PERFORMANCE AND PROTEOME ANALYSIS OF SELECTED SABAH DRYLAND RICE VARIETIES UNDER DROUGHT STRESS IN SILABUKAN SOIL

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important staple foods for over half of the world's population. The sustainability of rice production is negatively affected by increasing irrigation water scarcity. Dryland rice that is rain-fed and can be cultivated on dry land has lower irrigation water requirement compared to wetland paddy. Hence, it has a lot of potential to be commercialized as it has significantly lower irrigation water requirement. In this study, drought stress treatment was applied during 50% flowering stage. Performance of three (3) selected Sabah dryland rice varieties (Galigim, Hijau Manis and Kondinga) under well-watered and drought conditions were compared. Vegetative traits including plant height, number of tillers per plant and culm height were shown to be independent on drought stress treatment at 50% flowering stage. Nevertheless, yield and its associated traits including grain yield per plant, 1000-grain weight, number of filled grains per plant, percentage of filled grains per plant were significantly affected by the drought stress treatment applied at 50% flowering stage. Galigim was extremely sensitive to drought stress at 50% flowering stage where the highest relative reduction in grain yield per plant (61.14%) was observed. The rice variety Hijau Manis has shown the least adverse effect (13.57% reduction) on grain yield per plant under drought stress. The proteome pattern of the best performing Sabah dryland rice variety which was Hijau Manis under well-watered and drought conditions were compared and analyzed. Image analysis of the CBB stained gel showed that two (2) protein bands were present in the proteomic profile of Hijau Manis in response to drought stress. Besides, three (3) protein bands were up-regulated and ten (10) protein bands were down-regulated during drought stress. One of the protein bands was up-regulated significantly by 140% under drought stress. In conclusion, drought stress influences the yield and yield related traits significantly and the ability of dryland rice varieties to cope with drought stress varied remarkably. Specific proteins that were regulated under drought stress can be subjected to further analysis in the future. Proteins that were induced during drought stress could be involved in drought response.

PRESTASI DAN ANALISIS PROTEOME PADI HUMA SABAH TERPILIH PADA KEADAAN KEMARAU PADA TANAH SILABUKAN

ABSTRAK

Padi (Oryza sativa L.) adalah salah satu daripada makanan ruji yang paling penting bagi lebih separuh daripada penduduk dunia. Walau bagaimanapun, pengeluaran padi terjejas diseababkan kekurangan air. Padi huma yang boleh ditanam di atas tanah yang tidak ditakungi air mempunyai banyak potensi untuk dikomersialkan kerana ia mempunyai keperluan air pengairan yang lebih rendah. Dalam kajian ini, rawatan kemarau telah diberikan pada peringkat 50% berbunga. Prestasi padi huma Sabah terpilih (Galigim, Hijau Manis dan Kondinga) pada keadaan normal dan keadaan kemarau telah dibandingkan. Ciri-ciri vegetatif termasuk ketinggian tumbuhan, bilangan anak padi setiap tumbuhan, panjang tangkai dan ketinggian jelaga telah ditunjukkan tidak terjejas oleh tekanan kemarau pada peringkat 50% berbunga. Walau bagaimanapun, hasil dan ciri-ciri yang berkaitan dengannya termasuk hasil bijirin setiap tumbuhan, berat 1000-butir, bilangan isi bijirin setiap tumbuhan, peratusan penuh bijirin setiap tumbuhan telah ketara terjejas oleh rawatan tekanan kemarau digunakan pada 50% peringkat berbunga. Galigim amat sensitif kepada tekanan kemarau pada peringkat 50% berbunga di mana pengurangan relatif tertinggi dalam hasil bijirin setiap tumbuhan (61.14%). Hijau Manis telah menunjukkan kesan yang paling minimum (13.57% kekurangan) di bawah tekanan kemarau. Corak proteome Hijau Manis di bawah tekanan kemarau and keadaan normal telah dibandingkan. Analisis imej gel CBB menunjukkan bahawa dua (2) band protein telah hadir dalam profil proteomik daripada tindak balas Hijau Manis kepada tekanan kemarau. Salah satu band protein meningkat dengan ketara. Kesimpulannya, tekanan kemarau mempengaruhi hasil dan ciri-ciri hasil yang berkaitan.. Elektroforesis gel dua dimensi (2-D elektroforesis) dan spektrometri jisim boleh dijalankan pada masa hadapan untuk pengenalpastian dan pencirian protein yang berguna yang bertanggungjawab untuk toleransi kemarau tanaman padi tanah kering.

TABLE OF CONTENTS

Content		Page
DECLARATION		ii
VERIFICATION		iii
ACKNOWLEDGEMENT		iv
ABSTRACT		v
ABSTRAK		vi
TABLE OF CONTENTS		vii
LIST OF FIGURES		x
LIST OF SYMBOLS, UNITS, AND ABBREVIATIONS		xvi
CHAPTER 1	**INTRODUCTION**	
1.1	Background of the Study	1
1.2	Justification of the Study	3
1.3	Objectives of the Study	4
1.4	Hypothesis	4
CHAPTER 2	**LITERATURE REVIEW**	
2.1	Rice (*Oryza sativa* L.)	6
2.2	Rice Growth and Development	6
	2.2.1 Rice Vegetative Stage	7
	2.2.2 Rice Reproductive Stage	8
	2.2.3 Rice Ripening Stage	9
2.3	Rice Ecosystems	10
2.4	Production of Rice	11
2.5	Drought	12
	2.5.1 Effect of Drought to Rice Plants	12
	2.5.2 Drought in Malaysia	13
2.6	Drought Resistance Mechanisms	13
	2.6.1 Drought Escape	14
	2.6.2 Drought Avoidance	14
	2.6.3 Drought Tolerance	15
2.7	Physiological Mechanisms	15
2.8	Molecular Mechanisms	16
	2.8.1 Aquaporins	17
	2.8.2 Stress Proteins	17
	2.8.3 Signaling and Drought Stress Tolerance	18
2.9	Proteomics	19
	2.9.1 Importance of Proteomics	20
2.10	Steps in Proteomic Analysis	21
	2.10.1 Protein Extraction	21
	2.10.2 Protein Separation	22
CHAPTER 3	**METHODOLOGY**	
3.1	Location of Study	24
3.2	Duration of Study	24
3.3	Plant Materials	24
3.4	Preparation of Rain Shelter	24
3.5	Sanitization of Rain Shelter	25
3.6	Soil Preparation	25
3.7	Pot Preparation	25

3.8	Seed Germination Test	25
3.9	Germination of Dryland Rice	26
3.10	Nursery Stage of Dryland Rice	26
3.11	Establishment of Plant Growth	26
3.12	Experimental Design	26
3.13	Experimental Layout	27
3.14	Drought Stress Treatment	28
3.15	Temperature inside the Rain Shelter	29
3.16	Relative Water Content	29
3.17	Collection of Sample for Proteome Analysis	29
3.18	Parameters and Measurements	29
3.19	Pre-extraction Preparation	30
3.20	Protein Extraction	30
3.21	Protein Quantification	31
3.22	Protein Separation by SDS-PAGE	31
3.23	Proteomic Analysis	31
3.24	Statistical Analysis	31

CHAPTER 4 RESULTS

4.1	Performance of Dryland Rice under Normal and Drought Conditions	33
4.1.1	Plant Height	33
4.1.2	Number of Tillers per Plant	37
4.1.3	Number of Productive Tillers per Plant	40
4.1.4	Length of Panicles	44
4.1.5	Culm Height	47
4.1.6	Grain Yield per Plant	51
4.1.7	1000-Grain Weight	54
4.1.8	Number of Filled Grains per Plant	58
4.1.9	Number of Unfilled Grains per Plant	61
4.1.10	Number of Spikelets per Plant	65
4.1.11	Percentage of Filled Grains per Plant	68
4.1.12	Extrapolated Yield	72
4.1.13	Relative Water Content	75
4.2	One-Dimensional SDS-PAGE Analysis of Total Proteins in Flag Leaves of Hijau Manis	79
4.2.1	SDS-PAGE Proteomic Profiles of Hijau Manis' Flag Leaves	79
4.2.2	Proteomic Qualitative Analysis of Hijau Manis' Flag Leaves under Well Watered Condition and Drought Stress	80
	a. Image Analysis	80
	b. Statistical Analysis	81
4.2.3	Proteomic Quantitative Analysis of Hijau Manis' Flag Leaves under Well Watered Condition and Drought Stress	82

CHAPTER 5 DISCUSSION

5.1	Performance of Dryland Rice under Normal and Drought Conditions	84

5.1.1	Effect of Drought Stress on Vegetative Traits	84
5.1.2	Effect of Drought Stress on Yield and Its Associated Traits	85
5.1.3	Effect of Drought Stress on Relative Water Content of Rice Plants	86
5.2	One-Dimensional SDS-PAGE Analysis of Total Proteins in Flag Leaves of Hijau Manis	87
CHAPTER 6	**CONCLUSION**	
6.1	Conclusion	90
6.2	Suggestions and Recommendations	90
REFERENCES		91
APPENDICES		98

LIST OF FIGURES

Figure		Page
2.1	Development stages of the rice plant	6
2.2	Parts of a rice seedling	7
2.3	Parts of a tiller of rice	8
2.4	Structure of a rice grain	9
2.5	Steps in SDS-PAGE	22
3.1	Leaf rolling score	24
3.2	The layout of experimental design using completely randomized design (CRD)	27
4.1	Mean plant height of Galigim, Hijau Manis and Kondinga	34
4.2	Mean plant height under well-watered condition and drought stress	34
4.3	Mean plant height of Galigim under well-watered condition and drought stress	35
4.4	Mean plant height of Hijau Manis under well-watered condition and drought stress	35
4.5	Mean plant height of Kondinga under well-watered condition and drought stress	36
4.6	Relative percent change in plant height of Galigim, Hijau Manis and Kondinga under drought stress	36
4.7	Mean number of tillers of Galigim, Hijau Manis and Kondinga	37
4.8	Mean number of tillers under well-watered condition and drought stress	38
4.9	Mean number of tillers of Galigim under well-watered condition and drought stress	38
4.10	Mean number of tillers of Hijau Manis under well-watered condition and drought stress	39
4.11	Mean number of tillers of Kondinga under well-watered condition and drought stress	39

4.12 Relative percent change in number of tillers of Galigim, Hijau Manis and Kondinga under drought stress 40

4.13 Mean number of fertile tillers of Galigim, Hijau Manis and Kondinga 41

4.14 Mean number of fertile tillers under well-watered condition and drought stress 41

4.15 Mean number of fertile tillers of Galigim under well-watered condition and drought stress 42

4.16 Mean number of fertile tillers of Hijau Manis under well-watered condition and drought stress 42

4.17 Mean number of fertile tillers of Kondinga under well-watered condition and drought stress 43

4.18 Relative percent change in number of fertile tillers of Galigim, Hijau Manis and Kondinga under drought stress 43

4.19 Mean panicle length of Galigim, Hijau Manis and Kondinga 44

4.20 Mean panicle length under well-watered condition and drought stress 45

4.21 Mean panicle length of Galigim under well-watered condition and drought stress 45

4.22 Mean panicle length of Hijau Manis under well-watered condition and drought stress 46

4.23 Mean panicle length of Kondinga under well-watered condition and drought stress 46

4.24 Relative percent change in panicle length of Galigim, Hijau Manis and Kondinga under drought stress 47

4.25 Mean culm height of Galigim, Hijau Manis and Kondinga 48

4.26 Mean culm height under well-watered condition and drought stress 48

4.27 Mean culm height of Galigim under well-watered condition and drought stress 49

4.28 Mean culm height of Hijau Manis under well-watered condition and drought stress 49

4.29 Mean culm height of Kondinga under well-watered condition and drought stress 50

4.30 Relative percent change in culm height of Galigim, Hijau Manis and Kondinga under drought stress 50

4.31 Mean grain yield per plant of Galigim, Hijau Manis and Kondinga 51

4.32 Mean grain yield per plant under well-watered condition and drought stress 52

4.33 Mean grain yield per plant of Galigim under well-watered condition and drought stress 52

4.34 Mean grain yield per plant of Hijau Manis under well-watered condition and drought stress 53

4.35 Mean grain yield per plant of Kondinga under well-watered condition and drought stress 53

4.36 Relative percent change in grain yield of Galigim, Hijau Manis and Kondinga under drought stress 54

4.37 Mean 1000-grain weight of Galigim, Hijau Manis and Kondinga 55

4.38 Mean 1000-grain weight of dryland rice under well-watered condition and drought stress 55

4.39 Mean 1000-grain weight of Galigim under well-watered condition and drought stress 56

4.40 Mean 1000-grain weight of Hijau Manis under well-watered condition and drought stress 56

4.41 Mean 1000-grain weight of Kondinga under well-watered condition and drought stress 57

4.42 Relative percent change in 1000-grain weight of Galigim, Hijau Manis and Kondinga under drought stress 57

4.43 Mean number of filled grains per plant of Galigim, Hijau Manis and Kondinga 58

4.44 Mean number of filled grains per plant of dryland rice under well-watered condition and drought stress 59

4.45 Mean number of filled grains per plant of Galigim under well-watered condition and drought stress 59

4.46 Mean number of filled grains per plant of Hijau Manis under well-watered condition and drought stress 60

4.47 Mean number of filled grains per plant of Kondinga under well-watered condition and drought stress 60

4.48	Relative percent change in number of filled grains of Galigim, Hijau Manis and Kondinga under drought stress	61
4.49	Mean number of unfilled grains per plant of Galigim, Hijau Manis and Kondinga	62
4.50	Mean number of unfilled grains per plant of dryland rice under well-watered condition and drought stress	62
4.51	Mean number of unfilled grains per plant of Galigim under well-watered condition and drought stress	63
4.52	Mean number of unfilled grains per plant of Hijau Manis under well-watered condition and drought stress	63
4.53	Mean number of unfilled grains per plant of Kodinga under well-watered condition and drought stress	64
4.54	Relative percent change in number of unfilled grains of Galigim, Hijau Manis and Kondinga under drought stress	64
4.55	Mean number of spikelets per plant of Galigim, Hijau Manis and Kondinga	66
4.56	Mean number of unfilled grains per plant of dryland rice under well-watered condition and drought stress	66
4.57	Mean number of spikelets per plant of Galigim under well-watered condition and drought stress	67
4.58	Mean number of spikelets per plant of Hijau Manis under well-watered condition and drought stress	67
4.59	Mean number of spikelets per plant of Kodinga under well-watered condition and drought stress	60
4.60	Relative percent change in number of spikelets of Galigim, Hijau Manis and Kondinga under drought stress	60
4.61	Mean percentage of filled grains per plant of Galigim, Hijau Manis and Kondinga	69
4.62	Mean percentage of filled grains per plant of dryland rice under well-watered condition and drought stress	69
4.63	Mean percentage of filled grains per plant of Galigim under well-watered condition and drought stress	70
4.64	Mean percentage of filled grains per plant of Hijau Manis under well-watered condition and drought stress	70

4.65	Mean percentage of filled grains per plant of Kondinga under well-watered condition and drought stress	71
4.66	Relative percent change in percentage of filled grains of Galigim, Hijau Manis and Kondinga under drought stress	71
4.67	Mean extrapolated yield per plant of Galigim, Hijau Manis and Kondinga.	72
4.68	Mean extrapolated yield of dryland rice under well-watered condition and drought stress.	73
4.69	Mean extrapolated yield of Galigim under well-watered condition and drought stress.	73
4.70	Mean extrapolated yield of Hijau Manis under well-watered condition and drought stress.	74
4.71	Mean extrapolated yield of Kondinga under well-watered condition and drought stress.	74
4.72	Relative percent change in extrapolated yield of Galigim, Hijau Manis and Kondinga under drought stress.	75
4.73	Mean relative water content of Galigim, Hijau Manis and Kondinga	76
4.74	Mean relative water content of dryland rice under well-watered condition and drought stress	76
4.75	Mean relative water content of Galigim under well-watered condition and drought stress	77
4.76	Mean relative water content of Hijau Manis under well-watered condition and drought stress	77
4.77	Mean relative water content of Kondinga under well-watered condition and drought stress	78
4.78	Relative Percent change in relative water content of Galigim, Hijau Manis and Kondinga under drought stress	78
4.79	SDS-PAGE proteomic profiles of the proteins of Hijau Manis' flag leaves under water-watered condition (N) and drought stress (S) using equal volume of protein samples (25 µg/µL) with CBB staining method	79
4.80	SDS-PAGE proteomic profiles of the proteins of Hijau Manis' flag leaves under water-watered condition (N) and drought stress (S) using equal volume of protein samples (5 µg/µL) with silver staining method	80

4.81 Mean number of protein band under well-watered condition and drought stress with CBB staining method 81

4.82 Mean number of protein band under well-watered condition and drought stress with silver staining method 82

4.83 SDS-PAGE proteomic profiles of the protein labelled d of Hijau Manis' flag leaves under water-watered condition (N) and drought stress (S) using equal volume of protein samples (25 µg/µL) with CBB staining method (a) and silver-staining method (b) 83

4.84 Mean relative intensity of protein band labelled 'd' under well-watered condition and drought stress with CBB staining method 83

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

°C	Degree Celcius
$	Dollar
<	Less than
%	Percent
A	Galigim
ABA	Abscisic acid
AQP	Aquaporin
ANOVA	Analysis of variance
B	Hijau Manis
BSA	Bovine Serum Albumin
C	Kondinga
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
DTT	Dithiothreitol
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
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
N	Normal (well-watered)
nm	Nanometer
d^{-1}	Per day
PMSF	Phenylmethylsulfonyl fluoride
PAGE	Polyacrylamide gel electrophoresis
PVC	Polyvinyl chloride
RNA	Ribonucleic acid
RM	Ringgit Malaysia

S	Stressed
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 Agrilculture
UV	Ultraviolet
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Rice (*Oryza sativa* L.) is one of the most important staple foods of over half of the world's population (Akinbile, 2009). Globally, it ranks second after corn in terms of production of grain in 2012, based on a production amount of over 872 million metric tons (Statista, 2015). Currently, in Malaysia, there are more than 300,000 farmers involved in rice cultivation on 674,928 ha of land areas (Mohd Rashid and Mohd Dainuri, 2013). In Malaysia, rice is not only a staple food, but it also plays a major part in our national economic activity and a key source of employment and income for the rural population. However, the current country's National Self Sufficiency Level (SSL) of domestic rice production is about 71.4% and the balance relies on the importation of rice from other countries (Chamhuri *et al.*, 2014). Hence, to reduce the importation of rice and increase the National Self Sufficiency Level (SSL) of domestic rice production, it is essential to study the factors affecting rice production.

Water is one of the most important factors for sustainable rice production. The sustainability of rice production is negatively affected by increasing irrigation water scarcity. Climate change is predicted to be the main reason for the global increase in water scarcity. According to Sabar and Arif (2014), Intergovernmental Panel on Climate Change (IPCC, USA) has predicted that due to global warming, fluctuations in precipitation patterns around the world is causing extreme drought and floods. Drought is commonly defined as the inadequacy of water availability including period without significant rainfall that affects the crop growth (Hanson *et al.*, 1995) and it occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Singh *et al.*, 2012).

Drought stress is one of the major abiotic factors limiting rice production (Ding *et al.*, 2013).

Drought may delay the physiological development of rice plant and negatively affect the physical processes like photosynthesis, respiration, transpiration and translocation of assimilates to the grain (Davatgar *et al.*, 2009). Drought stress has effects on both morphological and physical traits and also yield. Water stress causes reduction in leaf area, cell size, and intercellular volume (Senanayake and Perera, 1997). A variety is considered as good if it shows more resistant to water flow from the stomata into the atmosphere. Water stress during vegetative growth tended to delay panicle initiation (Lilly and Fukai, 1994). Water stress at flowering greatly affects rice yield because it has diverse effect on pollination and causes flower abortion, grain abscission and increasing percentage of unfilled grain (Singh *et al.*, 2012).

Drought resistance of plants involves many complex traits reflected in morphological and physiological characteristics. Plants usually have different mechanisms combined to confer drought resistance. The mechanisms of drought resistance include drought escape, drought avoidance, drought tolerance and drought recovery, and drought avoidance and drought tolerance are among the two major mechanisms for drought resistance in rice (Nguyen *et al.*, 1997; Luo, 2010). These mechanisms are often regulated at the molecular levels. Various genes with diverse functions are induced or repressed by abiotic stresses (Shinozaki and Yamaguchi-Shinozaki, 2007). These gene products may function in stress response and tolerance at the cellular level. Significantly, improved plant stress tolerance will be resulted from the introduction of many stress-inducible genes via gene transfer (Umezawa *et al.*, 2006).

Drought stress is reported to inhibit the incorporation of amino acids into proteins and to cause a decrease in the protein content of the tissues (Sikuku *et al.*, 2012). Besides, drought stress also causes an increased synthesis of other specific proteins (Pessarakli, 2011). Hence, it is known that there will be changes in the pattern of protein synthesis in plants undergoing stress. Drought tolerance in rice can be improved by identifying 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 (Gupta *et al.*, 2015). In this study, the proteome pattern of three varieties of dryland rice which are Hijau Manis, Galigim and Kondinga under well-watered and drought conditions will be compared.

1.2 Justification of the Study

In Malaysia, rice is mostly cultivated in irrigated lowland paddy fields as wetland paddy. The irrigation water requirements of these fields are relatively high. Due to climate change, population growth, increasing urban and industrial demand for water, water pollution and water resource depletion, sustainability of rice production in this condition is threatened by increasing irrigation water scarcity. Hence, dryland rice that is rain-fed and can be cultivated on dry land without accumulation of water has a lot of potential to be commercialized as it has significantly lower irrigation water requirement. However, the research on dryland rice has been neglected because of its relatively low grain yields. The low grain yields of the dryland rice are attributed to the poor management by the farmers without any monitoring on plant nutrients and other critical aspects, such as weeds, disease and pest attacks (Hanafi *et al.*, 2009). Therefore, with good management practices, the grain yields of dryland rice varieties are expected to increase.

The agronomic information of Sabah dryland rice varieties Galigim, Hijau Manis and Kondinga are very limited as they are usually planted by the rural communities in Sabah. By doing performance tests for these dryland rice varieties, basic information like yield and harvesting time were obtained and this may assist farmers in planting these dryland rice varieties. The agronomic information obtained may also encourage further research to improve the yields of these dryland rice varieties. Besides, the dryland rice varieties were planted using Silabukan soil which is available in large area in Sandakan and is common in the Faculty of Sustainable Agriculture, Sandakan. The result may assist in future planting of these dryland rice varieties in the faculty.

Dryland rice varieties are known to be having better drought tolerance compared to wetland paddy. However, the drought tolerance for the dryland rice varieties Galigim, Hijau Manis and Kondinga were not well known. Our interest was to find out to what extend these Sabah dryland rice varieties can withstand less water environment. Proteome analysis is important because by comparing the proteome pattern of the best performing rice variety under normal and drought conditions, we can obtain information on gene regulation and explore genes that are responsible for their drought resistance. The genes responsible to the drought stress can be manipulated and be used to develop drought-resistant rice through genetic engineering and marker-assisted selection breeding in the future.

In this study, flag leaves of the selected Sabah dryland rice varieties were harvested for proteome analysis. Flag leaves were chosen as the plants part to be analyzed because flag leaves play a major role in synthesis and translocation of photoassimilates to the rice seeds, hence affecting grain yield (Sperotto *et al.*, 2013). Besides, another report showed that flag leaf contributed to 45% of rice grain yield (Abou-Khalifa *et al.*, 2008), so it was important for us to determine changes occur in flag leaves when the rice plants were under drought stress.

1.3 Objectives of the Study

This study was carried out to achieve the following objectives:

i. To compare the vegetative growth and yield performance of selected Sabah dryland rice varieties (Galigim, Hijau Manis and Kondinga) under normal and drought conditions.
ii. To compare the proteome pattern of the best performing Sabah dryland rice variety under normal and drought conditions.

1.4 Hypothesis

Hypothesis 1

H_0: There is no significant difference in the vegetative growth and yield performance of selected Sabah dryland rice varieties (Galigim, Hijau Manis and Kondinga) under normal and drought conditions.

H_1: There is a significant difference in the vegetative growth and yield performance of selected Sabah dryland rice varieties (Galigim, Hijau Manis and Kondinga) under normal and drought conditions.

Hypothesis 2

H_0: There is no significant difference in the proteome pattern of the best performing Sabah dryland rice variety under normal and drought conditions.

H_1: There is a significant difference in the proteome pattern of the best performing Sabah dryland rice variety under normal and drought conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice (*Oryza sativa* L.)

The cultivated rice (*Oryza sativa* L.) is an annual, monocot plant belongs to the Poaceae family, along with wheat, corn, millet, oats, barley, rye, and numerous others. It is a diploid species with 24 chromosomes (Chang and Bardenas, 1965). The rice plant can be characterized as an annual grass, with round, hollow, jointed culms, rather flat, sessile leaf blades, and a terminal panicle (Chang and Bardenas, 1965). It is the only cultivated cereal plant adapted to growing in both flooded and non-flooded soils. Grown under a wide range of climatic and geographical conditions on all five continents, it serves as an important staple food in many parts of the world.

2.2 Rice Growth and Development

The rice plant varies in size from dwarf mutants to floating varieties. The great majority of commercial varieties range from 1 to 2 m in height. The vegetative organs of rice plant consist of roots, culms and leaves. A branch of the plant bearing the culm, leaves, roots and often a panicle is a tiller (Chang and Bardenas, 1965). 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). Figure 2.1 characterizes the growth stages for rice plants.

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