



UNIVERSITI PUTRA MALAYSIA

**EXPRESSION PROFILE OF BOWMAN-BIRK PROTEASE INHIBITOR
GENE FROM MALAYSIAN RICE CULTIVARS (MR211&MR220) UNDER
DROUGHT STRESS**

WONG SIM YEE

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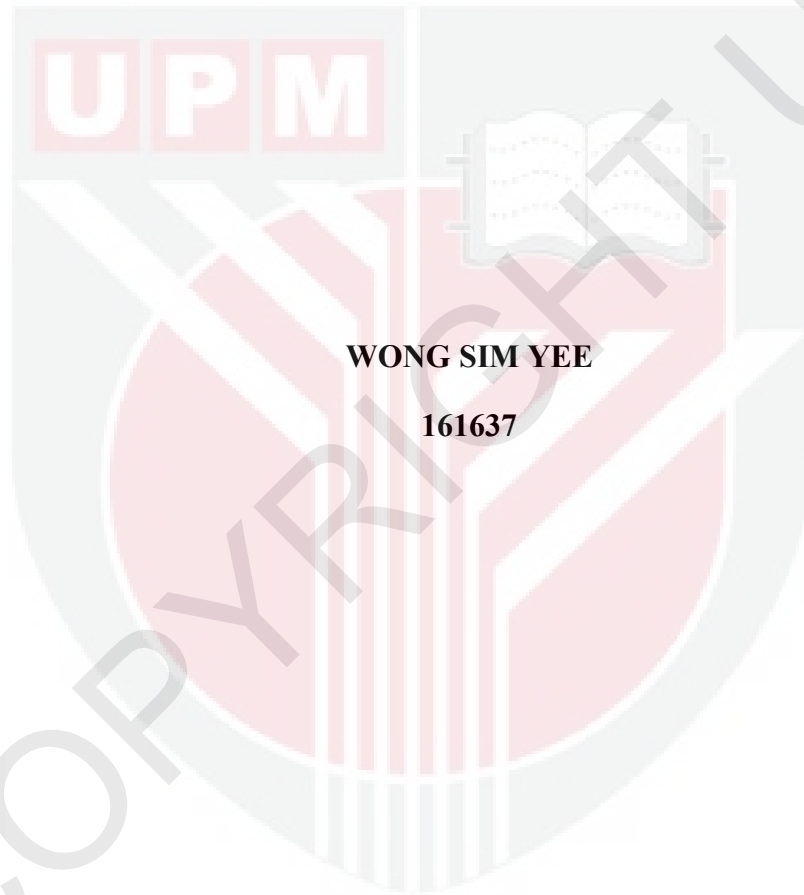


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2015

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan yang bertajuk **“Expression Profile of Bowman-Birk Protease Inhibitor Gene from Malaysian Rice Cultivars (MR211&MR220) Under Drought Stress”** telah disiapkan serta dikemukakan kepada Jabatan Biokimia, Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia oleh **WONG SIM YEE (161637)** sebagai syarat untuk kursus BCH 4999A (Projek).

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Tarikh:

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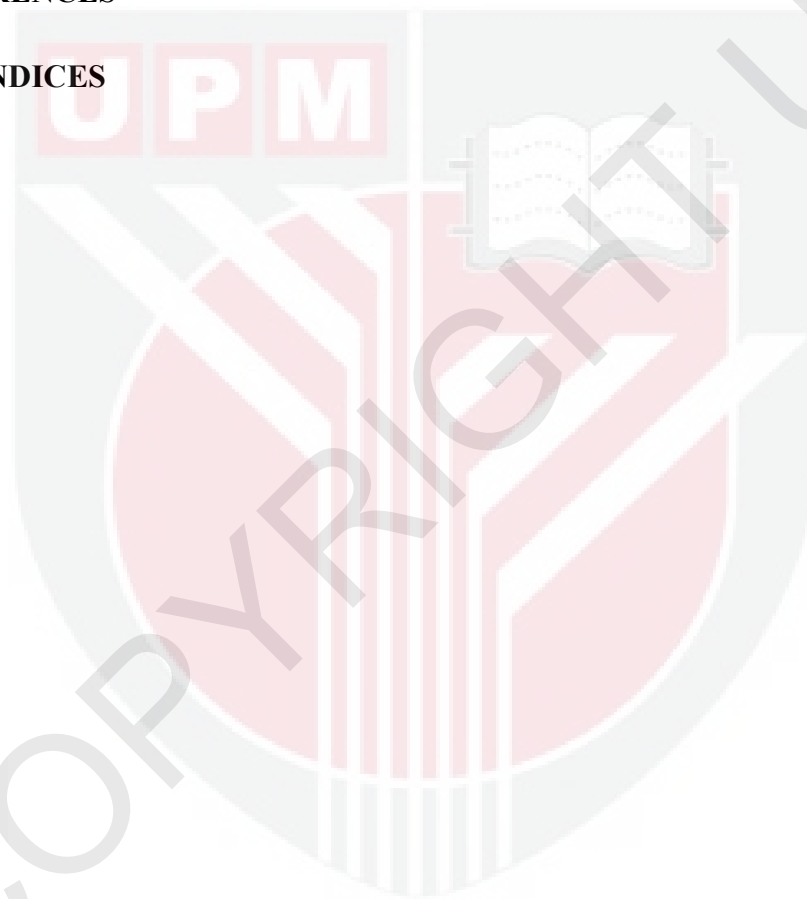
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LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percentage
BBI	Bowman-Birk protease inhibitor
bp	Base pair
BLAST	Basic Local Alignment Search Tool
dNTP	Deoxynucleotide triphosphates
ECER	East Coast Economic Region
EtBr	Ethidium bromide
g	Gram
IADA	Integrated Agriculture Development Area
KADA	Kemubu Agriculture Development Area
KETARA IADA	Kemasin Semerak IADA, North Terengganu
L	Litre
MARDI	Malaysian Agricultural Research and Development Institute
mg	Milligram
mM	Millimolar
mg/l	Milligram per litre
μl	Microlitre
nm	Nanometer
NCBI	National Centre for Biotechnology Information
PEG	Polyethylene glycol-6000
PIs	Protease Inhibitors
PCR	Polymerase Chain Reaction
ROS	Reactive oxygen species
RT-PCR	Reverse-transcription Polymerase Chain Reaction
T _m	Melting temperature

ABSTRACT

Drought adversely influences rice (*Oryza sativa* L.) growth and production. The understanding of rice plant responses toward drought stress can help to generate more drought-tolerant cultivars to achieve the full self-sufficiency level (SSL) in rice production by 2020 target in Malaysia. Currently rice researches have shown great interest in understanding plant protease inhibitors (PIs) as a way to improve plant resistance to drought. In this study, Malaysian rice cultivars i.e. MR211 and MR220 were treated with artificial drought stress induced by 6% PEG 6000 at 5 different time points. RT-PCR analysis was then performed to investigate the gene expression profile of Bowman-Birk type trypsin protease inhibitor (BBI) in both rice cultivars after treatment. The expression level of each DNA fragment was compared. The gene expression in both cultivars was up-regulated with the increase in drought stress exposure time until a peak level was reached. DNA sequence analysis showed that the isolated gene was about 300bp. Conserved domain with the protein superfamily which belongs to BBI was found in the isolated gene, proving that the BBI gene was identified from both cultivars. Besides, after 1 week of drought stress treatment, proline accumulation in both cultivars was also studied. Proline accumulated in both MR220 and MR211 under drought stress. BBI and proline accumulation help in drought-stress tolerance in MR211&MR220.

ABSTRAK

Kemarau mempengaruhi pertumbuhan dan produktiviti padi (*Oryza sativa* L.). Kefahaman tentang tindak balas padi terhadap kemarau dapat membantu untuk menghasilkan lebih banyak kultivar yang dapat bertahan ketika tekanan kemarau demi mencapai Kadar Sara Diri (SSL) yang penuh dalam pengeluaran padi sebelum tahun 2020 di Malaysia. Baru-baru ini, kajian padi menunjukkan minat dalam memahami penggunaan perencat protease dalam sistem pertahanan tumbuhan terhadap tekanan kemarau. Dalam kajian ini, kultivar padi Malaysia, iaitu MR211 dan MR220 telah dirawat dengan tekanan kemarau yang menggunakan 6% PEG 6000 pada 5 titik masa yang berlainan. Analisa RT-PCR kemudiannya dijalankan untuk mengkaji ekspresi profil gen perencat trypsin protease dari jenis Bowman-Birk (BBI) dalam kedua-dua kultivar selepas rawatan. Kadar ekspresi gen untuk setiap fragmen DNA dibandingkan. Ekspresi gen didalam kedua-dua kultivar menunjukkan peningkatan sejajar dengan peningkatan tempoh masa tekanan kemarau sehingga mencapai kadar maksima. Analisa jujukan DNA menunjukkan gen yang diasingkan mempunyai 300bp. Domain terpelihara serta superfamili protein kepunyaan BBI telah didapati dalam gen yang telah diasingkan dan ini membuktikan gen BBI telah dikenalpasti dalam kedua-dua kultivar. Selain itu, pengumpulan proline dalam kedua-dua kultivar juga dikaji selepas rawatan tekanan kemarau selama satu minggu. Proline berkumpul dalam kedua-dua MR211 dan MR220 ketika tekanan kemarau. Perencat protease Bowman-Birk dan pengumpulan proline dalam tumbuhan membantu pertahanan tumbuhan terhadap tekanan kemarau bagi MR211 dan MR220.

CHAPTER 1

INTRODUCTION

Protease is a proteolytic enzyme that hydrolyses the peptide bond within specific protein, maintaining protein turnover for the maintenance and survival of organisms together with specific protease inhibitors (PIs) (Habib & Fazili, 2007). Plant PIs are protein molecules that inhibit and regulate the proteolytic activities of their target proteases, producing a stable PI complex (Leung *et al.*, 2000). Several proteolytic enzymes such as endoproteases were found to be accumulated in plant during abiotic stress condition. Under adverse stress condition, plants will accumulate specific PIs such as cystatins to inhibit and regulate hydrolytic protein degradations (Contour-Ansel *et al.*, 2010). For instance, the over-expression of chymotrypsin-like protease inhibitor (OCPI1) gene in rice (*Oryza sativa*) exerted significant influence on improving drought resistance in rice (Huang *et al.*, 2007). This is due to uncontrolled protein degradation induced by abiotic stresses adversely influences plant growth and metabolism (Kidrič *et al.*, 2014). This in turn exerts a deleterious impact on global crop production since plants are exposed to various abiotic stresses.

Rice (*Oryza Sativa* L.) is important crop planted all over the world for human consumption. In Malaysia, the Agriculture and Agro-based Industry Ministry hopes that the country will achieve the target of 100% self-sufficiency level (SSL) in rice production by 2020 (“Full rice sufficiency by 2020 target”, 2015). Drought is one of the major abiotic stresses in plant world, so it is important that we understand the drought tolerance mechanism in rice plants. This can subsequently help to achieve

the target of 100% SSL in rice yield by increasing the productivity of drought-tolerant rice cultivar, especially in some areas with limited water availability for proper irrigation system.

The objectives of this study are:

- 1) To identify and characterize gene coding for Bowman-Birk type trypsin protease inhibitor from Malaysian rice cultivars (*Oryza Sativa* L. *ssp. Indica*), MR211 and MR220.
- 2) To investigate the gene expression profile of Bowman-Birk type trypsin protease inhibitor from Malaysian rice cultivars (*Oryza Sativa* L. *ssp. Indica*), MR211 and MR220 under drought stress treatment.
- 3) To study the proline accumulation in Malaysian rice cultivars (*Oryza Sativa* L. *ssp. Indica*), MR211 and MR220 under drought stress treatment.

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