



UNIVERSITI PUTRA MALAYSIA

**OPTIMIZATION OF 2-DIMENSIONAL GEL ELECTROPHORESIS FOR
PROTEOMIC STUDIES OF OIL PALM ROOTS**

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PROTEOMIC STUDIES OF OIL PALM ROOTS**

By

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Thesis Submitted to the Department of Cell and Molecular Biology,

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2D electrophoresis (2-DE) is a proteomic tool that has been widely used as this technique is capable of resolving and separating thousands of proteins in a single gel for parallel analysis. 2-DE allows the identification of individual protein and understanding of the host-pathogen interaction during their molecular crosstalks. In addition, this modification technique also allows the study of protein profiling of solid biological samples. However, the analytical conditions need to be optimized to achieve optimal parameters and procedures for 2-D gel images. In the present study, optimization was carried out which aimed to improve image quality, reproducibility and sensitivity of 2-D gel. Generally, focusing time and voltage of Isoelectric focusing (IEF) affect the reproducibility of 2-D gel images for protein analysis. It was found that increasing voltage and time for IEF running were optimal for 2-DE. Reducing development time, enhancing the washing step, prolong fixation to overnight and the use of freshly prepared reagents of silver staining produced better image resolution and enhanced sensitivity.

Keywords : 2D electrophoresis, optimization, isoelectric focusing (IEF), silver staining

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul Sebagai
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OPTIMIZATION OF 2-DIMENSIONAL GEL ELECTROPHORESIS FOR
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Elektroforesis 2D (2-DE) adalah teknik proteomik yang telah digunakan secara meluas kerana teknik ini mampu meleraikan dan memisahkan ribuan *protein* dalam gel tunggal untuk analisis selari. 2-DE membolehkan pengenalpastian *protein* individu dan pemahaman tentang interaksi perumah-patogen semasa perbincangan silang molekul mereka. Di samping itu, teknik ini juga membolehkan kajian profil *protein* daripada sampel biologi yang pejal dilakukan. Walau bagaimanapun, beberapa kondisi analisis perlu dioptimumkan untuk mencapai parameter dan prosedur yang optimum untuk 2-D imej gel. Dalam kajian ini, pengoptimuman telah dijalankan yang bertujuan untuk meningkatkan kualiti imej, kebolehulangan dan sensitiviti 2-D gel. Secara amnya, masa dan voltan Penumpuan isoelektrik memberi kesan kepada kebolehulangan 2-D gel imej untuk analisis protein. Untuk pewarnaan perak, kajian ini mendapati bahawa dengan mengurangkan masa untuk pembangunan gel, meningkatkan langkah membasuh gel, memanjangkan penetapan gel untuk semalaman dan penggunaan reagen yang baru disediakan menghasilkan resolusi imej yang lebih baik dan dapat meningkatkan sensitivity imej gel.

Keywords : Elektroforesis 2D, pengoptimuman, Penumpuan isoelektrik, pewarnaan perak

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APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the Degree of Bachelor of Science (HONS.) Cell and Molecular Biology. The member of the Supervisory Committee was as follows :

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DECLARATION

Declaration by undergraduate student

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This is to confirm that:

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LIST OF FIGURES

| | |
|--|----|
| Figure 1: 2-D gel images from two sets of isoelectro focusing conditions | 22 |
| Figure 2: 2-D gel images from two sets of silver staining conditions | 24 |
| Figure 3: Optimized 2-D gel image of protein extracts from oil palm | 25 |



LIST OF ABBREVIATIONS

| | |
|---------------------|---|
| 2-DE | Two-Dimesional Electrophoresis |
| SDS-PAGE | Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis |
| <i>G. boninense</i> | <i>Ganoderma boninense</i> |
| IPG | Immobilised pH gradients |
| IEF | Isoelectric Focusing |
| BSR | Basal Stem Rot |
| pI | Isoelectric point |
| DTT | Dithiothreitol |
| IAA | Iodoacetamide |

TABLE OF CONTENTS

| | |
|---|----------|
| ABSTRACT | i |
| ABSTRAK | ii |
| ACKNOWLEDGEMENT | iii |
| APPROVAL | iv |
| DECLARATION | v |
| LIST OF FIGURES | vi |
| LIST OF ABBREVIATIONS | vii |
| CHAPTER | |
| 1 INTRODUCTION | 1 |
| 2 LITERATURE REVIEW | |
| 2.1 Proteomics | 3 |
| 2.1.1 Contribution of Proteomics to the Study of Plant Pathogenic Fungi | 4 |
| 2.1.2 Approaches for proteomics study | 6 |
| 2.2 Oil Palm | 7 |
| 2.2.1 Importance of Oil Palm | 8 |
| 2.2.2 Basal Stem Rot Disease | 9 |
| 2.3 Two-Dimensional Electrophoresis (2-DE) for Proteomics | 10 |
| 2.3.1 The First Dimension: Isoelectric Focusing (IEF) | 11 |
| 2.3.2 The Second Dimension: Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) | 12 |
| 2.3.3 Silver Staining: The detection method | 13 |

| | | |
|----------|---|----|
| 3 | MATERIALS AND METHODS | |
| | 3.1 Protein Extraction | 14 |
| | 3.2 Protein Quantitation | 15 |
| | 3.3 Sample Preparation | 15 |
| | 3.4 The First Dimension of 2-DE: Isoelectric Focusing (IEF) | 15 |
| | 3.5 The Second Dimension of 2-DE: SDS-PAGE | 16 |
| | 3.5.1 Gel Casting | 16 |
| | 3.5.2 Immobiline DryStrip Gel Equilibration | 17 |
| | 3.5.3 SDS-PAGE | 17 |
| | 3.6 Detection of Proteins in Gels by Silver Staining | 18 |
| | 3.7 Image Acquisition | 19 |
| 4 | RESULTS AND DISCUSSION | |
| | 4.1 Protein extraction and Quantitation | 20 |
| | 4.2 Optimization of IEF | 20 |
| | 4.3 Optimization of Silver Staining | 23 |
| | 4.4 Optimized 2-DE for protein extracts from oil palm roots | 25 |
| | 4.5 Discussion | 26 |
| 5 | CONCLUSION AND RECOMMENDATION | 31 |
| 6 | REFERENCES | 32 |
| 7 | APPENDICES | 38 |

1.0 INTRODUCTION

As one of the largest world's producer of palm oil, the planted area of oil palm plantations in Malaysia had increased at a rapid pace. For years, oil palm has become the most important commodity crop in Malaysia because of its economic advantages. Unfortunately, the plantations are threatened by basal stem rot (BSR) disease, which cause damage to the oil palm and brought to half of the losses of the plantations. Mainly caused by the basidiomycetes fungus, *Ganoderma boninense* (*G. boninense*), currently available method for controlling the disease have been proven to be unsatisfactory as *G. boninense* has various resting stages.

Plant responds to pathogen infection by eliciting different defence mechanism. In view of this, the need for developing method for the detection of oil palm root proteins that playing key roles during the infection of *G. boninense* is highly important to avoid losses. In recent years, a number of proteomic studies have been carried out for the identification of such proteins. However, relatively little information is available regarding the studies of plant-pathogen interaction, particularly the oil palm, during an infection (Al-Obaidi, *et al.*, 2014).

Using one of the gel-based proteomic approach known as two-dimensional gel electrophoresis (2-DE) as the reliable technique, understanding of the interaction of plant-pathogen can be improved. Introduced by O'Farrell in 1975, 2-DE has become the most powerful technique in the analysis of complex protein mixtures as it capable of resolving thousands of proteins in a single gel and the ability to detect post- and co-translational modifications of proteins. In addition, the reliability and reproducibility of this technique have been proven as its instruments are capable of parallel running of dozens of 2-DE experiments (Roy *et al.*, 2014).

In addition, the capacity, sensitivity and reproducibility of 2D gels are greatly improved with the development of immobilised pH gradients (IPG) and pre-cast gradient polyacrylamide gels. However, a number of problems associated with the separation of proteins by 2DE still cannot be eliminated by this powerful technique. This study is conducted mainly to enhance the reproducibility of 2-DE by several optimization method for the use of future analyses.

Using protein extracts from oil palm root, this study aims for the optimization of 2-DE gel. It is hypothesized that manipulation of parameters of Isoelectric Focusing (IEF) and silver staining will produce a gel with higher reproducibility and sensitivity. The manipulation was done by increasing focusing voltage and time of IEF, in addition to optimize development time, washing step, fixation step and the use of freshly prepared reagents of silver staining.

In general view of proteomic field, the optimization of this technological advance is expected to resolve many problems regarding proteomic works and further help to secure yield of crop plants and to develop crop protection strategies, especially for the oil palm.

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