EFFECT OF DROUGHT ON AGRONOMIC CHARACTERISTICS AND PROTEOME PATTERNS OF SELECTED SABAH DRYLAND PADDY VARIETIES

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ABSTRACT

Drought is the major limiting factor to paddy production worldwide. Thus, drought tolerance emerges as one of the most important aspects of paddy breeding programs. A study on Sabah's local dryland paddy varieties were carried out using Silabukan soil as planting medium at Faculty of Sustainable Agriculture, Universiti Malaysia Sabah. This study aimed to compare the agronomic characteristics between Beruang, Kelopak and Tiga Bulan dryland paddy varieties under normal and drought stress conditions. Two cycles of drought treatments were applied at 50% heading stage to agronomic replicates. Completely randomized design was used in this study. Results showed that early maturing Kelopak variety performed the best under normal condition, exhibited the highest extrapolated yield (4.856 tonnes ha^{-1}). Drought stress at 50% heading stage led to a significant decrease in relative water content, percentage in filled grain and grain yield per plant. In this study, Tiga Bulan variety was the most drought-tolerant variety, which experienced the lowest percentage of yield reduction (28%) and gave the highest grain yield per plant (19.438 g $plant^{-1}$) under drought stress condition. Early flowering Tiga Bulan variety with slightly longer days to leaf rolling was selected for proteome analysis. Normal and completely rolled flag leaves of Tiga Bulan variety after first cycle of drought treatment at grain filling stage were subjected to Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis to compare their protein expressions. SDS-PAGE analysis revealed one and two bands were up- and downregulated significantly in response to drought respectively. These proteins may be involved in drought mechanism. Recent trend in climate change have predicted a further increase in drought intensity, therefore efforts to develop drought tolerant varieties are vital to sustain the paddy production worldwide.

KESAN KEMARAU TERHADAP CIRI-CIRI AGRONOMI DAN CORAK PROTEOME BAGI VARIETI PADI HUMA SABAH TERPILIH

ABSTRAK

Kemarau merupakan faktor utama yang menghadkan pengeluaran padi di seluruh dunia. Oleh itu, toleransi padi terhadap kemarau merupakan salah satu aspek yang terpenting dalam program pembiakan padi. Satu kajian berkenaan dengan varieti padi huma tempatan Sabah telah dijalankan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah. Tanah Silabukan digunakan sebagai medium tanaman dalam kajian ini. Objektif kajian ini adalah untuk membandingkan ciri-ciri agronomi antara varieti Beruang, Kelopak dan Tiga Bulan dalam keadaan normal dan tekanan akibat daripada kemarau. Dua kitaran rawatan kemarau telah diberikan kepada replikasi agronomi pada peringkat 50% pembungaan. Susunan reka bentuk Rawak Lengkap digunakan dalam kajian ini. Hasil kajian menunjukkan bahawa varieti terbaik dalam keadaan normal adalah varieti Kelopak yang berbunga awal dan menunjukkan unjuran hasil yang tertinggi (4.856 tan ha^{-1}). Rawatan kemarau pada peringkat 50% pembungaan menyebabkan penurunan bererti bagi kandungan air relatif, peratusan bijiran penuh dan hasil bijiran. Dalam kajian ini, varieti Tiga Bulan mempunyai ciri toleransi kemarau yang terbaik, di mana varieti ini mengalami peratusan pengurangan hasil yang terendah (28%), malahan memberikan hasil bijirin yang tertinggi (19.438 g $pokok^{-1}$) dalam keadaan tekanan akibat daripada kemarau. Varieti Tiga Bulan dipilih untuk analisis proteome kerana berbunga lebih awal dan menunjukkan tempoh masa yang panjang untuk perggulungan daun. Daun bendera bagi varieti Tiga Bulan yang normal dan bergulung sepenuhnya berikutan rawatan kemarau yang pertama pada peringkat pegisian bijiran tertakluk kepada analisis Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) untuk membandingkan ekspresi proteome mereka. Analisis SDS-PAGE menunjukkan peningkatan intensiti satu band dan pengurangan intensiti dua band bererti sebagai tindak balas terhadap tekanan kemarau. Protein yang dikenalpasti ini mungkin terlibat dalam mekanisme kemarau. Trend terkini dalam perubahan iklim meramalkan peningkatan berterusan intensiti kemarau pada masa akan datang, oleh yang demikian, usaha untuk memperkenalkan varieti yang toleran terhadap kemarau adalah penting untuk mengekalkan pengeluaran padi di seluruh dunia.

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2ME	2-Mercapto-Ethanol
AcDP	Acetone Dried Powder
ANOVA	Analysis Of Variance
cm	centimetre
$cmol_c\ kg^{-1}$	centimoles of positive charge per kg of oven dry soil
CRD	Completely Randomized Design
CBB	Coomassie Brilliant Blue
DAS	Days After Sowing
°C	degree Celsius
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
FSA	Faculty of Sustainable Agriculture
g	gram
g	gravitational force
kDa	kilodaltons
kg	kilogram
LSD	Least Significant Difference
MPa	Megapascal
µL	microlitre
mg	milligram
mL	millilitre
mm	millimetre
mM	millimolar
M	molar
M_r	Molecular weight
MOP	Muriate of Potash
N	Nitrogen
%	percentage
PWP	Permanent Wilting Point
P	Phosphorus
P_2O_5	Phosphorus pentoxide
K	Potassium
K_2O	Potassium oxide
RO	Reverse Osmosis
rpm	revolutions per minute
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SAS	Statistical Analysis System
TCA	Trichloroacetic Acid
TSP	Triple Superphosphate
v/v	volume/ volume
H_2O	Water
w/v	weight/ volume

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

Climate change, increasing population pressure, and shrinking water resources are currently the major global concerns. These events often lead to drought events, which adversely impact the crop production and food security worldwide (IPCC, 2007). Paddy, being one of the world's most vital staple food crop, is very prone to drought stress.

In the past, change in climate possessed severe impacts in Malaysia. One of the notable impacts was the occurrence of drought events. As an evidence, 1997/98 El Nino related drought event caused severe impacts to the environment, economic and social activities of the country (Simon, 2014). Water shortages experienced during this drought event had significantly reduced the paddy production and affected the welfare of paddy farming community.

Given that there are about 674,000 hectares of paddy fields (both wetland and dryland paddy), producing about 2.6 million tonnes of paddy annually in Malaysia (DOA Peninsular Malaysia, 2014), the occurrence of drought would have a threatening impact on the country's food production. Climatic changes such as uncertainty of rainfall and uneven distribution of rainwater are expected to increase the frequency of drought events in rain-fed paddy-growing areas in the coming years (Barh, 2014). The yield of rain-fed paddy is estimated to decline significantly following these drought events (Rice Today, 2009).

In the East Malaysia particularly Sabah, dryland paddy cultivation is mostly practiced by the rural communities as a self-sustaining agricultural activity. Beruang, Kelopak and Tiga Bulan are among the Sabah's indigenous rain-fed dryland paddy varieties. The grain yield of dryland paddy varieties are much lower as compared to that of wetland (Hanafi *et al.*, 2009), however, these varieties have been widely recognized to be more drought tolerant than wetland paddy (Ding *et al.*, 2013), as well as highly valued for their unique aroma, grain quality and palatability (Siambun, 2003).

Silabukan soil in Sandakan consists of acrisols, which are acidic soils with a layer of clay accumulation (Panagos *et al.*, 2011). Agricultural uses on acrisols are often limited since it lacks of plant nutrients, high in aluminium toxicity, slaking or crusting easily and highly prone to erosion (ISRIC, 2015).

In this study, drought stress was applied during the 50 percent heading (agronomy analysis) and grain filling (proteomic analysis) stages since these two stages of paddy are highly sensitive to drought stress (He and Serraj, 2012). By comparing the agronomic characteristics of three different rain-fed dryland paddy varieties under drought stress condition, responses of these varieties towards drought stress and superior variety suitable to be planted using Silabukan soil in Sandakan can be identified.

Proteins are the building blocks of all cells, where the biological functions are exerted. Proteins are needed to carry out dynamic processes of life maintenance, defense, replication and reproduction (Breda *et al.*, 1999). Proteomics is defined as the study of structures and functions of proteins (Graves and Haystead, 2002). Since proteome is dynamic, its analysis is often used to monitor global changes in the protein expression of drought affected crops (Salekdeh *et al.*, 2002), particularly in paddy (Komatsu and Tanaka, 2005).

Since drought tolerance is among the important paddy breeding goals (Kumar *et al.*, 2007), the discovery of drought responsive proteins would be useful in the development of high yielding-drought tolerant paddy varieties. In this study, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was used to compare and analyze the effect of drought in the proteomic expressions of dryland paddy variety.

1.2 Justification

In Sabah, research on rain-fed dryland paddy varieties has often been neglected due to its low yield as compared to wetland paddy. This contributed to the scanty agronomy information available for Beruang, Kelopak and Tiga Bulan dryland paddy varieties. However, without these information, efforts in enhancing the quality and yield of these paddy varieties will be difficult to conduct. Thus, in this study, valuable information on the agronomic characteristics of these dryland paddy varieties were provided. These information will be able to assist paddy farmers in the design of planting manuals and the selection of these varieties for planting using Silabukan soil in Sandakan.

Proteomic analysis for dryland paddy varieties in response to drought stress has not been well investigated. Alterations in cellular protein expression are responsible for drought tolerance in dryland paddy. The identification of drought responsive proteins using molecular approach able to assist paddy breeding projects worldwide, by improving the drought tolerance of high-yielding but drought-susceptible paddy varieties.

In this study, flag leaf was used for proteomic analysis since it is the major carbohydrate producer, and photosynthetic carbohydrates are the primary source of paddy's grain yield. Since leaf rolling is most noticeable on flag leaves, leaf rolling scale was used as an indicator for drought stress treatment. Two staining methods, namely Coomassie Brilliant Blue (CBB) and silver staining, were compared in this study to obtain an optimized method for proteomic analysis of dryland paddy plants.

This study also demonstrated the feasibility of rain-fed dryland paddy cultivation in Silabukan associations of Sabah. Superior variety suitable to be planted using Silabukan soil was identified among three different dryland paddy varieties in this study as well.

1.3 Objectives

(1) To compare the agronomic characteristics between Beruang, Kelopak and Tiga Bulan dryland paddy varieties under normal and drought stress conditions using Silabukan Soil.

(2) To compare the proteome patterns expressed using SDS-PAGE of dryland paddy variety that exhibited the longest day to leaf rolling under normal and drought stress conditions using Silabukan Soil.

1.4 Hypotheses

H_0 = There is no significant difference in the agronomic characteristics between Beruang, Kelopak and Tiga Bulan dryland paddy varieties under normal and drought stress conditions using Silabukan Soil.

H_1 = There is a significant difference in the agronomic characteristics between Beruang, Kelopak and Tiga Bulan dryland paddy varieties under normal and drought stress conditions using Silabukan Soil.

H_0 = There is no significant difference in the proteome patterns expressed using SDS-PAGE of dryland paddy variety that exhibited the longest day to leaf rolling under normal and drought stress conditions using Silabukan Soil.

H_1 = There is a significant difference in the proteome patterns expressed using SDS-PAGE of dryland paddy variety that exhibited the longest day to leaf rolling under normal and drought stress conditions using Silabukan Soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Paddy

Paddy (*Oryza sativa* L.), in the family of Poaceae, is an annual, semi aquatic grass primarily cultivated worldwide for rice production (OECD, 1999). Paddy is an autogamous plant that propagates via seeds (OECD, 1999). It can be cultivated as wetland or dryland paddy (Hanafi *et al.*, 2009). The genome size of paddy is smaller as compared to other cereals, thus it is used as an ideal model plant for molecular studies of monocots (Ali and Komatsu, 2006).

Since paddy is a C3 plant, its photorespiration rate is high (Sage, 2004) and water use efficiency is low. Thus, paddy generally is vulnerable to water stress (Karki *et al.*, 2013). Besides, most of the paddy varieties are classified as short-day plants, which means flowering only takes place when daylength is shorter than a certain critical daylength (less than 10 hours) (Eckardt, 2000).

2.2 Morphology of Paddy

According to Hardke (2013), paddy is an annual, monocotyledon grass that forms a fibrous root system; bears erect, hollow, jointed culms (up to 1.2 m tall); develops narrow, long flat leaves (up to 30 cm long and ~1.2 cm broad) with parallel veins that joined to the leaf sheaths with collars; forms sickle-shaped, hairy auricles and small ligules; produces erect or nodding terminal panicles, each of which bears a number of long, flat and one-flowered spikelets (~7 mm long).

Paddy forms multiple tillers, each of which consists of a culm and leaves, with or without panicles. Panicles emerge within a flag-leaf sheath on the uppermost node of a culm and bear spikelets (reproductive structures), whereby each spikelet consists of a single floret (six stamens and a plumose ovary with two branches) and two glumes, enclosed by a rigid lemma. Grain, which consists of an embryo, endosperm, pericarp and testa, and surrounded by hull (lemma and palea) develops from each spikelet (OECD, 1999).

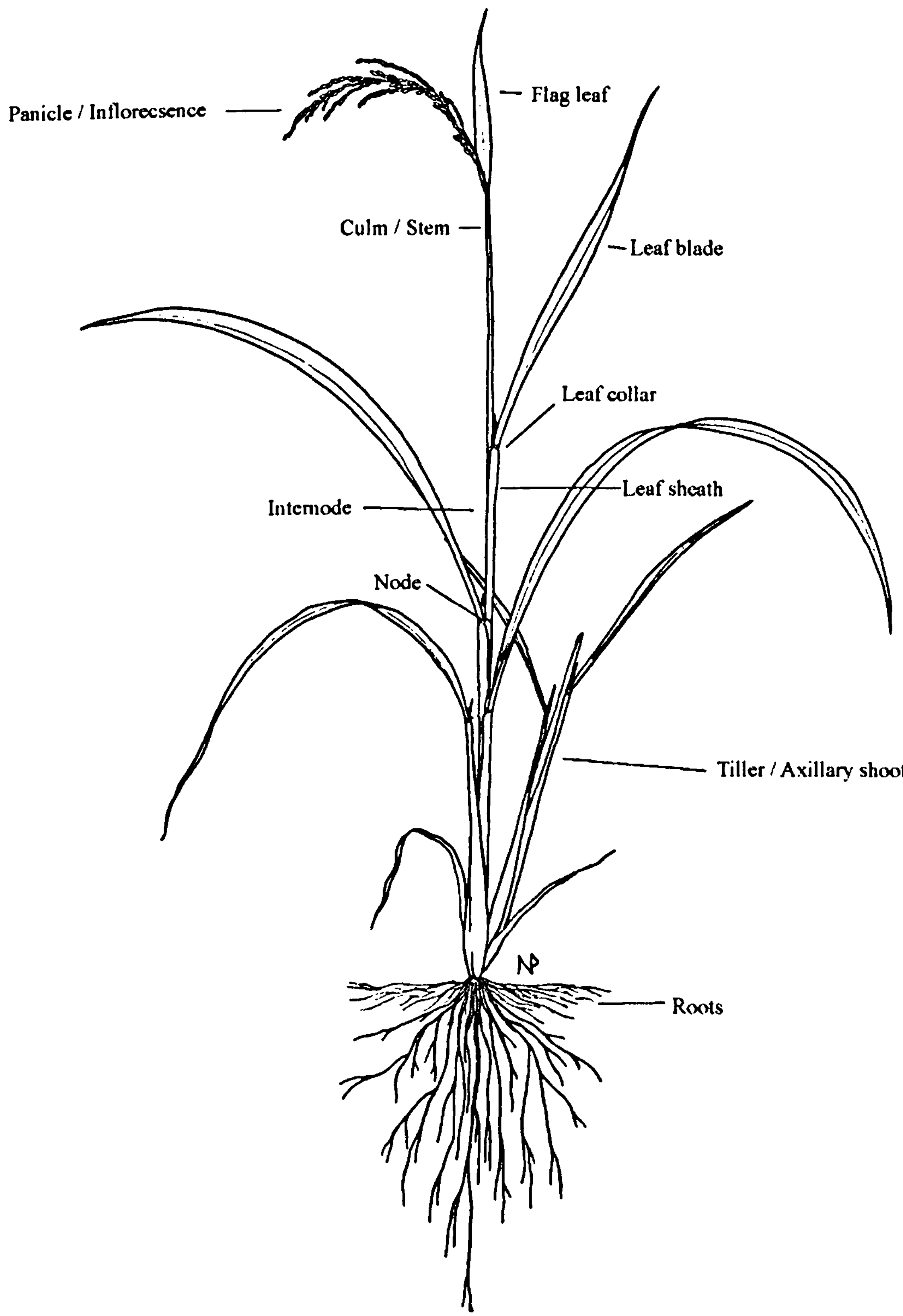


Figure 2.1 Morphology of Paddy
Source: Polato, n.d.

2.2.1 Flag Leaf

Flag leaf (uppermost leaf) is the last mature leaf before flowering takes place in paddy (IRRI, 2007). Typical length of flag leaf ranges between 100 to 300 mm; whereas its width ranges between 10 to 25 mm (Pask *et al.*, 2011). It is the major carbohydrate producer in paddy plant, whereby it provides more than 50% of the photosynthetic products mainly to the panicle (Zhang *et al.*, 2015). IRRI (2007) reported that flag leaf contributes largely to the grain filling process in paddy.

According to Yue *et al.* (2006), size and shape of flag leaf influence the generation of photosynthetic products and thus are directly responsible for the yield potential of paddy. It was found that the flag leaf area was positively correlated with the potential yield and number of spikelet per panicle of paddy.

2.3 Growth Phases of Paddy

According to Hardke (2013), growth of paddy can be divided into three different phases, namely vegetative (germination to panicle initiation (PI)), reproductive (PI to heading) and grain filling and ripening (heading to maturity) (Figure 2.2).

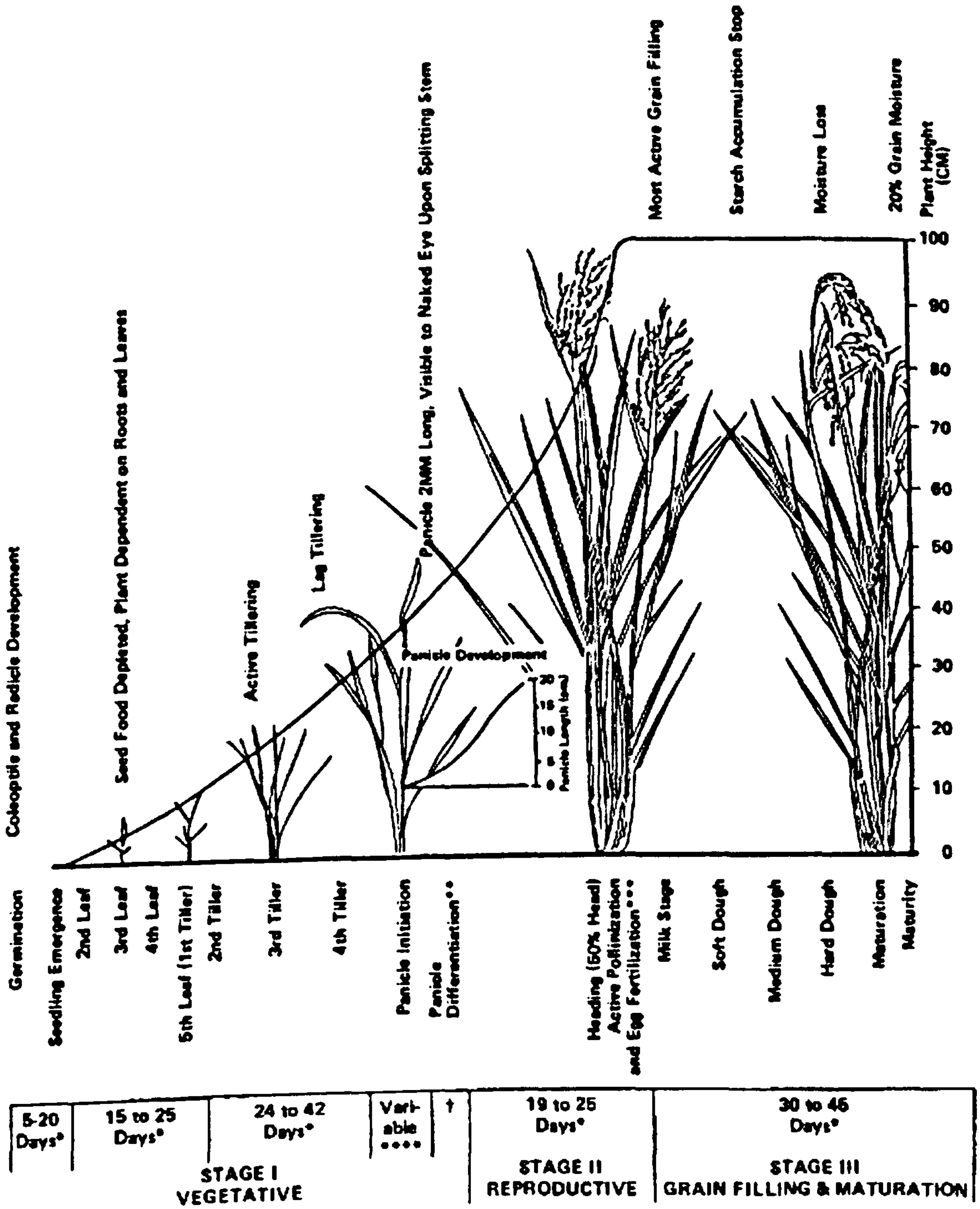
2.3.1 Reproductive Phase of Paddy

Phenomena such as culm elongation, decrease in tiller number, booting, emergence of flag leaf, heading and flowering occur during the reproductive phase of paddy (Hardke, 2013). This phase which usually lasts for approximately 35 days in most paddy varieties, is known to be the most sensitive towards drought stress (Maisura *et al.*, 2014).

Heading is the period when the panicle begins to exsert from the boot, and normally occurs for 10 to 14 days. 50% heading is defined as the time when 50 percent of the panicles have at least partially exserted from the boot. Flowering or anthesis (opening and closing of the spikelet) generally begins upon panicle exsertion and due to that, it is considered synonymous with heading (Hardke, 2013).

2.3.2 Grain Filling and Ripening Phase of Paddy

This phase extends from flowering to physiological maturity and is characterized by grain growth (Fageria, 2014). Physiological maturity is defined as the attainment of maximum seed dry weight (Li *et al.*, 2011). Grain filling and ripening can be divided into four stages, namely milk, soft dough, hard dough and maturity stage (Hardke, 2013). Grain filling process can be severely affected by drought, low solar radiation, N deficiency, adverse temperature and panicle blast (Wada *et al.*, 2011).



†3 to 5 days.
*Under warm conditions use the lower number of days and for cool conditions use the larger number of days.
**The reproductive stage begins with panicle initiation.
***Stage III begins when 50% of the florets are pollinated.
****Variable time - 0 to 25 days (dependent upon variety).

Figure 2.2 Growth Phases of Paddy
Source: Hardke, 2013

2.4 Dryland Paddy

In Malaysia, dryland paddy refers to rain-fed paddy that is planted on aerobic rice soil either in upland or lowland dry areas during main season (1st August to 28/29 February of the following year) (IRRI, 2007; DOA, 2014). Since rainwater is the only water source for dryland paddy, thus site selection is vital for its optimum growth, whereby areas with frequent and well-distributed rains (more than 1200 mm/ annum) are preferable for its cultivation (Toktok, 2003).

The recommended planting distance for dryland paddy is 20cm (between rows) x 20cm (between plants) or 30cm x 30cm (Oikeh *et al.*, 2008). Besides, Siambum (2003) reported the use of 30 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1} as basal dressing, and 30 kg N ha^{-1} as top dressing for her experiment on Sabah's dryland paddy varieties.

2.4.1 Sabah's Dryland Paddy Varieties

In Sabah, cultivation of dryland paddy varieties is usually practiced by the rural communities on hill slopes, mainly for subsistence farming (Hanafi *et al.*, 2009). Even though dryland paddy varieties are popular among the local farmers owing to their desirable qualities such as unique fragrance, grain quality and palatability, however, these varieties have not been commercialized due to their low and unstable grain yields (Mariam *et al.*, 1991; Siambum, 2003). Kelopak (AN 1211) is one of the early-flowering and high-yielding dryland paddy varieties in Sabah (Siambum, 2003).

Table 2.1 Agronomic parameters of Kelopak dryland paddy variety planted in the main season on a hill slope plot at Tamu Darat Agriculture Station, Kota Belut over three consecutive years (1998-2000)

Agronomic Parameter	Kelopak (AN 1211)
Culm Height (cm)	114.50
Panicle number per hill	7
Panicle length (cm)	25.00
Day to flower (days)	77
Number of spikelet per panicle	119-160
1000-grain weight	20.23-25.00
Percentage of ripened grain (%)	65.47-80.80
Harvest index (%)	33.90-42.83
Yield (t ha^{-1})	1.94

Source: Siambum, 2003

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