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THE ROLE OF NITRIC OXIDE IN *Fusarium oxysporum* f.sp. *cubense* – BANANA INTERACTION

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THE ROLE OF NITRIC OXIDE IN *Fusarium oxysporum* f.sp. *cubense* – BANANA INTERACTION

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Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfilment of the requirement for the degree of Cell and Molecular Biology

The Role of Nitric Oxide in *Fusarium oxysporum* f.sp. *cubense* – Banana Interaction

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June 2015

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Nitric oxide (NO) is involved in many physiological responses in plants including defense mechanism against biotic and abiotic stresses. NO is known to be an important signaling molecule in protein S-nitrosylation which involves modification of cysteine thiols to generate S-nitrosothiol (SNO). Protein extraction from *Fusarium oxysporum* f. sp. *cubense* (*Foc*)-banana root tissue was performed by using a modified TCA-acetone extraction method. Detection of S-nitrosylated proteins in the protein extract following biotin switch assay results in detection of a single band approximately at 52 kDa. Berangan banana seedlings which was treated with NO donor, nitrosogluthathione (GSNO) and NO scavenger, (2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide) cPTIO prior to inoculation with *Foc* indicated that disease progression in GSNO treated Berangan banana seedlings was delayed. On the other hand, disease progression was shown to be accelerated in cPTIO-treated Berangan banana seedlings. This study shows that NO plays important role in basal resistance against *Foc*.

Abstrak tesis yang dikemukakan kepada Jabatan Biology Sel dan Molekul sebagai memenuhi keperluan untuk ijazah Biologi Sel dan Molekul

Peranan Nitrik Oksida di dalam Interaksi *Fusarium oxysporum* f.sp. *cubense* – Pisang

Oleh

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Nitrik oksida (NO) terlibat dalam pelbagai tindak balas fisiologi di dalam tumbuh-tumbuhan termasuk mekanisme pertahanan terhadap tekanan biotik dan abiotik. NO telah dikenali sebagai molekul isyarat penting dalam protein yang menjalani proses nitrosilasi di mana proses ini melibatkan pengubahsuaian tiol sisteina bagi penghasilan S-nitrosotiol (SNO). Pengekstrakan protein daripada tisu akar pisang Berangan telah dilakukan dengan menggunakan kaedah pengekstrakan TCA-aseton yang telah diubah suai. Pengesanan protein yang telah menjalani proses nitrosilasi dijalankan setelah ekstrak protein tersebut menjalani analisis penukaran biotin dan hanya satu “band” protein yang dapat dikesan kira-kira pada saiz 52 kDa. Anak benih pisang Berangan yang dirawat dengan penderma NO iaitu, nitrosogluthathione (GSNO) dan, penyah NO iaitu, (2- (4-Carboxyphenyl) -4,4,5,5-tetramethylimidazoline-1-oxyl-3-oksida) cPTIO sebelum inokulasi dengan *Foc*

menunjukkan bahawa perkembangan penyakit bagi anak benih pisang Berangan yang dirawat dengan GSNO adalah lebih lambat. Sebaliknya, perkembangan penyakit adalah lebih cepat bagi anak benih pisang Berangan yang dirawat dengan cPTIO. Kajian ini menunjukkan bahawa NO memainkan peranan yang penting dalam ketahanan dasar terhadap *Foc*.

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 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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LIST OF ABBREVIATIONS

NO	Nitric oxide
SNO	S-nitrosothiol
GSNO	nitrosogluthathione
cPTIO	2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide
Foc	*Fusarium oxysporum* f.sp. *cubense*
MMTS	Methyl methanethiosulfonate
Biotin-HPDP	N-[6-(biotinamido)hexyl]-3'-(2'pyridyldithio)propionamide
HR	Hypersensitive response
PAMP	Pathogen-associated molecular pattern
PTI	PAMP-triggered immunity
ETI	Effector-triggered immunity
ROS	Reactive oxygen species
SA	Salicyclic acid
JA	Jasmonic acid
Cys	Cysteine
O^2	Oxygen
CA	Carbonic anhydrase
GSNOR	Nitrosogluthathione reductase
NHR	Non-host resistance
SNA	Spezieller nahrstoffarmer agar

DMSO	Dimethyl sulfoxide
PBS	Phosphate-buffered saline
TCA	Tricholoacetic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
EDTA	Ethylenediaminetetraacetic acid
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpolypyrrolidone
RuBisCo	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SD	Standard Deviation

CHAPTER 1

1.0 INTRODUCTION

In Malaysia, banana is the second most widely cultivated fruit crop. Besides contributing to high balance of trade for its annual production, banana also has various nutritional values which are important for human health. Despite the importance of banana in Malaysia agriculture, banana crops are facing a decrement in production because of increasing threat of disease Fusarium wilt (also known as Panama disease), and this disease is caused by a fungus known as *Fusarium oxysporum* f.sp. *cubense* (*Foc*) (TengkuAb. Malik et al., 2013).

During plant defense response, plant will produce reactive nitrogen intermediate which is nitric oxide (NO). In order to protect itself from harmful effect caused by NO, plant will produce glutathione to react with NO and produce nitrosoglutathione (GSNO) which acts as a mobile store of NO activity. The NO group within the GSNO can be delivered to proteins, and can react with cysteine to produce S-nitrosothiol (SNO). This process is called S-nitrosylation which acts as an important mechanism to regulate protein function (Astier et al., 2011). The SNO compound is being produced in plants even if there is no infection, but the production is in a low level. Upon infection of pathogen on plants, the production level of SNO will be increased (Feechan et al., 2005). Hence, the level of SNO production in normal and infected plant can be measured and analyzed as SNO would be easier to obtain because it is attached to the protein. Studies on S-nitrosylation in banana plant have never been carried out up until now. Hence, S-nitrosylated proteins in banana are not yet determined.

It is hypothesized that NO is involves in basal resistance against *F. oxysporum* and S-nitrosylation can be induced when there is infection in banana by *F. oxysporum*.

In this research, biotin switch technique (BST) will be used to identify and quantify the endogenous protein of SNO as biotin switch assay has never been done using protein extract from banana. Three principle steps are involved in BST which are blocking of the free cysteine of proteins, reduction of SNO to thiols and labeling the resulting free thiols with biotin. Once biotinylated, the SNO proteins will be immunodetected by using antibodies raised against biotin.

The objectives of this research were to determine the effect of NO on Fusarium wilt progression in banana seedlings, optimize the protein extraction from banana root tissue and to optimize biotin switch assay for specific detection of S-nitrosylated proteins from banana including optimization of SDS-PAGE analysis.

CHAPTER 6

6.0 REFERENCES

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