GROWTH PERFORMANCE, SOIL CHEMICAL PROPERTIES AND PROTEOME ANALYSIS OF TR-8 PADDY VARIETY UNDER DIFFERENT APPLICATION RATE OF CHITOSAN AND NPK FERTILIZER IN SILABUKAN SOIL

WOO MUN KIT

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ABSTRACT

A study on the effect of different application rate of chitosan and NPK to the growth performance and proteome pattern of TR-8 paddy variety was conducted. NPK fertilizer was applied at (0 kg N ha^{-1}, 0 kg P_2O_5 ha^{-1}, 0 kg K_2O ha^{-1}), (90 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, 70 kg K_2O ha^{-1}), (130 kg N ha^{-1}, 70 kg P_2O_5 ha^{-1}, 110 kg K_2O ha^{-1}) and (170 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 150 kg K_2O ha^{-1}) in 3 split applications. Chitosan was applied in the soil at 0%, 0.1%, 0.2% and 0.3% (w/w) of soil respectively. Agronomic parameters and soil samples were analyzed at harvesting stage while proteome analysis was conducted using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 2 different root samples at the age of 90 days. The results showed that chitosan and NPK fertilizer did not show any interaction on the vegetative growth and yield components in paddy and also soil chemical properties. The effect of chitosan alone did not contribute to the growth and yield of paddy. NPK fertilizer, on the other hand, did affect the growth and yield significantly when applied to those without fertilization. The soil nitrogen and soil pH was not affected by NPK fertilizer while soil available phosphorus was significantly affected by NPK fertilizer. This showed that NPK fertilizer played an important role on the growth performance in paddy. Based on SDS-PAGE analysis, there were total of 14 protein bands detected. Two of the bands showed two-folds in protein expression in roots with and without treatments. The expression could be plant response to nutrient stress and defense mechanisms. Overall, fertilization at 170 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1} gave the best growth performance. However, application at 130 kg N ha^{-1}, 70 kg P_2O_5 ha^{-1} and 110 kg K_2O ha^{-1} could have similar effect and this could help farmers to reduce input cost on fertilizer.

PRESTASI PERTUMBUHAN, SIFAT-SIFAT KIMIA TANAH DAN ANALISI PROTEOME VARIETI PADI TR-8 DI BAWAH KADAR APLIKASI KITOSAN DAN BAJA NPK YANG BERBEZA DALAM TANAH SILABUKAN

ABSTRAK

Satu kajian tentang kesan kitosan dan baja NPK dalam kadar yang berbeza terhadap prestasi pertumbuhan dan corak proteome padi varieti TR-8 telah dijalankan. Baja NPK telah digunakan pada kadar (0 kg N ha^{-1}, 0 kg P_2O_5 ha^{-1}, 0 kg K_2O ha^{-1}), (90 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, 70 kg K_2O ha^{-1}), (130 kg N ha^{-1}, 70 kg P_2O_5 ha^{-1}, 110 kg K_2O ha^{-1}) dan (170 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 150 kg K_2O ha^{-1}) dalam 3 pecahan. Kitosan diaplikasikan dalam tanah pada kadar 0%, 0.1%, 0.2% dan 0.3% berat tanah. Parameter agronomik dan sampel tanah telah dianalisis pada tahap kematangan sementara analisis proteome telah dijalankan dengan menggunakan sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) ke atas 2 sampel akar yang berbeza pada usia 90 hari. Keputusan menunjukkan kitosan dan baja NPK tidak menunjukkan interaksi atas pertumbuhan vegetatif dan komponen hasil padi dan juga sifat-sifat kimia tanah. Kitosan dengan sendirinya tidak menyumbang kepada pertumbuhan vegetatif dan hasil padi. Manakala, baja NPK memberi kesan perbezaan ketara antara tumbuhan yang dibaja dengan yang tidak dibaja. Nitrogen dan pH tanah tidak dipengaruhi manakala fosforus tanah dipengaruhi secara ketara oleh baja NPK. Ini menunjukkan baja NPK memainkan peranan yang penting terhadap prestasi pertumbuhan padi. Berdasarkan analisis SDS-PAGE, sejumlah 14 band protein telah dikesan. Dua band daripada jumlah band tersebut menunjukkan 2 kali ganda dalam ekspresi protein akar dengan dan tiada rawatan. Ekspresi tersebut mungkin adalah respons tumbuhan ke atas stres nutrisi dan mekanisasi pertahanan. Secara keseluruhannya, pembajaan pada 170 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} dan 150 kg K_2O ha^{-1} memberikan prestasi pertumbuhan yang terbaik. Walaubagaimanapun, aplikasi pada 130 kg N ha^{-1}, 70 kg P_2O_5 ha^{-1} dan 110 kg K_2O ha^{-1} boleh memberikan kesan yang sama dan ini akan membantu para petani untuk mengurangkan kos input baja.

TABLE OF CONTENTS

Content **Page**

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percent
µ	Micro
AcDP	Acetone Dried Powder
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
BSA	Bovine Serum Albumin
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
K	Potassium
kDa	Kilodalton
kg	Kilogram
kg ha^{-1}	Kilogram Per Hectare
L	Litre
LC-MS	Liquid Chromatography Mass Spectrometry
M	Molar
m^2	Square Meter
mg	Milligram
mL	Millilitre
mM	Millimolar
MOP	Muriate of Potash
N	Nitrogen
nm	Nanometer
°C	Degree Celcius
P	Phosphorus
ppm	Part Per Million
rpm	Rotation Per Minute
SAS	Statistical Analysis System
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
TSP	Triple Super Phosphate
V	Volt
v/v	Volume Over Volume
v/w	Volume Over Weight
w/w	Weight Over Weight

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Background

Paddy is one of the most cultivated crops as staple food in most part of the world especially in various regions in Asia. According to Hays *et al.* (2005), paddy areas in Southeast Asia (SEA) comprise about 30% of the world total rice production in which rice serves as a food crop and export commodity in the region. In Malaysia, cultivation of wetland paddy uses approximately 674,000 hectares of land with total paddy production of 2.6 million metric tonnes in 2013 (Department of Agriculture, 2014). By comparing with the total paddy production of 2.5 million metric tonnes in 2011, the production of paddy is indeed increasing every year. Despite the increase in paddy production, Malaysia's rice yield per capita declines every year (Sung, 2011). This demands better performances of the paddy crops in terms of growth and yield in order to secure self-sufficient of rice to people.

Since then, various different conventional approaches have been used to improve the performance of paddy. Although there is significant improvement of paddy production through increase in fertilizer application rate, it is costly and may be a burden to small holders and farmers. Non-conventional approaches such as biotechnology and genetic engineering to produce many new varieties have been studied in the last decade. Furthermore, application of plant growth regulator has been one of the important practices as it is convenience, labour and cost efficiency (Mondal *et al.*, 2012). It is reported that plant growth regulator is used for better growth and yield of the crops.

The effects of chitosan on growth performances and development in many different plant species have been studied recently. Chitosan is a natural amino polysaccharides derived from chitin which is extracted from the main structural component found in most exoskeleton of crustaceans such as shrimps, crab and lobster as well as in fungal cell walls (Deepmala *et al.*, 2014). Chitin and chitosan are co-polymers found together naturally. They are environmentally friendly and easily degradable. Chitosan has a wide range of application and benefits in agriculture. A few studies have reported that application of chitosan is able to improve the growth performances and yield of paddy (Boonlertnirun *et al.*, 2007; Hadwiger, 2013). According to Boonlertnirun *et al.*, (2008), chitosan greatly helps in agriculture even without using chemical fertilizer by increasing the microbes in soil in large scale and converts organic form of nutrients into inorganic forms through mineralization in which can be easily absorbed by the plant roots, so that the more nutrients can be absorbed by the plant for growth.

However, research works of chitosan as a soil amendment on the growth and yield of paddy are rare compared to those in which chitosan is used as foliar application for growth performance, yield, plant protection and environmental stresses resistance in crops such as paddy (Boonlertnirun *et al.*, 2007; Boonlertnirun *et al.*, 2008), okra (Mondal *et al.*, 2012) and mung bean (Mondal *et al.*, 2013). This study was to investigate the potential of chitosan as soil amendment to the growth performance and yield of paddy in combination of NPK chemical fertilizer. Furthermore, proteome analysis can provide information which assists the improvement of growth performance and yield of paddy when applied with both treatments (Barh, 2014).

1.2 Justification

Paddy is the most important staple food in Malaysia. As the population increases, the demand is also increases and hence improvement of the paddy production is necessary to meet the market demand. Due to the limitation in increasing the planted land area, farmers tend to increase the fertilizer application rate in order to increase the yield. This causes the cost of input to be increase drastically and may be a burden to most of the farmers. Furthermore, excessive fertilizer application especially nitrogen promotes lodging and diseases in paddy. In fact, excessive nitrogen can result in yield reduction as much as applying too little nitrogen (Stevens and Dunn, n.d.). Therefore, other alternative is needed to improve the production of paddy while avoiding excessive fertilizer application to the crop. TR-8 paddy variety was used for this study due to its commercial value and it is widely consumed by people in Sabah.

Meanwhile, chitosan is readily available either in processed form or in raw material. For instance, in Sabah where it is one of the major in sea production in Malaysia especially in shrimp farming which accounting for 42% of total shrimp farming areas (Hashim and Kathamuthu, 2005) has abundant raw materials for chitosan production, which is a way to create an added value to the materials. Furthermore, readily made chitosan is not very costly. Since it is eco-friendly and easily degradable, this can be a new research platform to improve the performance of the crop without harming the environment.

Silabukan soil is an unfertile soil, thus it is suitable to be tested with various soil amendments and fertilizers on the growth performance of paddy. Furthermore, the result data obtained from this study can be referred and used in Faculty of Sustainable Agriculture on the paddy project as well as in the local areas around since the soil series in this area is Silabukan association series. Thus, this study would be carried out to determine the optimum application rate of chitosan incorporated with NPK fertilizer in the soil and the result could be used as reference to improve the paddy production. Proteome analysis could be used to identify protein related to growth performance. Root was used as it is the main part of plant in nutrient absorption and is the first part of the plant to contact with the nutrient sources from NPK fertilizer and chitosan in the soil.

1.3 Significance of Study

This study would provide information on the growth performance and yield of TR-8 paddy variety planted in Silabukan soil using chitosan together with NPK fertilizer. The result of this study could be used as reference for any paddy project in Faculty of Sustainable Agriculture and also to farmers who are planting paddy in Silabukan soil. This study aimed to reduce the input of NPK fertilizer by adding with chitosan which is cheap and easily available. The proteome analysis would help researchers to study on proteins that are affected in response to chitosan that related to growth.

1.4 Objectives

The objectives for this study are:

1) To determine the effect of different application rate of chitosan and NPK fertilizer on the growth performances and yield of TR-8 paddy variety in Silabukan soil.

2) To determine the effect of different application rate of chitosan and NPK fertilizer on the soil chemical properties in Silabukan soil.

3) To investigate the proteome expression pattern of different levels of chitosan and NPK fertilizer rate on TR-8 paddy variety in Silabukan soil.

1.5 Hypotheses

H_{O1}: There is no significant effect in different rate of chitosan and NPK fertilizer on the growth performances and yield of TR-8 paddy variety in Silabukan soil.

H_{A1}: There is significant effect in different rate chitosan and NPK fertilizer on the growth performances and yield of TR-8 paddy variety in Silabukan soil.

H_{O2}: There is no significant effect in different rate chitosan and NPK fertilizer on the soil chemical properties in Silabukan soil.

H_{A2}: There is significant effect in different chitosan and NPK fertilizer rates on the soil chemical properties in Silabukan soil.

H_{O3}: There is no significant difference in proteome expression under different application rate of chitosan and NPK fertilizer on the growth performances and yield of TR-8 paddy variety in Silabukan soil.

H_{A3}: There is a significant difference in proteome expression under different application rate of chitosan and NPK fertilizer on the growth performances and yield of TR-8 paddy variety in Silabukan soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Paddy

Paddy is a grass plant that belongs to the family Gramineae, subfamily Oryzoideae, tribe Oryzeae and genus *Oryza*. There are two cultivated species of paddy; *Oryza sativa* (L) and *Oryza glaberrima* (steud). *Oryza sativa* is widely grown in most part of the world including Asia, parts of Europe and America whereas *Oryza glaberrima* is restricted in Africa (Panda, 2010). *Oryza glaberrima* differs from *Oryza sativa* mainly in a lack of secondary branching on the primary branches of the panicle and in minor differences related to pubescence on the lemmas and length of the ligule (Chang and Bardenas, 1965).The cultivated species of *Oryza sativa* can be further divided into three subspecies; *indica*, *japonica* and *javanica* based on the morphological and physiological characteristics. *Oryza sativa* is a diploid species with 24 chromosomes (2n=24).

2.1.1 Paddy Morphology

The cultivated paddy is generally characterized as a semiaquatic annual grass with round, hollow, narrow and jointed culms together with rather flat leaves and sessile leaf blades and a terminal panicle. The roots are fibrous with rootlets and root hairs. At maturity, the paddy plant has a main stem and a number of tillers. The paddy culm (stem) consists of a series of nodes and internodes arranged in alternate orders. Tillers which are the side shoots are produced from the basal nodes on the main culm known as primary tillers which give out secondary tillers and then secondary tillers branch into tertiary tillers. The leaves consist of blade and the leaf sheath which wraps the culms and are borne at an angle of every node. The uppermost leaf below the panicle is known as flag leaf and the number of leaves on the stem decreases from main culm to primary tillers and to secondary tillers and then to tertiary tillers. Each panicle has spikelets (grains).

The grain, known as caryopsis is a dry one-seeded fruit, with its pericarp fused with the seed coat. The seed consists mainly of husk, pericarp, endosperm and embryo. The surface contains several thin layers of differentiated tissues that enclose the embryo and endosperm. Studies have described the morphological nature of each part in the paddy embryo (Panda, 2010). The cotyledon (scutellum) is a fleshy, shield-shaped which provide food for the germinating embryo. The face of the scutellum is differentiated into a columnar epithelium with elongated cells in contact with endosperm which absorb food from the cotyledon. A poorly developed vascular system consisting of procambial stands is at the middle of the scutellum. The endosperm made up the major part of the seed. The aleurone layer serves as the outer layer of the cells of the endosperm and in cross section, these cells are irregularly hexagonial to polygonal (Panda, 2010). The protein of the seed mostly contained in the aleurone layer. A paddy grain weighs about 10 to 45 mg at 0% moisture content. The grain length, width and thickness vary widely among varieties. The husk weight constitutes about 20% of the total grain weight.

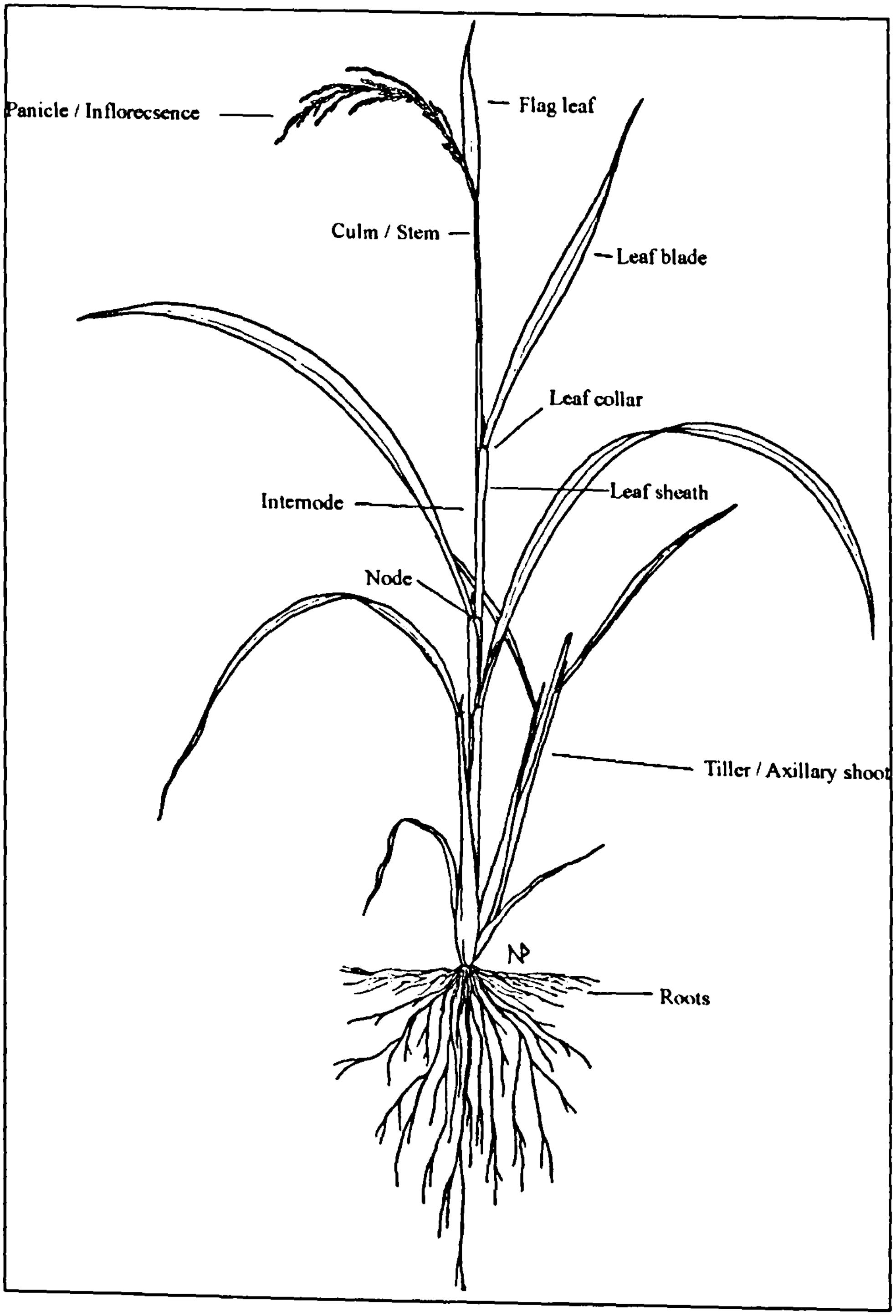

Figure 2.1 Morphology of paddy
Source: Polato, from http://archive.gramene.org/

2.2 Paddy Growth and Development

The growth of paddy can be physiologically divided into three phases; vegetative phase, reproductive phase and ripening phase (Moldenhauer and Slaton, 2001; Panda, 2010).

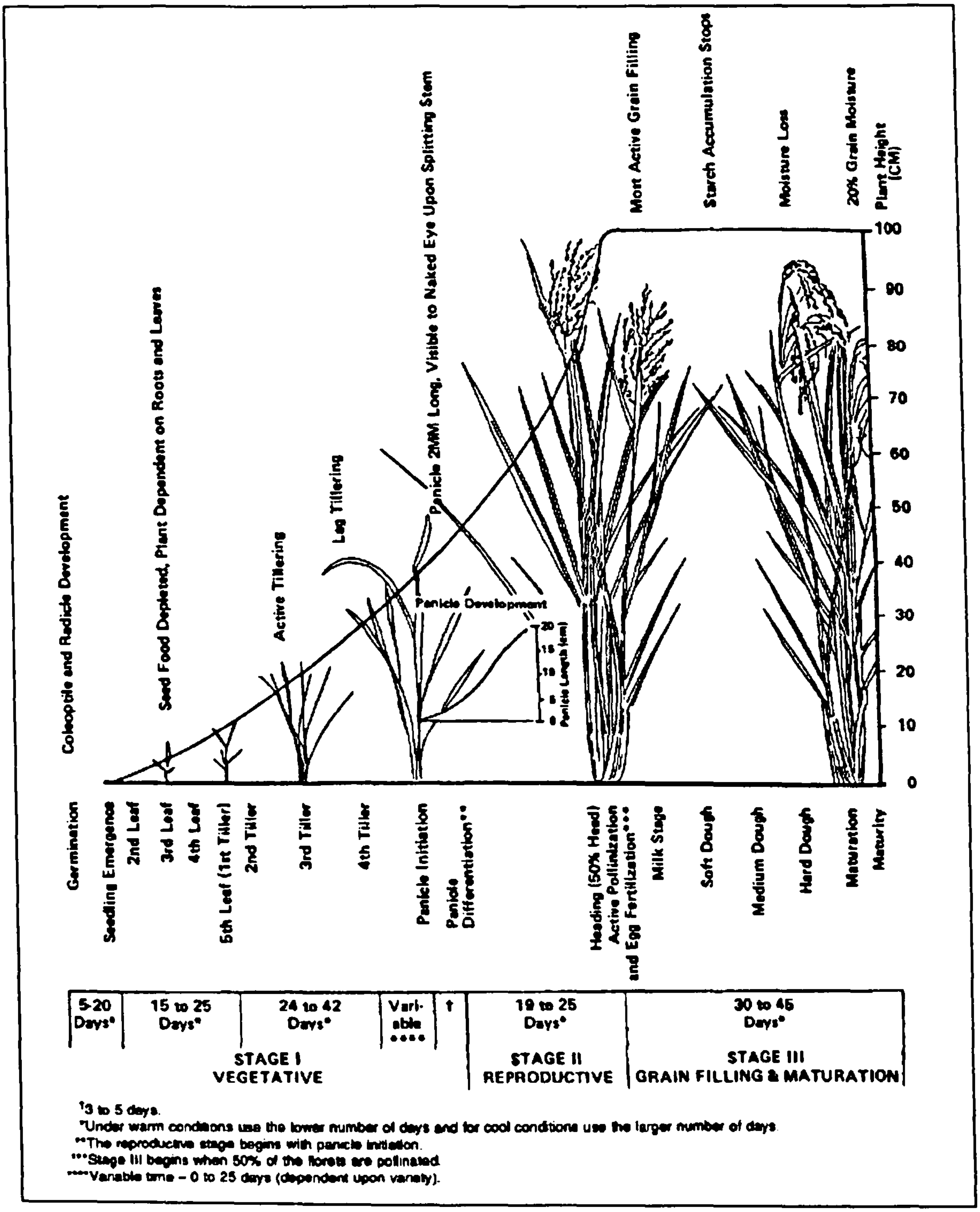

Figure 2.2 Growth development of paddy
Source: Moldenhauer and Slaton, 2001

2.2.1 Vegetative Phase

Vegetative phase is characterized by a gradual increase in plant height and leaf emergence at regular intervals (Moldenhauer and Slaton, 2001). It is divided into four stages known as seedling stage, transplanting stage, tillering stage and vegetative lag

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