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Differentially Expressed Proteins from Roots of Healthy and Diseased *Piper nigrum*

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ABSTRACT

Introduction: Diseased *Piper nigrum* plants have affected pepper berry production in Sarawak farms, contributing to the economic loss for farmers that depend on this species for their income.

Objective: This study was performed to analyse the protein profiles of healthy and diseased *P. nigrum* roots and to identify differentially expressed proteins.

Methods: Two methods of protein extraction were compared, and the extracted proteins were quantified through two quantitative assays: the Bradford and BCA assays. The Phenol/SDS extraction method was significantly better compared to the phenol method.

Results: The SDS-PAGE analysis revealed two differently expressed unique proteins bands with the molecular size of approximately 20 kDa and 12.5 kDa present in the sample of diseased plants. These differentially expressed proteins are likely to be pathogenesis-related (PR) proteins which are produced as a response to infection.

Conclusion: These PR proteins can potentially be used as markers or be developed further to allow plants to gain systemic resistance against diseases.

Key Words: BCA assays, Pathogenesis-related proteins, Phenol extraction, *Piper nigrum*, Proteomics

INTRODUCTION

Piper nigrum plant known as black pepper is a type of woody perennial climber with dilated or swollen nodes and stipules.¹ Peppers from Indian origins are widely grown in pepper producing countries such as Thailand and Malaysia because of high quality and yield. The pepper berry has been used as a spice and seasoning due to its pungent flavour and aromatic scent. In addition to the main usage as spice and seasoning, different parts of the plant have been used for the extraction of the secondary metabolites. These metabolites were used in drugs, preservative, insecticidal and larvicidal control agents.² For example, studies have shown that *P. nigrum* affects the digestive system, where piperine increases the production of saliva and gastric secretions.^{3,4}

Through a proteomic study of *P. nigrum* roots, this study aims to identify proteins which are expressed as a response towards disease and infection. The previous study from

leaves has revealed the role of different pathogenesis-related (PR) proteins and enzymes in the disease reaction of black pepper concerning *Phytophthora capsici* foot rot infection.⁵ Nowadays, advancements in proteomic studies have allowed the quantitative and qualitative separation of complex protein mixtures, which makes it possible to monitor dynamic protein expression in plant growth and metabolism by using two-dimensional electrophoresis (2-DE) and mass spectrometry analysis.⁶⁻⁹

The study hypothesis is that there are differently expressed proteins in *P. nigrum* plants when compared between the healthy and foot rot diseased roots caused by *Phytophthora*. In this study, two different extraction protocols were evaluated. Furthermore, two protein quantification methods, the Bradford assay and Bicinchoninic acid assay were compared. Lastly, the protein profiles of the healthy and diseased samples were compared to identify any differentially expressed proteins.

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