                                                                                                       |          |
| <b>I INTRODUCTION.....</b>                                                                                           | <b>1</b> |
| 1.1 Introduction .....                                                                                               | 1        |
| 1.2 Research objectives .....                                                                                        | 2        |
| 1.3 Scope of Research .....                                                                                          | 3        |
| <b>II LITERATURE REVIEW.....</b>                                                                                     | <b>4</b> |
| 2.1 Overview of date palm ( <i>Phoenix dactylifera</i> L.) and situation of date palm<br>in Thailand / Cannabis..... | 4        |
| 2.2 Biology and Reproductive System of Date Palm / Cannabis.....                                                     | 5        |
| 2.3 Date Palm Sex Determination .....                                                                                | 7        |
| 2.4 Polymerase Chain Reaction (PCR): Principles and Limitations.....                                                 | 10       |
| 2.5 Principles and Mechanisms of LAMP .....                                                                          | 13       |
| 2.6 Diagnostic Applications and Performance of LAMP .....                                                            | 16       |
| 2.7 Phenol Red indicator .....                                                                                       | 17       |
| 2.8 Cryoprotectants and Stabilizers in Freeze-Drying.....                                                            | 18       |
| 2.9 Role of Sugars in Lyophilization of Molecular Kits.....                                                          | 20       |
| 2.9.1 Stresses During Lyophilization and the Need for Stabilizers.....                                               | 21       |
| 2.9.2 Mechanisms of Sugar-Mediated Stabilization .....                                                               | 21       |

## TABLE OF CONTENTS (Continued)

|                                                                           | Page      |
|---------------------------------------------------------------------------|-----------|
| 2.9.3 Common Sugars Used in Lyophilization .....                          | 22        |
| 2.9.4 Formulation Considerations for Molecular Kits.....                  | 23        |
| 2.10 Trehalose: Structure, Protective Mechanisms, Benefits, and           |           |
| Enzyme Stabilization in LAMP assay.....                                   | 24        |
| 2.10.1 Structure of Trehalose.....                                        | 24        |
| 2.10.2 Protective Mechanisms of Trehalose .....                           | 24        |
| 2.10.3 General Benefits and Applications of Trehalose .....               | 25        |
| 2.10.4 Enzyme Stabilization in LAMP Assays .....                          | 26        |
| <b>III RESEARCH METHODOLOGY .....</b>                                     | <b>27</b> |
| <b>3.1 Cannabis.....</b>                                                  | <b>27</b> |
| 3.1.1 Sample.....                                                         | 27        |
| 3.1.2 DNA Extraction .....                                                | 27        |
| 3.1.3 Specificity Testing of DNA markers for Cannabis sex using PCR ..... | 28        |
| <b>3.2 Date Palm .....</b>                                                | <b>28</b> |
| 3.2.1 Sample .....                                                        | 28        |
| 3.2.2 DNA Extraction.....                                                 | 29        |
| 3.2.3 Specificity Testing of DNA markers for Date Palm sex using PCR..... | 29        |
| 3.2.4 Development of LAMP technique for determining the sex of            |           |
| date palm LAMP primer design.....                                         | 30        |
| 3.2.5 LAMP Reaction and Detection.....                                    | 30        |
| 3.2.6 Lyophilization process.....                                         | 32        |
| 3.2.7 Testing of lyophilized product .....                                | 33        |
| 3.2.8 Stabilized Freeze-Dried LAMP.....                                   | 33        |
| 3.2.9 Properties of Date Palm sex identification kits.....                | 33        |
| <b>IV RESULTS AND DISCUSSION .....</b>                                    | <b>34</b> |
| <b>4.1 Cannabis.....</b>                                                  | <b>34</b> |

## TABLE OF CONTENTS (Continued)

|                                                                                                     | Page      |
|-----------------------------------------------------------------------------------------------------|-----------|
| 4.1.1 Samples.....                                                                                  | 34        |
| 4.1.2 Comparison of MADC2 and SCAR119 markers.....                                                  | 34        |
| 4.1.3 MADC2 marker amplification products.....                                                      | 36        |
| 4.1.4 Simple and Rapid DNA Extraction.....                                                          | 37        |
| <b>4.2 Date Palm.....</b>                                                                           | <b>39</b> |
| 4.2.1 Samples .....                                                                                 | 39        |
| 4.2.2 DNA Extraction.....                                                                           | 40        |
| 4.2.3 Specificity Testing of DNA markers for Date Palm sex using PCR .....                          | 41        |
| 4.2.4 Development of LAMP technique for determining the sex of<br>date palm LAMP primer design..... | 42        |
| 4.2.5 LAMP Reaction and Detection.....                                                              | 43        |
| 4.2.6 Lyophilization process and testing of lyophilized product .....                               | 50        |
| 4.2.7 Stabilized Freeze-Dried LAMP.....                                                             | 54        |
| 4.2.8 Properties of Date Palm sex identification kits.....                                          | 58        |
| <b>V CONCLUSION.....</b>                                                                            | <b>61</b> |
| REFERENCES .....                                                                                    | 62        |
| BIOGAPHY .....                                                                                      | 78        |

## LIST OF TABLES

| Table                                                                         | Page |
|-------------------------------------------------------------------------------|------|
| 3.1 Primers used for Cannabis sex determination .....                         | 28   |
| 3.2 Primer sequence for Date Palm sex determination and their reference ..... | 29   |

## LIST OF FIGURES

| Figure                                                                                                                                                               | Page |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 2.1 Phenol red molecules combine with H <sup>+</sup> produced by DNA amplification, resulting in changes in molecular structure.....                                 | 17   |
| 4.1 Collection of cannabis leaf samples with known sex from SUT Cannabis for molecular analysis.....                                                                 | 34   |
| 4.2 Visualization of PCR products for sex determination of cannabis by MADC2 (A) and SCAR119 (B) primers.....                                                        | 35   |
| 4.3 Visualization of PCR products for sex determination of cannabis by MADC2 primer.....                                                                             | 36   |
| 4.4 Fresh leaf samples from cannabis seedlings of 5 mg, along with a ten-fold dilution, prepared used as simple DNA extraction method.....                           | 38   |
| 4.5 Visualization of PCR Products for Sex Determination of Cannabis Using the MADC2 Primer from simply and Rapidly Extracted DNA.....                                | 39   |
| 4.6 Collected sample of young date palm leaves of both known and unknown sex .....                                                                                   | 39   |
| 4.7 DNA extraction process and DNA concentration checking.....                                                                                                       | 41   |
| 4.8 Sex verification of Date Palm seedlings using multiplex PCR.....                                                                                                 | 42   |
| 4.9 LAMP-based Sex Determination of Date Palm using Commercial Buffer Set.....                                                                                       | 44   |
| 4.10 Illustrates the colorimetric results of the LAMP reaction following incubation and agarose gel electrophoresis (2%) for male-specific primers of date palm..... | 45   |
| 4.11 Effect of initial at pH 7.8 and 8.0 and varying Phenol Red concentration (0.125 mM and 0.250 mM) on LAMP amplification and visual detection.....                | 47   |
| 4.12 Illustrates the colorimetric results of the LAMP reaction following incubation and agarose gel electrophoresis (2%) .....                                       | 48   |
| 4.13 Performance evaluation of homemade <i>Bst</i> DNA polymerase (3.41 pmol/                                                                                        |      |

## LIST OF FIGURES (Continued)

| Figure                                                                                                                                                                                                    | Page |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| reaction) in the LAMP assay.....                                                                                                                                                                          | 50   |
| 4.14 Compares the LAMP reaction with 5% sucrose and trehalose and the<br>reaction without added sugars .....                                                                                              | 51   |
| 4.15 The results of the LAMP reaction before and after lyophilization.....                                                                                                                                | 52   |
| 4.16 Shows further testing of the date palm sex identification assay on<br>multiple individual samples .....                                                                                              | 53   |
| 4.17 Shows the results of testing the freeze-dried date palm sex identification<br>kit after storing it at different temperatures (-20°C, 4°C, and Room<br>Temperature) for 7 and 14 days,.....           | 56   |
| 4.18 Shows the results of testing the freeze-dried date palm sex identification<br>kit after storing it at different temperatures (-20°C, 4°C, and Room<br>Temperature) for 21, 28 day and 2 months ..... | 57   |
| 4.19 Shows the results of testing the freeze-dried date palm sex identification<br>kit after storing it at different temperatures (-20°C, 4°C, and Room<br>Temperature) for 6 month.....                  | 58   |
| 4.20 Date palm sex determination and fieldwork by using the "Date Palm Sex<br>Determination LAMP Kit" .....                                                                                               | 59   |
| 4.21 The MANDate date palm gender test kit was honoured with two gold<br>awards at the Kaohsiung International Invention and Design EXPO (KIDE).....                                                      | 60   |

## LIST OF ABBREVIATIONS

|         |   |                                               |
|---------|---|-----------------------------------------------|
| M       | = | Molar                                         |
| mM      | = | Milli Molar                                   |
| mg      | = | Milligram                                     |
| µg      | = | Microgram                                     |
| min     | = | Minute                                        |
| mL      | = | Milliliter                                    |
| g       | = | Gram                                          |
| gDNA    | = | Genomic DNA                                   |
| kg      | = | Kilogram                                      |
| M       | = | Mole                                          |
| mg/ml   | = | Milligram per milliliter                      |
| ml      | = | Milliliter                                    |
| mM      | = | Millimolar                                    |
| mm      | = | Millimeter                                    |
| ng      | = | Nanogram                                      |
| nt      | = | Nucleotide                                    |
| pmol    | = | Picomole                                      |
| rpm     | = | Revolutions per minute                        |
| µg/ml   | = | Microgram per milliliter                      |
| µl      | = | Microliter                                    |
| µm      | = | Micrometer                                    |
| GPAT3   | = | Glyceraldehyde-3-phosphate dehydrogenase gene |
| LOX 5.1 | = | Lipoxygenase gene                             |
| h       | = | Hour                                          |

## LIST OF ABBREVIATIONS (Continued)

|        |   |                                        |
|--------|---|----------------------------------------|
| U.S.A. | = | United States of America               |
| dNTPs  | = | Deoxyribonucleoside triphosphates      |
| PCR    | = | Polymerase chain reaction              |
| LAMP   | = | Loop-mediated isothermal amplification |