EFFECT OF WATER DEPTHS ON THE GROWTH, YIELD, AND PROTEOME PATTERN OF LOWLAND RICE IN SILABUKAN SOIL

LIEW XI YUN

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SAYA: LIEW XI YUN SESI PENGAJIAN: ~~I 2015/2016~~ 2012-2016

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LIEW XI YUN
BR12110048
30 NOVEMBER 2015

VERIFIED BY

1. Assoc. Prof. Dr. Azwan bin Awang
SUPERVISOR

PROF. MAD[illegible]AN AWANG
TIMBALAN [illegible]DEMIK & HEP)
FAKULTI PER[illegible]N LESTARI
UMS KAMPUS SANDAKAN

2. Assoc. Prof. Datuk Hj. Mohd. Dandan @ Ame bin Hj. Alidin
CO-SUPERVISOR

PROF. MADYA HJ. MOHD. DANDAN @
AME HJ. ALIDIN
FELO KANAN
FAKULTI PERTANIAN LESTARI
UMS KAMPUS SANDAKAN

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ABSTRACT

Effects of deeper ponding water depth on plant growth have received renewed attention because of the subsurface irrigation and water table conditions in large sized rice fields. Moreover, rice plants are sensitive to water stress and it will influence the rice yield produced. A study was conducted to evaluate the effect of different water depths on the growth, yield, and proteome pattern of TR8 and TR9 rice varieties in Silabukan soil. The reasons to carry out this study were because there is variation in water depth due to irregularity of levelling, different cultural practices of farmers as they tend to keep different water depths in planting wetland rice, and the increasing of ponding water especially during rainy seasons. In this study, TR8 and TR9 rice varieties were planted in three water depths of 5, 10, and 15 cm with three replicates each under rain shelter covered by zinc plates and net. Rice roots of TR9 rice variety in 5 cm (control) and 15 cm water depths (stressed condition) were harvested at late booting stage for proteome analysis. Trichloroacetic (TCA) acetone method was used for protein extraction. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was done to compare protein profiling in these two water depths with three biological replicates. Results indicated that there was no interaction between different water depths with different rice varieties on the growth and yield. However, TR9 rice variety was found to have better yield than TR8 rice variety under different water depths in Silabukan soil. The mean extrapolated yield of TR9 rice variety showed 0.9 tons/ha more than TR8 rice variety. In SDS-PAGE analysis, one band was found differently expressed in 15 cm water depth. This band could be related to the mechanism of rice crops during waterlogging stress.

KESAN KEDALAMAN AIR TERHADAP PERTUMBUHAN, HASIL, DAN CORAK PROTEOME PADI SAWAH DALAM TANAH SILABUKAN

ABSTRAK

Kesan daripada kedalaman air tinggi atas pertumbuhan tumbuhan telah mendapat perhatian baru disebabkan oleh pengairan subpermukaan dan keadaan aras air di sawah padi yang bersaiz besar. Padi sawah adalah sensitif terhadap stres air dan air akan menjejas hasil padi sawah. Satu kajian telah dijalankan untuk mengkaji kesan kedalaman air yang berbeza terhadap pertumbuhan, hasil, dan corak proteome pada padi varieti TR8 dan TR9 dengan menggunakan tanah Silabukan. Kajian ini dijalankan disebabkan oleh ketidakrataan tanah yang akan menyebabkan perbezaan kedalaman air bertakung, amalan petani yang mengekalkan kedalaman air yang berbeza semasa penanaman padi, dan peningkatan kedalaman air bertakung semasa musim hujan. Dalam kajian ini, kedalaman air setinggi 5, 10, dan 15 cm telah digunakan. Setiap kedalaman air mempunyai tiga replikasi. Kajian ini telah dijalankan di bawah pelindung hujan yang dilindungi dengan plat zink dan jala. Akar padi varieti TR9 dalam kedalaman air 5 cm (kawalan) dan 15 cm (keadaan stres) telah dituai pada tahap bunting akhir untuk analisis proteome. Kaedah trichloroasetik (TCA) aseton telah digunakan dalam ekstrasi protein. Natrium dodecyl sulfat polyacrylamide gel elektrophoresis (SDS-PAGE) telah dijalankan untuk membandingkan profile protein dalam kedua-dua kedalaman air tersebut dengan menggunakan tiga replikasi biologikal. Berdasarkan hasil kajian ini, tiada perbezaan bererti yang didapati daripada interaksi antara kedalaman air yang berlainan dengan varieti padi sawah yang berlainan ke atas pertumbuhan dan hasil padi. Walaubagaimanapun, padi varieti TR9 didapati mempunyai hasilan yang lebih banyak daripada padi varieti TR8. Hasil ekstrapolasi padi variety TR9 menunjukkan 0.9 tan/ha lebih daripada padi varieti TR8. Dalam analisis SDS-PAGE, satu jalur protein berbeza telah didapati dalam kedalaman air 15 cm. Jalur protein tersebut diramalkan adalah berkaitan dengan mekanisasi padi sawah semasa takungan air yang berlebihan.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

1-DE	**One-dimensional electrophoresis**
2-DE	Two-dimensional electrophoresis
2-ME	2-mercaptoethanol
AAS	Atomic absorption spectrometer
AcDP	Acetone dried powder
ANOVA	Analysis of variance
BSA	Bovine serum albumin
CBB	Coomassie Brilliant Blue
CEC	Cation exchange capacity
$cmol_c\,kg^{-1}$	Centimole per kilogram
CRD	Completely randomized design
C.V.	Coefficient variation
FSA	Faculty Of Sustainable Agriculture
g	Acceleration (for centrifugation)
HSD	Tukey's Studentised Range
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometry
MS	Mass spectrometry
PAGE	Polyacrylamide gel electrophoresis
ppm	Parts per million
ROL	Radial oxygen loss
SAS	Statistical Analysis Software
SDS	Sodium dodecyl suphate
TCA	Trichloroacetic acid
UMS	Universiti Malaysia Sabah
WAT	Week after transplanting
X	Times

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Background

Rice (*Oryza sativa* L.) is an important cereal crops in the world. It is the staple food in nearly half of the world's population especially in Asian counties. Rice is planted in areas of high population density and fast population growth (Srivastava and Mahapatra, 2012). In Malaysia, rice crop occupies third position in the most widely planted crop after oil palm and rubber in 2013 which having an overall planted area of 674,332 ha (Department of Agriculture Peninsular Malaysia, 2014a). Out of the overall planted area, 606,846 ha (90%) was planted with lowland rice (wetland rice). The largest state in Malaysia which was planted with lowland rice was Kedah with the overall planted area of 210,327 ha (35%). Whereas, in Sabah, there was an overall of 33,317 ha (5%) area planted with lowland rice.

Rice is usually grown under shallow flooded condition but is also cultured with several meters deep floodwaters, and at the opposite extreme, as an upland rice. Submergence or flooded soil practices are commonly followed in lowland rice cultivation under assured rainfall or irrigated conditions for obtaining better production. The flooded soil condition provides benefits during rice cultivation. According to Buresh *et al.* (2008), around 95% of the world's rice production occurs with soil submerged. In Malaysia, three-quarters of the total fresh water supply is used for irrigated agriculture and more than 90% of that is used in lowland rice cultivation (Teh, 1998). However, lowland rice requires different amount of water at different growth stages. In addition, different water depths will affect the performance of different varieties of rice crops especially their yields. Panda (2010) reported that yields of non-flooded condition rice crops are lower than those rice crops which were grown in flooded soil by 50%. However, rice crops production would only be maximised with optimum water depths.

Waterlogging or excess water is one of the abiotic stress that will affect the growth of plants, which include also rice crops. It is referring as a condition of the soil in which excess water limits gas diffusion (Setter and Waters, 2003). Aerenchyma formation under waterlogged condition has been reported in rice crops (Justin and Armstrong, 1991). This aerenchyma formation in root is a strategy of adaptation to waterlogging. On the other hand, the identification of the proteins that are responsive to abiotic stresses is an essential step towards understanding of the molecular mechanisms underlying the stress responses (Khan and Komatsu, 2004; Liu and Xue, 2006).

Proteomics allow global investigation of structural, functional, abundance, and interactions of proteins at a given time point (Ghosh and Xu, 2014). Boyer (1982) estimated that about 70% of the potential yield is lost due to unfavourable physiochemical environments, even in developed agricultural systems. To meet these challenges, genes and proteins that control the architecture of crop plants and their tolerance or resistance to stress in a wide range of environments need to be identified and characterised to facilitate improvements in crop productivity (Komatsu *et al.*, 2007). Besides, Agrawal *et al.* (2009), also reported that proteomics has been well established as a tool for unraveling global changes in protein profiles as to investigate the "stress" response in crops.

Rice is the crop that needed the proper waterlogged condition for growth and it can be grown on almost all soil types (Panda, 2010). In Sabah, there are four soil associations on mudstone and minor sandstone namely Lungmanis association, Silabukan association, Kalabakan association, and Mawing association (Sabah Forestry Department, 2005). By running of simple ball squeeze test, the soil of Silabukan association forming ribbon and resists breaking (Appendix A1). Therefore, soil of Silabukan association will be referred as fine texture (clayey) soil. According to Hillel (2008), clay is an earthy material that is soft and moldable when wet. Clay particles absorb water, and forming hydration envelopes that cause the soil to well upon wetting and then shrink upon drying.

Soil of Silabukan association is found belongs to the Ultisols and Alfisols orders (Panagos *et al.*, 2011; Selvaradjau *et al.*, 2005). Soil from Ultisols is from fairly intense weathering and leaching process that result in a clay-enriched subsoil dominated by minerals. Whereas, Alfisols are formed from the weathering processes that leach clay minerals and other constituents (Hillel, 2008).

1.2 Justification

This study aims to compare the growth, yield, and proteome pattern of TR8 and TR9 lowland rice varieties under three different water depths (5 cm, 10 cm, and 15 cm) using Silabukan soil.

Water is one of the most important factors influencing the distribution of rice in the world and its performance. In fact, wetland rice needed waterlogged condition for its survival. Flooded soil is favourable for tiller production, vegetative and reproductive growth, and ultimate yields. According to Mikkelsen and Datta (1991), the benefits from the flooded soil are the enhanced availability of nutrients, especially nitrogen (N), phosphorus (P), iron (Fe), and manganese (Mn), enhanced nitrogen fixation, less competition from weeds, and favourable microclimate regulation. They have pointed out that with an adequate water supply, continuous flooding with 5 cm to 7 cm of standing water is the ideal water depth on most soil for the best moisture supply. At this water depth, weeds and insects are controlled significantly with granular chemicals and high nutrient availability with minimum loss of nutrients from fertilizer and soil. Based on the report by Tuong and Bouman (2003), rice is very sensitive to water stress and the attempts to use improper water depths in rice production may result in yield reduction and may threaten food security in Asia.

According to Srivastava and Mahapatra (2012), moisture stress reduces the crop yield most during the critical growth stages. Normally, rice crops showing sensitivity to moisture stress during the formation of the reproductive organs and flowering. Excess water such as improper waterlogging condition during the growth of rice crops hampers rooting and decreases tiller production (Srivastava and Mahapatra, 2012). Tillering is the most important yield attributing factor of rice plant among all the yield attributes whereas the plant height is an index of growth of a crop (Panda, 2010). Waterlogging problems happened especially during raining season. In Peninsular Malaysia, raining season happens especially during April until October while east coast tends to have rainy period in November until February. Continuous rainy days cause the water depth of ponding water increased in rice fields. Besides, the use of different water depths by farmers will cause them to encounter the negative effect during rice crops planting (Abdul *et al.*, 2005). Submergence due to waterlogging up to 50% plant height at any growth stage showing reducing number of yields by one-fourth (Pande *et al.*, 1979). According to Panda (2010), deep submergence of rice up to 15 cm causes greater percolation losses and resulting high leaching losses of mobile nutrients, particularly on coarse textured soils.

Based on the study by Ghosh and Xu (2014), abiotic stress responses in plants occur at various organ levels among which the root specific processes are of particular importance. They have pointed out that, root will absorb water and nutrients from soil and transport to the other plant parts under normal growth condition, playing pivotal roles in maintaining cellular homeostasis. However, root's role will be altered during stress period when roots are forced to adopt several structural and functional modifications. Therefore, this has increased the interest on carry out proteome analysis on the root part in this study.

Excess water will cause problem to rice crops. Therefore, rice crops which are tolerate to excess water are needed. The understanding and the improvement on rice crops especially on their tolerance on abiotic stresses cannot be obtained by studying on the genome solely. The rice crops which are tolerant to excess water can be developed by genetic engineering with the help of proteomics. The study of proteins is important as proteins are the main components of the physiological metabolic pathways of cells (Abhilash, 2008). According to Graves and Haystead (2002), proteins will alter in response to a variety of intracellular and extracellular signals. Therefore, as the environment condition has changed, proteins will alter. From here, the effect of different water depths of submerged soil to the rice variety chosen can be differentiated by comparing their proteome pattern analysis. As a technique, proteomics is advantaged over other "omics" tools such as genomics and metabolomics since proteins are the key players in majority of cellular events (Ghosh and Xu, 2014).

As the area of this study is mainly with soil from the Silabukan association, the decision has made as to utilise the available soil source in this area and to make this study as a reference for those who are particularly planting of lowland rice using Silabukan soil. According to British Government Overseas Development Administration in Sabah (1974) and Sabah Forestry Department (2005), Silabukan association occurs at the north and west of Sandakan, from the Lokan River to Gomantong, from between

Sg Pin and Sg Lamag westwards to Sg Malua, in the Bangan Basin, and in the Inarat lowlands (Appendix A2).

1.3 Significance of the Study

This study may contribute to those scholars in agronomy or soil science as well as to practicing farmers, particularly in planting TR8 and TR9 rice varieties using Silabukan soil. This study will reveal the effects of different water depths of ponding water on Silabukan soil to the growth and yields of TR8 and TR9. The proteome pattern analysis at late booting stage of the selected rice variety allowed more understanding of the mechanisms involved in response to waterlogging. Proteomics enable us to observe and compare the total protein expressed in the root cells under different water depths for the chosen rice variety. The reason to carry out this study is because there is variation in water depth due to irregularity of levelling. In addition, farmers tend to practice different water depths in planting wetland rice crops, and furthermore the water depth of the ponding water will increase especially during rainy season. In Sabah, flooding due to heavy rain tend to occur normally in November until February. According to the report by Davies (2014), Kota Kinabalu saw 147 mm of rainfall in the 24 hours period between 6 and 7 October 2014. Due to that, parts of Kota Kinabalu, Penampang, Inanam, and Tuaran were said to be under one meter of water.

1.4 Objectives

The objectives of this study are:

1. To compare the effects of different water depths of ponding water to the growth and yield of TR8 and TR9 lowland rice varieties using Silabukan soil.
2. To compare root proteome pattern of the selected lowland rice variety between the normal condition and the stressed condition at late booting stage.

1.5 Hypotheses

Hypothesis 1:

H_0: There is no significant difference on the growth and yield between TR8 and TR9 rice varieties under different depths of ponding water using Silabukan soil.

H_1: There is significant difference on the growth and yield between TR8 and TR9 rice varieties under different depths of ponding water using Silabukan soil.

Hypothesis 2:

H_0: There is no significant difference for the root proteome pattern of the selected rice variety between the normal condition and the stressed condition at late booting stage.

H_1: There is significant difference for the root proteome pattern of the selected rice variety between the normal condition and the stressed condition at late booting stage.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice

The dominant rice, *Oryza sativa* which is now cultivated worldwide, is belongs to family Gramineae. According to Chatterjee (1948), there are altogether 23 species of genus *Oryza* of which 21 are wild and two cultivated species, namely *Oryza sativa* and *Oryza glaberrima*. *Oryza sativa* is grown in all rice growing area while *Oryza glaberrima* is confined to the West Africa only (Panda, 2010).

2.1.1 Rice Botany

Rice is from the family of Gramineae, sub-family of Oryzoideae, tribe of Oryzeae, and genus of *Oryza*. Out of the two cultivated species, *Oryza sativa* can be further divided into three sub-species namely indica, japonica, and javanica. These three sub-species are differ from their morphological, physiological characteristics, and their geographical distribution.

2.1.2 Rice Morphology

Rice crops are annual plants with round, hollow, jointed culms, rather flat leaves directly attached to the nodes of the stem, and a terminal panicle. Under favourable conditions, rice crops may grow more than one year (Chang *et al.*, 1965). The vegetative organs of rice crops are composed of the roots, culms, and leaves; while the floral organs are comprising of the panicles, which are actually a group of spikelets on the uppermost node of the culm.

The seeds which are lack of dormancy will germinate immediately upon ripening. The germination of the rice seeds is defined when the appearance of white tip of the coleoptile, and subsequent growth of the coleoptile. When the grain germinates, the sheath or the coleorhiza enveloping the primary root in the embryo protrudes first.

The coleoptile emerges ahead of the coleorhiza. The radicle will then break through the coleorhiza (Figure 2.1). This is then followed by two or more secondary embryonic roots, all of which develop lateral roots. The embryonic roots later die and are replaced by adventitious roots.

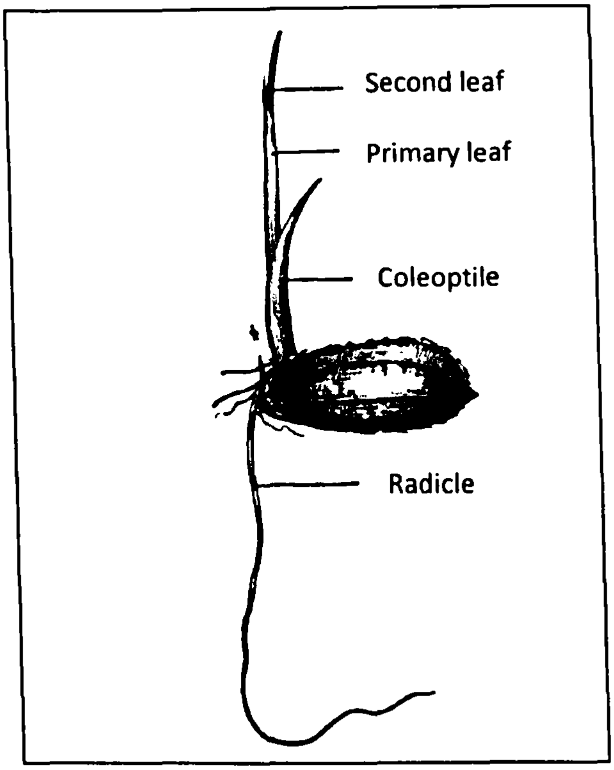

Figure 2.1 Parts of a young seedling germinated under light
Source: Chang *et al.*, 1965

The roots of rice crops are fibrous, and consist of rootlets and root hairs. The embryonic roots have few branches when it germinates and live only for a short time after germination. After that, the embryonic roots are replaced by the secondary adventitious roots which are produced from the underground nodes of the young culms and are freely branched. The coarse adventitious roots will form in whorls from the nodes above ground level as the rice crops continue to grow.

The culm of rice crops is made up of a series of node and internodes in an alternate order. The node bears a leaf and a bud. The bud may later grow into a tiller. The mature internode is hollow, finely grooved and hairless on the outer surface. Tillers

The panicles (Figure 2.3) also known as the inflorescence of the rice crops are a group of spikelets which are located at the uppermost node of the culm. The primary panicle branch can be divided into secondary and sometimes tertiary branches. The panicle branches bear spikelets.

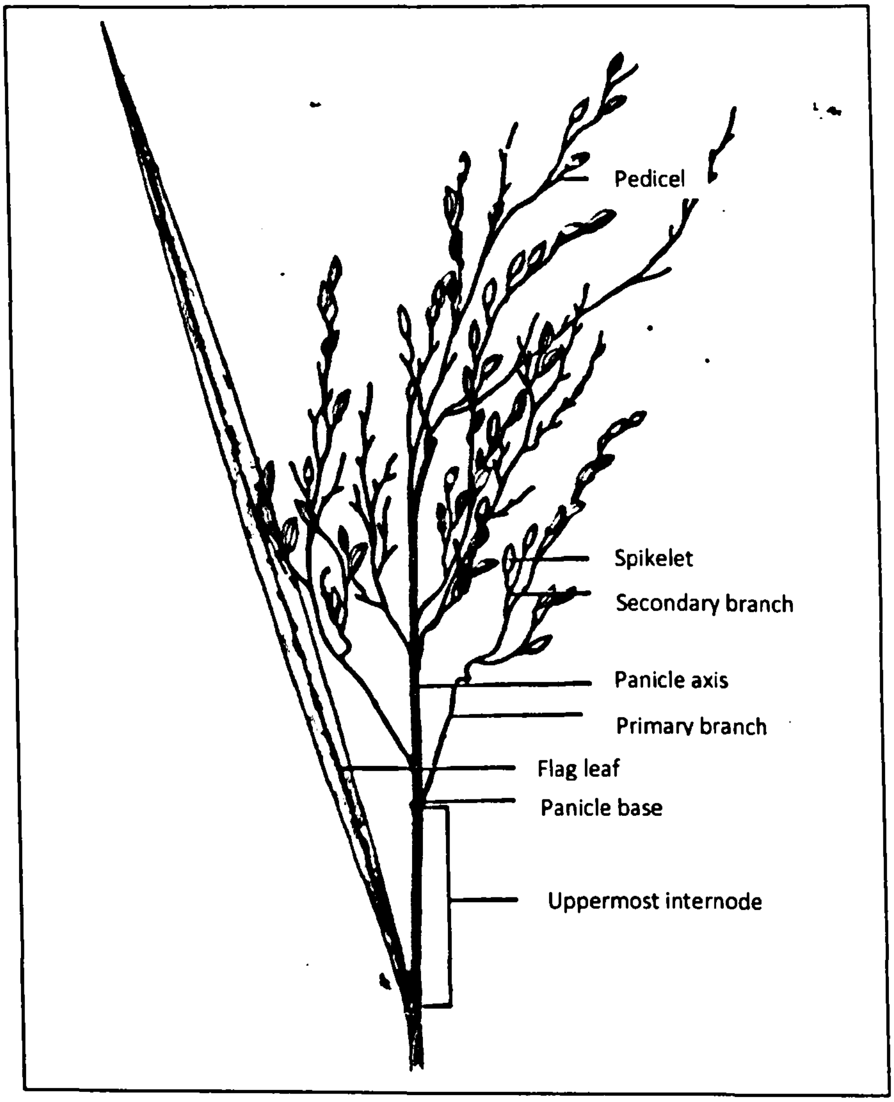

Figure 2.3 Component parts of a panicle
Source: Chang *et al.*, 1965

The spikelets (Figure 2.4) consists of two "outer glumes" (sterile lemmas) with all other floral parts lying in between or above them. The spikelets will become mature and develop into grains. The flower of the spikelets consists of six stamen and a pistil.

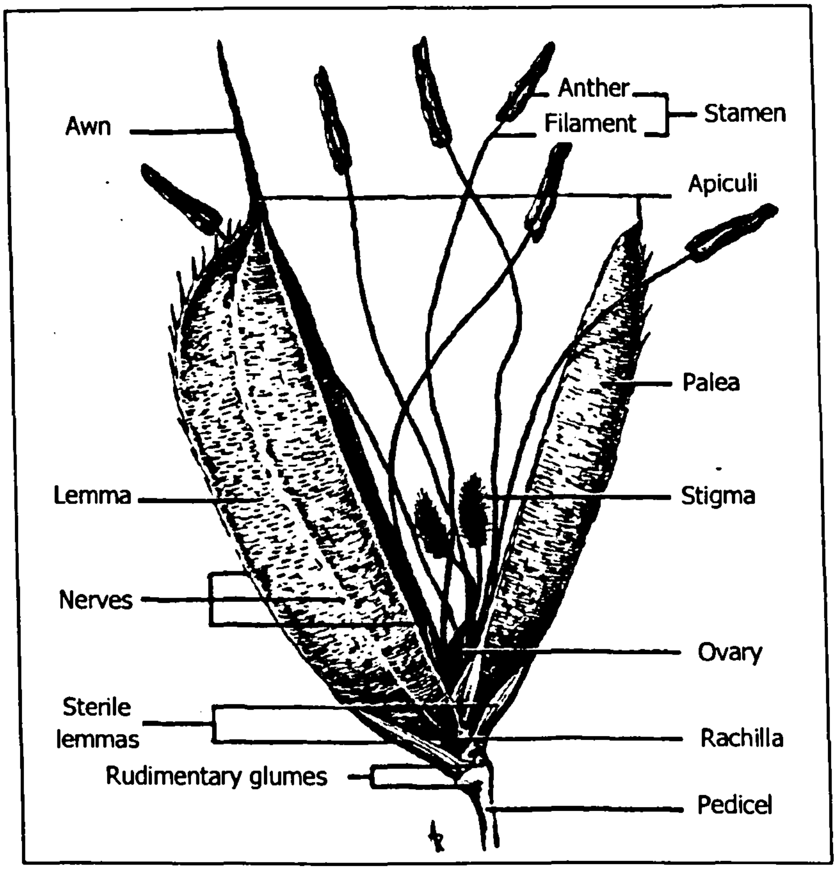

Figure 2.4 Parts of a spikelet
Source: Chang *et al.*, 1965

The rice grain (Figure 2.5) which are the product of pollination and fertilization. It is composed of the ripened ovary, the lemma, palea, and others. The dehulled rice grain or caryopsis is known as brown rice. When the bran of the brown rice has removed, it is known as white rice.

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