PROTEOME PATTERN ANALYSIS OF TQR-1 PADDY LEAVES UNDER DROUGHT STRESS

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Abstract

Rice (*Oryza sativa* L.) is one of the world's most important food crops, especially in Asia. Of the numerous factors affecting rice yield, water stress is one of the most important. In this study, the initial genes response in TQR-1 rice variety were identified by comparing and analyzing the proteome pattern between a drought stress and well-watered condition (control), together with different duration of drought exposure. Two-week-old rice seedlings were exposed to drought conditions for three, six, nine, twelve and fifteen days. As the results, approximately 200 bands were detected in all samples, and nine of them that were differ qualitatively between each control and stress samples. The expression intensity of the five most interesting proteins was also studied and their pattern varied from each other. The proteome analysis revealed that drought stress causes changes in the protein pattern expression of this paddy leaves.
Abstrak

Padi (Oryza sativa L.) merupakan makanan yang sangat penting di seluruh dunia, terutamanya di Asia. Dalam banyak faktor yang mempengaruhi hasil padi, tekanan air merupakan yang paling penting. Dalam kajian ini, gen awal yang bertindak balas dalam padi jenis TQR-1 telah dikenal pasti dengan membandingkan dan menganalisis corak proteome antara tekanan kekurangan air dan keadaan air yang cukup (kawalan), bersama-sama dengan tempoh pendedahan kekurangan air yang berbeza. Benih padi yang berusia dua minggu telah didedahkan kepada keadaan kekurangan air kepada tiga, enam, sembilan, 12 dan 15 hari. Hasilnya, terdapat sembilan gelung protein yang menunjukkan variasi kualitatif antara kumpulan kawalan dan tekanan, juga lebih 200 gelung protein yang telah terekspresi secara keseluruhan. Tahap ekspretasi untuk lima protein yang paling menarik telah dipelajari dan menunjukkan corak yang berbeza untuk setiap satu. Analisis corak protein telah menunjukkan tekanan kekurangan air mengubah corak ekspretasi protein di dalam daun jenis padi ini.
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<td>°C</td>
<td>Degree Celcius</td>
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<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>2-DE</td>
<td>Two-dimensional gel electrophoresis</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<td>ABA</td>
<td>Abscisic Acid</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>APX</td>
<td>Ascorbate peroxidases</td>
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<tr>
<td>BRT</td>
<td>Basal Root Thickness</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CAT</td>
<td>Chloramphenicol acetyltransferase</td>
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<tr>
<td>COR</td>
<td>Cold regulated</td>
</tr>
<tr>
<td>CRD</td>
<td>Completely Randomized Design</td>
</tr>
<tr>
<td>DAT</td>
<td>Days after transplanting</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOA</td>
<td>Department of Agriculture</td>
</tr>
<tr>
<td>DS</td>
<td>Drought stress</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ERD</td>
<td>Early response to dehydration</td>
</tr>
<tr>
<td>ESTs</td>
<td>Expressed sequence tags</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation</td>
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<td>FSA</td>
<td>Faculty of Sustainable Agriculture</td>
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<td>g</td>
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<tr>
<td>GE</td>
<td>Electrophoresis system</td>
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<tr>
<td>GR</td>
<td>Glutathione reductase</td>
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<tr>
<td>ha</td>
<td>Hectare</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
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<tr>
<td>Hsps</td>
<td>Heat-shock proteins</td>
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<tr>
<td>IPG</td>
<td>Immobilized pH gradient</td>
</tr>
<tr>
<td>KCI</td>
<td>Potassium Chloride</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>KIN</td>
<td>Cold inducible</td>
</tr>
<tr>
<td>LEA</td>
<td>Late Embryogenesis Abundant</td>
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<tr>
<td>μL</td>
<td>Micro liter</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>Mha</td>
<td>Million hectares</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>MPSS</td>
<td>Massive Parallel Signature Sequencing</td>
</tr>
<tr>
<td>mRNAs</td>
<td>messenger Ribonucleic acid</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>Mt</td>
<td>Metric ton</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<tr>
<td>OA</td>
<td>Osmotic adjustment</td>
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<tr>
<td>PMSF</td>
<td>Phenylmethanesulfonyl fluoride</td>
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<tr>
<td>POD</td>
<td>Peroxidases</td>
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<td>RAB</td>
<td>Responsive to ABA</td>
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<td>RD</td>
<td>Responsive to dehydration</td>
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<td>Abbreviation</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<td>RT-Qpcr</td>
<td>Real-time reverse-transcription Polymerase Chain Reaction</td>
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<td>SAGE</td>
<td>Serial Analysis of Gene Expression</td>
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<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate polyacrylamide gel electrophoresis</td>
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<td>SOD</td>
<td>Superoxide dismutases</td>
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<td>TCA</td>
<td>Trichloroacetic acid</td>
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<td>UMS</td>
<td>Universiti Malaysia Sabah</td>
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<tr>
<td>v/v</td>
<td>Volume for volume</td>
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<tr>
<td>WW</td>
<td>Well-watered</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight for volume</td>
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<td>x-ray</td>
<td>X- radiation</td>
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CHAPTER 1
INTRODUCTION

1.1 Background of the Study

Rice (*Oryza sativa*) is the most common staple food in Asia, including Malaysia. Most of cultivated rice has a semi-aquatic ancestry and therefore extremely sensitive to water shortages. This type of paddy is known as lowland paddy. When soil water content drops below saturation, this variety develops symptoms of water stress.

Many elements need to be controlled when dealing with paddy cultivation, where water is very crucial for maintaining sustainable production especially in the traditional growing areas. There are many factors that contribute to water problems of paddy cultivation such as poor irrigation management and climate change. Some studies addressed climate change impacts on rice yields, which vary greatly, in South and Southeast Asia (Matthews *et al.*, 1994). Farmers’ adaptation practices to cope with the agricultural vulnerability due to climatic change are not found adequate and satisfactory (Alam *et al.*, 2011, 2012). Their adaptation methods are based only on their ideas or reactions. As a result, only 30.3% farmers believe that they have been able to properly cope with climatic vulnerabilities (Alam *et al.*, 2012).

Furthermore, drought stress is the most devastating abiotic factor that affects rice yields severely. It has been estimated that losses due to drought stress are about 18 Mt annually (O’Toole, 2004). In Asia alone, it is estimated that a total of 23 Mha of rice fields are drought prone (Pandey *et al.*, 2009). It has been shown that drought
stress during the crop flowering stage disrupts photosynthesis and transfer of stored carbohydrates into grains, which is the reason for the reduced grain number and weight (Reynolds et al., 2011). The reduction was found to be more severe when the stress occurred at the early grain filling stages rather than at later stages (Stone et al., 1995).

Generally three mechanisms are involves that can help to mitigate the effect of drought stress which are drought escape, drought avoidance and drought tolerance. A proper timing of lifecycle, resulting in the completion of the most sensitive developmental stages while water is abundant, is considered to be a drought escape strategy. Avoiding water-deficit stress with a root system capable of extracting water from deep soil layers, or by reducing evapotranspiration without affecting yields, is considered as drought avoidance.

This mechanism can be expressed even in the absence of stress and are then considered constitutive. Drought tolerance in the other hand is a mechanism such as osmotic adjustment (OA) whereby a plant maintains cell turgor pressure under reduced soil water potential. It is the results of a response triggered by drought stress itself and are therefore considered adaptative.

When the stress is terminal and predictable, drought escapes through the use of shorter duration varieties is often the preferable method of improving yield potential. Drought avoidance and tolerance mechanisms are required in situations where the timing of drought is mostly unpredictable.

At the molecular level, there are abundant proteins responsible for better water uptake by paddy but are yet to be discovered. These entire proteins surely have important role, especially helping in mechanisms against water stress. Because plant response to water stress is complex, however, the specific functions of many of these proteins are still unknown (Cushman and Bohnert, 2000; Bray, 2002). Many of the traits that explain plant adaptation to drought are those determining plant development and shape, like phenology, the size and depth of the root system, xylem properties or the reserves storage.
Since genetic diversity is the basis of plant breeding, understanding and assessing them is important for crop management, crop improvement by selection, use of crop germplasms, detection of genome structure, and transfer of desirable traits to other plants (Arshney et al., 2008; Sasaki, 2005). Furthermore, with present technology, we are able to run protein analysis and observe the proteome pattern of any organism.

1.2 Justification of the study

This study outlined proteome patterns differences in TQR-1 paddy variety subjected to water deficient and well-watered conditions over different durations of stress.

Malaysia is having two types of climate conditions, which are hot and rainy throughout the year, growing paddy has to follow the climate trend as well. Since water is highly required in lowland paddy cultivation (about 2000 liter water are required), this study is crucial as water deficiency will affect the sustainable production of rice, which can cause yield declines.

Furthermore, water situation in the country has changed from one of relative abundance to one of scarcity lately. Population growth, urbanization, industrialization and the expansion of irrigated agriculture are imposing rapidly growing demands and pressures on the water resources. Hence, water management is becoming highly comprehensive and complicated due to large concentrations of population, commercial activities and industries around the country.

Regardless of the water situation nowadays, paddy production however, must increase dramatically over the years to meet the food needs. Therefore, producing more rice with less water is a formidable challenge to the agriculture industry nowadays. The TQR-1 paddy variety is used because it is one of the high yield varieties suitable for the local environment in Sabah (New Sabah Times, 2009).

The study of proteomes is chosen as it gives a better understanding of an organism than genomics, since proteins represent the actual functional molecules in a
The ultimate goal of this study is to find genes that are related to drought so that this can be used to develop a drought resistant paddy through genetic engineering.

1.3 Research objectives
The objectives for this study were:

i. To compare the proteome pattern of TQR-1 paddy variety under well watered and water stress conditions

ii. To compare the proteome pattern of TQR-1 paddy variety for different durations of water stress conditions

1.4 Hypotheses

Hypothesis 1

H₀: There were no significant differences in proteome pattern of TQR-1 paddy variety between normal and water stress conditions

H₁: There were significant differences in proteome pattern of TQR-1 paddy variety between normal and water stress conditions

Hypothesis 2

H₀: There were no significant differences in proteome pattern of TQR-1 paddy variety for different durations of water stress

H₁: There were significant differences in proteome pattern of TQR-1 paddy variety for different durations of water stress


CHAPTER 2
LITERATURE REVIEW

2.1 Paddy Crop Cultivation

2.1.1 World Paddy Crop Cultivation

Half of world's populations are depending on rice for daily diet, accounts for around 23% of the global calorie intake (Li et al., 2011; Bernier et al., 2008). It has been estimated that rice supplies 35% to 60% of the total calorie intake at any given day in Asia where 90% of world's rice is grown (Khush, 1997). About 90% of rice is grown and consumed in Asia, with China and India being the largest Asian producers, accounting collectively for nearly half of world production and consumption in 2005-2010 (Ramziath and John, 2012) (Figure 2.1).

![World rice production in 2005-2010](image)

Source: Production, Supply and Distribution data base, U.S Department of Agriculture (USDA)
Increasing population pressure, global warming and unpredictable rainfall patterns have induced severe drought spells in the major rice growing areas of the world in past few years. To meet the growing demands of global population rice productivity needs to be significantly increased. In order to producing high quality of rice, the agronomic requirement and the molecular level of the paddy plants must be well studied.

Many of rice are semi aquatic plant that can be grown under irrigated as well as rainfed conditions. Poehlman and Sieper (1995) described four different types of rice growing environments: irrigated, rainfed lowland, deep-water and rainfed upland. Irrigated rice is by far the most common rice ecosystem constituting 55% of the world area providing 75% of the rice production globally (Khush, 1997). Of the total 150.1 Mha of world rice acreage, the rainfed rice shares 30.9% whereas upland rice is about 9% (Bernier et al., 2008). Rainfed lowland rice occupies about 32.1% of the total rice grown area in Asia and 35.4% in Africa (Figure 2.2).

![Map of worldwide rice cultivation](image)

**Figure 2.2** Worldwide areas of rice cultivation

Source: International Rice Research Institute (IRRI)

Bangladesh, Indonesia, and Philippines are the areas that plant the most upland rice, but the yield is so low, about one ton per ha on average and highly variable.
Although upland rice constitutes a relatively small proportion of the total rice area worldwide, it is the predominant method of rice cultivation in Latin America and West Africa for about 75% and 50% of rice area, respectively (Gupta and O'Toole, 1986).

### 2.1.2 Paddy Crop Cultivation in Malaysia

In Malaysia, nearly all states of Malaysia's are composed of land dedicated to rice cultivation alone (Table 2.1). This shows that rice is a crucial part of daily diet. The average Malaysian citizen consumes 82.3 kilograms of rice per year. In 1998, Malaysia produced 1.94 million metric tons of rice (Malaysia Agriculture, 2010). The production volume has changed little over the years since 2000. In 2007, Malaysia produces about 2.3 MT of paddy, most are produced in Peninsular. The cultivation and production of paddy in Sabah and Sarawak is much lower. In Sabah alone, rice production is only at 30% SSL, half the target set by the government at 60% SSL (The Borneo Post, 2012).

### Table 2.1 Hectare of Overall Planted Area, Average Yield, Production of Paddy and Rice by Season and State, Malaysia in 2011.

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<td></td>
<td>Padi (TAN METR)</td>
<td>Padi (TAN METR)</td>
<td>Ribas (TAN METR)</td>
</tr>
<tr>
<td></td>
<td>(ha)</td>
<td>(TAN METR)</td>
<td>(TAN METR)</td>
</tr>
<tr>
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<td>3,022</td>
<td>3,796</td>
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<tr>
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<tr>
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<td>Pahang</td>
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<td>3,246</td>
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<td>82,150</td>
<td>3,937</td>
<td>323,445</td>
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<tr>
<td>Perlis</td>
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<td>4,460</td>
<td>232,674</td>
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<td>P. Pinang</td>
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<td>5,607</td>
<td>144,013</td>
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<td>242,669</td>
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Source: Department of Agriculture Peninsular Malaysia
Hence, with this production trends, Malaysia take an alternative to import rice from nearby countries to support the citizen needs. The situation caused Malaysia to become one of the major importers of rice as they increase year by year. Major suppliers are Thailand and Vietnam, while Pakistan and India supply specialty rice.

In addition, local rice price is normally higher than international rice price. This somehow caused the government or BERNAS happily import the rice from international markets. World rice production is around 370 million-tons per year (milled), while traded rice is only about 5% of total production. Because of the small volume for export market, price can rise sharply when something happens to the production of exporter or major consuming countries. For example in 2008 the price rose sharply causing the domestic rice price to increase almost double.

Therefore, Malaysia should not rely on the import rice which will influence the local rice supplies and price if the major country suppliers decided to stop their exports. Plus, the increasing population is calling for more research and technological advancement to increase rice production for consumption within the nation (Selamat et al., 2009).

2.2 Economical Value of Paddy

Food security, which is the condition of continuously having enough food to provide adequate nutrition for a healthy life for now and following generation, is a critical issue in the developing world. About 3 billion people, nearly half the world’s population, depend on rice for survival. Therefore, there is no doubt of major economic value of rice, feeding more than half of world population. It is the predominant dietary energy source for 17 countries in Asia and the Pacific, nine countries in North and South America and eight countries in Africa.

Rice provides 20% of the world’s dietary energy supply, while wheat supplies 19% and maize 5% (FAO, 2004). As of 2009 world food consumption of rice was 531.6 million metric tons of paddy equivalent (354,603 of milled equivalent), while the far largest consumers were China consuming 156.3 million metric tons of paddy equivalent (29.4% of the world consumption) and India consuming 123.5 million metric tons of
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