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JUDUL: DROUGHT EFFECT ON PROTEOME PATTERN OF LEAVES OF TR-8 RICE VARIETY

IJAZAH: IJAZAH SARJANA MUDA SAINS PERTANIAN DENGAN KEPUJIAN (PENGELUARAN TANAMAN)

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DROUGHT EFFECT ON PROTEOME PATTERN OF LEAVES OF TR-8 RICE VARIETY

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DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other university.

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ABSTRACT

Rice (*Oryza sativa* L.) is the most important food crops which provide a staple diet for almost half of the world's population. However, drought stress resulted from global climatic change severely limits the productivity of lowland irrigated rice. Proteins that are responsible for drought stress will express differently during drought which might be contributed to drought resistant mechanism and could led to the development of drought resistant rice by genetic engineering or marker-assisted selection breeding. In this study, proteome pattern of TR-8 variety rice between well-watered condition and drought stress condition was analyzed and compared. Four-week-old rice seedlings were exposed to drought condition for three, six, nine, twelve, and fifteen days. The proteins were extracted from leaves, separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and stained with silver nitrate solution. The relative water contents (RWC) under well-watered condition for fifteens days were similar and consistent, however, the relative water content under drought stress condition declined dramatically after six days drought stress. The rice seedlings reached their permanent wilting point after 15 days of drought stress. Qualitative variation on the protein expression by three and five protein bands were observed between well-watered and drought-stressed rice seedlings respectively. Protein bands under twelve days drought stress showed the most qualitative variation. Two-dimensional electrophoresis and staining with Coomassie Briiliant Blue can be conducted for future improvement.

ABSTRAK

Padi (Oryza sativa L.) adalah tanaman makanan yang paling penting dan merupakan makanan ruji kepada hampir separuh daripada penduduk dunia. Walau bagaimanapun, perubahan iklim global mengakibatkan keadaan kekurangan air dan mengehadkan produktiviti padi sawah. Protein yang berperanan penting untuk tekanan kekurangan air akan berbeza semasa dalam keadaan kekurangan air. Protein ini berkemungkinan membantu dalam mekanisme tahan kekurangan air dan pembangunan varieti padi yang tahan kekurangan air. Dalam kajian ini, corak proteome padi TR-8 antara keadaan yang normal dan keadaan tekanan kekurangan air telah dibandingkan dan dianalisis. Benih padi yang berumur empat minggu telah didedahkan kepada keadaan kekurangan air untuk tiga, enam, sembilan, dua belas, dan lima belas hari. Protein telah diekstrak daripada daun, dipisahkan oleh natrium sulfat dodecyl gel elektroforesis (SDS-PAGE), dan diwarnakan dengan agentum nitrat. Kandungan air relatif untuk benih padi yang berada dalam keadaan yang normal adalah konsistent tetapi kandungan air relatif untuk padi dalam keadaan kekurangan air menurun secara mendadak selepas pada hari keenam. Terdapat tiga gelung protein untuk padi yang berada dalam keadaan normal dan lima band protein dalam keadaan kekurangan air menunjukkan variasi kualitatif. Gelung protein untuk padi yang berada dalam kekurangan air menunjukkan variasi kualitatif yang paling ketara. Elektroforesis dua dimensi dan pewarnaan dengan menggunakan Coomassie Briiliant Blue adalah dicadangkan untuk penambahbaikan pada masa akan datang.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

°C	Degree Celcius
$	Dollar
<	Less than
%	Percent
ABA	Abscisic acid
AcDP	Acetone Dried Powder
AQP	Aquaporin
ANOVA	Analysis of variance
BSA	Bovine Serum Albumin
CDPK	Calcium dependent protein kinase
Ca^{2+}	Calcium ion
CGPRT	The Regional Co-ordination Centre for Research and Development of Coarse Grains, Pulses, Roots and Tuber Crops in the Humid Tropics of Asia and the Pacific
Cm	Centimeter
CRD	Completely Randomized Design
DAT	Days after transplanting
DS	Drought stress
EDTA	Ethlylenediaminetetraacetic acid
ENSO	El Niño – South Oscillation
FAO	Food and Agriculture Organization of the United Nation
FAOSTAT	Food and Agriculture Organization Statistical Database
g	Gram
HSP	Heat Shock Protein
ha	Hectare
IRRI	International Rice Research Institute
KADA	Kemubu Agriculture Development Authority
kg	Kilogram
km^2	Kilometer square
LEA	Late embryogenesis abundant
MS	Mass spectrometry
mRNA	Messenger ribonucleic acid
m^3	Meter cube
µL	Microliter
mA	milliampere
mm	Millimeter
mM	Millimolar
mL	Milliliter
mts	Mitochondrial enzymatic reduction activity
M	Molar
MADA	Muda Agriculture Development Authority
nm	Nanometer
NOAA	National Oceanic and Atmospheric Administration
d^{-1}	Per day
PMSF	Phenylmethylsulfonyl fluoride
PAGE	Polyacrylamide gel electrophoresis
PVC	Polyvinyl chloride
RNA	Ribonucleic acid
RTBV	Rice Tungro Baciiliform Virus

RM	Ringgit Malaysia
SSL	Self-sufficiency level
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
$t\ ha^{-1}$	Tonne per hectare
TF	Transpiration Factors
TCA	Trichloroacetic acid
2DE	Two dimensional electrophoresis
UNEP	United Nations Environment Programme
US	United States
USDA	United States Department of Agriculture
UV	Ultraviolet
w/v	Weight per volume
WW	Well-watered

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Human and livestocks rely on agriculture for food to survive. Therefore, the most fundamental importance of agriculture is food production. However, food productivity is decreasing due to detrimental effects of biotic and abiotic stresses (Anjum *et al.*, 2011). Food security in the world is challenged by increasing food demand and threatened by declining water availability. In order to guarantee food supply for the increasing world population, exploiting yield potential and maintaining yield stability of crops in environmental stresses condition are urgent tasks.

One of the important food crops is rice. Rice (*Oryza sativa* L.) is one of the most widely consumed cereal crops, providing a staple diet for almost half of the world's population (Song *et al.*, 2003). In Malaysia, rice is the third most important crop after oil palm and rubber in terms of production and is mainly grown in the eight granary areas in Peninsular Malaysia (Azmi and Mashhor, 1995). The total rice growing area in Malaysia is about 676,700 hectares which produce 2.091 million tonnes of rice (FAO, 2002). Malaysia has an average annual rice production of about 26,000 metric tonnes per year (FAOSTAT, 2009).

Rice is a semi-aquatic species that is typically cultivated under partially flooded condition. Irrigated rice, in particular, is a heavy consumer of fresh water and is less efficient in the way it uses water compared to other crops (Sariam *et al.*, 2002). Therefore, water is essential for rice cultivation and its supply in adequate quantity is one of the most important factors in rice production. Rice is sensitive to drought stress especially for irrigated lowland rice cultures due to its limited adaptation to water deficit condition. Approximately 30% of 154 million hectares of the world's rice farmland are at a low elevation and irrigated by rain (Bailey-Serres *et al.*, 2010). Rice grown in Malaysia is mainly under flood irrigation. There are some 564,000 hectares of wet paddy land in Malaysia, of which 322,000 hectares is capable of double cropping. In Sabah, there is about 52,000 hectares of land are cultivated with wet paddy with an average yield of about 2.8 tonnes ha^{-1} (Department of Agriculture, Sabah, 2000). In addition, upland rice also relies strictly on rainfall as a source of water although it may requires less water supply than irrigated rice.

Global climatic changes such as dry spell, heat waves, and uneven precipitation patterns limit water availability for farming (Bates *et al.*, 2008). Changes in temperature increase water losses from lakes and reservoirs and raise evapotranspirative demand for water. Rising temperatures therefore increase crop water demand, deplete soil water and increase irrigation demand (Singh *et al.*, 1996; Peng *et al.*, 2004). The depletion of ground water resources and rising soil salinity are pushing some rice-cropping systems away from traditional paddy land to aerobic fields (Delmer, 2005; Bernier *et al.*, 2008).

Drought stress has a tremendous effect on agriculture, thus negatively influencing human activities (NOAA, 2003). Recently, it is reported that drought resulted in extensive fires in North and Central America as well as other regions of the world. In addition, a drought in northern China in 1876 dried up crops in extensive regions, causing millions of human death. In Russia, drought incidence during 1921 producing up to five millions deaths in the Volga river basin. According to World Health Organization, drought is the cause of death for about half the people killed by natural disasters.

Drought which is the most important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factors (Shao *et al.*, 2009). It is estimated that drought stress reduced the average loss of 17% - 70% of grain yield (Nouri-Ganbalani *et al.*, 2009). United Nations Environment Programme reported that agricultural activities account for approximately 75% of the global water consumption and in developing countries, 90% of water is used for irrigation (UNEP, 2009).

Malaysia receives an annual average rainfall of more than 2500 mm, mainly due to the Southwest and Northeast monsoons. The average annual water resources on a total land mass of 330,000 km^2 amount to 990 billion m^3 (Tubiello, 2005). However, 360 billion m^3 or 36% returns to the atmosphere as evapotranspiration, 566 billion m^3 or 57% appear as surface run off and the remaining 64 billion m^3, or 7% go to the recharge of groundwater. Of the total 566 billion m^3 of surface runoff, 147 billion m^3 are found in Peninsular Malaysia, 113 billion m^3 in Sabah and 306 billion m^3 in Sarawak (Wong *et al.*, 2010).

In addition, the El Niño – Southern Oscillation (ENSO) largely influences the climate variability over Malaysia and the greater Southeast Asian region (Tangang, 2001; Tangang and Juneng, 2004; Juneng and Tangang, 2005). El Niño is a band of anomalously warm ocean water temperatures that periodically develop off the Pacific coast of South America. Extreme climate change pattern oscillations fluctuates weather across the Pacific Ocean which results in fluctuating droughts, floods, and crop yields in varying regions of the world. The 1997-1998 low rainfalls leading to drought conditions associated with the ENSO event affected some parts of Malaysia such as Selangor, Kuala Lumpur Federal Territory, Penang, Kedah, Kelantan, Sarawak, and Sabah which causes disrupted water supply in water utilities and irrigated agriculture (Ahmad and Low, 2001). In addition, the ENSO event during 1997-1998 resulted extensive fires in more than one million hectares of forests in Sabah. The highest temperature of 40.1°C was recorded in Chuping, Perlis in 1998 and the lowest rainfall was in Tawau, Sabah in 1997 with 1151 mm annual rainfall (Malaysian Meteorological Department, 2010).

Plants respond to adapt to stresses in order to survive. These stresses induce various biochemical and physiological responses in plants. Response of plants to water deficit varies depending on cultivar genetics, developmental stage, intensity, and duration of stress (Eskandari and Kazemi, 2010; Ahmadizadeh *et al.*, 2011). Plants responses' to drought are complex and different mechanisms are adopted by plants when they encounter drought (Levitt, 1980; Jones, 2004). A set of genes is transcriptionally activated, leading to new proteins in vegetative tissue under drought stress. Gene expression is regulated in physiological responses to drought stress and its pattern may vary from tissue to tissue. Thus, protein in different tissues differ both qualitatively and quantitatively.

Some proteins may be synthesized or decrease, with or without induction of unique stress proteins. Proteins that increase during stress include late embryogenesis abundant (LEA) proteins, proteins responsive to abscisic acid, osmotins, heat shock proteins and pathogen related proteins. For instance, late embryogenesis abundant (LEA) proteins which are highly accumulated in the plant embryos (Dure *et al.*, 1981; Galau *et al.*, 1986) and expressed at basal level are induced to high levels during osmotic and drought stress (Ingram and Bartels, 1996). These stress-induced proteins are the primary defence barrier to prevent the loss of intercellular water during adverse conditions. The stress-induced proteins allow plants to make biochemical and structural adjustments that enable them to cope with stress (Shinozaki and Yamaguchi-Shinozaki, 2000).

Several studies proved that the changes in gene expression at transcript level do not often correspond with the changes at protein level (Gygi *et al.*, 1999). Since proteins are direct effectors of plant stress response, investigation of changes in plant proteome is highly important. The investigation of proteins that are responsive to abiotic stresses is crucial for the understanding of molecular mechanisms that underlying the stress response (Khan and Komatsu, 2004; Liu and Xue, 2006). It is helpful in improving drought tolerance in rice by identify the potential protein markers whose changes in abundance can be associated with quantitative changes in some physiological parameters used for a description of genotype's level of stress tolerance. In this study, the proteome pattern rice under drought stress will be compared by using one dimension sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

1.2 Justification of the Study

One of the significance of this study is the exploration of genes that are responsible to drought resistant from Sabah rice variety is not well known. There is still lacking of information of what Sabah rice variety that can withstand to drought. As a preliminary study, the TR-8 rice variety is chosen.

This is because TR-8 or 'Seri Aman' is a high quality rice variety that is able to produce between five to seven tonnes per hectare of yield and highly potentially to produce ten tonnes of rice per hectare. It is also resistant to red disease attributed to the Rice Tungro Bacilliform Virus (RTBV) and also other major diseases in rice such as neck rots and bacterial blight disease.

TR-8 rice strain is cultivated in lowland irrigated farming systems. Hence, drought stress can impair the growth and yield of this rice variety. Comparing the proteome pattern during drought stress of this rice with its normal condition could give information on gene regulation during drought stress which could led to the development of drought resistant rice variety. The study of comparing the proteome pattern of rice strain TR-8 under drought stress and normal conditions helps to discover its drought resistance mechanisms. The proteins that are responsible for gene manipulation to drought stress can be used to develop drought-resistant rice through genetic engineering or marker-assisted selection breeding.

Plants respond to stress in several stages, depending on the stress duration and stress intensity. The interest of this study is the changes of genes and protein level during drought stress. Each stage of plant response results in different genes and protein level which could be seen by the unique protein profile or proteome pattern. The proteome pattern is important for protein identification and quantification for gene discovery as well as molecular breeding in relation to stress. This is because proteins not only include enzymes catalyzing changes in metabolite levels, but they also regulate plant stress response at transcript and protein levels. Proteins have also direct stress-acclimation functions leading to changes in plasma membrane, cell cytoplasm, cytoskeleton, and affinity of cell cytoplasm to water. Changes in protein accumulation under stress are interrelated to plant phenotypic response to stress, determining plant tolerance to stress. Therefore, studies of plant reaction to stress condition at protein level significantly contribute to our understanding of physiological mechanisms underlying plant stress tolerance.

1.3 Objectives of the Study

There are three objectives in this study which include:

i. To compare the proteome pattern of TR-8 rice variety between drought stress and normal condition

ii. To compare the proteome pattern of TR-8 rice variety under different duration of drought stress

iii. To observe the morphological changes (leaf colour, leaf wilting and roots growth) of TR-8 rice variety under different duration of drought stress

1.4 Hypothesis

Hypothesis 1

H_0: There is no significant difference in proteome pattern of TR-8 rice variety between drought stress condition and normal condition.

H_1: There is a significant difference in proteome pattern of TR-8 rice variety between drought stress condition and normal condition.

Hypothesis 2

H_0: There is no significant difference in proteome pattern of TR-8 rice variety under different duration of drought stress.

H_1: There is a significant difference in proteome pattern of TR-8 rice variety under different duration of drought stress.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Drought is a major limitation on rice production especially lowland irrigated rice which requires large water consumption. The El Niño phenomenon in Malaysia during 1999 severely impacted the agricultural production. When plants are subjected to drought stress, they will develop resistance mechanisms and respond to stress at morphological, physiological, and molecular level. Differential-expression proteomics based on comparison of different proteomes composition of stress and non-stress plants might help to develop drought-tolerant variety.

2.2 Rice (*Oryza sativa* L.)

Rice is the major staple food for a large proportion of the world's population. On an average, rice accounts for 27% of all cereal grains production worldwide which ranks second only to wheat at 30% (Smith, 1995). Rice is an annual, monocot plant that belongs to the Poaceae family. The most cultivated species are *Oryza sativa*, the Asian rice and Oryza *glaberrima*, the African rice. *Oryza sativa* is cultivated in is most of the region the world while *Oryza glaberrima* is cultivated only in South Africa. The growth duration of the rice plant is three to six months, depending on the variety and the environment under which it is grown. Rice plant growth can be divided into three agronomic stages of development which are vegetative stage (germination to panicle initiation), reproductive stage (panicle initiation to heading), and maturation or ripening stage (grain filling, heading to maturity). During vegetative stage, the rice plants are highly sensitive to stress at young seedlings stage (Flower and Yeo, 1981). A given level of drought at vegetative stage can cause reduction in yield (O'Toole and Baldia, 1982). The morphology of rice plants in vegetative stage is shown in Figure 2.1.

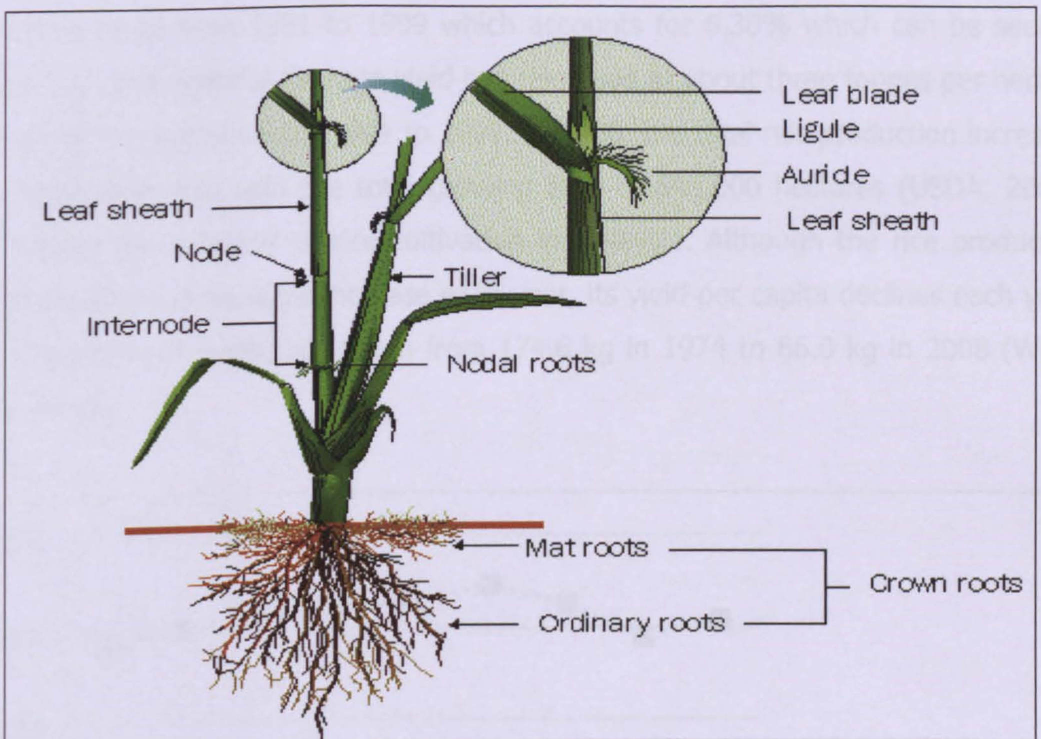

Figure 2.1 The morphology of rice plants in vegetative stage (tillering plants)
Source: Retrieved from www.ikisan.com, March 2014

2.3 Rice Production in Malaysia

Rice (*Oryza sativa* L.) is a highly protected crop in a strategically important industry as a staple food in Malaysia. It ranks the third most important crop in Malaysia after oil palm and rubber in terms of production. Malaysia produces about two million tonnes of rice annually which are mainly grown in the eight granary areas in Peninsular Malaysia with total area of approximately 209,300 hectares (Azmi and Mashhor, 1995). The eight granary areas have been reserved purposely for wetland cultivation by the government in the National Agriculture Policy. The two major rice growing areas are Muda Agriculture Development Authority (MADA) and Kemubu Agriculture Development Authority (KADA).

Rice is grown in Malaysia mainly under flooded irrigation. Rice area and production increased slightly from 1991 to 1999 with the net increase of 0.15% in annual planted area, 0.69% in total production, and 0.53% in average yield which can be seen in the Figure 2.2 (Department of Agriculture Sabah, 1999). This modest increase in production is due to more to an increase in productivity than an increase in cultivation area. However, the net increase of average yield in Sabah show a dramatic increasing trend from 1991 to 1999 which accounts for 6.30% which can be seen in Figure 2.3. The national average yield has remained at about three tonnes per hectare with minor fluctuations from 1991 to 1999. In 2006, the total rice production increased to 2,154,000 tonnes with the total growing area of 645,000 hectares (USDA, 2008). This shows the potential of rice cultivation in Malaysia. Although the rice production and productivity in Malaysia increase each year, its yield per capita declines each year. The rice yield per capita has fallen from 174.6 kg in 1974 to 86.0 kg in 2008 (World Bank, 2010).

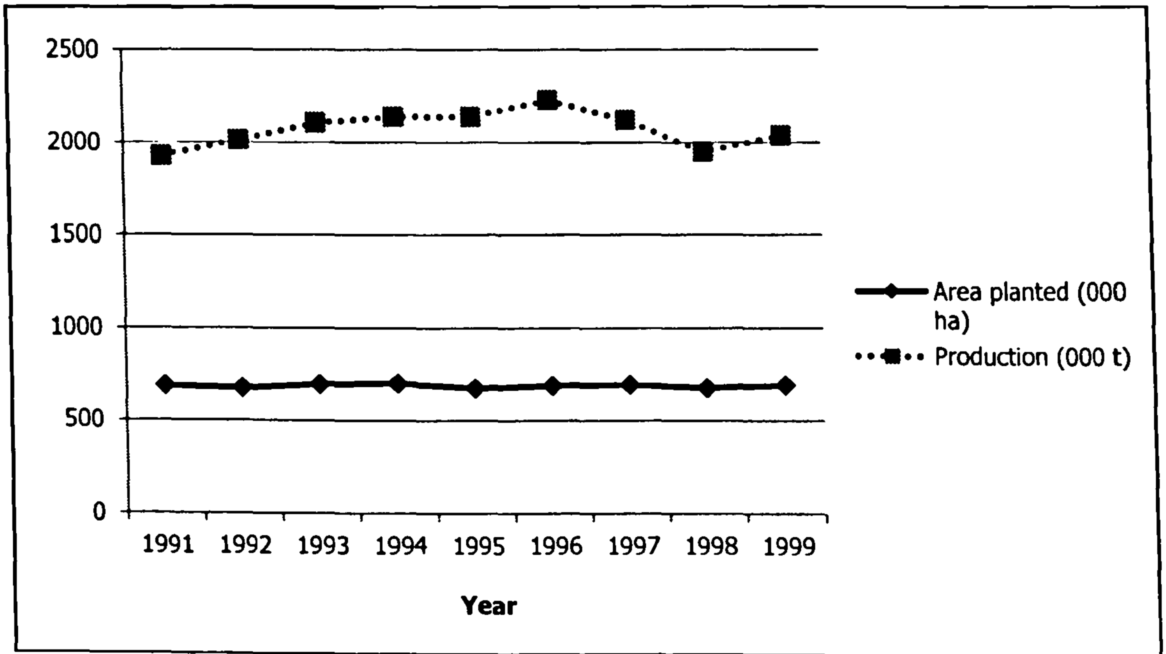

Figure 2.2 Trends in planted area and paddy production for Malaysia during 1991 to 1999

Source: Paddy Statistic 1999, Department of Agriculture, Malaysia

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