Faculty of Resource Science and Technology

Characterising Upland Rice Landraces from the North-Western Region of Sarawak

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Oryza sativa L. or commonly known as rice belongs to the family of Poaceae. In Malaysia, rice is normally cultivated either as lowland or upland rice. Commonly, upland rice is cultivated for domestic consumption in Sarawak. There has been no prior documentation of upland rice in Sarawak. It has many good characteristics including colourful grain and good fragrance. In this study, 22 upland rice accessions from the North-Western region of Sarawak were characterised. The present study was undertaken with the objectives to characterised morphological traits, anatomical traits, as well as genetic diversity using Simple Sequence Repeat (SSR) marker and *matK* barcoding gene. A total of ten accessions were further selected at S_1 generation and the morphological traits were characterised. The qualitative traits observed on the first generation of 22 upland rice accessions *viz*., blade colour, ligule colour, auricle colour, panicle type and grain colour while the quantitative traits *viz*., heading days (94-126 days), flowering days (127-153 days), culm length (40.7 – 115.7 cm), tiller number (2-6 tillers), panicle number (1- 7 panicles), and percentage of filled grain (40-90%) were useful for characterisation as they exhibited variations. There was a positive correlation between the percentage of filled grains with the number of panicle and number of tillers. Due to certain constraint, only selected morphological traits were observed and leaf anatomy was done for accessions in S_1 generation. Qualitative trait such as blade colour and ligule colour, while quantitative traits *viz*., plant height ($p < 0.01$), culm length ($p < 0.01$) and number of tillers ($p < 0.01$) were found exhibited variations between the accessions in S_1 generation. The leaf, midrib and root anatomy of the accessions in first generation and S_1 generation have the same fundamental anatomical structure. Leaf anatomy revealed that all the accessions contained silica body, which may correlate with resistance to fungal diseases and insect pests. There was no variation in

terms of cell arrangement in leaves, midrib, and root of both first and S_1 generation. A total of 39 SSR markers distributed across the 12 chromosomes were screened for polymorphism. A set of eight SSR primer pairs were polymorphic. A total of 18 alleles were detected across the 22 upland rice accessions, with an average of 2.57 alleles per SSR marker. Number of effective alleles was 1.64 in the present study. The average of polymorphism information content value was 0.67, which revealed that the SSR markers used in this study were highly informative. It is concluded that the studied accessions were low in diversity based on the value of Nei's gene diversity and Shannon's Information Index. A dendrogram was constructed using UPGMA analysis and revealed the clustering of accessions into two clusters. The clustering analysis did not show clear clustering of 22 upland rice accessions according to their morphological traits nor geographical origin. Barcoding gene marker *matK* revealed the accessions could be assigned to one clade along with the 22 lowland rice accessions from UNIMAS collection. Also in the cluster were 94 rice accessions from different origins and three accessions of *Oryza rufipogon*. The clustering categorise the accessions based on their *matK* sequence. In conclusion, this study observes the variations of Sarawak upland rice. A set of collection at S_1 generation is now available for further characterisation and may become breeding lines to improve the quality and yield of Sarawak upland rice.

Keywords: Sarawak, upland rice, morphological, anatomical, microsatellite, *matK*

Pencirian Padi Bukit Tradisional dari Wilayah Barat-Laut Sarawak

ABSTRAK

Oryza sativa L. atau lebih dikenali sebagai padi tergolong dalam keluarga Poaceae. Di Malaysia, padi lazimnya ditanam sama ada sebagai padi sawah atau padi bukit. Lazimnya, padi bukit diusahakan untuk kegunaan rumah di Sarawak. Setakati ni, tiada rekod dokumentasi atau kajian dibuat mengenai padi bukit di Sarawak walaupun mempunyai ciri-ciri yang baik termasuk bijiran yang berwarna-warni dan wangian yang baik. Dalam kajian ini, 22 aksesi padi bukit dari wilayah Barat Laut Sarawak telah dicirikan. Kajian ini dijalankan dengan objektif untuk mencirikan ciri-ciri morfologi, ciri-cirianatomi, serta kepelbagaian genetik menggunakan penanda Simple Sequence Repeat (SSR) dan gen barcoding matK. Sebanyak sepuluh aksesi telah dipilih pada generasi S_1 dan ciri-ciri morfologi telah dicirikan.Ciri-ciri morfologi yang diperhatikan pada 22 capaian padi tanah tinggi iaitu warna bilah, warna ligule, warna daun telinga,warna biji, hari tajuk (94-126 hari), hari berbunga (127-153 hari), jenis malai, panjang batang (40.7-115.7 cm), nombor anak (2-6 anak), nombor malai (1-7 malai) dan peratusan biji terisi (40 – 90%) ,berguna untuk pencirian kerana ia mempamerkan variasi. Bilangan bijirin yang diisi kelihatan meningkat secara positif apabila ciri-ciri berkaitan hasil seperti bilangan malai dan bilangan anak benih meningkat. Disebabkan oleh kekangan tertentu, hanya ciri morfologi terpilih diperhatikan dan anatomi daun dilakukan untuk aksesi dalam generasi S_1. Ciri-ciri seperti warna bilah, warna ligule, ketinggian tumbuhan ($p < 0.01$) , panjang batang ($p < 0.01$) dan bilangan anak benih ($p < 0.01$) didapati berbeza-beza antara aksesi dalam generasi S_1.Anatomi daun, pelepah dan akar aksesi pada generasi pertama dan generasi S_1 mempunyai struktur anatomi asas yang sama. Anatomi daun mendedahkan bahawa semua aksesi mempunyai kandungan badan silika, yang mungkin berkorelasi

dengan ketahanan terhadap penyakit kulat dan perosak serangga.. Tiada variasi dari segi susunan sel pada daun, pelepah, dan akar kedua-dua generasi pertama dan S_1. Sebanyak 39 penanda SSR yang diedarkan merentasi 12 kromosom telah disaring untuk polimorfisme. Satu set lapan pasangan primer SSR adalah polimorfik. Sebanyak 18 alel telah dikesan merentasi 22 aksesi padi bukit, dengan purata 2.57 alel setiap penanda SSR Bilangan alel berkesan ialah 1.64 dalam kajian ini. Purata nilai kandungan maklumat polimorfisme ialah 0.67, yang menunjukkan bahawa penanda SSR yang digunakan dalam kajian ini adalah sangat bermaklumat. Disimpulkan bahawa aksesi yang dikaji adalah rendah dalam kepelbagaian berdasarkan nilai kepelbagaian gen Nei dan Indeks Maklumat Shannon. Dendrogram telah dibina menggunakan analisis UPGMA dan mendedahkan pengelompokan aksesi kepada dua kelompok. Analisis pengelompokan tidak menunjukkan pengelompokan yang jelas bagi 22 aksesi padi tanah tinggi mengikut ciri morfologi mahupun asal geografi. Penanda gen matK mendedahkan aksesi telah dikelompokkan dalam satu kelompok besar bersama dengan 22 aksesi padi sawah daripada koleksi UNIMAS. Turut dalam kluster itu ialah 94 aksesi padi dari tempat yang berbeza dan tiga aksesi Oryza rufipogon. Pengelompokan mengkategorikan aksesi berdasarkan kumpulan genom. Kesimpulannya, kajian ini merekodkan variasi padi bukit Sarawak. Satu set koleksi pada generasi S_1 kini tersedia untuk pencirian lanjut dan mungkin menjadi garis pembiakan untuk meningkatkan kualiti dan hasil padi tanah tinggi Sarawak.

Kata kunci: *Sarawak, padi bukit, morfologi, anatomi, SSR, matK*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
AC	Auricle Colour
ANOVA	Analysis of Variance
BC	Blade Colour
bp	Base Pair
C	Degree Celsius
CD	Culm Diameter
Ch	Chromosome
CL	Culm Length
CTAB	Cetrimonium bromide
DID	Drainage and Irrigation Department
DNA	Deoxyribonucleic Acid
Dntp	Deoxynucleoside triphosphate
DOA	Department of Agriculture
DOS	Department of Statistics
EPS	Expected Product Size
EtBr	Ethidium bromide
FD	Flowering Days
FLR	Fine Lateral Root
GC	Grain Colour
GL	Grain Length
GPS	Global Positioning System
GuC	Guard Cell

GW	Grain Width
h	Nei’s Gene Diversity
HCL	Hydrochloric acid
HD	Heading Days
I	Shannon’s Diversity Index
IRRI	International Rice Research Institute
ISSR	Inter Simple Sequence Repeat
kg/year	Kilograms Per Year
LC	Ligule Colour
LEPC	Length of Long Cell
LL	Leaf Length
LP	Length of Panicles
LS	Ligule Shape
LSC	Length of Stomata Complex
LW	Leaf Width
MARDI	Malaysian Agricultural Research and Development Institute
MEGA	Molecular Evolutionary Genetic Analysis
ML	Maximum Likelihood
Ne	Number of Effective Alleles
NP	Number of Panicle
NT	Number of Tillers
PCR	Polymerase Chain Reaction
PFG	Percentageof Filled Grains
PH	Plant Height
PIC	Polymorphic Information Content

PT	Panicle Type
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SB	Secondary Branching
SC	Seed Colour
SCS	Subsidiary Cell Shape
SD	Stomata Density
SEA	Southeast Asia
SH	Seedling Height
SL	Seed Length
SNP	Single Nucleotide Polymorphism
spp.	Species (plural)
SPSS	Statistical Package for the Social Sciences
SS	Seed Shape
SSR	Simple Sequence Repeat
SuC	Subsidiary Cell
SW	Seed Width
Ta	Annealing Temperature
Ta	Annealing Temperature
TAE	Tris Acetate EDTA
TLR	Thick Lateral Root
TRIS	Trisaminomethane
UNIMAS	Universiti Malaysia Sarawak
unit/mm^2	Unit Per Millimetres Square
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

WEPC	Width of Long Cell
WSC	Width of Stomata Complex

CHAPTER 1

INTRODUCTION

1.1 Study Background

Sarawak, the country's largest state, has a low and inconsistent grain production when compared to paddy produced from granary areas in Peninsular Malaysia. Sarawak generated less than 2.0 metric tonnes per hectare, but Peninsular Malaysia produced more than 5.0 metric tonnes per hectare (Omar et al., 2019). A few strategies such as rice bowl project (Anonymous, 2012) and increasing the rice planting area (Wahab, 2017) was implemented to improve the rice production in Sarawak. Besides, rice breeding is one of the good strategies to increase rice production. For example, hybrid rice offers a lot of improvement in rice yield performance, considering the ability of this variety type is more resistant to the pests and diseases (Rahim et al., 2021).

This was shown by a successful trial carried by Engkelili Agriculture Department in collaboration with Bayer CropScience in 2015. Arize 6444 Golden hybrid rice was cultivated in the trial by farmers. Tanjung Saduru Drainage and Irrigation Department (DID) Scheme was chosen as the planting site, located at Ili Batang Ai, Sri Aman Division (DID, 2015; Mail, 2015). The Minister of Modernisation of Agriculture had specified in a press statement that the yield from the hybrid variety was 5.7 tons per ha in 2015, which doubled the production as compared to previous harvest. The variety was resistance to many plant diseases and only took around 140 days of production period as compared to 170 days of other rice varieties (DID, 2015; Mail, 2015). Increasing rice production in Sarawak as well as Malaysia in a sustainable manner is crucial to meet the increasing

population from 29.7 million in 2020 to 33.1 million in May 2022 and 34.2 million is forecasted for 2023 (DOSM, 2022).

Rice is normally cultivated as wetland rice in Peninsular Malaysia. Most granary areas were in states such as Kedah, Selangor, Pulau Pinang, Perak, Terengganu and Kelantan (DOSM, 2021). Rice grown in those granary areas contributed approximately 70% of the domestic supply (Herman et al., 2015; Omar et al., 2019). Meanwhile, Sabah and Sarawak are mostly cultivating upland rice. Upland or dryland rice is planted in field with naturally rain-fed, well-drained soils with no surface water accumulation and having undulated topography (Department of Agriculture, 2005). In Sarawak, upland rice is known by the local people as ‘padi huma’ or ‘padibukit’ (Yusop et al., 2021). Most farmers in Sarawak still practising traditional cultivation method. Karubi and Peter (2021) stated, upland rice planting in Serian division, Sarawak, still apply shifting cultivation, using slash-and-burn technique.

Department of Agriculture stated that Sarawak is rich in rice diversity. A total of 1652 accessions of Sarawak rice were collected from ten different divisions of Sarawak (Yeo et al., 2018). Hanafi et al. (2009) stated that certain upland rice has desirable characteristics such as shape, good fragrance, colour, and texture that are useful for rice breeding programmes. Hanafi et al. (2009) had concluded that the upland rice has potential to produce higher yield with proper management, not only through the application of fertilizers but also cultural practices. However, upland rice is considered as a home-consumption crop. Therefore, available records for upland rice varieties or landraces are limited, leads to lack of information on Sarawak upland rice.

1.2 Problem Statement

Sarawak is a state with diverse types of rice. The success of a breeding program relies heavily on the diversity. Despite the fact that Sarawak is rich in rice biodiversity, the assessment of their agronomical traits and genetic diversity which may provide basic information that is useful for the future breeding program is still unavailable. This is because upland rice cultivation is practiced mostly by the communities in the rural areas of Sarawak for their own consumption and have not been commercialized (Hanafi et al., 2009), thus, limited reports or research were carried out on upland rice landraces. As a result, little is known about upland rice in terms of agronomical performance level, and morphological and anatomical characteristics.

Moreover, using these materials for breeding is challenging due to purity. The different landraces are usually planted separately either in one plot with no clear boundary or in different plots but very near to each other. The worst case would be having a mixture of different landraces in one plot (Yeo et al., 2018). For farmers who are planting rice for own consumption, purity of seeds may not be important for them. The impurity will complicate the attempt of rice breeders to use the materials for breeding. This is because great variations will be observed when characterizing the different traits of a landrace by using a batch of impure seeds. At the same time, this will complicate the comparisons of traits between landraces or varieties.

Another problem is that the nomenclature of the landraces is based on the name given by farmers (Yeo et al., 2018). The rice landraces were named by the farmers based on their characteristics, origin, or by maintaining the ancestral name. The landraces with different names might sometimes belong to the same variety/landrace when some of these varieties were introduced to places with different ethnic backgrounds. Problems arise when

landraces having the same morphological characteristics were given different names or landraces having different morphological characteristics were given the same name by the farmers. This will lead to confusion. The recognized traditional method for variety identification is by evaluating the morphological characteristics. This method has been highly successful and efficient. However, using morphological data alone can be challenging due the tendency of certain traits may be altered by the environmental factors and close morphological similarities (Nadeem et al., 2018). Anatomical characterisation may provide additional data to support the morphological data. Systemic anatomists rely on anatomical traits although no character is absolutely invariable. Some anatomical traits are more stable and it is on those that are less plastic as it is not really affected by environmental condition (Chimezie et al., 2016).

However, morphological and anatomical features are insufficient to characterise a plant. For better determining of genetic variation, molecular methods have gained the attention as they are being independent of biotic-abiotic stress (Keramas et al., 2004). The utilisation of molecular markers system has effectively been used to evaluate the gene diversity. To date, different types of DNA markers have been used in discriminating plant varieties. One of these markers is Simple Sequence Repeat (SSR) marker. SSR marker is well known and have been used in numerous studies due to their abundancy, high polymorphism rate and well distributed throughout the genome (Miah et al., 2013). It is effectively used for assessing genetic diversity among closely related rice cultivars compared to other molecular markers (Sohrabi et al., 2013).

Besides SSR, barcoding marker may be used as a character to distinguish varieties as demonstrated by De Mattia et al. (2011). The author manages to discriminate up to cultivar level by using *matK* and *trnH-psbA* barcoding genes, supporting Soltis et al.

(1998) whose stated that *matK* have higher specific level of accuracy at the level of species. According to Hilu et al. (2003), phylogenetic studies using *matK* produce phylogenetic tree that are stronger compared to other gene. In the end, the morphological and anatomical data together with molecular data will be able to distinguish the differences in rice accessions.

1.3 Objectives

Keeping in view of the points stated above, the present study was carried out with the following objectives:

i. To document the morphological and anatomical characters of 22 upland rice accessions selected from 11 rice landraces collected from three divisions in the North-West region of Sarawak.

ii. To establish the genetic relatedness of the Sarawak 22 upland rice accessions selected from 11 rice landraces collected from the North-West region of Sarawak using SSR molecular markers.

iii. To establish the genetic relatedness of the Sarawak 22 upland rice accessions selected from 11 rice landraces collected from the North-West region of Sarawak using *matK* barcoding markers.

CHAPTER 2

LITERATURE REVIEW

Rice is the second most important crop after wheat (*Triticum aestivum*) (Rajamoorthy et al., 2015). Over 80% of the world's rice was consumed by the people in the Asian countries (Omar et al., 2019). Today, it is grown in over 100 countries with Asia contributed 90% of the total global production (Fukugawa & Ziska, 2019). Rice was classified primarily as a tropical and sub-tropical crop. Rice is usually grown under diverse condition (Ahmadi et al., 2014) and International Rice Research Institute (IRRI, unpublished) has stated that rice is generally grows in four ecosystems, which were rain-fed upland or dryland, rain-fed lowland (wet rice), irrigated lowland (flooded), and deep-water (floating). In Sarawak, Sang et al. (2018) stated that the rice were mainly cultivated in all those different ecologies, except deep-water (floating) cultivation method. Upland or dryland rice is planted in fields with naturally well drained soils and no surface water accumulation. Rice is direct seeded in non-flooded, well-drained soil or on level to steeply sloping hills (Teo, unpublished).

2.1 Rice, *Oryza sativa* L.

Rice or *Oryza sativa* belongs to the Poacea family or commonly known as the grass family (USDA, 2006). According to Peterson (2003), the grass family is the fourth largest flowering plant family and contains about 11 000 species in 800 genera worldwide. According to Ge et al., (1999), the genus *Oryza* contains 26 recognized species, of which 23 are wild species and the other three are cultivated species which are *Oryza sativa (Asian)*, *Oryza glaberrima* (African) and *Oryza meridionalis* (Australia). All species have

been classified into six different diploid genome (Table 2.1). *Oryza sativa* is the most widely grown species in Asian, North & South American, European Union, Middle Eastern & African countries.

Table 2.1: List of *Oryza* Species with Different Types of Diploid Genome (Ge et al., 1999)

Species	Genome	Species	Genome
Oryza sativa	AA	*Oryza latifolia*	CCDD
Oryza glaberrima	AA	*Oryza minuta*	BBCC
Oryza glumipatula	AA	*Oryza alta*	CCCD
Oryza longistaminata	AA	*Oryza brachyantha*	FF
Oryza nivara	AA	*Oryza australiensis*	EE
Oryza rufipogon	AA	*Oryza meyeriana*	GG
Oryza barthii	AA	*Oryza coarctata*	HHKK
Oryza meridionalis	AA	*Oryza neocaledonica*	GG
Oryza punctata	BB	*Oryza granulata*	GG
Oryza officinalis	CC	*Oryza longiglumis*	HHJJ
Oryza rhizomatis	CC	*Oryza ridleyi*	HHJJ
Oryza eichingeri	CC	*Oryza schlechteri*	Unknown
Oryza grandiglumis	CCDD	*Oryza malampuahaensis*	Unknown

2.1.1 Rice Domestication

Few hypotheses have been proposed, suggesting either a single or multiple origin of cultivated rice from its wild ancestors. According to Sang and Ge (2007), the monophyletic hypothesis proposes that Asian cultivated rice arose from a single wild rice lineage and then diverged into several groups, including *japonica* and *indica*. Based on the study carried by Kovach et al. (2007), the *indica* group is more closely related to the annual wild rice, *Oryza nivara*. This species adapted to seasonally dry habitats and are well known to contribute gene for resistance to biotic stresses; grassy stunt virus, bacterial leaf blight, sheath blight, neck blast, drought and heat for rice improvement (Guttikonda et al., 2018). Apart from that, loci for yield, seedling vigour, quality and biofortification were also reported from *O.nivara*. Whereas *O. rufipogon*, is more closely related to *japonica*. *O.*

rufipogon is a perennial species that is widely distributed throughout South and Southeast Asia, Papua New Guinea, Southern China and Northern Australia (Sang & Ge, 2007; Vaughan & Tomooka, 2008). This species adapted to wet habitats where it is commonly found in a marsh or aquatic habitats. *O. rufipogon* serves as a source of valuable gene pool as well as quantitative trait loci (QTL) for components of yield and tolerance to biotic and abiotic stresses (Neelam et al., 2018). This species is widely accepted as the progenitor of *O. sativa*, and often being used to accommodate the wild Asian A-genome taxonomical group. *Indica* and *japonica* have clearly diverged in morphological and agronomic traits, in physiological and biochemical characteristics, as well as their genomic structure due to rice domestication (Yang et al., 2014). However, the strong evidence to support any of those hypotheses made for rice domestication is still lacking.

2.2 Rice Industry Overview

Countries in Asia consumed more than 80% of the world's rice and demand is expected to continue to rise. Southeast Asia (SEA) region has been the centre of the world's rice economy for the past century (Omar et al., 2019), with Thailand and Vietnam being the region's top rice exporters. Thailand contributed up to 15.1% of the world's total export of rice, followed by Vietnam and Cambodia contributed 7.4% and 1.9%, respectively in 2020 (Workman, 2021). Some SEA countries such as Indonesia, Malaysia and the Philippines rely on imports to supplement the shortage of supply from domestic production. According to TrendEconomy (2021), Philippines imported 4.5% of the world's total rice import followed by Malaysia and Indonesia with 3.1% and 1.0%, respectively in 2020.

2.2.1 Rice Production in Malaysia

Rice being the staple food of Malaysia is one of the country's most important crop. Rice is a highly protected food crop and strategically important industry in Malaysia economic development. In 2020, Malaysia estimated to consist almost 700 thousand hectare of paddy planted area, 74.6% were in Peninsular Malaysia, followed by Sarawak (19.3%) and 6.1% were in Sabah. Mainly, Peninsular Malaysia had contributed more than 85% of Malaysia's total production of rice, with the remaining were produced by Sabah and Sarawak (DOSM, 2021). Kedah, one of the rice bowls in Malaysia, held 31.0% of the planted paddy area and become the largest state in Malaysia that contributed 36.9% of total rice production Malaysia.

The Malaysia rice production was recorded 24.4% in 2020, which was the highest in recent years (DOSM, 2021). Although it shows an increase in number, Malaysia recorded still 72% self-sufficient, eight percent short from the target (Chan, 2020). According to Adnan and Nordin (2020), Malaysia consumes 110kg/year of paddy per capita. With the amount of total rice production in 2020, it is insufficient to meet local demands. Consequently, the rice import has considerably increased from 330 336 tonnes in 1990 to 1.1 million tonnes in 2020 (DOSM, 2021) to cope with the rising population and demands. Malaysian paddy industry as country imports 30% of its overall consumption from different part of the world. DOSM stated that the rice imports in 2020 was valued at RM 2.5 billion, rose 32.1% compared to the previous year. The main suppliers of imported rice for Malaysia were Vietnam (42.0%) and India (27.5%) followed by Pakistan (10.9%), Myanmar (6.9%), Thailand (6.6%) and Cambodia (5.5%) as stated in (DOSM, 2021)

2.2.2 Rice in Sarawak

In Malaysia, rice has been cultivated as lowland and upland rice. The cultivation of upland rice was normally practice by the rural community in Sarawak and Sabah (Hanafi et al., 2009). Upland rice is commonly known by the local people as “padi huma” or “padi bukit”. Upland rice in Sarawak were mainly cultivated for self-consumption rather than income generating (Ho & Wasli, 2015). For decades, the Dayak tribe, including the Ibans' and the Bidayuhs' in Sarawak has practice shifting cultivation of upland rice, one of the most important crops as it is their staple food (Kleinman et al.,1995; Yusop et al., 2021).

Certain upland rice cultivars offer appealing qualities, notably in terms of smell, colour, size, and form. These characteristics lead to its popularity as an organic product among farmers and health-conscious customers (Hanafi et al., 2009). However, due to their poor grain yields, these upland rice cultivars have not been marketed. According to Mariam et al. (1991), research on upland rice has been ignored due to low and variable grain yields, despite the fact that it is widely produced in the country's interior.

Low grain yields of upland rice are related to inadequate management by farmers throughout the cultivation stage, in which fields are left unattended after planting without monitoring of plant nutrients and other essential elements such as weeds, insect-pest attacks and diseases (Hanafi et al., 2009). With excellent management practises, the application of sufficient plant nutrients and water, as well as the management of weeds, diseases, and insect-pests, grain yields of upland rice varieties are predicted to grow (Hanafi et al., 2009). All of these factors are so critical in achieving greater yields.

Besides good management practices, various efforts from the government in the rice industry to ensure food security and protect farmer’s welfare. One of the priorities of Department of Agriculture Sarawak is to improve rice productivity in Sarawak to make

Sarawak as one of the rice bowls for Malaysia (The Borneo Post, 2012) and to increase the self-sufficiency level to 60% by 2030, for rice production. (The Dayak Daily, 2022). According to Deputy Minister for Modernisation of Agriculture and Regional Development in The Dayak Daily (2022), SSL for rice production only reach 38% in Sarawak with the rest imported. Rice breeding is one of the strategies for Sarawak to increase the rice production of both state and country.

Rice breeding is the alteration of the plant by stacking desirable traits to create a better variety. Nowadays, consumers often demand for more superior quality rice such as better aroma, taste and nutrient content. To the farmers, besides high resistance or tolerance to biotic and abiotic stresses, high yield of harvested rice is often thought to be one of the main reasons for the choice of rice variety to plant (Laborte et al., 2015).

As Sarawak is rich in rice diversity, the hidden potential of the local rice is yet to be discovered. A total of 1652 accessions of Sarawak rice landraces were deposited in the gene bank of Agriculture Research Centre Semonggok, Department of Agriculture Sarawak. According to Teo (n.d), the rice landraces are of different grain sizes, shapes and colours which are very valuable for rice breeding.

2.3 Morphological Structure of Rice (*Oryza sativa* L.)

Rice phenology is generally divided into three main crops phase: (i) vegetative phase, including germination, seedling, tillering and jointing stages which took around 45-100 days; (ii) reproductive phase, including booting, heading and flowering stages; around 35 days (iii) maturation phase, including milk, dough grain and maturity stages of rice, around 30 days (Moldenhauer & Slaton, 2001; Nelson et al., 2014). When germination

starts, the vegetative phase begins, and it ends with panicle initiation (reproduction phase). Initiation starts when the plant starts to develop panicle. A reproductive phase is commonly associated with the formation of the panicle and grain. The ripening or grain-filling phase begins after anthesis and ends at maturation (Moldenhauer &Gibbons, 2003). The rice plant has a main stem and a number of tillers at maturity. Each productive tiller bears a terminal flowering head or known as panicle. The morphology of rice is divided into the vegetative part and reproductive part.

2.3.1 Vegetative Organs of Rice (*Oryza sativa* L.)

The vegetative organs of *Oryza sativa* consist of leaves, culm and roots (Chang & Bardenas, 1965). Leaf is the determinate organ that serves as a main photosynthetic structure of plants (Piazza et al., 2005). During the vegetative phase, rice leaves appear approximately every four days and every seven days during reproductive phase Nelson et al. (2014). Rice plants produce ten or more foliage leaves before entering the reproductive phase. The mature rice leaf is strap-like, with three separate areas along the proximal-distal axis. Lamina or leaf blade is the distal region of a leaf, where photosynthesis mostly occur (Figure 2.1). The proximal area is the leaf sheath, which protects the shoot apex and younger leaves from physical harm. The boundary of the blade and sheath consists of three distinct parts: the lamina joint (collar), the ligule and the auricle (Figure 2.1). Whitish area at the blade's base that bends the leaf blade toward the abaxial side is known as lamina joint (Figure 2.1). In mature leaves, the ligule is membranous and acuminate, frequently splitting into two segments, whilst auricles are a pair of tiny appendages with long hairs located at the leaf edges (Figure 2.1).

In rice, Wu et al. (2016) stated that the flag leaf (FL) acts as a functional leaf in the plant (Figure 2.1). FL plays an important role in synthesis and translocation of photo assimilates in the rice, affecting the grain growth and development in rice plant. This is due to the position of the FL that is the closest to the panicle of the rice plant. Besides, an ideal blade shape is beneficial to the yield of rice and have a marked impact on light energy utilization (Zhu et al., 2009; Jiajia et al., 2020). The varieties with high yield potential usually have leaves that were erect, short, narrow, thick and dark green. Thick leaves have high chlorophyll and nitrogen (N) photosynthetic enzymes content per unit leaf area.

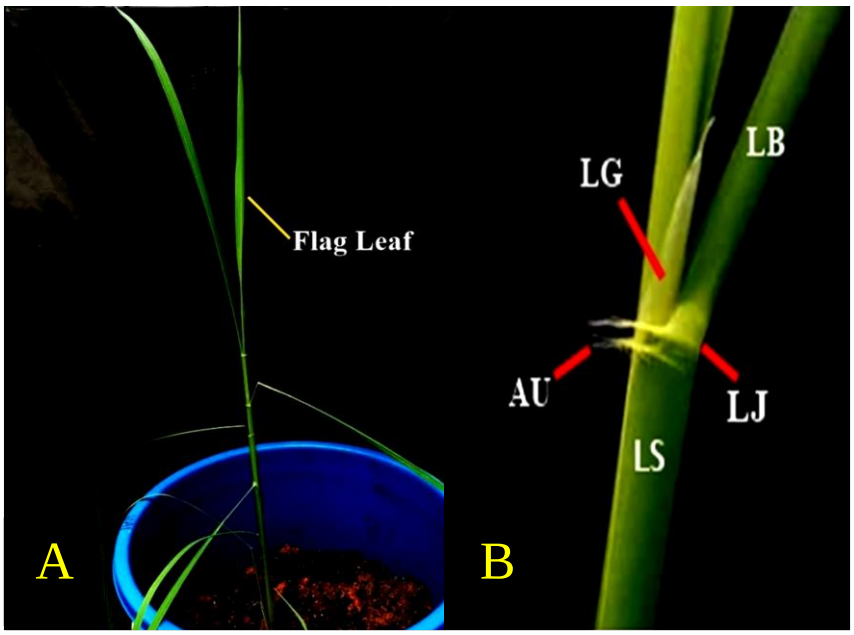

Figure 2.1: Few General Structures Observed in *Oryza sativa* L. **(**A) Flag Leaf of *Oryza sativa*, (B) Enlarge Image of Leaf Part. **AU**: Auricle, **LS**: Leaf sheath, **LJ**: Lamina Joint, **LG**: Ligule and **LB**: Leaf Blade.

Culm is a jointed stem of rice composed of nodes and internodes. A culm's internodes vary in length, often increasing from the bottom to the top internodes. Lower internodes near the culm's base are short and thickened into a solid segment. Internodes vary in cross-sectional size as well. Lower internode diameters are often bigger and thicker

than upper internode diameters. Under rapid increases in water level, certain deep-water rice types can lengthen their lower internodes by more than 30 cm each. Tillers emerge in an alternative manner from the main culm. Culm is a supporting organ that is essential in the movement and storage of water and nutrients(Wei et al., 2011). According to Stern et al. (2003), rice culms consist of three tissues which are mechanical tissue (sclerenchyma cells), conducting tissue (vascular bundles) and group parenchyma tissue.

Root systems play a crucial role in supporting plant, synthesizing hormones and water and nutrient absorption (Kano et al., 2011). Rice plants forms fibrous root systems (Figure 2.2) consist of an ephemeral seminal root, nodal roots and lateral root (Gu et al., 2017). The seminal roots originate from the embryo and persist only for a short time after germination and are sparsely branched (Chang & Bardenas, 1965). Nodal roots (NR) are the roots that develop from the basal nodes above the soil surfaces and often found in rice cultivars growing at water depths above 80 cm (Chang & Bardenas, 1965; Gu et al., 2017). For lateral roots, it can be classified as fine lateral roots (FLR) or thick lateral roots (TLR). However, rice roots seldom exceed a depth of 40 cm in flooded soil. This is due to the fact of limited oxygen diffuse through the aerenchyma to supply the growing root tips.

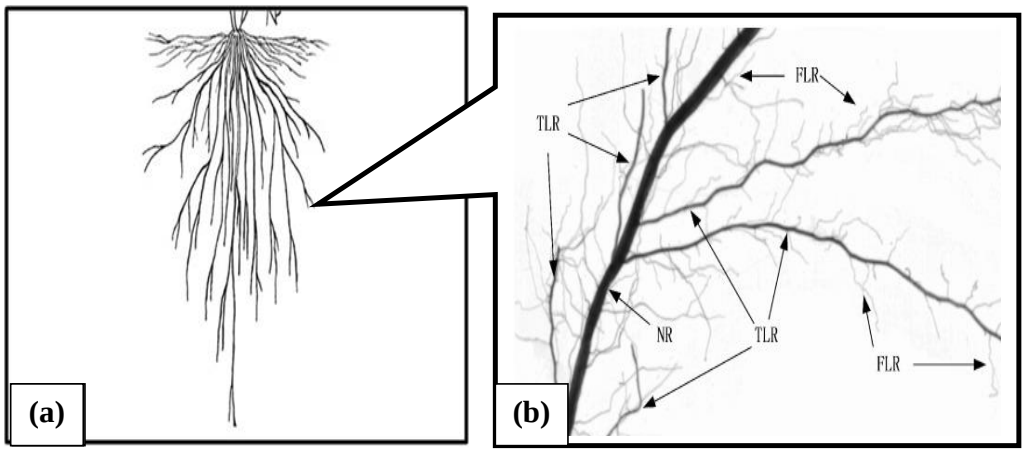

Figure 2.2: General Structure of *Oryza sativa* L. Root (a) Fibrous roots part of *Oryza sativa* L. (b) Nodal Root (NR), Fine Lateral Root (FLR) and Thick Lateral Root (TLR). Adapted from (Gu et al., 2017).

2.3.2 Floral Organs of Rice (*Oryza sativa* L.)

The floral organs are modified shoots (Chang & Bardenas, 1965). Panicle, spikelets, and flowers are the floral organs. The panicle is the name given to the rice inflorescence. Rice inflorescences, like those of Sorghum, Panicum, and Avena, are classified as racemes because spikelets are generated on lateral branches rather than the main axis. Due to is conical form, the rice inflorescence is also known as a panicle. In grasses, the main axis of the inflorescence is called the rachis (Bell, 1991) as shown in Figure 2.3. Lateral branches that are attached directly to the rachis are called primary (rachis) branches (Figure 2.3). The panicle branches in a racemose manner, with each node on the main axis giving birth to the major branches, which in turn bear the secondary branches, and the secondary branches bearing the spikelets. Rice inflorescences have ten or more major branches with roughly 150 spikelets. Different rice varieties differ greatly in the length, shape and angle of the primary branches, and in the weight and density of panicle. Panicle traits such as size and structure are one of the crucial determinants of grain yield and quality (Mo et al., 2012).

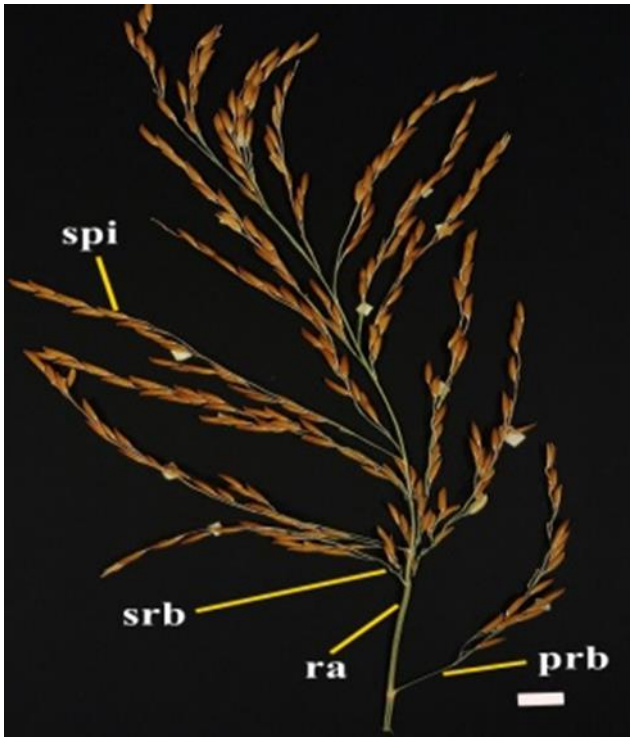

Figure 2.3: General Structure of *Oryza sativa* L. Panicle. Abbreviations: **ra**: rachis, **spi**: spikelet, **prb**: primary branch, **srb**: secondary branch. White Bar indicates 1 cm.

Tanaka et al. (2012) stated that a spikelet is the structural unit of inflorescence. It is composed of florets and glumes. The rice spikelets have three florets which are subtended by two tiny glumes. A spikelet consists of a minute axis (rachilla) on which a single floret is borne in the axis of 2-ranked bracts. The bracts of the lower pair of rachilla, being always sterile, are the sterile lemma. The upper bracts or the flowering glumes consist of the lemma and palea. According to Lombardo and Yoshida (2015), palea's feature have a hook-shaped marginal structure where its lateral region curls outwardly. This is to allow the palea to lock into the inwardly curled facing lemma. The spikelet is detached from the pedicel during natural shattering or the threshing process at the junction of the lower sterile lemma and the facet. As it disarticulates from the pedicel, the base of the lower sterile lemma appears horizontal or oblique.

According to Rost (1997), flower development in rice can be divided into two stages which are pre-flowering stage and flowering stage. The general structure for the flower of rice is shown in Figure 2.4. The flower consists of six stamens and one central pistil (Yamaguchi et al., 2004). The six stamens are made up of two-celled anthers with a thin filament, while the pistil has one ovule. The short rice style has a dry and plumose stigma. The rice fruit is a caryopsis in which a single seed is bonded with the ripening ovary's wall (pericarp), generating a seed-like grain. The grain is the mature ovary, complete with the lemma, palea, rachilla, sterile lemmas, and awn.

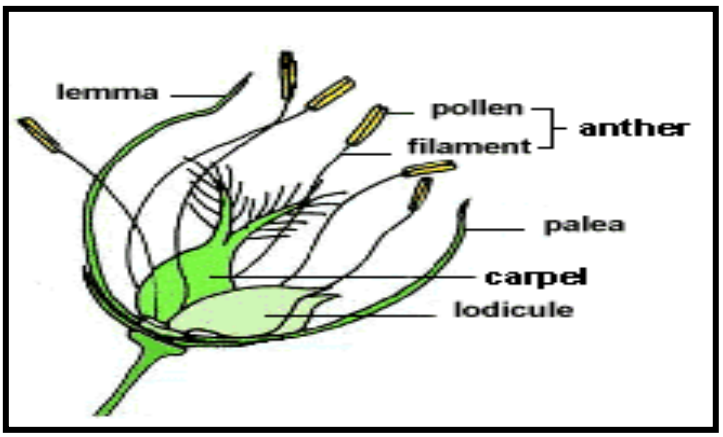

Figure 2.4: Flower Part of *Oryza sativa* L. (Rost, 1997)

2.4 Morphological Characterisation of Rice (*Oryza sativa* L.)

Morphological characteristics in various parts of plant have been used traditionally in delineating landraces or varieties in general (Chang & Bardenas, 1965; Suriyagoda et al., 2011; Ray et al., 2013). Kikkawa (1912) was the earliest that working on detailed classification of rice varieties based on physiological and anatomical characters. In 1913, leaf sheaths colour and grain dimensions were used in classified Indian rice varieties (Graham, 1913). Following this, rice was first divided by Kato et al. (1928) into two types, *japonica* and *indica* based on morphology and sexual affinity. In the classification of

Bengal rice, anthocyanin pigmentation of leaf sheath, stigma and apiculus colour were used (Sethi & Saxena, 1930; Ram & Chetty, 1934).

Traditionally, morphological characters of various parts of plants have been used to distinguish the plant from each other. Other than the morphological traits use for characterisation in the previous sections, grain characters are also important in the seed certification process to control field and seed standards. Several grain characters, such as grains per panicle, number of panicles, grain yield, weight and seed shape were reported as valuable traits in identifying rice hybrids (Geetha et al., 1994; Kalaichelvan, 2009). The earliest attempt at a detailed classification of rice varieties based on grain characters was that of Naidu et al. (1986). The author characterised 20 rice varieties as bold, slender and medium classes based on grain length to breadth ratio. In addition, characters *viz*., colour of hulled grain, length, shape, width, 100 grain weight, presence or absence of pearl spots and shape of pearl spot. Aidy et al. (2000) suggested that subspecies *japonica*, *indica* and *japonica*/*indica* can be distinguished from others using morphological traits such as days to 50% flowering, leaf, plant height, 1000 grain weight, grain length and width, phenol test and suggested the usefulness in differentiating the parental lines of rice hybrids.

Yield-related traits which shows co-efficient variation such as the spikelet per panicle, panicle weight, 100 grain weight and grain yield has been used to characterised rice in Sarma et al. (2004). In line with this study, Zafar et al. (2004) recorded high genetic variation among the rice landraces using both quantitative and qualitative traits. Few workers suggested that morphological trait and development phase such as plant height and days to 50% flowering contributed to genetic divergence (Bose & Pradhan, 2005; Singh et al., 2006). The characterisation based on yield-related traits are crucial to select lines that

can be used as potential parents in parents with desirable traits for future hybridization programme (Ogunbayo et al., 2005).

Rice grain quality of Pakistan local rice germplasm has been evaluated by Siddiqui et al. (2007) and revealed that the variations present in grain size, shape and weight correlated with the attitude of the sampling site. In line with this study, Zeng et al. (2007) has analysed that the genetic diversity of Yunnan rice landraces based on morphological, isozyme and SSR variations. The study also confirmed that Yunnan is the center of genetic differentiation of two Asian sub-species, which were *japonica* and *indica*. The genetic variability of South American wild rice has been assessed using several morpho-agronomic traits (Veasey et al., 2008).

Sohrabi et al. (2012) has evaluated a total of 50 upland rice in Malaysia. The author managed to classify the studied rice into six group cluster according to their quantitative morphological traits. Recently, Ruslan et al. (2018) managed to distinguish two rice varieties, MR269 and MR220 by using seed width as a parameter. Although characterisation and assessment of variability of plants is the basic criteria to provide fundamental information for breeding programmes, the characterisation of upland rice on the basis of morphological characteristics in Sarawak has yet to be done.

2.5 Anatomical Study of *Oryza*

Plant anatomy is the study of the tissue and cell structure of plant organ (Simpson, 2019). Plant anatomy can provide valuable characteristics in phylogenetic analysis. The anatomical characteristics may vary among the species; hence, it can help to support the morphological and molecular data available.

The leaf structure studies in (Chatterjee et al., 2016) revealed the basic anatomical features of a rice leaf, such as the position of the mesophyll cells between the veins, the presence of bundle sheath cells surrounding the veins, the presence of stone cells above and below each vascular bundle, and the presence of bulliform cells at the upper middle portion in between two adjacent veins, were consistent across all wild and cultivated species. Typically, an *Oryza* leaf has 7-8 inter-veinal mesophyll cells and 12-13 bundle sheath cells around any minor vein via general observation. The transverse sections of *Oryza* leaves were observed to varied in terms of leaf thickness, epidermal curvature, bulliform size, vein spacing, mesophyll size and number, and bundle sheath cell size and number were all substantially different across species.

Stomatal number and size diversity was identified in several *Oryza* species and *Oryza* complexes (Chatterjee et al., 2020). The author observed the stomatal features in the *Oryza* family vary depending on the *Oryza* genetic complex. For example, the *sativa* complex has the most stomatal number variety, but the *officinalis* complex has higher stomatal size diversity. Combining structural data with the *Oryza* phylogeny indicated that diversification in the rice family has tended to increase stomatal density rather than stomatal size (Chatterjee et al., 2020). They also observed different stomatal features in *Oryza sativa* and its wild species. During the vegetative stage, the wild and domesticated species are morphologically identical and difficult to distinguish one from the other. With the anatomical characters, it can help to identify this spp. even at vegetative stage.

Root anatomical characters also had been used in identification of rice varieties or landraces (Kondo et al., 2000). Idio and Jayeola (2020) were able to distinguish *Oryza glaberrima, Oryza barthii* and *Oryza longistaminata* from each other based on the root anatomy. Closed vascular system in stele was observed with the present of both

protoxylem and metaxylem in all species but vary in terms of structures. *O. barthii* were having two metaxylem and numerous protoxylem. *O. glaberrima* was found having one metaxylem and numerous. Four metaxylem and numerous protoxylem were observed in *O. longistaminata.*

2.6 Molecular Characterisation

Although using both quantitative and qualitative morphological characterisation to study diversity is commonly used in the past, these conventional techniques are less reliable as it is often predisposed by the environmental factors and limited in number of traits, often not associated with important economic traits (Nadeem et al., 2018). Fingerprinting using DNA molecular markers offer several advantages over conventional method. It is rapid, remain unaffected by the environment factors, less laborious, specific, sensitive and have been proven as an efficient tool for crop germplasm characterisation, collection, and management (McCouch et al., 1997; Magistrado et al., 2001; Keramas et al., 2004). Collard et al. (2005) stated that DNA markers also play crucial roles in agriculture for the construction of linkage map for crops Ulukan (2009) stated that it had been used widely for crop improvement specifically in wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), soybean (*Glycine max*) and capsicum (*Capsicum* L.).

In early 1980, Restriction Fragment Length Polymorphism (RFLP) was developed as a new marker system (Botstein et al., 1980). According to Zhu et al. (2011), PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) utilizes conventional PCR techniques to amplify a large amount of specific DNA sequence to be digested with restriction endonucleases into smaller polymorphic fragments. It has been identified as DNA markers used in rice for the cultivar identification and intra cultivar

polymorphism (McCouch et al., 1988; Olufowote et al., 1997). RFLP has been tested on 70 varieties by Wang and Tanksley (1989) and the finding result shows that 83% of the varieties could be uniquely identify. This technique however has some drawbacks which were labour intensive, require high quality and large quantity of DNA and time-consuming.

Numerous molecular markers *viz*., Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR), Simple Sequence Repeat (SSR) etc. were developed ever since it has been proven to be an effective tool in the assessment of genetic relationship within and among species, where differentiation among accessions can be revealed at the DNA level. RAPD, AFLP and SSR have been used frequently used earlier in characterizing and fingerprinting in different crop species. Some of the brief work pertaining to rice by using SSR marker are highlighted below.

Among various PCR-based markers, microsatellites, or Simple Sequence Repeat (SSR) are very well known and commonly used for genome mapping, fingerprinting, varietal identification, diversity etc. (Bhattarai et al., 2021) as it effectively used for assessing the genetic diversity among closely related rice cultivar (Spada et al., 2004) and distantly related genotypes (Giarrocco et al., 2007). This is due to the ability of SSR that can detect high level of polymorphism at multiple loci and serve as a major source of genetic variation, thus aiding individual identification. In 1991, Condit and Hubbel was the first that made a report of microsatellites in plant. In the report, they suggested that SSR are abundant in plant system (Condit & Hubbel, 1991). In 2000, the locus specific markers such as SSR got its preferential application in cultivar identification in many crops such as

potato (*Solanum tuberosum*) (Dangi et al., 2001), apple (*Malus pumila*) (Moriya et al., 2011), wheat (Zhu et al., 2011) and rice (Rahman et al., 2012).

In rice, SSR marker is found abundantly and well distributed throughout the genome (Miah et al., 2013). The codominant characteristics of the SSR marker and their well-known map positions on the rice genome level reveal high polymorphism among different plants (Garcia et al., 2004). SSR have been used widely in rice genetic studies as they are able to detect high levels of allelic diversity (McCouch et al., 1997; Zhang et al., 2011) as well as fingerprinting and cultivar identification of rice (Sarao et al., 2009).

2.6.1 Previous Study Using Simple Sequence Repeat Marker

Few studies were carried out to evaluate genetic diversity of Malaysian rice using SSR markers. In 2011, the genetic diversity of 53 Sarawak rice originating from Southern Sarawak was genotyped using 54 pairs of SSR marker (Lee et al., 2011). The results had classified the rice into two main groups with six sub clusters and show a wide range of similarity values which indicate a high degree of diversity among cultivars. The number of markers used by Lee et al. (2011) was able to determine the allelic diversity and able to detect the differences among the Sarawak rice cultivars. However, it is insufficient to distinguish the 53 Sarawak rice landraces.

Sohrabi et al. (2013) studied the genetic divergence of 50 Malaysian upland rice, 24 from Peninsular Malaysia and another 26 accessions from Sabah (West Malaysia) using ten sets of SSR markers distributed across the rice genome. As a result, a total of 49 alleles were observed across the 50 accessions. A few accessions in this study had been identified to be the potential parents for future hybridization breeding program in Malaysian environment. Another study was carried out by Noorzuraini et al. (2013) to assess the diversity of Malaysian rice germplasm accessions for drought tolerant grain yield

Quantitative Traits Loci (QTLs). A total of 80 Malaysian rice accessions were selected from the previous drought field screening studies and tested on 119 SSR markers. Based on 45 markers that linked to drought grain yield QTLs, the presence of QTLs was assessed in which five drought grain yield QTLs were present in the 80 Malaysian rice accessions. Those identified rice accession in this study could be useful for future references in breeding programme to develop drought tolerance rice variety.

Another study using SSR markers is on the assessment of 73 rice germplasm originating from India, the Philippines, China, and Malaysia, by using a set of 24 mapped SSR markers (Nayak et al., 2014). All 73 genotypes originating from different geographical regions form well-defined and distinct groups in the cluster analysis, indicating association between the SSR locus and the geographic origin of the genotypes study. Mahsuri, the genotype originated from Malaysia were clustered in one group with 'Lalat' (origin: India) with 78% similarity. The genotype had long-duration high-yielding rice and tolerance drought.

Aljumaili et al. (2018) analysed the genetic diversity of 53 (Sarawak: 10, Peninsular Malaysia: 10, Sabah: 30) aromatic local rice accessions including three local check varieties (MR219, MRQ74 and MR253) collected from the Malaysian Agricultural Research and Development Institute (MARDI) using a total of 32 polymorphic microsatellite markers. The result shows a high level of diversity among the 53 accessions and the dendrogram constructed shows that the accessions from the same regions were most likely clustered in one group together supporting the relationship between the molecular groupings and their source of collection. A total of seven accessions in this study have been identified as diverse accessions and suitable as a parent in the future breeding aromatic rice program.

Recently, 21 Malaysia rice cultivars from MARDI were characterized by using a total of 27 sets of microsatellite primers (Supari et al., 2019) and were grouped into 3 main clusters. Thus, the review on the available literature disclosed that, the information on the ability of SSR markers to determine the identity of closely related rice cultivars and distantly related genotypes are crucial to use the markers for characterisation, particularly in rice genotypes.

Undoubtedly, these studies provide initial insights into the promising materials that will be used in the breeding programme however, to date, there is less effort that has been made in fingerprinting Sarawak local upland rice landraces using SSR Markers, which has promising traits.

2.7 Deoxyribonucleic Acid of Barcoding Plant

Accurate classification and identification of plant species in large number through traditional macroscopic and microscopic characterisation remains a challenge even for specialist taxonomists. Deoxyribonucleic acid (DNA) barcoding is the other possible approach and play a decisive role in assisting classification and identification. Paul Herbert and colleagues published a paper and were the first to propose the use of short DNA sequences for biological identification in 2003. A part of mitochondrial DNA region cytochrome oxidase subunit 1 (CO1) was used and it was considered as the first example of animal identification method (Herbert et al., 2003). The term 'DNA Barcoding' as mentioned in (Herbert et al., 2003) refers to a short gene sequence from a signature region of the genome which was utilized as a marker to make species-level identifications.

The study was soon followed by the study of several taxa using DNA barcoding, particularly in soil meiofauna (Blaxter et al., 2004), marine organisms (Shander & Willasen, 2005), fishes (Yancy, 2008; Ward et al., 2009), freshwater meiobenthos

(Markmann & Tautz, 2005). The study was even carried out on extinct bird using DNA barcoding by Lambert et al. (2005). Although CO1 has been proven as an effective barcode for species identification in many animal groups, CO1 however was insufficiently variable to distinguish species among the plant taxa (Cowan & Fay, 2012). This is due to low substitution rate of mitochondrial DNA in plants (Chase et al., 2005).

A variety of loci including coding genes and non-coding spacers in the nuclear and plastid genomes have been suggested as DNA barcodes for plants (Figure 2.5). Several studies have assessed the efficacy of plant DNA barcoding (Lahaye et al., 2008; Kress et al., 2009; Burgess et al., 2011). For example, DNA barcoding has been used in various plant products such as medicinal plants (Chen et al., 2010; Mankga et al., 2013), commercial kitchen spices (de Mattia et al., 2011), tea plants (Stoeckle et al., 2011) and recently *matK*, *trnL-trnF*, *trnH-psbA*, *rbcL* and *ITS* gene sequence have successfully been used for DNA barcoding of several plant species.

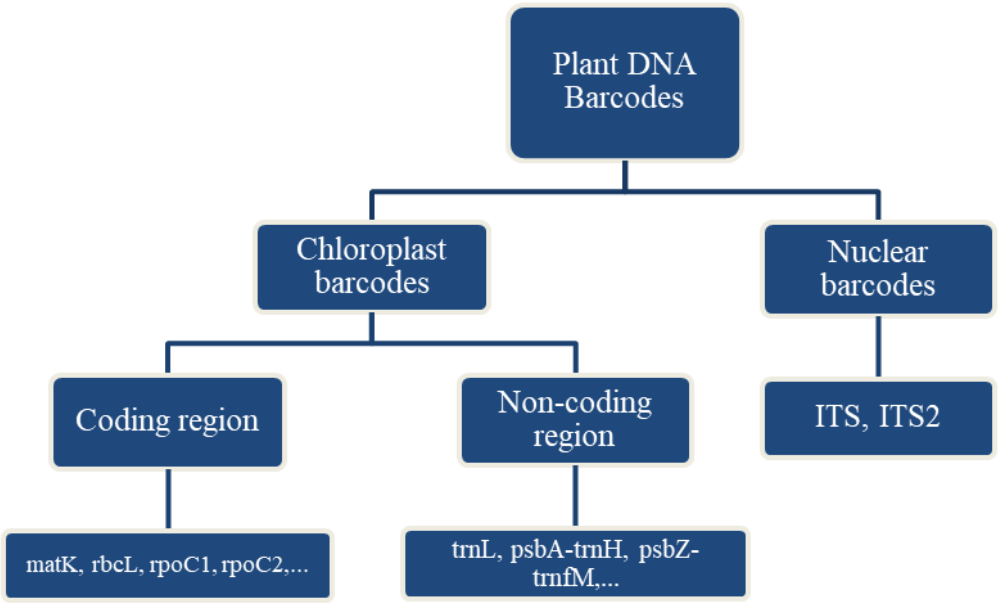

Figure 2.5: Schematic Illustrations of Employed Plant DNA Barcode Markers. Modified from Ali et al. (2007)

2.7.1 Maturase-K gene

According to Fujii et al. (2002), the chloroplast genome is a useful subject for evolutionary and phylogenetic study as it is mostly conserved, has without recombination, haploid, maternally inherited, and present in multiple copies per cell. The chloroplast *matK* marker consist of a 1500 base pair (bp), encodes a maturase like protein, involved in Group II intron splicing between the 50 and 30 exons of *trnK* (Figure 2.6) in the large single copy region of the chloroplast genome of most of the green plants (Sugita et al., 1985; Hilu & Liang., 1997; Steane, 2005). The *matK* genes are considered to be one of the most informative loci which have ideal size, high rate of substitution, large proportion of variation at nucleic acid level at first and second codon position, low transition/transversion ratio and the presence of mutationally conserved sectors. These features of *matK* gene are exploited to resolve phylogenetic relationship at different taxonomic levels in different plant groups (Olmstead & Palmer, 1994; Hilu et al., 2003).

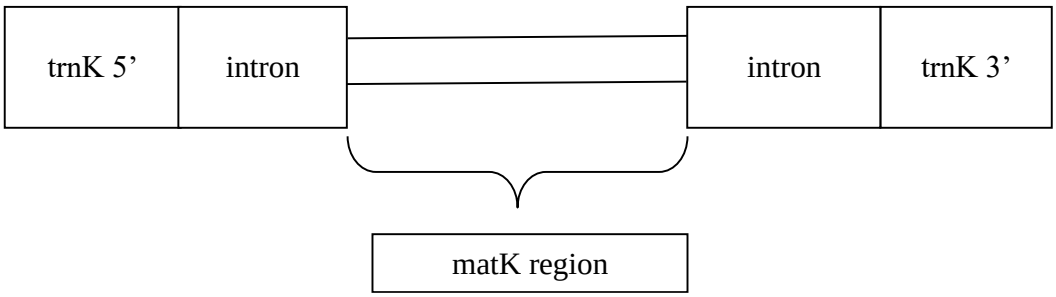

Figure 2.6: Diagrammatic Representation of *matK* Gene Located Between trnK 5' and trnK 3'. (Adapted from Hilu et al., 1999).

Phylogenetic analysis of a data set composed of *matK*, *rbcL*, and *trnT-F* sequences from basal angiosperms demonstrated that *matK* contributes more parsimony informative characters and significantly more phylogenetic structure on an average per parsimony-informative site than the highly conserved chloroplast gene *rbcL* (Muller et al., 2006). Sequence information from *matK* alone has generated phylogenies as robust as those

constructed from data sets comprised of 2-11 other genes combined (Hilu et al., 2003). Further, the molecular data generated from *matK* has been used to resolve phylogenetic relationships at taxonomic levels (Johnson & Soltis, 1994; Hayashi & Kawano, 2000; Hilu et al., 2003). The *matK* gene stands out among plastid genes used in plant systematic for its distinct mode of evolution. The rate of substitution in *matK* is three times higher at the nucleotide level and six times higher at the amino acid level than that of *rbcL* which denoting it as a rapidly evolving gene (Johnson & Soltis, 1994; Olmstead & Palmer, 1994; Soltis & Soltis, 2004).The *matK-trnK* gene complex can be applied to resolve the phylogenetic relationship between taxa as well as to infer evolutionary relationships.

2.7.2 Previous Study on Rice using Barcoding *matK*

The application of DNA barcoding using *matK* marker were widely used on rice identification and phylogenetic analysis. Phylogenetic relationships were inferred using nucleotide sequences of the chloroplast gene *matK* for 26 species representing 11 genera of the tribe Oryzeae and three outgroup species by Ge et al. (2002). Analyses of the sequence data indicated that species of Oryzeae form a strongly supported monophyletic group, concordant with previous morphological and anatomical evidence and highlighted that *Porteresia coarctata* has a high affinity with *Oryza* species and should be treated as a member of the genus *Oryza* rather than an independent monotypic genus.

In 2013, the phylogenetic relationship study of *Oryza sativa* L. cultivars in Southern Thailand based on *matK* gene sequence were studied by Klomklao et al. (2013). This study shows the 50% majority consensus tree showed that the 18 rice cultivars were divided into three groups. The study concluded that the *matK* gene and phylogeny of 18 rice cultivars are useful for future conservation and breeding program for improvement of

rice cultivars. Zodinpuii et al. (2013) analysed the genetic relatedness of genus *Oryza*, both wild and cultivated rice, from Eastern Himalayan region by using chloroplast *matK* gene. The result shows all *Oryza* species groups were clearly distinguished. The species belonging to the *Oryza* AA genome group clustered together and were separated from those belonging to the other genome groups (BB, BBCC, CC/CCDD, EE, GG, HHJJ and FF).

Patil et al. (2015) studied the phylogenetic relationship of ten Indian indigenous aromatic rice and its wild relatives along with the progenitors's grasses families using nucleotide sequences of the chloroplast gene *matK*. As a result, phylogenetic tree of aromatic rice with its wild relatives and grasses progenitors showed that aromatic rice was more closely related to *Oryza sativa* ssp. *japonica* group. The genus *Oryza* was divided into two main clades and evolved from completely different group of grasses families. *Oryza brachyantha* has high similarity with grasses and should be treated as a progenitor for wild *Oryza* species.

A study was conducted between local rice varieties based on *matK* and *rbcL* gene to unveil the relationship between local rice varieties provided from East Java, Banten, and Samarinda based on *matK* and *rbcL* genes (Anggraini et al., 2020). The result shows the phylogenetic analysis using *matK* proved that rice varieties from East Java were closely related to rice varieties from Banten rather than to rice varieties from Samarinda. Meanwhile the same study using the *rbcL* gave inconsistent results. Whilst various phylogenetic study of rice has been reported, no work has yet to focus on Sarawak upland rice landraces using *matK* barcoding marker.

CHAPTER 3

MATERIALS AND METHODS

3.1 Field Sample Collection

A total of 11 upland rice landraces (Table 3.1) were collected from three different divisions in Sarawak (Figure 3.1). Basic information such as the sampling location coordinates, division and the local names of the paddy are furnished in Table 3.1.

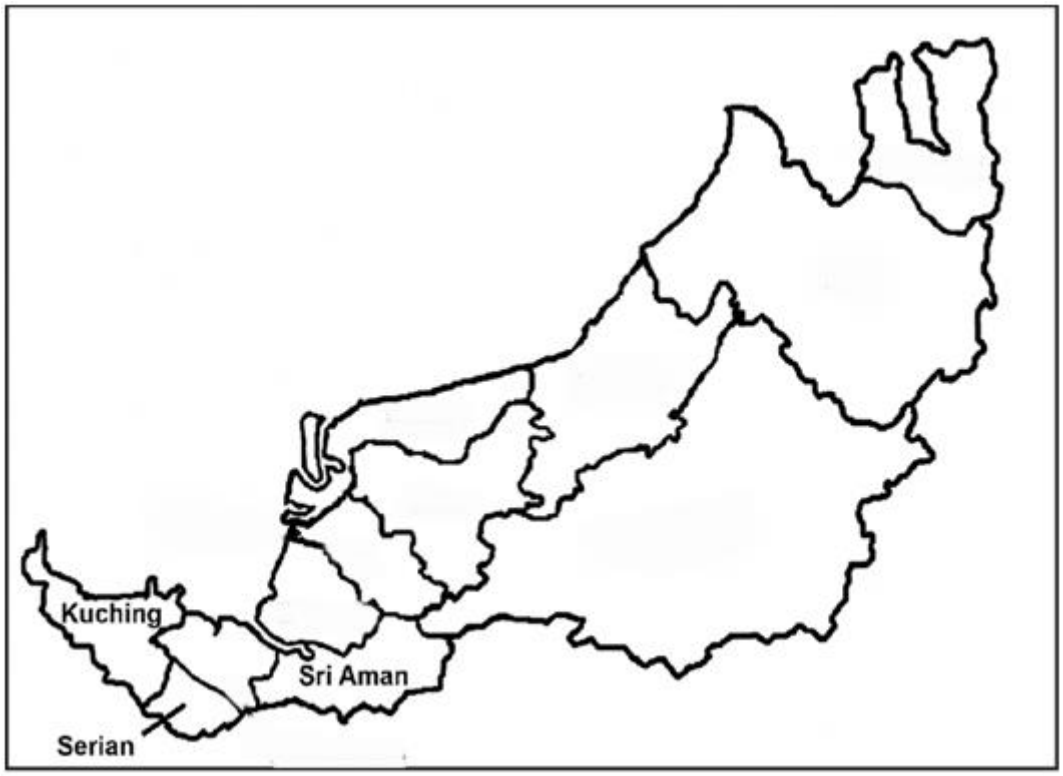

Figure 3.1: Sampling location of the three division along the North-Western region of Sarawak. Modified from Dow (2007)

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Table 3.1: List of the Collected Landraces with Its Locality, GPS and Division

Landrace	Location	GPS	Division
UNISR 2018-06 Padi Belawi Pandan	Kg Paon Gahat	0° 57' 0" North, 110° 39' 0" East	Serian
UNISR 2018-10 Padi Belawi	Kg Paon Gahat	0° 57' 0" North, 110° 39' 0" East	Serian
UNIPDW 2018-42 Padi Merjat	Kg TabuanRabak	1° 18' 39" North, 110° 20' 59" East	Kuching
UNIPDW 2018-16 Padi Merjat	KgMambong	1° 22' 0" North, 110° 21' 0" East	Kuching
UNIPDW 2018-17 Padi Pandan Wangi	Kg Pesak	1° 45' 0" North, 110° 46' 0" East	Kuching
UNISRA 2018-36 Padi Badawi	Kg Sungai Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman
UNISRA 2018-39 Padi Sempang	Kg Melugu Tengah	1° 06'14" North, 111°23'57" East	Sri Aman
UNISRA 2018-41 Padi Mawang	Kg Melugu Tengah	1° 06' 14" North, 111° 23' 57" East	Sri Aman
UNISRA 2018-29 Padi Limbang	Kg Sungai Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman
UNISRA 2018-30 Padi Chelum	Kg Sungai Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman
UNISRA 2018-31 Padi Bario	Kg Sungai Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman

*Kg indicates Kampung, GPS indicates Global Positioning System.

3.2 Germinating Seed

A total of 10 seeds per landraces were germinated by three different batches (Table 3.2) in the distilled water. The germinated seeds were transplanted into the planting medium consisted of 3:2:1 soil mixture (topsoil: organic compost: sand) once the radicle and plumule were formed. Each seed per landrace was considered as one accession. In total, there were 110 accessions (ten accessions per landrace).

Table 3.2: Germination Date for 11 Collected Landraces

Landrace	Germination Date
UNISR 2018-06 Padi Belawi Pandan	26.10.2018
UNISR 2018-10 Padi Belawi	26.10.2018
UNIPDW 2018-16 Padi Merjat	26.10.2018
UNIPDW 2018-17 Padi Pandan Wangi	26.10.2018
UNISRA 2018-29 Padi Limbang	11.11.2018
UNISRA 2018-30 Padi Chelum	11.11.2018
UNISRA 2018-31 Padi Bario	11.11.2018
UNISRA 2018-36 Padi Badawi	11.11.2018
UNISRA 2018-39 Padi Sempang	30.11.2018
UNISRA 2018-41 Padi Mawang	30.11.2018
UNIPDW 2018-42 Padi Merjat	30.11.2018

3.3 Deoxyribonucleic Acid Extraction

Young leaf samples, about 4-5 cm were collected from the 110 accessions. DNA was extracted using Cetyltrimethylammonium bromide (CTAB) protocol by Doyle and Doyle (1987). The concentration and quality of extracted DNA was measured using Single Drop Spectrophotometer (ND-2800-ODJ Nano DOT Nucleic Acid Analyser; Hercuvan). About 3 µL of DNA sample was loaded into 0.8% of 1X-TAE (Tris base, glacial acetic acid and 0.5 M EDTA, pH 8.0) agarose gel. The DNA samples were electrophoresed on for 30 minutes at a voltage of 90V.The integrity of the DNA was visually checked using Ethidium bromide (EtBr) staining in 1% 1X-TAE (Tris Base, glacial acetic acid and 0.5M EDTA, pH 8.0) agarose gel. The gel was visualised under ultraviolet transilluminator.

3.4 Pre-Selection using Simple Sequence Repeat Markers

A total of 12 Simple Sequence Repeat (SSR) markers were randomly chosen representing the 12 chromosomes of rice. The markers were tested on two randomly selected accessions. Four SSR markers (Table 3.3), which showed polymorphisms, were chosen for pre-selection to reduce the number of accessions for morphological and anatomical observations. Polymerase Chain Reaction (PCR) amplification was performed following Zhu et al. (2012) in a 20 µL volume containing 5 ng of genomic DNA, 0.2 µM each of forward and reverse primers, 0.2 mM dNTPs, 1X PCR buffer (750 mM Tris, pH 8.8, 200 mM KCl and 50 mM $(NH_4)_2SO_4$), 2.5 mM $MgCl_2$ and 1 unit of Taq DNA polymerase (First Base Laboratories Sdn Bhd, Malaysia). The PCR started with DNA denaturation at 94°C for 4 minutes followed by 40 cycles of repeated PCR amplification with the following parameters in sequential order: denaturation at 94°C for 45s, annealing temperature for each SSR following Table 3.2 for 45s, primer extension at 72°C for 1 minute with a final extension at 72°C for 10 minutes. The amplified PCR products were separated in 2% agarose gel prepared in 1X TAE buffer. The gel was run in 1X TAE buffer at a voltage of 90 V. The agarose gel was stained after completely electrophoresed with EtBr solution and visualised.

Table 3.3: List of SSR Markers used for Pre-Selection.

Marker	Primer sequence (5' to 3')	Ch	Ta (°C)	Expected Product Size (bp)
RM1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	1	55	90-120
RM279	F: GCGGGAGAGGGATCTCCT R: GGCTAGGAGTTAACCTCGCG	2	55	170-190
RM489	F: ACTTGAGACGATCGGACACC R: TCACCCATGGATGTTGTCAG	3	55	250-270
RM335	F: GTACACACCCACATCGAGAAG R: GCTCTATGCGAGTATCCATGG	4	55	100-200

*Ch: Chromosome, Ta: Annealing temperature, EPS: Expected product size. Reference: Gramene https://archive.gramene.org/markers/microsat/all-ssr.html

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3.5 Accession Characterisation

The genotype of the different accessions based on the four polymorphic SSR markers were used to select accessions for further evaluation in net house located at Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. Accessions with polymorphic genotype per landrace were chosen for morphological and anatomical characterisation. Only one individual was selected randomly for landrace having only one genotype. Each of the selected accessions was transplanted into pots with the drainage holes at the bottom to drain the excess water (Size: 5 Gallon, 33 cm X 31 cm) containing 3:2:1 soil mixture (topsoil: organic compost: sand). The nitrogen, phosphorus and potassium (NPK) fertiliser (17.5: 15.5: 10) was used for the fertilisation. Ten grams of fertiliser was applied per pot per application. The first fertiliser application took place at roughly the same time with transplanting. The second application took place about 30-40 days after the first application. The third application took place during the flowering stage. The watering was done once a day using tap water.

3.5.1 S_1 Generation Observation

At S_1 generation, a total of ten accessions were selected randomly for morphological and anatomical data collection. Due to certain constraints such as limited time and space, only three replicates per accession were planted and observed. Plants of S_1 generation were prepared as described in previous section. The plants were arranged randomly in a net house.

3.5.2 Morphological Observation

The morphological traits of both first generation and S_1 generation were recorded. A total of 15 traits selected from the International Research Rice Institutes guidelines (IRRI, 1980) and 10 additional traits were added for observation. All recorded characters, methods and criteria are shown in Table 3.4. Statistical analysis was not performed on the quantitative traits of the first generation as each of the accession was considered as one unique individual, hence no replications. Statistical analysis was only performed on quantitative traits of S_1 generations using IBM SPSS Statistics version 20.0 software (SPSS Inc., Chicago, IL, USA). Data was tested for normality and one way analysis of variance (ANOVA) was performed.

Table 3.4: The 25 Morphological Traits Selected Based on IRRI Guidelines Including Ten Additional Traits.

Observed Traits	Trait Code	Recording Standard
Seedling height	SH	Measured for height at the 5-leaf stage. Height was recorded in centimetres from the base of the shoot to the tip of the tallest leaf blade.
*Plant height	PH	Height was recorded in centimetres from the base of the stem to the tip of the tallest leaf blade. Time: Before flowering period.
Heading days	HD	Heading day is characterised by the emergence of the first panicle.
*Flowering days	FD	Flowering day is recorded when the flower opens, and the pollination takes place.
Culm length	CL	Culm length was measured in centimetres from the base of the stem to the base of the panicle Time: After heading.
Culm diameter	CD	Data were taken in centimetres from the outer diameter of the culms measured at the midportion of the culm. Time: Flowering period.
*Number of tillers	NT	Numbers of tillers were taken by counting the maximum number of tillers per plant.
Leaf length	LL	Measured in centimetres from the topmost leaf blade below the flag leaf on the main culm. Time: Late vegetative stage, before panicle initiation.
Leaf width	LW	Measured in centimetres from the topmost leaf blade below the flag leaf on the main culm, at the widest portion of the blade. Time: Late vegetative stage, before panicle initiation.
Blade colour	BC	Six broad classes of blade colour were recognised following (IRRI, 1980) guidelines: (1) pale green, (2) green, (3) dark green, (4) purple tips, (5) purple margins. (6) purple blotch. Leaf Colour Chart developed by IRRI, and Philippines Rice Research Institute (PhilRice) was used to score the colour on the sixth leaf. Time: Late vegetative stage, before panicle initiation.
Ligule shape	LS	Shape observation was made on random ligule. Three classes were given: (1) acute to acuminate, (2) 2-cleft and (3) truncate. Time: Late vegetative stage, before panicle initiation.
Ligule colour	LC	Colour observation was made on random ligule. Three classes of ligule colours were recognized: (1) white, (2) purple lines and (3) purple. Time: Late vegetative stage, before panicle initiation
Auricle colour	AC	Colour observation was made on random auricles. Colours of auricles were classified as: (1) white, (2) purple and (3) pale green. Time: Late vegetative stage, before panicle initiation.

Table 3.4 continued

Panicle type	PT	Panicles were classified according to their mode of branching, angle of primary branches and spikelet density: (1) compact, (2) intermediate and (3) open. Time: Near maturity.
*Number of panicles	NP	The panicle number of the plant was taken as the maximum of the panicle numbers extracted from the plant.
Length of panicles	LP	Measured in centimetres from the base to the tip of one panicle from the main tiller. Time: Near maturity.
Secondary branching	SB	Secondary branches of the main panicle from the main tiller bearing the spikelet were classified as: (0) absent, (1) light and (2) heavy.
*Percentage of filled grains	PFG	Measured by counting the number of fully filled grains per panicle multiplied by a factor of 100 over the total number of filled grains per panicle of the main panicle from productive tiller (Wiansamut et al., 2013).
*Seed shape	SS	Four classes were given: (1) round, (2) oblong, (3) elliptic and (4) linear.
*Seed length	SL	Measured in centimetres as the distance from the base of the lowermost sterile lemma to the tip (apiculus) of the fertile lemma or palea whichever is longer, mean length for 100 of seed were recorded.
*Seed width	SW	The widest portion of seed middle section measured in centimetres, mean width for 100 of seeds were recorded.
Grain length	GL	Measured in centimetres from one tip to another end, mean length for 10 grains were recorded.
Grain width	GW	The widest portion of grain middle section was measured in centimetres, mean length for 10 grains were recorded.
*Seed colour	SC	Colour of 10 random seeds husk were classified as: (1) yellow, (2) pale yellow, (3) blackish yellow and (4) golden brown.
*Grain colour	GC	Colour of 10 random grains were classified as: (1) milky white, (2) reddish brown, (3) dark brown, (4) black, (5) greenish white and (6) light reddish brown.

Remarks. * Additional traits made upon observation

3.5.3 Anatomical Observation

Anatomical characteristics of first generation and S_1 generation were recorded. Anatomical samples of plant materials of first generation such as leaves, midribs and roots

were prepared based on Cutler et al. (1978) with modifications. Meanwhile, in S_1 generation, only leaf samples were observed. For the preparation of leaf sample, the 5th eaf was selected and hand-sectioned by using a scalpel. Only the middle part of the leaf was used for uniformity. The leaf section was soaked in 70% bleach solution (Clorox) to remove the chlorophyll. The leaf section was rinsed with distilled water and stained using Safranin O. Then, the stained leaf section was dehydrated by soaking in 50% ethanol for 3-5 minutes and rinsed with distilled water. The dehydration process was repeated with 60%, 70%, 80%, 90% and 95% ethanol. The midrib of the 5th leaf was hand-sectioned using a microtome blade. The section was processed using the same protocol as described above except for, Alcian Blue was used for staining and a drop of hydrochloric acid (HCl) was added into 70% ethanol which was used for dehydration purpose. For root, midsection of root was selected randomly and hand-sectioned using microtome blade and processed as described for leaf midrib.

Microscopic slides were prepared using wet mount techniques (Karuppaiyan and Nandini, 2006). A drop of distilled water was pipetted onto the sample at the centre of the slide. Cover slip was lowered carefully onto the slide to avoid air bubbles. Excess water on the slide was drawn out with paper towel. For leaf samples, at least three slides were observed and two slides for midrib and root samples. Clear cross-sectioned of leaf, midrib and root tissues were photographed (Magnification: 40x) using Olympus DP72 camera attached to Olympus BX151 light microscope.

Epidermal characters of leaves such as length and width of long cell, the shape, arrangement and cell outline were studied under compound microscope. The measurement and characteristic of stomatal complex which comprises of size of stomata (length and

width) and shape of subsidiary cells were recorded. The stomata density (SD) was calculated based on Equation 3.1 (Paul et al., 2017).

$$\text{SD over 1 mm}^2 = S \times (pi)\ r^2 \qquad \text{Equation 3.1}$$

S are the number of stomata in microscopic view field, pi= 3.142, r = radius.

For midrib, the data of length and width were measured, and shape was described. Diameter of the root and stele were recorded. A Java-based image processing program called Image J was used to process all anatomical measurement (Schneider et al., 2012). The collected data of three replicates per accession for S_1 generation were analysed statistically using Student's t-test (IBM SPSS Statistics version 20.0 software). Wilcoxon Signed Rank Test was used to compare the anatomical characters between abaxial and adaxial leaf surfaces.

3.6 DNA Barcoding and Population Study

Molecular characterisations were made using two markers, which were Simple Sequence Repeat (SSR) and maturase-K (mat-K) gene marker.

3.6.1 Simple Sequence Repeat Genotyping

In addition to the four SSR markers used for pre-selection of accessions above, another 35 SSR markers were tested for polymorphism (Table 3.5). The details for these markers can be obtained from RiceGenes database (www.gramene.org). Similar PCR amplification method described in Section 3.4 was applied. The amplified bands were visually scored as present (1) or absence (0) separately for each primer pair. The scores of the band were pooled to create a binary data matrix. Polymorphic Information Content (PIC) was calculated using the Equation 3.2 following Anderson et al. (1993).

$$PICi = 1 - \Sigma Pij^2, \quad \text{Equation 3.2}$$

where ‘i’ is the total number of alleles detected for SSR marker and Pij is the frequency of the jth allele for marker i.

Number of alleles, number of effective alleles (*Ne*), Nei’s Gene Diversity (*h*) and Shannon’s Information Index (*I*) were calculated in Genetic Analysis in Excel (GenAIex) v.65 package (Peakall & Smouse, 2012)

Table 3.5: List of SSR Markers Tested on the Selected Accessions

Marker	Primer sequence (5'to 3')	Chromosome	Ta (°C)	Expected Product Size (bp)
RM226	F: GAAGCTAAGGTCTGGGAGAAACC R: AATGGCCTTAACCAAGTAGGATGG	1	55	280-350
RM207	F: CCATTCGTGAGAAGATCTGA R: CACCTCATCCTCGTAACGCC	2	55	N/A
RM514	F: AGATTGATCTCCCATTCCCC R: CACGAGCATATTACTAGTGG	3	55	N/A
RM567	F: ATCAGGGAAATCCTGAAGGG R: GGAAGGAGCAATCACCACTG	4	55	280-400
RM317	F: CATACTTACCAGTTCACCGCC R: CTGGAGAGTGTCAGCTAGTTGA	4	55	160
RM335	F: GTACACACCCACATCGAGAAG R: GCTCTATGCGAGTATCCATGG	4	55	100-200
RM229	F: CACTCACACGAACGACTGAC R: CGCAGGTTCTTGTGAAATGT	4	55	100-120
RM166	F: GGTCCTGGGTCAATAATTGGGTTACC R: TTGCTGCATGATCCTAAACCGG	4	61	321-410
RM108	F: TCTCTTGCGCGCACACTGGCAC R: CGTGCACCACCACCACCACCAC	4	67	N/A
RM413	F: GGCGATTCTTGGATGAAGAG R: TCCCCACCAATCTTGTCTTC	5	53	N/A
RM480	F: GCTCAAGCATTCTGCAGTTG R: GCGCTTCTGCTTATTGGAAG	5	58	N/A
RM17	F: TGCCCTGTTATTTTCTTCTCTC R: GGTGATCCTTTCCCATTTCA	12	55	190-220

Table 3.5 continued

RM164	F: TCTTGCCCGTCACTGCAGATATCC R: GCAGCCCTAATGCTACAATTCTTC	5	58	246-300
RM250	F: GGTTCAAACCAAGCTGATCA R: GATGAAGGCCTTCCACGCAG	5	57	170-190
RM469	F: AGCTGAACAAGCCCTGAAAG R: GACTTGGGCAGTGTGACATG	6	55	100-120
RM454	F: CTCAAGCTTAGCTGCTGCTG R: GTGATCAGTGCACCATAGCG	6	55	N/A
RM276	F: CTCAACGTTGACACCTCGTG R: TCCTCCATCGAGCAGTATCA	6	59	100-180
RM125	F: ATCAGCAGCCATGGCAGCGACC R: AGGGGATCATGTGCCGAAGGCC	7	63	130-600
RM455	F: AACAACCCACCACCTGTCTC R: AGAAGGAAAAGGGCTCGATC	7	55	140
RM481	F: TAGCTAGCCGATTGAATGGC R: CTCCACCTCCTATGTTGTTG	7	59	190-300
RM47	F: ACTCCACTCCACTCCCCAC R: GTCAGCAGGTCGGACGTC	7	59	N/A
RM152	F: GAAACCACCACACCTCACCG R: CCGTAGACCTTCTTGAAGTAG	8	53	140
RM447	F: CCCTTGTGCTGTCTCCTCTC R: ACGGGCTTCTTCTCCTTCTC	8	53	100-600
RM519	F: AGAGAGCCCCTAAATTTCCG R: AGGTACGCTCACCTGTGGAC	12	55	120-140

Table 3.5 continued

RM210	F: TCACATTCGGTGGCATTG R: CGAGGATGGTTGTTCACTTG	8	57	120-180
RM444	F: GCTCCACCTGCTTAAGCATC R: TGAAGACCATGTTCTGCAGG	9	55	190-280
RM215	F: CAAAATGGAGCAGCAAGAGC R: TGAGCACCTCCTTCTCTGTAG	9	56.7	150
RM244	F: CCGACTGTTCGTCCTTATCA R: CTGCTCTCGGGTGAACGT	10	55	N/A
RM228	F: CTGGCCATTAGTCCTTGG R: GCTTGCGGCTCTGCTTAC	10	55	N/A
RM552	F: CGCAGTTGTGGATTTCAGTG R: TGCTCAACGTTTGACTGTCC	11	55	200-210
RM206	F: CCCATGCGTTTAACTATTCT R: CGTTCCATCGATCCGTATGG	11	58.1	N/A
RM257	F: CAGTTCCGAGCAAGAGTACTC R: GGATCGGACGTGGCATATG	11	55	150-200
RM224	F: ATCGATCGATCTTCACGAGG R: TGCTATAAAAGGCATTCGGG	11	55	150-200
RM21	F: ACAGTATTCCGTAGGCACGG R: GCTCCATGAGGGTGGTAGAG	11	51	140-180
RM247	F: TAGTGCCGATCGATGTAACG R: CATATGGTTTTGACAAAGCG	12	55	140-190
RM561	F: GAGCTGTTTTGGACTACGGC R: GAGTAGCTTTCTCCCACCCC	-	55	N/A

*N/A represents not available. Ta indicates annealing temperature. EPS indicates expected product size.
Reference: https://www.gramene.org

3.6.2 Cluster Analysis Based on Simple Sequence Repeat Marker

A dendrogram were constructed based on genetic distances matrices using Unweighted Pair Group Method with Arithmetic Means (UPGMA) generated from Molecular Evolutionary Genetic Analysis (MEGA-X) v.10.1 software.

3.6.3 Amplification of Maturase-K Barcoding Marker

The *matK* region was amplified by using forward primer 5'-TAATTAAGAGGATTCACCAG-3' and reverse primer 5'-ATGCAACACCCTGTTCTGAC-3'. The reaction volume was 25 µL volume containing 3-5 ng of genomic DNA, 0.5 µM of each forward and reverse primers, 0.2 mM dNTPs, 1X PCR buffer, 1.5 mM $MgCl_2$ and 2.5 unit of Taq DNA polymerase (First Base Laboratories) following (Patil et al., 2015). The temperature cycles were programmed as at 94 °C for 4 min for initial denaturation, denaturation at 94 °C for 2 mins, annealing at 57.8 °C for 2 mins, extension at 72 °C for 2 mins for 35 cycles, followed by 72 °C for 15 mins for final extension. The PCR products were electrophoresed on 1.5% agarose gels along with 100 bp ladder (First Base Laboratories Sdn Bhd, Malaysia). The gel was then stained with EtBr and visualized using UV transilluminator.

PCR products of *matK* were purified using QIAquick Gel Extraction Kit (Qiagen, Germantown, MD, USA) following the manufacturer's manual. All purified products were sent for sequencing by Apical Scientific Sdn Bhd, Malaysia.

3.6.4 Cluster Analysis Based on Barcoding Marker

The *matK* sequences from the 22 upland accessions were manually edited in BioEdit software. The *matK* sequences for 94 accessions of *Oryza sativa*, three accessions of *Oryza rufipogon*, two accessions of *Oryza granulata*, one accession of *Oryza australiensis*, three *Oryza officinalis* and three accessions of *Oryza punctata* were downloaded from National Center of Biotechnology Information (NCBI) as shown in Table 3.6. The 106 sequences were analysed in MEGA-X v. 10.1 with 22 upland rice accessions along with 22 lowland rice accessions from UNIMAS collection. A Maximum Likelihood (ML) phylogenetic tree was constructed using MEGA-X v. 10.1. The best-fit substitution models, Tamura 3-parameters + discrete gamma distribution evolutionary rates among sites (T92+G) were used. The stability of internal nodes was assessed by bootstrap analysis with 1000 replicates bootstrap values.

Table 3.6: List of Species, Origin and Database Accessions Number from NCBI used in the Genetic Study.

Species	ID Code	Country of Origin	NCBI Accession Number
Oryza sativa	OS India 1	India	KT894765.1
Oryza sativa	OS India 2	India	KT894764.1
Oryza sativa	OS India 3	India	KT894763.1
Oryza sativa	OS India 4	India	KT894762.1
Oryza sativa	OS India 5	India	KT894761.1
Oryza sativa	OS India 6	India	KT894760.1
Oryza sativa	OS India 7	India	KT894759.1
Oryza sativa	OS India 8	India	KT894758.1
Oryza sativa	OS India 10	India	KT894756.1
Oryza sativa	OS India 11	India	KF731111.1
Oryza sativa	OS India 12	India	KF731110.1
Oryza sativa	OS India 13	India	KF731109.1
Oryza sativa	OS India 14	India	KF731108.1
Oryza sativa	OS India 15	India	KF731107.1
Oryza sativa	OS India 16	India	KF731106.1
Oryza sativa	OS India 17	India	KF731105.1
Oryza sativa	OS India 18	India	KF731104.1
Oryza sativa	OS India 19	India	KF731103.1
Oryza sativa	OS India 20	India	KF731102.1

Table 3.6 continued

Oryza sativa	OS India 21	India	KF731101.1
Oryza sativa	OS India 21	India	KF731101.1
Oryza sativa	OS India 22	India	KF731100.1
Oryza sativa	OS India 23	India	KF731099.1
Oryza sativa	OS India 24	India	KF731098.1
Oryza sativa	OS India 25	India	KF731097.1
Oryza sativa	OS India 26	India	KF731096.1
Oryza sativa	OS India 27	India	KF731095.1
Oryza sativa	OS India 28	India	KF731094.1
Oryza sativa	OS India 29	India	KF731093.1
Oryza sativa	OS India 30	India	KF731092.1
Oryza sativa	OS India 31	India	KF731091.1
Oryza sativa	OS India 32	India	KF731090.1
Oryza sativa	OS India 33	India	KF731089.1
Oryza sativa	OS India 34	India	KF731088.1
Oryza sativa	OS India 35	India	KF731087.1
Oryza sativa	OS India 36	India	KF731086.1
Oryza sativa	OS India 37	India	KF731085.1
Oryza sativa	OS India 38	India	KF731084.1
Oryza sativa	OS India 39	India	KF731083.1
Oryza sativa	OS India 40	India	KF731082.1
Oryza sativa	OS India 41	India	KF731081.1
Oryza sativa	OS India 42	India	KF731080.1
Oryza sativa	OS India 43	India	KF731079.1
Oryza sativa	OS India 44	India	KF731078.1
Oryza sativa	OS India 45	India	KF731077.1
Oryza sativa	OS India 46	India	KF731074.1
Oryza sativa	OS India 47	India	KF731073.1
Oryza sativa	OS India 48	India	KF731072.1
Oryza sativa	OS India 49	India	KF731071.1
Oryza sativa	OS India 50	India	KF731070.1
Oryza sativa	OS India 51	India	KF731069.1
Oryza sativa	OS India 52	India	KF731068.1
Oryza sativa	OS India 53	India	KF731067.1
Oryza sativa	OS India 54	India	KF731066.1
Oryza sativa	OS India 55	India	KF731065.1
Oryza sativa	OS India 56	India	KF731064.1
Oryza sativa	OS India 57	India	KF731063.1
Oryza sativa	OS India 58	India	KF731062.1
Oryza sativa	OS India 59	India	KF731061.1
Oryza sativa	OS India 60	India	KF731060.1
Oryza sativa	OS India 61	India	KF731059.1
Oryza sativa	OS India 62	India	KF731058.1
Oryza sativa	OS India 63	India	KF731057.1

Table 3.6 continued

Oryza sativa	OS India 64	India	KF731056.1
Oryza sativa	OS India 65	India	KF731055.1
Oryza sativa	OS India 66	India	KF731054.1
Oryza sativa	OS India 67	India	KF731053.1
Oryza sativa	OS India 68	India	KF731052.1
Oryza sativa	OS India 69	India	KF731051.1
Oryza sativa	OS India 70	India	KF731050.1
Oryza sativa	OS India 71	India	KF731049.1
Oryza sativa	OS India 72	India	KF731048.1
Oryza sativa	OS India 73	India	KF731047.1
Oryza sativa	OS India 74	India	KF731046.1
Oryza sativa	OS India 75	India	KF731045.1
Oryza sativa	OS India 76	India	KF731044.1
Oryza sativa	OS India 77	India	KF731043.1
Oryza sativa	OS India 78	India	KF731042.1
Oryza sativa	OS India 79	India	KF731041.1
Oryza sativa	OS India 80	India	KF731040.1
Oryza sativa	OS India 81	India	KF731039.1
Oryza sativa	OS India 82	India	KF731038.1
Oryza sativa	OS India 83	India	KF731037.1
Oryza sativa	OS India 84	India	KF731036.1
Oryza sativa	OS India 85	India	KF731035.1
Oryza sativa	OS India 86	India	KF731034.1
Oryza sativa	OS India 87	India	KF731033.1
Oryza sativa	OS India 88	India	KF731032.1
Oryza sativa	OS India 89	India	HG794000.1
Oryza sativa	OS Australia 1	Australia	KU923990.1
Oryza sativa	OS Australia 2	Australia	KU923989.1
Oryza sativa	OS Australia 3	Australia	KU923988.1
Oryza sativa	OS Australia 4	Australia	KU923987.1
Oryza sativa	OS China 1	China	AF148650.1
Oryza sativa	OS Unknown	Unknown	EU434287.1
Oryza rufipogon	O. rufipogon 1	Unknown	EU434286.1
Oryza rufipogon	O. rufipogon 2	India	FJ908261.1
Oryza rufipogon	O. rufipogon 3	China	AF148651.1
Oryza punctata	O. punctata 1	Unknown	KP864529.1
Oryza punctata	O. punctata 2	Unknown	KP864528.1
Oryza punctata	O. punctata 3	Unknown	KP864527.1
Oryza officinalis	O. officinalis 1	Philippines	AF148658.1
Oryza officinalis	O. officinalis 2	China	KP121859.1
Oryza officinalis	O. officinalis 3	Papua New Guinea	KP121858.1
Oryza australiensis	O. australiensis 1	Australia	AF148667
Oryza granulata	O. granulata 1	China	AF148674.1
Oryza granulata	O. granulata 2	Vietnam	KP121857.1

CHAPTER 4

RESULTS

4.1 Pre-Selection of Rice Accessions using SSR Marker

A total of 22 accessions out of 110 accessions (Table 4.1) which show polymorphisms for the four SSR markers (RM1, RM279, RM489 and RM335) were selected for further morphological, anatomical, and molecular analysis. The 22 selected accessions were designated as UNIMAS-01 – UNIMAS-22 (Table 4.1). The ten selected accessions from the first generation were designated as listed in Table 4.2.

Table 4.1: SSR Genotype of 22 Selected Upland Rice Accessions based on Four SSR Markers.

Landrace	Selected Accession	Marker			
		RM1	RM279	RM489	RM335
UNISR 2018-06 Padi Belawi Pandan	UNIMAS-01	0	1	1	N/A
	UNIMAS-02	1	1	1	N/A
UNISR 2018-10 Padi Belawi	UNIMAS-03	1	N/A	1	N/A
UNIPDW 2018-16 Padi Merjat	UNIMAS-04	1	N/A	1	N/A
	UNIMAS-05	3	N/A	1	N/A
	UNIMAS-06	0	N/A	1	N/A
	UNIMAS-07	2	N/A	1	N/A
UNIPDW 2018-17 Padi Pandan Wangi	UNIMAS-08	1	0	1	1
	UNIMAS-09	1	1	1	0
	UNIMAS-10	1	1	1	2
	UNIMAS-11	1	0	0	3
	UNIMAS-12	1	1	1	1
	UNIMAS-13	1	1	1	4
	UNIMAS-14	1	1	2	1
UNISRA 2018-29 Padi Limbang	UNIMAS-15	1	N/A	1	N/A

Table 4.1 continued

UNISRA 2018-30 Padi Chelum	UNIMAS-16	1	N/A	1	N/A
UNISRA 2018-31 Padi Bario	UNIMAS-17	1	N/A	1	N/A
	UNIMAS-18	0	N/A	1	N/A
UNISRA 2018-36 Padi Badawi	UNIMAS-19	1	N/A	1	N/A
UNISRA 2018-39 Padi Sempang	UNIMAS-20	1	1	1	N/A
UNISRA 2018-41 Padi Mawang	UNIMAS-21	1	1	1	N/A
UNIPDW 2018-42 Padi Merjat	UNIMAS-22	1	1	1	N/A

*Number scored for each accession and SSR marker represents grouping made based on allele scoring. N/A indicates not available.

Table 4.2: List of 11 Collected Landraces, 22 Chosen Accessions in First Generation and Ten Accessions Observed at S_1 Generation.

Landrace	First generation Accession	Selected S_1
UNISR 2018-06 Padi Belawi Pandan	UNIMAS-01	UNIMAS-01-S1
	UNIMAS-02	UNIMAS-02-S1
UNISR 2018-10 Padi Belawi	UNIMAS-03	UNIMAS-03-S1
UNIPDW 2018-42 Padi Merjat	UNIMAS-22	N/A
UNIPDW 2018-16 Padi Merjat	UNIMAS-04	UNIMAS-04-S1
	UNIMAS-05	N/A
	UNIMAS-06	N/A
	UNIMAS-07	UNIMAS-07-S1
UNIPDW 2018-17 Padi Pandan Wangi	UNIMAS-08	UNIMAS-08-S1
	UNIMAS-09	UNIMAS-09-S1
	UNIMAS-10	N/A
	UNIMAS-11	N/A
	UNIMAS-12	UNIMAS-12-S1
	UNIMAS-13	N/A
	UNIMAS-14	N/A
UNISRA 2018-36 Padi Badawi	UNIMAS-19	N/A
UNISRA 2018-39 Padi Sempang	UNIMAS-20	N/A

Table 4.2 continued

UNISRA 2018-41 Padi Mawang	UNIMAS-21	N/A
UNISRA 2018-29 Padi Limbang	UNIMAS-15	N/A
UNISRA 2018-30 Padi Chelum	UNIMAS-16	N/A
UNISRa 2018-31 Padi Bario	UNIMAS-17 UNIMAS-18	UNIMAS-17-S1 UNIMAS-18-S1

*N/A indicates not available

4.2 Morphological Characterisation of the Rice Accessions

A total of 15 traits selected from the International Rice Research Institutes guidelines and another 10 additional traits (Total= 25 traits) were recorded for the first-generation rice accession. Due to certain constraints, only 11 morphological traits were recorded for S_1 generation.

4.2.1 Qualitative Traits

Nine qualitative traits of 22 rice accessions in the first generation are furnished in Table 4.3 while four qualitative traits for ten rice accessions in S_1generation are furnished in Table 4.4. The leaf blade colour (BC) showed variations among the accessions in first generation (Figure 4.1). Three classes of BC were recognized (Figure 4.1) among the 22 accessions in first generation: four accessions were pale green, 12 accessions were green and the remaining accessions were dark green (Table 4.3). Meanwhile, two classes of BC were recognized in S_1 (Table 4.4). Only one accession was scored as '2' (green) and the remaining were dark green.

The ligule shape (LS) ranged from acute to acuminate. Variations were observed in ligule colour (LC) and auricle colour (AC). All accessions in first generation were having white LC and AC (Figure 4.1), except for one accession, UNIMAS-07 (Table 4.3) having purple ligule and auricle (Figure 4.1). In S_1 generation, UNIMAS-07-S1 (Table 4.4) was showing purple ligule and auricle and the rest of the accession had white LC and AC.

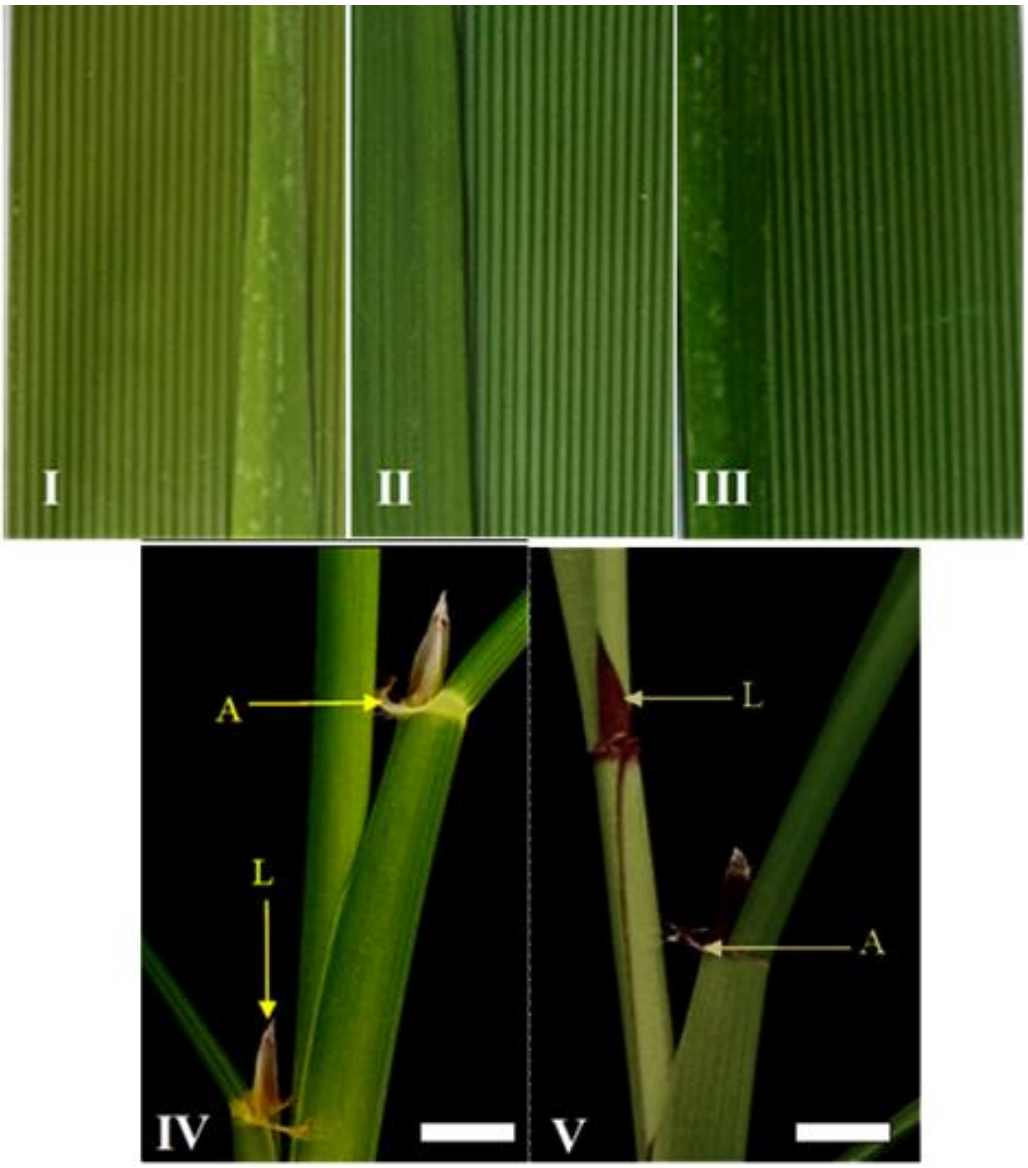

Figure 4.1: Representative Photo of Blade Colour, Auricles and Ligules. **I**: Pale green BC, **II**: Green BC, **III**: Dark Green BC, **IV:** White auricle and ligule, **V**: Purple auricle and ligule. Abbreviations: **A**- Auricle, **L**- Ligule. White Scale Bar represents 1 cm.

Out of the 22 accessions of the first generation, one accession (UNIMAS-22) did not produce any panicle during the period of observation. The types of panicle (PT) separated the accessions into three different groups (Figure 4.2). Out of 21 accessions in first generation, 14 were having intermediate PT and six accessions exhibited open PT (Table 4.3). Only one accession, which was UNIMAS-15 were having compact PT (score 1). Secondary branches (SB) in the panicle were found absent in eight accessions in first generation (Figure 4.3). Eight accessions were having light SB and the remaining five accessions (UNIMAS-07, UNIMAS-08, UNIMAS-15, UNIMAS-17, and UNIMAS-18) were having heavy SB (score 2) as shown in Figure 4.3.

Out of 21 accessions with panicle, variations were found in the seed shape (SS) as shown in Table 4.3. Two different classes of SS were recorded (Figure 4.4). There were 20 accessions (95%) were having linear SS. Only UNIMAS-16 was having elliptic SS (5%). Variation was also observed among the accession of first generation in seed colour (SC). Three different groups of colours were observed among the accessions (Figure 4.4). A majority, 13 out of 21 (62%) accessions were having yellow husk. Five other accessions (24%) were having blackish yellow husk and the remaining three accessions (UNIMAS-12, UNIMAS- 19 and UNIMAS-21) were having golden brown husk (14%).

The grain colour (GC) had variations (Table 4.3) and the accessions were grouped into four different groups (Figure 4.5). A total of 15 accessions were having milky white GC. Four accessions (UNIMAS-08, UNIMAS-11, UNIMAS-13 and UNIMAS-14) presented reddish-brown GC. One accession (UNIMAS-16) was observed having black grain. Another accession, UNIMAS- 07 was having dark brown GC.

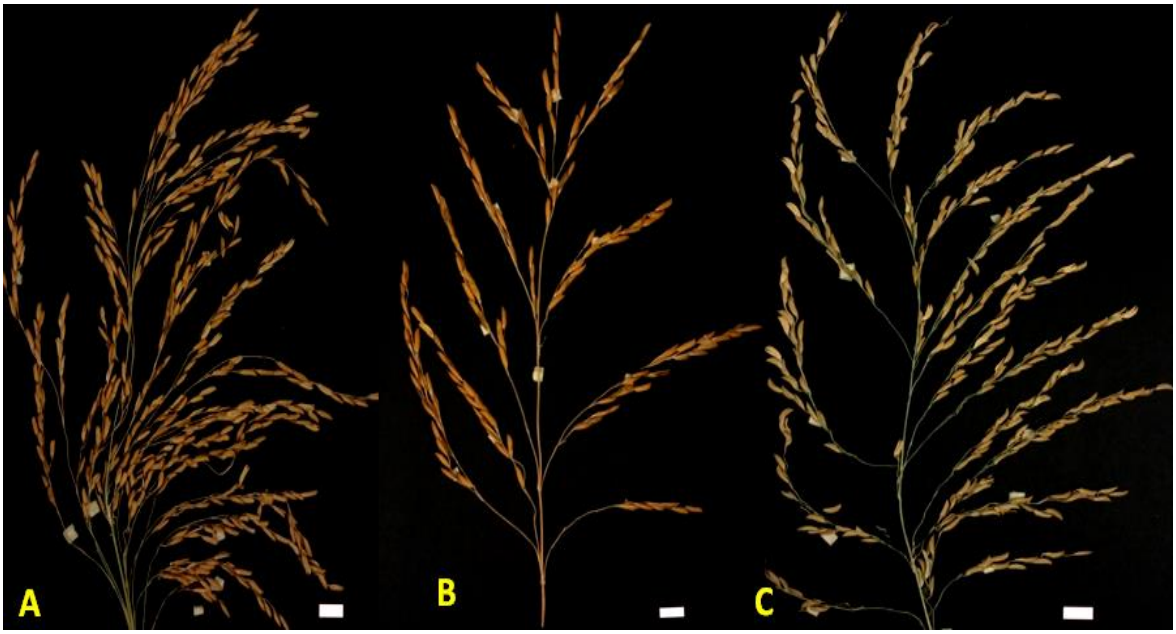

Figure 4.2: Representative Photos for Type of Panicle (PT). **A**: Compact Panicle, **B**: Open Panicle, **C**: Intermediate Panicle. Remarks: White Bar Indicates Scale= 1 cm.

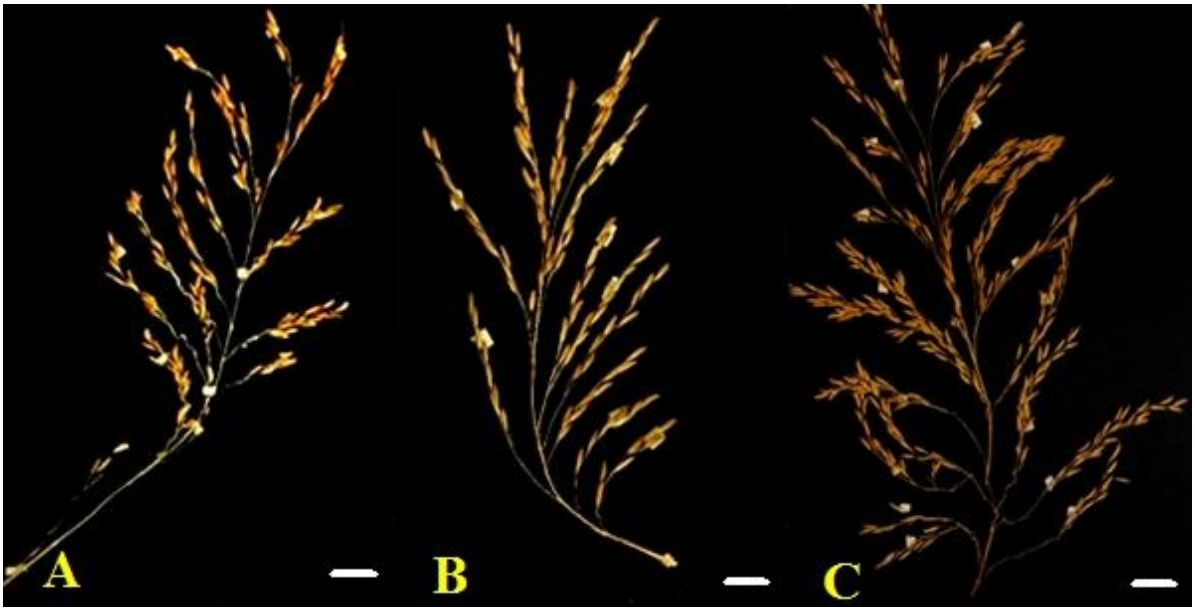

Figure 4.3: Representative Photos for Type of Secondary Branching (SB). **A**: Absent, **B**: Light, and **C**: Heavy. Remarks: White Bar Indicates Scale= 1 cm.

Figure 4.4: Representative of Seed Shape (SS) and Seed Colour (SC)of the Accessions in this Study. **I**: Linear SS, Golden brown SC, **II**: Elliptic SS, Yellow SC, **III**: Linear SS, Blackish Yellow SC. White Bar Indicates Scale= 1 cm.

Figure 4.5: Representative of Grain Colour (GC) of the Accessions in the Study. **A**: Reddish-brown GC, **B**: Dark-brown GC, **C**: Milky white GC, **D**: Blackish GC. White Bar Indicates scale= 1 cm

Table 4.3: Nine Qualitative Traits of 22 Upland Rice Accessions in First Generation.

Accession	BC	LS	LC	AC	PT	SB	SS	SC	GC
UNIMAS-01	3	1	1	1	2	0	4	1	1
UNIMAS-02	1	1	1	1	3	0	4	1	1
UNIMAS-03	2	1	1	1	2	0	4	1	1
UNIMAS-04	2	1	1	1	2	0	4	1	1
UNIMAS-05	2	1	1	1	2	0	4	1	1
UNIMAS-06	2	1	1	1	2	1	4	1	1
UNIMAS-07	2	1	3	2	2	2	4	1	3
UNIMAS-08	2	1	1	1	2	2	4	1	2
UNIMAS-09	2	1	1	1	2	1	4	1	1
UNIMAS-10	2	1	1	1	2	1	4	1	1
UNIMAS-11	3	1	1	1	2	1	4	1	2
UNIMAS-12	2	1	1	1	2	0	4	1	1
UNIMAS-13	2	1	1	1	2	1	4	1	2
UNIMAS-14	2	1	1	1	3	1	4	1	2
UNIMAS-15	1	1	1	1	1	2	4	1	1
UNIMAS-16	3	1	1	1	3	1	3	1	4
UNIMAS-17	1	1	1	1	3	2	4	1	1
UNIMAS-18	3	1	1	1	3	2	4	1	1
UNIMAS-19	1	1	1	1	3	1	4	4	1
UNIMAS-20	2	1	1	1	2	0	4	3	1
UNIMAS-21	3	1	1	1	2	0	4	4	1
UNIMAS-22	3	1	1	1	N/A	N/A	N/A	N/A	N/A

*N/A indicates data not available. BC: Blade colour (Scale: 1; Pale green, 2; Green, 3; Dark green, 4; Purple tips, 5; Purple margins, 6; Purple blotch), LS: Ligule shape (Scale: 1;Acute to Acuminate, 2; 2-Cleft, 3; Truncate), LC: Ligule colour (Scale: 1; White, 2; Purple Lines, 3; Purple), AC: Auricle colour (Scale:1; White, Or 2; Purple, 3; Pale Green), PT: Panicle type (Scale: 1; Compact, 2; Intermediate, 3; Open), SB: Secondary branching (Scale: 0; Absent, 1; Light, 2; Heavy), SS: Seed shape (Scale: 1; Round, 2; Oblong, 3; Elliptic, 4; Linear), SC: Seed colour (Scale: 1; Yellow, 2; Pale yellow, 3; Blackish yellow, 4; Golden brown), GC: Grain colour (Scale: 1; Milky white, 2; Reddish brown, 3; Dark brown, 4; Black, 5; Greenish white, 6; Light reddish brown)

Table 4.4: Four Qualitative Traits Observed at Ten Accessions in S_1 Generation

Accession	BC	LS	LC	AC
UNIMAS-01-S1	2***	1	1	1
UNIMAS-02-S1	3*	1	1	1
UNIMAS-03-S1	3	1	1	1
UNIMAS-04-S1	3	1	1	1
UNIMAS-07-S1	3**	1	$3^{!}$	$2^{!!}$
UNIMAS-08-S1	3	1	1	1
UNIMAS-09-S1	3**	1	1	1
UNIMAS-12-S1	3**	1	1	1
UNIMAS-17-S1	3**	1	1	1
UNIMAS-18-S1	3**	1	1	1

*BC: Blade colour (Scale: 1; pale green, 2; green, 3; dark green, 4; purple tips, 5; purple margins, 6; purple blotch), *=Represents one out of three replicates was having pale green BC, **=Represents one out of three replicates was having green BC, *** = Represents one out of three replicates was having dark green BC. LS: Ligule shape (Scale: 1; Acute to acuminate, 2; 2-cleft, 3; truncate), LC: Ligule colour (Scale: 1; white, 2; purple lines, 3; purple), != Represents one out of three replicates was having white LC, AC: Auricle colour (Scale: 1; white, or 2; purple, 3; pale green),$^{!!}$= Represents one out of three replicates was having white AC.

4.2.2 Quantitative Traits

A total of 16 quantitative morphological traits of the 22 accessions in first generation are furnished in Table 4.5. Due to certain constraints, only seven quantitative morphological traits (Table 4.6) are recorded in S_1 generation.

The plant height was purposely measured at the five leaves stage. This is to have early indication whether there were any accessions having faster vegetative development than others, which can be further investigated in the future. Plant height at five leaves stage or seedling height (SH) of first generation (Table 4.5) ranged from 41.1 cm (UNIMAS-16) to 54.5 cm (UNIMAS- 08). While in S_1, the mean value (Table 4.6) of SH ranged from 37.67 cm (UNIMAS-17-S1) to 50.40 cm (UNIMAS-12-S1). There was no significant difference in SH between accessions at S_1 generation (Table 4.6).

At mature stage, the plant height (PH) of first generation (Table 4.5) ranged from 90.9 cm (UNIMAS-09) to 201.2 cm (UNIMAS-07). For S_1 generation, the mean value of PH ranged from 133.87 cm (UNIMAS-03-S1) to 210.73 cm (UNIMAS-07-S1) as shown in Table 4.6.

Culm length (CL) measurements for first generation (Table 4.5) were ranging from 40.7 cm (UNIMAS-02) to 115.7 cm (UNIMAS-22). For S_1 generation, the CL was statistically different (Table 4.6) between accessions ranging from 78.13 cm (UNIMAS-18-S1) to 124.70 cm (UNIMAS-07-S1).

Culm diameter (CD) for first generation (Table 4.5) ranged from 2.7 cm (UNIMAS-17) to 4.1 cm (UNIMAS-19). In S_1generation, the mean value of CD ranged from 2.93 cm (UNIMAS-03-S1) to 3.97 cm (UNIMAS-02-S1) and the statistical analysis showed no significant difference between the accessions (Table 4.6).

In case of tiller number (NT), the accessions in the first generation were found to have tillers ranging from two to six tillers per plant (Table 4.5). The mean value of NT in S_1 generation ranged from 2.00 to 6.67 per plant (Table 4.6). Statistical analysis showed significant differences between the accessions.

The leaf length (LL) (Table 4.5) observed among the accessions in the first generation ranging from 42.3 cm (UNIMAS-02) to 72.0 cm (UNIMAS-19) while the mean value of the LL in S_1 ranging from 60.2 cm (UNIMAS-12-S1) to 91.03 cm (UNIMAS-09-S1) as shown in Table 4.6. Statistical analysis of LL showed significant difference between the accessions in S_1 generation (Table 4.6).

Leaf width (LW) of the accessions in first generation (Table 4.5) ranged from 1.4 cm (UNIMAS-04) to 2.1 cm (UNIMAS-03). In S_1 generation, significant difference was

recorded for LW (Table 4.6). Accession UNIMAS-04-S1 was having the narrowest LW with the mean value of 1.4 cm.

The heading days (HD), as shown in Table 4.5, the accessions in the first generation ranged from 94 days (UNIMAS-18) to 126 days (UNIMAS-02 and UNIMAS-12). The flowering days (FD) took around 127 days (UNIMAS-10 and UNIMAS-15) to 153 days (UNIMAS-02) after germination.

The number of panicle (NP) and length of panicle (LP) of the accessions in first generation ranged from 1 (UNIMAS-20) to 7 (UNIMAS 04 and UNIMAS-06) panicles per plant and 23.9 cm (UNIMAS-20) to 39.8 cm (UNIMAS-14), respectively (Table 4.5).

The percentage of filled grain (PFG) per accession in first generation, ranged from 40% (UNIMAS-05) to 90% (UNIMAS-15) grains per accession. The seed length (SL) in first generation ranged from 0.7 cm to 1.0 cm with the seed width (SW) ranged from 0.2 cm to 0.3 cm (Table 4.5). The grain length (GL) and grain width (GW) of the 22 accessions in first generation were ranged from 0.6 cm to 0.9 cm, and 0.15 cm to 0.2 cm, respectively (Table 4.5).

Table 4.5: The 16 Quantitative Traits of 22 Upland Rice Accessions in First Generation

Landrace	Acc.	SH (cm)	PH (cm)	CL (cm)	CD (cm)	NT	LL (cm)	LW (cm)	HD (days)	FD (days)	NP	LP (cm)	PFG (%)	SL (cm)	SW (cm)	GL (cm)	GW (cm)
UNISR 2018-06 Padi Belawi Pandan	UNIMAS-01	46.3	127.3	65.7	3.2	4	54.1	1.8	124	151	5	33.5	69%	1.0	0.2	0.7	0.2
	UNIMAS-02	50.1	154.7	109.8	3.3	3	42.3	1.9	126	153	4	30.3	70%	0.9	0.2	0.7	0.2
UNISR 2018-10 Padi Belawi	UNIMAS-03	47.1	139.4	88.9	3.1	5	67.8	2.1	112	140	5	24.5	65%	0.8	0.2	0.6	0.2
UNIPDW 2018-16 Padi Merjat	UNIMAS-04	45.1	171.0	95.2	3.0	6	69.1	1.4	106	128	7	25.7	69%	1.0	0.2	0.7	0.2
	UNIMAS-05	44.9	172.1	90.1	2.9	5	70.1	1.8	103	132	3	26.7	40%	1.0	0.2	0.7	0.2
	UNIMAS-06	43.2	169.1	99.1	2.8	6	65.1	1.9	107	141	7	25.8	85%	0.9	0.2	0.7	0.2
	UNIMAS-07	44.9	201.2	89.1	2.9	3	69.1	2.0	112	145	5	29.8	82%	0.8	0.3	0.6	0.2
UNISR 2018-17 Padi Pandan Wangi	UNIMAS-08	54.5	185.6	100.7	3.3	2	58.2	1.8	112	142	6	27.9	72%	0.9	0.2	0.8	0.2
	UNIMAS-09	46.1	90.9	88.9	3.2	3	56.7	1.9	114	143	3	33.3	61%	0.7	0.2	0.6	0.2
	UNIMAS-10	49.1	149.9	85	3.4	2	57.4	1.7	102	127	4	32.1	64%	0.9	0.2	0.8	0.2

Table 4.5 continued

UNISR 2018-17 Padi Pandan Wangi	UNIMAS-11	47.2	159.4	92.1	3.4	3	58.1	1.7	103	128	5	34.6	71%	0.8	0.2	0.7	0.2
	UNIMAS-12	48.1	159.9	87.1	3.1	2	58.7	1.5	126	146	2	24.7	64%	0.8	0.2	0.6	0.2
	UNIMAS-13	46.1	159.1	99.1	3.2	2	60.5	1.8	104	130	3	30.2	69%	0.9	0.2	0.6	0.2
	UNIMAS-14	49.1	163.5	102.1	3.3	3	63.4	1.8	99	129	3	39.8	86%	0.8	0.2	0.6	0.2
UNISRA 2018-29 Padi Limbang	UNIMAS-15	43.1	169.3	95.0	3.2	3	65.9	1.7	104	127	3	28.8	90%	0.9	0.2	0.7	0.15
UNISRA 2018-29 Padi Chelum	UNIMAS-16	41.1	167.3	80.8	2.9	4	70.0	1.9	101	131	3	26.3	77%	0.9	0.2	0.7	0.2
UNISRA 2018-31 Padi Bario	UNIMAS-17	44.6	147.8	76.4	2.7	3	61.0	1.8	107	137	3	27.9	89%	1.0	0.2	0.6	0.2
	UNIMAS-18	46.1	139.9	40.7	3.1	3	70.2	1.9	94	128	5	25.4	84%	0.9	0.2	0.9	0.2
UNISRA 2018-36 Padi Badawi	UNIMAS-19	45.3	120.9	64.5	4.1	3	72.0	1.7	107	132	2	29.9	67%	0.9	0.2	0.7	0.2

Table 4.5 continued

UNISRA 2018-41 Padi Mawang	UNIMAS -21	49.2	138.5	56.9	3.7	4	60.5	1.7	111	147	3	24.2	45%	0.8	0.3	0.6	0.2
UNIPDW 2018-42 Padi Merjat	UNIMAS -22	49.4	175.2	115.7	3.8	4	64.0	1.8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

*N/A indicates data not available. Acc indicates accessions. SH: Seedling height in centimetre, PH: Plant height in centimetre, CL: Culm length in centimetre, CD: Culm diameter in centimetre, NT: Number of tillers, LL: Leaf length in centimetre; LW: Leaf width in centimetre, HD: Heading days, FD: Flowering days, NP: Number of panicles, LP: Length of panicle in centimetre, PFG: Percentage of filled grains, SL: Seed length, SW: Seed width, GL: Grain length, GW: Grain width.

Table 4.6: The Seven Quantitative Traits Characterised for the Ten Accessions in S_1 Generation

Landrace	Acc.	SH (cm)	PH (cm)	CD (cm)	CL (cm)	NT	LL (cm)	LW (cm)
UNISR 2018-06 Padi Belawi Pandan	UNIMAS -01-S1	49.00^{a} ± 5.01	158.37^{a} ± 13.18	3.10^{a} ± 0.30	99.60^{cd} ± 4.61	4.67^{cd} ± 0.42	80.37^{ab} ± 8.45	1.63^{ab} ± 0.16
	UNIMAS -02-S1	48.90^{a} ± 5.01	153.03^{a} ± 13.18	3.97^{a} ± 0.30	98.78^{bcd} ± 4.61	3.33^{abc} ± 0.42	79.37^{ab} ± 8.45	2.00^{bc} ± 0.16
UNISR 2018-10 Padi Belawi	UNIMAS -03-S1	48.13^{a} ± 5.01	133.87^{a} ± 13.18	2.93^{a} ± 0.30	85.53^{abc} ± 4.61	6.00^{de} ± 0.42	66.23^{ab} ± 8.45	1.90^{abc} ± 0.16
UNIPDW 2018-16 Padi Merjat	UNIMAS -04-S1	44.80^{a} ± 5.01	158.17^{a} ± 13.18	3.57^{a} ± 0.30	95.00^{bcd} ± 4.61	6.67^{e} ± 0.42	70.17^{ab} ± 8.45	1.40^{a} ± 0.16
	UNIMAS -07-S1	44.53^{a} ± 5.01	210.73^{b} ± 13.18	3.83^{a} ± 0.30	124.70^{e} ± 4.61	2.33^{a} ± 0.42	88.00^{ab} ± 8.45	2.37^{c} ± 0.16
UNISR 2018-17 Padi Pandan Wangi	UNIMAS -08-S1	46.70^{a} ± 5.01	179.60^{ab} ± 13.18	3.67^{a} ± 0.30	100.70^{cd} ± 4.61	2.00^{a} ± 0.42	81.17^{ab} ± 8.45	1.83^{abc} ± 0.16
	UNIMAS -09-S1	45.23^{a} ± 5.01	178.17^{ab} ± 13.18	3.77^{a} ± 0.30	110.17^{de} ± 4.61	3.00^{ab} ± 0.42	91.03^{b} ± 8.45	1.90^{abc} ± 0.16
	UNIMAS -12-S1	50.40^{a} ± 5.01	147.97^{a} ± 13.18	3.13^{a} ± 0.30	85.77^{abc} ± 4.61	3.00^{ab} ± 0.42	60.20^{a} ± 8.45	1.70^{ab} ± 0.16
UNISRA 2018-31 Padi Bario	UNIMAS -17-S1	37.67^{a} ± 5.01	161.17^{a} ± 13.18	3.53^{a} ± 0.30	83.00^{ab} ± 4.61	2.33^{a} ± 0.42	73.30^{ab} ± 8.45	1.80^{ab} ± 0.16
	UNIMAS -18-S1	45.17^{a} ± 5.01	150.03^{a} ± 13.18	3.33^{a} ± 0.30	78.13^{a} ± 4.61	4.00^{bc} ± 0.42	67.10^{ab} ± 8.45	1.73^{ab} ± 0.16

*Difference in morphological traits between accession were compared using Variance Analysis (ANOVA) followed by Tukey HSD Post Hoc Test ($P<0.05$). The different alphabet indicates significant difference.SH: Seedling height, PH: Plant height, CL: Culm length, CD: Culm diameter, NT: Number of tillers, LL: Leaf length; LW: Leaf width.

4.3 Characterisation Based on Anatomical Traits

A total of six traits of epidermal cell were recorded in first-generation (Table 4.7) and S_1 generation (Table 4.8) of rice accessions. Meanwhile, the traits of midrib and root were only observed for the accessions in first generation.

4.3.1 Epidermal Traits

Table 4.7 shows that the length of the long cells (LEPC) on abaxial surface ranged from 58.73 µm (UNIMAS-05) to 138.876 µm (UNIMAS-13). On adaxial surface (Table 4.7), the length ranged from 47.65 µm (UNIMAS-07) to 97.83 µm (UNIMAS-08).

The LEPC of the abaxial epidermis observed in S_1 generation showed no significant difference between the accessions (Table 4.8). The mean value of the LEPC ranged from 67.52 µm (UNIMAS-12-S1) to102.08 µm (UNIMAS-08-S1) in Table 4.8. On adaxial surface, however, significant difference (P-value= 0.049) of LEPC between the accessions were recorded (Table 4.8), ranged from 71.67 µm (UNIMAS-03-S1) to 108.96 µm (UNIMAS-18-S1). The LEPC when compared between abaxial and adaxial surface did not show significant different (Table 4.8).

The width of the long cell (WEPC) on abaxial and adaxial surface (Table 4.7) of accessions in the first generation were ranging from 11.23 µm (UNIMAS-21) to 23.90 µm (UNIMAS-16) and 8.98 µm (UNIMAS-13) to 21.03 µm (UNIMAS-03), respectively. In the S_1 generation, the WEPC of abaxial and adaxial surface (Table 4.8) had a mean value ranged from 14.13 µm to 19.57 µm and 13.86 µm to 19.33 µm, respectively. Statistical analysis showed no significant difference between accessions on abaxial or adaxial surface. The WEPC when compared between abaxial and adaxial surface also did not show significant different (Table 4.8).

Large difference in the epidermis tissue were not detected in the present study in all the studied accessions. The long cells of abaxial and adaxial leaf surfaces were rectangular in shape (Figure 4.6). Cells and appendages including long and short cell (comprised of silica bodies and phellem cell) and stomata with guard cells were observed. The arrangement of cells was linear elongate with alternating long and short cells (Figure 4.6).

The lateral walls showed that the cell outline was undulated, and papillae were found presence in both first and S_1 generation (Figure 4.6).

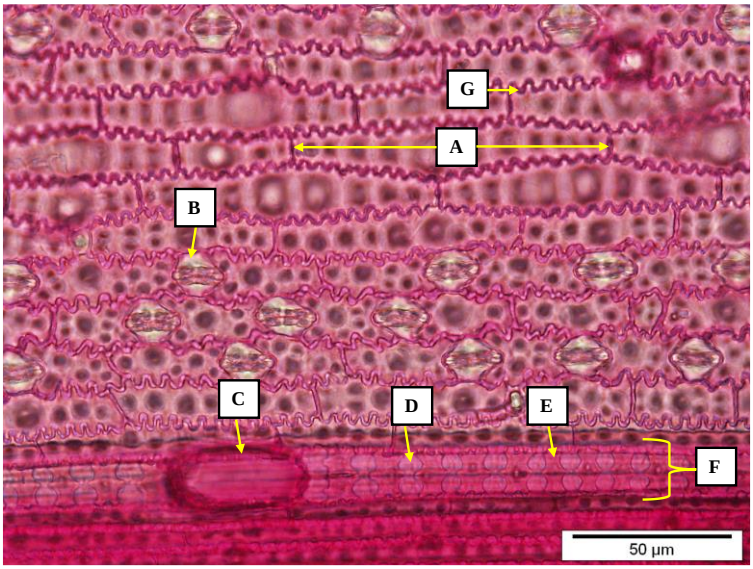

Figure 4.6: Representative of Epidermal Structure in Different Accessions from this Study. **A**: Rectangular long cell; **B**: Stomatal complex; **C**: Trichome; **D**: Silica cell; **E**: Phellem cell; **F**: Short cell row comprised of silica cell and phellem cell; **G**: Undulated long cell outline. Magnification: 20x. Scale Bar: 50 µm

4.3.2 Diversity in Stomatal Complex

Stomatal complex was composed of two dumbbell-shaped guard cell (GuC) which were flanked by two subsidiary cells (SuC) as shown in Figure 4.7. SuC did not show variation in shape (Table 4.7 and Table 4.8). All accessions in first generation and S_1 generation were having triangular shape of SuC (Figure 4.7).

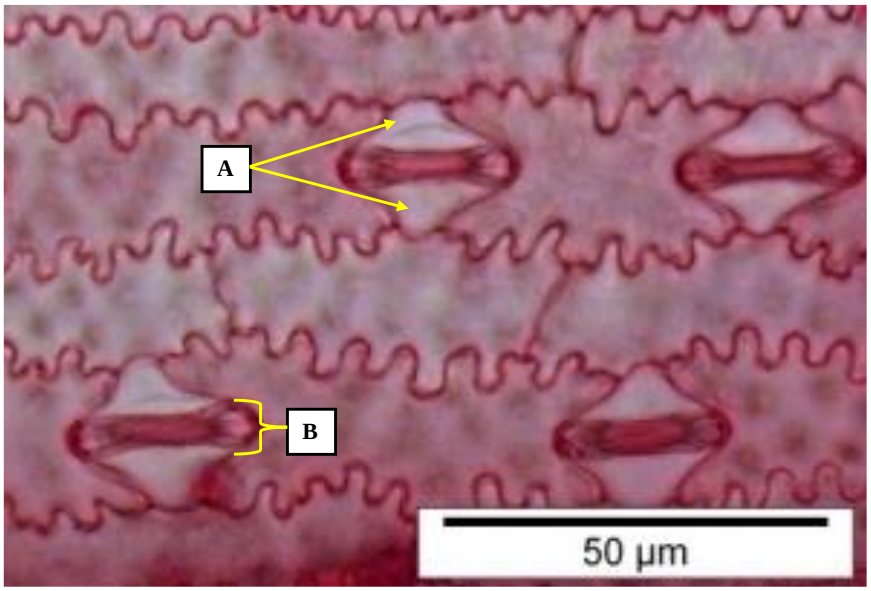

Figure 4.7: Representative of Stomatal Complex Observed in Different Accessions from this Study. **A**: A Pair of Triangular-Shaped Subsidiary Cells; **B**: A Pair of Guard Cell. Magnification: 20x. Scale Bar: 50 µm

The accessions in the first generation showed the length of stomatal complex (LSC) on abaxial and adaxial surface ranged from 13.86 µm (UNIMAS-02) to 23.77 µm (UNIMAS-09) and 14.20 µm (UNIMAS-02) to 24.09 µm (UNIMAS-19), respectively (Table 4.7). The width of stomatal complex (WSC) on abaxial and adaxial surface (Table 4.7), ranged from 10.86 µm (UNIMAS-02) to 18.51 µm (UNIMAS-19) and 10.55 µm (UNIMAS-02) to 19.33 µm (UNIMAS-19), respectively.

In S_1 generation, the mean value of the LSC on abaxial and adaxial surface (Table 4.8) ranged from 18.28 µm (UNIMAS-09-S1) to 22.99 µm (UNIMAS-18-S1) and 19.19 µm (UNIMAS-12-S1) to 23.78 µm (UNIMAS-18-S1), respectively. The mean value for the WSC ranged from 14.79 µm (UNIMAS-01-S1) to 18.27 µm (UNIMAS-04-S1) on abaxial surface (Table 4.8). The LSC when compared between abaxial and adaxial surface did not show significant different (Table 4.8). WSC on adaxial surface (Table 4.8) ranged from 13.86 µm (UNIMAS-09-S1) to 16.17 µm (UNIMAS-17-S1). Statistical analysis

showed that there was no significant difference of stomata size on both abaxial and adaxial surface between the accessions. However, the WSC when compared between abaxial and adaxial surface was significantly different with the P-value of .005 (Table 4.8).

Table 4.7 shows that the stomata density (SD) on the leaf of accessions in the first generation ranging from 269 units/mm^2 (UNIMAS-03) to 423 units/mm^2 (UNIMAS-02) on the abaxial surface. SD on the adaxial surface (Table 4.7) ranged from 223 units/mm^2 (UNIMAS-17) to 392 units/mm^2 (UNIMAS-12). In S_1 generation, significant difference was not observed for SD in abaxial surface (Table 4.8), ranging from 195 units/mm^2 (UNIMAS-01-S1) to 282 units/mm^2 (UNIMAS-09-S1). The SD on adaxial surface, however, was significantly different between the accessions (Table 4.8), with a range of 126 units/mm^2 (UNIMAS-18-S1) to 344 units/mm^2 (UNIMAS-09-S1).Meanwhile, the SD when compared between abaxial and adaxial surface did not show significant difference (Table 4.8).

4.3.3 Midrib of 22 Upland Rice Accessions at First Generation

General structure of midrib observed on the accessions in this study has vascular bundle, sclerenchyma strand and parenchyma (Figure 4.8). The shape of midrib across the 22 accessions at the first generation revealed that there was no variation. All accessions were having concave-shaped midrib for abaxial side and flat-shaped midrib for adaxial side. The midrib width (Table 4.9) for the 22 accessions ranged from 321.00 µm (UNIMAS-15) to 2188.99 µm (UNIMAS-19).

4.3.4 Root and Stele of 22 Upland Rice Accessions at First Generation

The microscopic observation on root anatomy of the accessions at first generation showed no variations in types of plant tissue and arrangement of roots between the 22 accessions in first generation. The anatomical features of the transverse sections of roots, revealed tissue such as epidermis, exodermis, sclerenchyma layer, mesodermis and well-developed aerenchyma (Figure 4.9). The root diameter of the 22 accessions (Table 4.9) ranged from 778.00 µm (UNIMAS-02) to 1427.50 µm (UNIMAS-17). Other than that, the stele of the root was having vascular cylinder which comprises of endodermis, fibre, pericycle,metaxylem, protoxylem and phloem (Figure 4.9). Stele diameter (Table 4.9) of the accessions in this study ranging from 213.55 (UNIMAS-22) µm to 399.91 µm (UNIMAS-13).

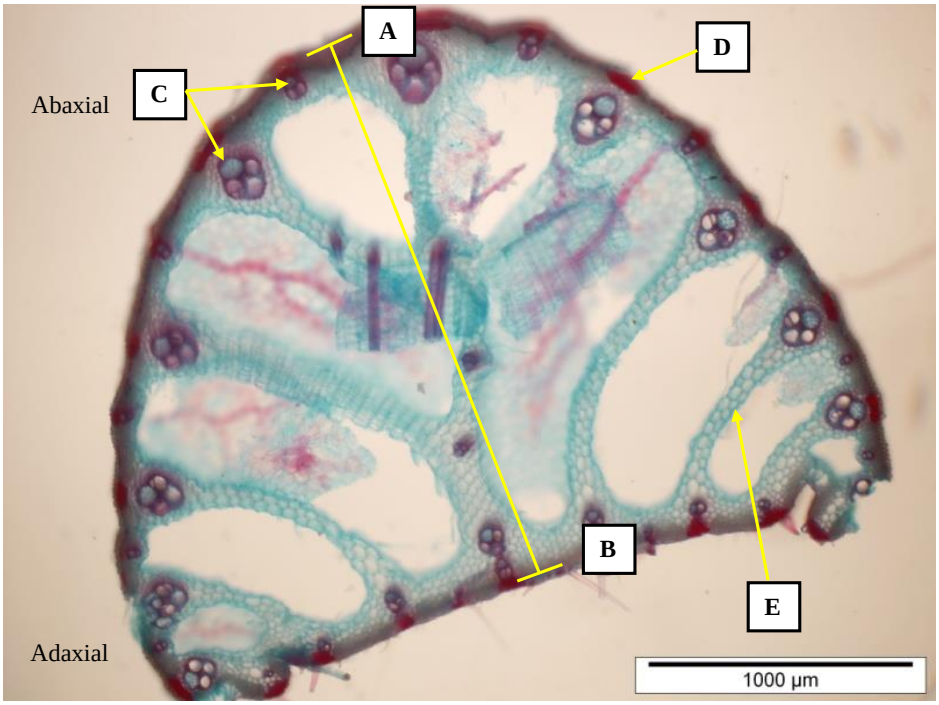

Figure 4.8: Representative Midrib Anatomical Structure Observed in Different Accessions from this Study. Midrib Width Measurement are Measured from Point **A** to **B** Using Image J, **C**: Vascular Bundle; **D**: Sclerenchyma Strand; **E**: Parenchyma. Magnification: 10x. Scale Bar: 1000 µm.

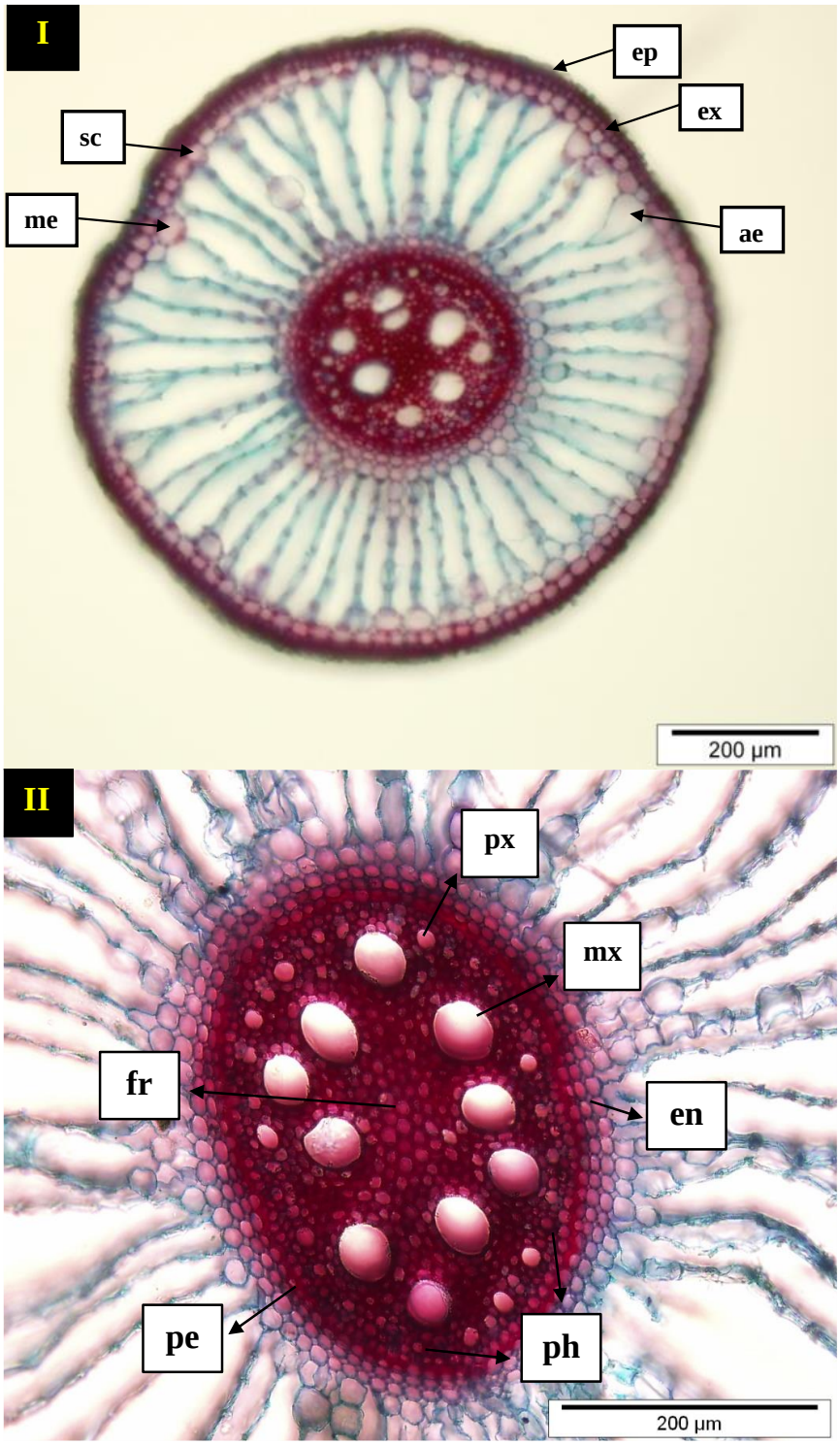

Figure 4.9: Representative of Root and Stele Observed in Different Accessions from this Study. **I**: Root Anatomical Structure, **II**: Enlargement Of Root Stele. Abbreviations: **Ep**- Epidermis, **Ex**- Exodermis, **Sc**- Sclerenchyma Layer, **Me**- Mesodermis, **Ae**- Aerenchyma, **Mx**- Metaxylem, **Px**- Protoxylem, **Ph**- Phloem, **En**- Endodermis, **Fr**- Fibre, **Pe**- Pericycle. Magnification: **I** = 10x, **II** = 40x. Scale Bar: **I&II** = 200 µm

Table 4.7: Epidermal Traits of First Generation for Abaxial and Adaxial Surface

Accession	Abaxial						Adaxial					
	LEPC (µm)	**WEPC (µm)**	**SCS**	**LSC (µm)**	**WSC (µm)**	**SD (unit/ mm^2)**	**LEPC (µm)**	**WEPC (µm)**	**SCS**	**LSC (µm)**	**WSC (µm)**	**SD (unit/ mm^2)**
UNIMAS-01	75.88	14.50	2	18.66	12.94	369	70.75	14.72	2	18.74	16.65	338
UNIMAS-02	69.09	16.23	2	13.86	10.86	423	50.16	13.15	2	14.20	10.55	285
UNIMAS-03	75.41	16.48	2	17.08	12.76	269	62.84	21.03	2	17.77	12.93	269
UNIMAS-04	66.06	17.85	2	19.29	13.43	346	68.36	13.33	2	20.71	14.70	369
UNIMAS-05	58.73	14.59	2	18.72	12.85	392	69.52	13.59	2	19.55	17.75	300
UNIMAS-06	73.11	18.84	2	20.37	13.65	408	54.68	11.47	2	20.68	17.61	331
UNIMAS-07	85.82	16.26	2	20.17	17.01	400	47.65	15.99	2	20.41	13.00	354
UNIMAS-08	92.81	18.33	2	19.46	14.04	285	97.83	16.24	2	20.89	11.55	331
UNIMAS-09	79.98	12.81	2	23.77	14.57	323	78.52	13.53	2	20.38	12.88	362
UNIMAS-10	84.06	16.71	2	20.66	15.36	392	64.08	10.83	2	19.01	12.40	315
UNIMAS-11	79.18	16.71	2	21.48	14.95	362	96.03	15.74	2	21.19	16.44	331
UNIMAS-12	84.46	17.47	2	17.52	14.28	385	73.63	12.48	2	15.36	17.74	392
UNIMAS-13	138.88	12.50	2	21.37	14.03	354	67.68	8.98	2	16.13	12.56	292
UNIMAS-14	96.10	19.87	2	20.35	14.29	292	89.48	12.31	2	14.60	14.60	285
UNIMAS-15	73.54	15.76	2	19.63	16.74	377	72.98	13.18	2	20.07	12.89	238
UNIMAS-16	89.78	23.90	2	18.80	17.26	362	74.71	20.18	2	21.58	16.25	354
UNIMAS-17	69.178	13.64	2	20.16	15.16	392	78.46	17.48	2	17.59	16.432	223
UNIMAS-18	84.13	13.69	2	18.52	13.10	346	78.27	19.23	2	17.58	14.599	308
UNIMAS-19	81.16	23.70	2	22.76	18.51	377	85.41	16.32	2	24.09	19.334	277
UNIMAS-20	67.93	17.43	2	17.66	15.53	377	94.81	17.05	2	17.64	13.35	285
UNIMAS-21	64.25	11.23	2	19.56	13.26	369	66.64	15.51	2	18.97	14.873	300
UNIMAS-22	79.92	14.86	2	19.96	12.45	392	70.22	13.24	2	17.05	13.357	315

*LEPC: Length of epidermal cell in micrometre; WEPC: Width of epidermal cell in micrometre; SCS: Subsidiary cell shape (1=ellipsoid, 2=triangular), LSC: Length of stomatal complex in micrometre; WSC: Width of stomatal complex in micrometre; SD: Stomata density in millimetre per square.

Table 4.8: The Epidermal Traits of the Ten Accessions at S_1 generation for Abaxial and Adaxial Surface

Surface	Accession	LEPC (µm)	WEPC (µm)	SCS	LSC (µm)	WSC (µm)	SD (unit/ mm)
Abaxial	UNIMAS-01-S1	87.25 [a]	16.27 [a]	2	21.51[a]	14.79[a]	195[a]
	UNIMAS-02-S1	82.09 [a]	17.88 [a]	2	21.61[a]	16.64[a]	259[a]
	UNIMAS-03-S1	98.86 [a]	17.96 [a]	2	21.59[a]	17.17[a]	215 [a]
	UNIMAS-04-S1	86.65 [a]	19.57 [a]	2	21.84[a]	18.27[a]	256 [a]
	UNIMAS-07-S1	93.87 [a]	18.83 [a]	2	19.83[a]	17.67[a]	262 [a]
	UNIMAS-08-S1	102.08[a]	14.13 [a]	2	22.15[a]	17.12[a]	246 [a]
	UNIMAS-09-S1	91.53 [a]	17.21 [a]	2	18.28[a]	15.37[a]	282 [a]
	UNIMAS-12-S1	67.52 [a]	19.23 [a]	2	20.98[a]	16.78[a]	225 [a]
	UNIMAS-17-S1	87.67 [a]	17.33 [a]	2	22.11[a]	18.09[a]	223 [a]
	UNIMAS-18-S1	87.78 [a]	15.86 [a]	2	22.99[a]	16.73[a]	236 [a]
Adaxial	UNIMAS-01-S1	96.67[a]	14.25[a]	2	20.15[a]	14.23[a]	218[abc]
	UNIMAS-02-S1	92.86[ab]	18.42[a]	2	23.15[a]	15.69[a]	249[abc]
	UNIMAS-03-S1	71.67[a]	13.86[a]	2	21.20[a]	14.87[a]	205[ab]
	UNIMAS-04-S1	83.35[ab]	18.29[a]	2	21.45[a]	15.26[a]	254[abc]
	UNIMAS-07-S1	92.39[ab]	17.51[a]	2	22.27[a]	15.94[a]	238[abc]
	UNIMAS-08-S1	94.90[ab]	19.33[a]	2	21.79[a]	15.02[a]	256[bc]
	UNIMAS-09-S1	86.31[ab]	17.37[a]	2	19.79[a]	13.86[a]	344[c]
	UNIMAS-12-S1	82.70[ab]	17.16[a]	2	19.19[a]	14.92[a]	210[ab]
	UNIMAS-17-S1	95.20[ab]	16.23[a]	2	22.85[a]	16.17[a]	151[ab]
	UNIMAS-18-S1	108.96[b]	15.60[a]	2	23.78[a]	15.22[a]	126[al]
Mean ± Std. Deviation		-1.97 ± 13.88	0.63 ± 2.43	N/A	-0.27 ± 1.35	1.74 ± 0.6[!!]	14.80 ± 47.80

*Difference in anatomical traits between accession for abaxial and adaxial surface were compared using Variance Analysis (ANOVA) followed by Tukey HSD post hoc test ($P<0.05$). The different alphabet indicates significant difference among the accessions. **!!** indicates significant difference between abaxial and adaxial surfaces LEPC: Length of epidermal cell in micrometre, WEPC: Width of epidermal cell in micrometre, SCS: subsidiary cell shape (1=Ellipsoid, 2=Triangular), LSC: Length of stomata complex in micrometre, WSC: Width of stomata complex in micrometre; SD: Stomata density in millimetre per square. Std.: Standard

Table 4.9: Mean Value of Root Diameter, Stele Diameter and Width of Midrib in First Generation

Accession	Width of Midrib (µm)	Root Diameter (µm)	Stele Diameter (µm)
UNIMAS-01	1010.45	1190.90	322.635
UNIMAS-02	1072.14	778.00	247.348
UNIMAS-03	863.88	1330.60	308.633
UNIMAS-04	1023.95	908.56	229.498
UNIMAS-05	1264.66	904.00	307.476
UNIMAS-06	780.55	910.56	315.171
UNIMAS-07	1428.88	1265.37	300.364
UNIMAS-08	835.13	1158.67	347.46
UNIMAS-09	1314.01	1147.38	326.77
UNIMAS-10	1008.17	1068.35	255.00
UNIMAS-11	929.75	1294.10	333.91
UNIMAS-12	1092.56	826.83	328.97
UNIMAS-13	1066.98	1300.31	399.91
UNIMAS-14	1159.23	1123.48	311.32
UNIMAS-15	777.50	1316.00	353.25
UNIMAS-16	1051.38	1051.59	304.10
UNIMAS-17	1164.47	1427.47	285.56
UNIMAS-18	1094.68	832.83	228.31
UNIMAS-19	2188.99	985.96	246.86
UNIMAS-20	885.94	877.86	313.34
UNIMAS-21	1006.42	898.89	295.48

4.4 SSR Markers Genotyping

Out of 39 primer pairs, eight primers (20.5%) had clear DNA amplification and displayed polymorphic banding pattern. The eight primers were RM1, RM489, RM552, RM444, RM257, RM164, RM481 and RM166 (Table 4.10). The primers with poor amplifications were excluded from the present study.

Table 4.10: Eight SSR Markers which Displayed Clear Banding Patterns on 22 Upland Rice Accessions.

Marker	Sequence (5' to 3')	Annealing temperature (°C)
RM1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	55
RM489	F: ACTTGAGACGATCGGACACC R: TCACCCATGGATGTTGTCAG	55
RM552	F: CGCAGTTGTGGATTTCAGTG R: TGCTCAACGTTTGACTGTCC	55
RM444	F: GCTCCACCTGCTTAAGCATC R: TGAAGACCATGTTCTGCAGG	55
RM257	F: CAGTTCCGAGCAAGAGTACTC R: GGATCGGACGTGGCATATG	55
RM164	F: TCTTGCCCGTCACTGCAGATATCC R: GCAGCCCTAATGCTACAATTCTTC	58
RM481	F: TAGCTAGCCGATTGAATGGC R: CTCCACCTCCTATGTTGTTG	59
RM166	F: GGTCCTGGGTCAATAATTGGGTTACC R: TTGCTGCATGATCCTAAACCGG	61

4.4.1 Polymorphism of SSR Markers

A total of 18 different and reproducible alleles were detected from the eight microsatellite markers loci. A representation of polymorphisms observed between the accessions is depicted in Figure 4.10 - Figure 4.12. Each marker generated two to three alleles with an average value of 2.25 (Table 4.12) and the smallest size was observed in RM1 (90 bp-100 bp) as shown in Figure (4.11) and the largest size of allele belonged to RM166, ranges from 321 bp to 410 bp (Figure 4.12).

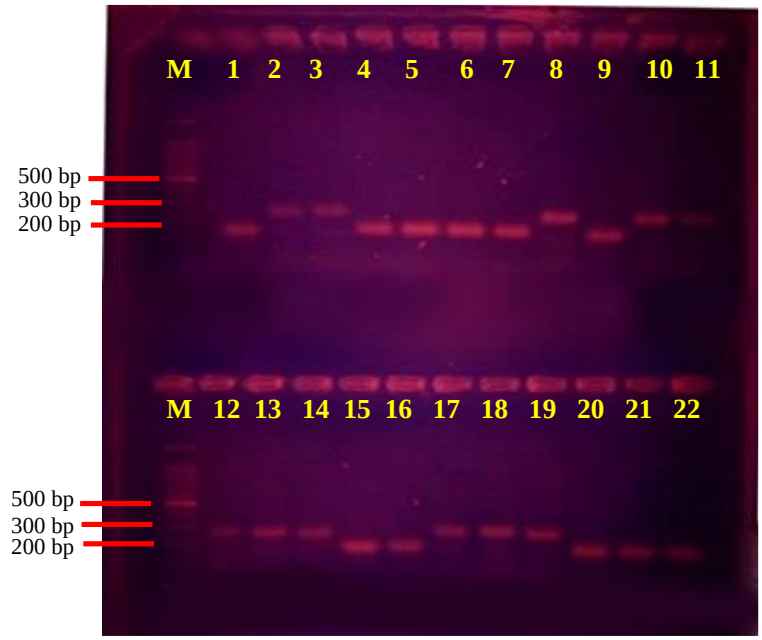

Figure 4.10: SSR Banding Pattern of the 22 Upland Rice Accessions Amplified Using Primer RM444. Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22

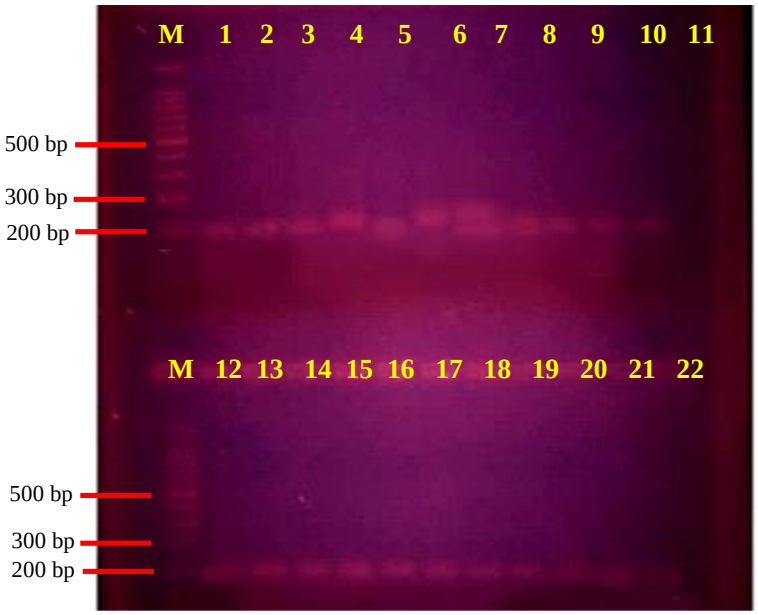

Figure 4.11: SSR Banding Pattern of the 22 Upland Rice Accessions Showing Smallest BasePair (bp). Size Of Alleles Amplified using Primer RM1.Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22

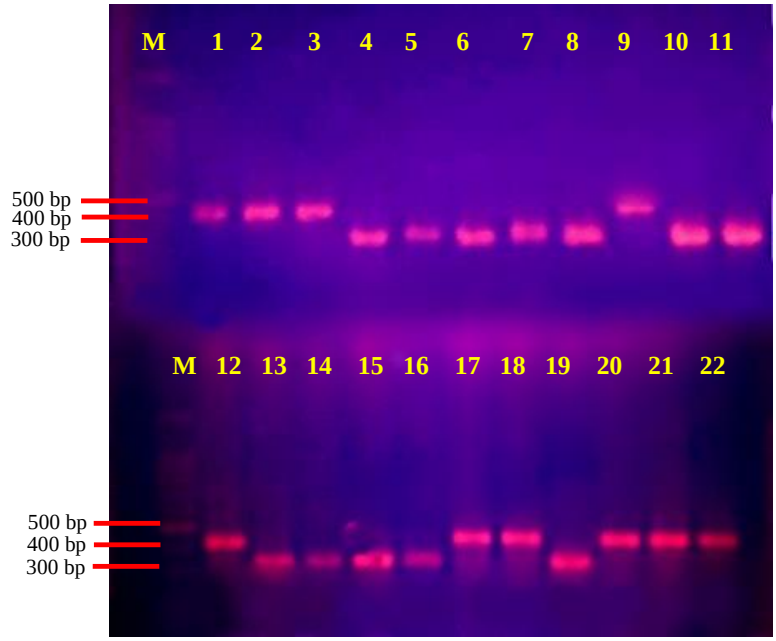

Figure 4.12 : SSR Banding Pattern of the 22 Upland Rice Accessions Showing Biggest BasePair (bp). Size of Alleles Amplified using Primer RM166.Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22

4.4.2 PIC Values and Genetic Diversity

The polymorphic information content (PIC) values of the eight SSR markers ranged from 0.25 to 0.88. The highest PIC value (0.88) was recorded for RM257. RM552 had the lowest PIC value (0.25). The average PIC value of the eight SSR markers for 22 accessions was 0.67. Table 4.11 shows the summary of genetic analysis of the 22 rice accessions based on the SSR markers genotype. The numbers of alleles observed were ranging from two to three alleles per marker. The observed grand mean number of alleles and the effective number of allelles (*Ne*) in the 22 accessions of the first generation were 2.25 and 1.64, respectively (Table 4.11). Nei's gene diversity (*h*) ranged from 0.22 (RM552) to 0.50 (RM444 and RM166) with an average value of 0.37 (Table 4.11). Shannon's Information Index (*I*), ranges from 0.36 to 0.69, with an average of 0.55.

Table 4.11: Allelic Diversity of the Eight Polymorphic SSR Markers Used in the Study

Marker	No. of alleles	PIC	*Ne*	*h*	*I*
RM1	3	0.77	1.31	0.23	0.39
RM489	2	0.65	1.42	0.30	0.47
RM552	2	0.25	1.32	0.22	0.36
RM444	2	0.75	2.00	0.50	0.69
RM257	2	0.88	1.66	0.40	0.59
RM164	2	0.67	1.66	0.40	0.59
RM481	2	0.71	1.77	0.43	0.63
RM166	3	0.75	1.98	0.50	0.69
MEAN	2.25	0.67	1.64	0.37	0.55

* **PIC**: Polymorphic Information Content, ***Ne***: Number of effective alleles, ***h***: Nei's gene diversity, ***I***: Shannon's Information Index

4.4.3 Cluster Analysis based on SSR Markers

A UPGMA dendrogram was constructed for the 22 accessions using the MEGA-X v.10.1 program based on the eight SSR markers genotype (Figure 4.13). The UPGMA dendrogram classified the 22 rice accessions into two major clusters, Cluster I and Cluster II. Cluster I was further divided into two sub-clusters, Sub-Cluster IA and Sub-Cluster IB as shown in (Table 4.12). Sub-Cluster IA had the highest number of accessions with 11 rice accessions. Sub-Cluster IB comprised of six accessions. Cluster II consisted of five accessions. The dendrogram tree did not show clear clustering pattern according to their morphological traits nor division.

Table 4.12: Cluster for 22 Upland Rice Accessions based on Eight Polymorphic SSR Markers

Cluster	Sub Cluster	Number of Accessions	Accessions
Cluster I	Sub Cluster IA	11	UNIMAS-13-UP-KCH UNIMAS-14-UP-KCH UNIMAS-11-UP-KCH UNIMAS-10-UP-KCH UNIMAS-08-UP-KCH UNIMAS-19-UP-SRI UNIMAS-17-UP-SRI UNIMAS-18-UP-SRI UNIMAS-12-UP-KCH UNIMAS-02-UP-SER UNIMAS-03-UP-SER
	Sub Cluster IB	6	UNIMAS-09-UP-KCH UNIMAS-20-UP-SRI UNIMAS-01-UP-SER UNIMAS-15-UP-SRI UNIMAS-21-UP-SRI UNIMAS-22-UP-KCH
Cluster II	-	5	UNIMAS-16-UP-SRI UNIMAS-06-UP-KCH UNIMAS-05-UP-KCH UNIMAS-04-UP-KCH UNIMAS-07-UP-KCH

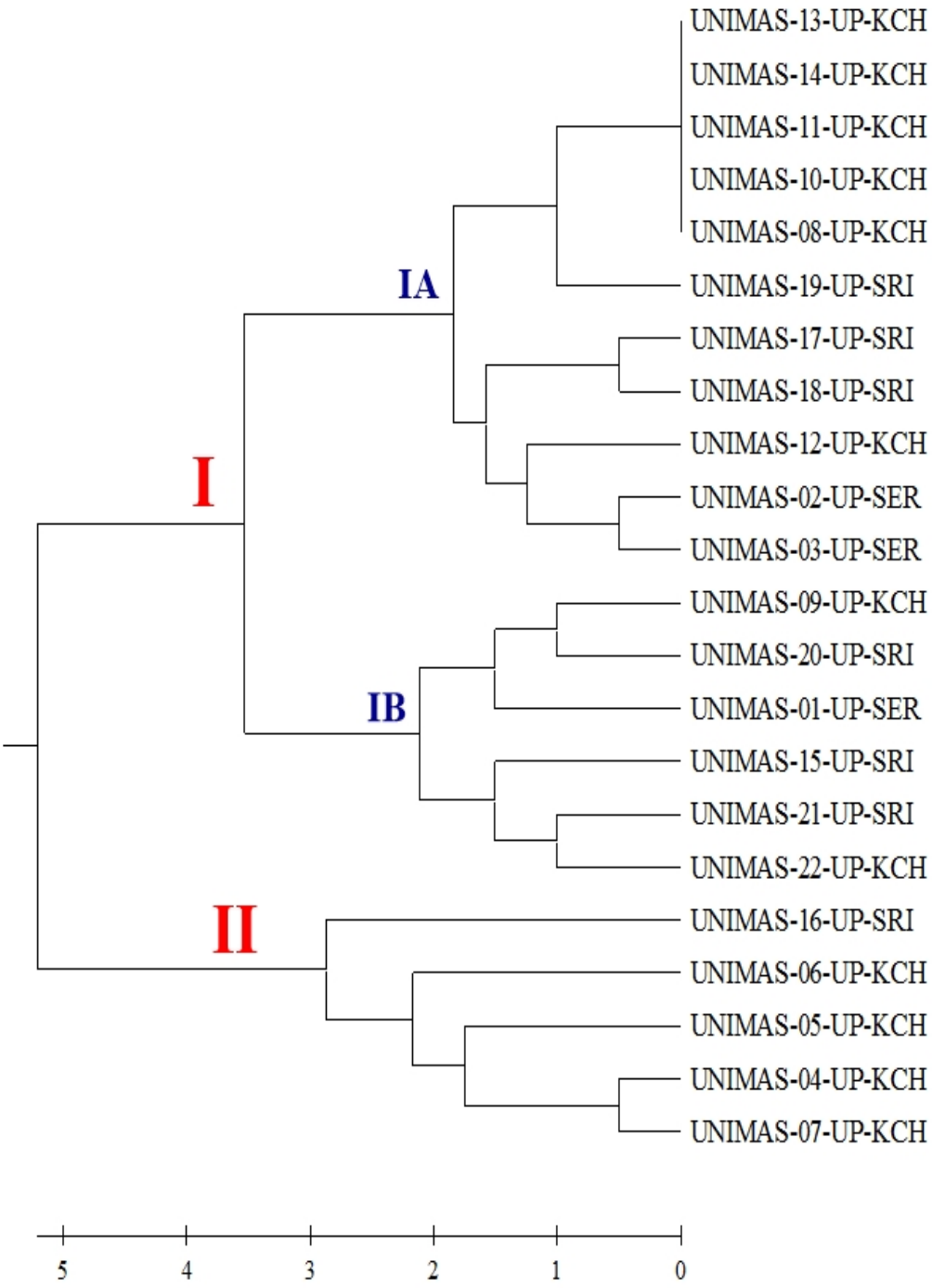

Figure 4.13: Cluster Analysis of 22 Upland Rice Accessions Based on Eight Polymorphic Simple Sequence Repeat Markers. **I**: Cluster I, **IA**: Sub Cluster IA, **IB**: Sub Cluster IB, **II**: Cluster II.

4.5 Sequence Characteristics and Genetic Diversity of Maturase-K gene

Approximately, bright and sharp bands of 1500 bp of plastid *matK* gene fragment from the 22 rice accessions were amplified (Figure 4.14). The phylogenetic information generated by MEGA-X v.10.1showed the range of sequence length was 1490 bp to1500 bp. The aligned sequences resulted in the final data matrix with 1470 bp with no alignment gaps. Number of segregating sites showed a value of 140.

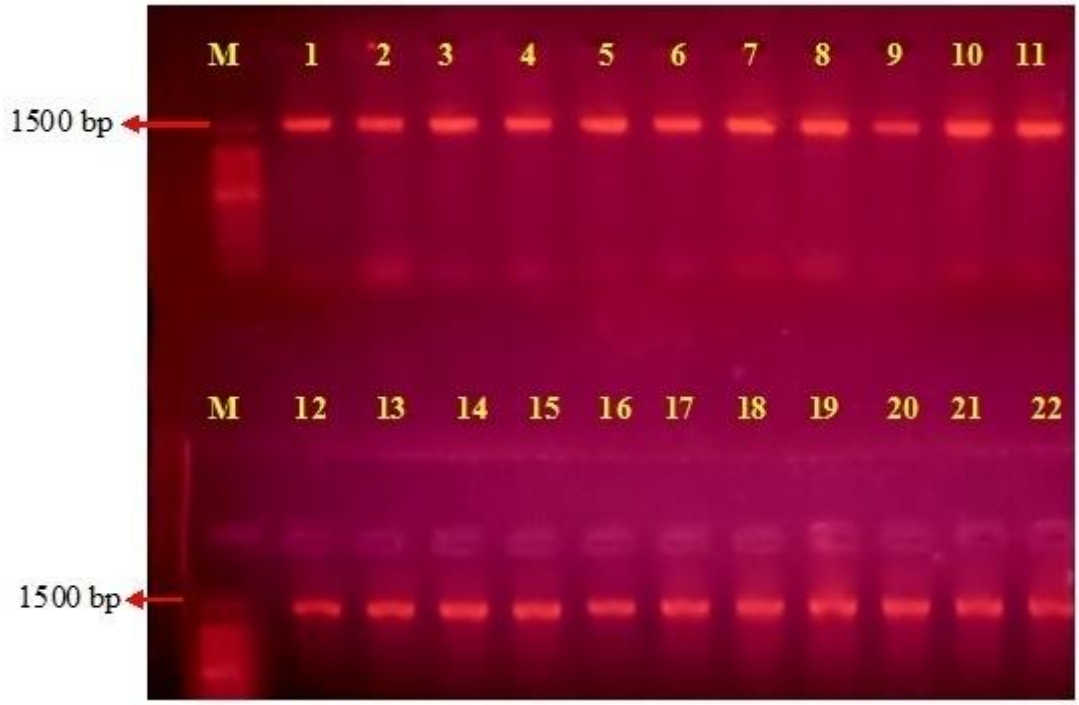

Figure 4.14: Amplification of 22 Rice Accessions Employing *matK* Gene Specific Primer. Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22.

4.5.1 Phylogenetic Analysis Based on Chloroplast Marker, Maturase-K

The phylogenetic tree constructed one big cluster based on the chloroplast *matK* (Figure 4.15). All the 22 upland accessions were grouped in Cluster I (Table 4.13) with 100% bootstrap value, together with 22 lowland accessions from UNIMAS collections. Also in the same cluster, there were 94 accessions of *Oryza sativa* which mostly originated

from India (88 accessions), followed by Australia (4 accessions), China (1 accession) and one from unknown origin. Other than *O. sativa*, three accessions of *O. rufipogon* were grouped together in the big cluster (Table 4.13). Outgroup were consisted of four species (Table 4.13) *viz*., *O. punctata*, *O. granulata*, O. *australiensis*, and *O. officinalis*. The clustering pattern showed the accessions were grouped based on their genome.

Table 4.13: Cluster for 22 Upland Rice Accessions based on Chloroplast *matK*

Cluster	Species	Country of Origin	No of Accession
I	*Oryza sativa*	India	88
	Oryza sativa	China	1
	Oryza sativa	Australia	4
	Oryza sativa	Unknown	1
	*Oryza sativa**	Malaysia	22
	*Oryza sativa***	Malaysia	22
	Oryza rufipogon	Unknown	1
	Oryza rufipogon	China	1
	Oryza rufipogon	India	1
	Oryza punctata	Unknown	1
	Oryza punctata	Unknown	1
Outgroup	*Oryza granulata*	Unknown	1
	Oryza granulata	China	1
	Oryza australiensis	Vietnam	1
	Oryza officinalis	Australia	1
	Oryza officinalis	Phillipines	1
	Oryza officinalis	China	1
	Oryza punctata	Papua New Guinea	1

*Lowland Rice Accessions from UNIMAS collections, **Upland Rice Accessions from UNIMAS collections.

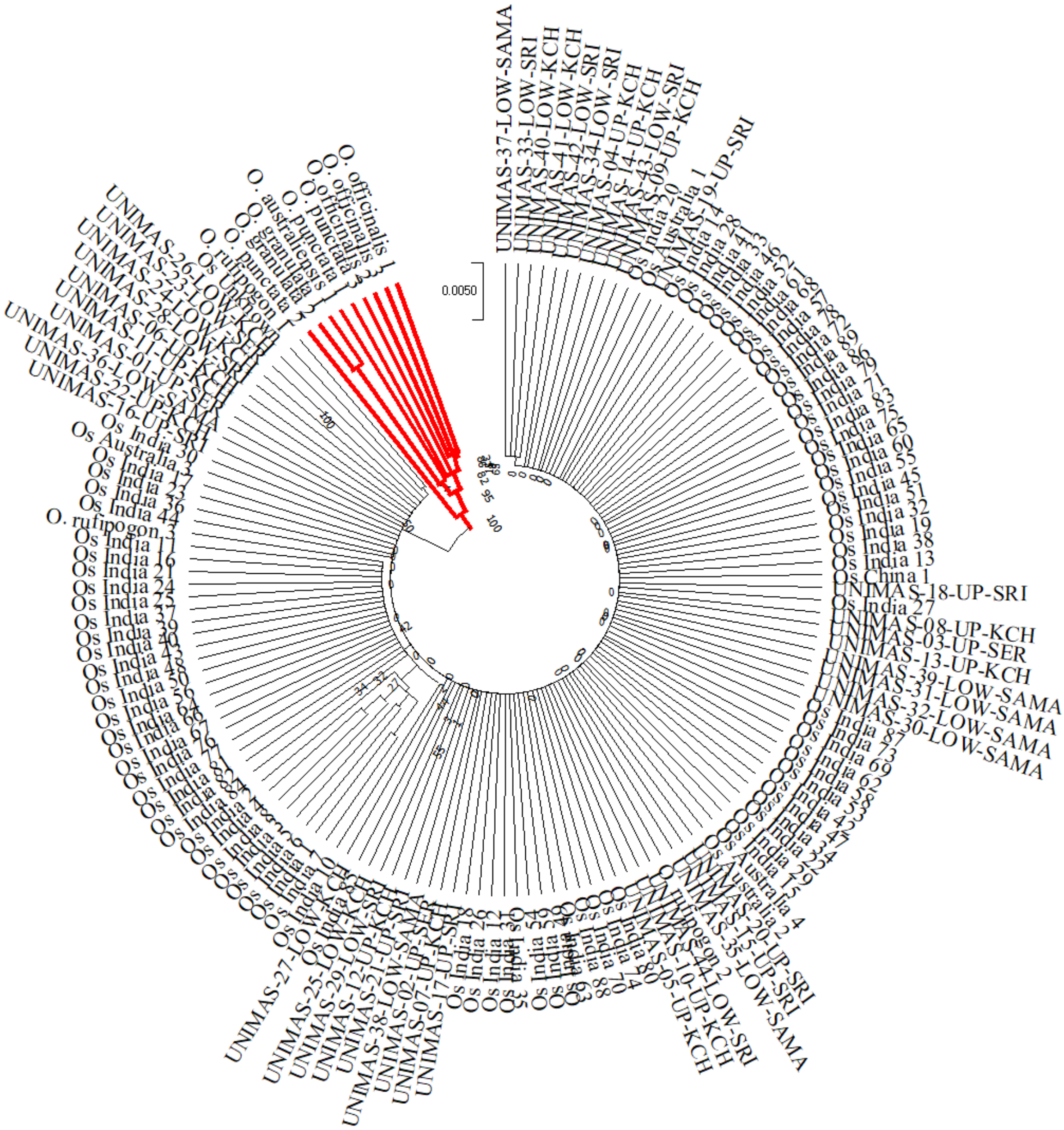

Figure 4.15: Phylogenetic Tree of 22 Upland Rice Accessions based on Chloroplast *matK* using Maximum Likelihood Analysis. Best-Fit Substitution Models: Tamura 3-Parameters + Discrete Gamma Distribution Evolutionary Rates Among Sites (T92+G) with 1000 Replicates Bootstrap Value in MEGA-X v.10.1.

CHAPTER 5

DISCUSSION

For crop breeding programmes, it is important to select suitable genotypes, to create varieties with better traits which contribute to yield, quality and resistance to biotic and abiotic stresses. The characterisation of different varieties or accessions is a necessary step to establish their identity. Characterisation may be defined as the scoring of characters that can be easily detected and have high heritability based on the form and structure of the organism, especially their external form (Perrino & Monti, 1988, quoted by Woyessa, 2006).

Basic objective in the present study is to document the traits that help to distinguish accessions from each other. In addition, the knowledge of existing plant genetic diversity is crucial for effective management of crop genetic resources. Characterisation is usually based on morphological, biochemical, physiological and molecular characteristics (Mondini et al., 2009).

5.1 Characterisation Based on Morphological Traits

5.1.1 Characterisation of Qualitative Traits

Qualitative traits are considered as marker characters in the identification of landraces of rice, which are less independent to the environmental responses (Singh & Mishra, 2013; Singh et al., 2014). According to Wang et al. (2011), leaf traits are very important as it primarily involved in photosynthesis and transpiration which determine the yield performance in crops.

In the present study, it is observed that the accessions were grouped into three different classes of blade colour (BC) in first generation, and two groups were formed on

S_1 generation. Although it varied between the accessions, this trait will not be reliable for identification as it is affected by environmental conditions (Kooistra, 1964) and the degree of the greenness of the leaves could be influenced by fertiliser applications or the nitrogen status content of the leaf (Singh et al., 2002). Based on Leaf Colour Chart, pale green BC highlights nitrogen deficiency conditions. Green BC indicates elevated dose of nitrogenous fertilization. High rate of nitrogenous content resulting in dark green BC (Bhupenchandra et al., 2021). The BC of this study may suggest there are variations in soil nitrogenous content even though equal amount of fertiliser was applied.

Ligule is present in all accessions, both in first and second generation. There is no variation in ligule shape (LS) between the accessions in first and S_1 generation. Ligule is acute to acuminate in shape following the descriptions used in IRRI (1980) guidelines. The morphology of ligule is important in identification of grass species, also vital for identifying individual species living in non-optimal conditions (Neumann, 1938; Zuloaga et al., 1998; Fuente & Ortunez, 2001). Ligule colour (LC) and auricle colour (AC) are white in colour except for accession UNIMAS-07 which shows purple colour in the first generation. The purple or red colour is results from the expression of $BKPK10_{450}$ belonging to a member of flavonoid 3'-hydroxylase which is important for anthocyanin biosynthesis as stated in Phanchaisri et al. (2012) and Semsang et al. (2012). In S_1 generation, two out of three replicates of UNIMAS-07 expressed purple ligule and auricle, while one replicate expressed white ligule and auricle. This variation observed in S_1 generation may suggest the variation is due to environmental effect. Another possibility is that the trait is segregating, however, the current observation in triplicates is insufficient to provide evidence of segregation.

The panicle architecture has directly influenced the rice yield. It is one of the key targets of high-yield rice breeding (Agata et al., 2020). Panicle branching patterns are mainly regulated by the number of primary and secondary branches, which directly influence the total grain number (Agata et al., 2020). The stronger the branching pattern; the higher the yield, because grain number is associated with several morphological components of panicle architecture. Study stated that there are significant linear correlations between the total spikelet numbers per panicle with total primary branch per panicle (Tay & Othman, 1992). In this study, UNIMAS-08, UNIMAS-15, UNIMAS-17 and UNIMAS-18, in the first generation, have heavy secondary branches (Table 4.4) and recorded high percentage of filled grain (PFG), ranging from 72% to 90%. PFG seems to be lower in those accessions with secondary branching (SB) found absent, ranging from 40% to 69%. The difference between accessions should be validated statistically using seeds from the later selfing generation. The number of grains per panicle could be used as selection criteria in rice breeding (Halil & Necmi, 2005).

Panicle type of rice refers to the mode of branching, the angle of the primary branches and the spikelet density (IRRI, 2009). Based on panicle type, three groups were obtained, compact panicles, intermediate type of panicles and open panicles. One accession (UNIMAS-15) was observed to have compact panicles. Crop breeders usually selectively breed for a compact panicle type; open panicle type is actively selected against, for reasons of maximizing crop grain production, and harvest (Kalaichelvan, 2009). Hence UNIMAS-15 with compact panicle type and heavy secondary branches can be considered for Sarawak rice breeding program for the purpose of increasing rice production.

Seed shape (SS) are useful in identifying rice hybrids and are also valuable in the seed certification process to control field and seed standards (Geetha et al., 1994;

Kalaichelvan, 2009). Studied accessions were classified into two groups according to their SS where majority were having linear SS and only one accession were elliptic SS (UNIMAS-16). SS is an important factor that determines grain appearance, cooking quality and grain yield. Seeds having linear shape were more likely to produce long grain.

In the present study, the seed colours (SC) of the accessions were grouped into three group which were yellow, blackish-yellow and golden brown. This morphological trait is the most important basis for traditional classification and folk nomenclature (Lei et al., 2018). According to Pascual et al. (1993), SC of rice could by influenced by the environmental conditions during ripening besides the genetic effect. Thus, this character can only be used in broad classification of varieties.

Grain colour (GC) is a heritable character and has been used to categorise the rice varieties by several workers (Rosta, 1975; Maeda et al., 2014). In the present study, the GC of the 21 accessions was grouped mostly as milky white, followed by reddish-brown, black and dark brown. Similar result was obtained by Chong et al. (2018) where they characterized Sabah's traditional rice varieties. The author observed almost half of the studied rice was having milky white GC, followed by reddish-brown, purplish black, reddish-orange and orange GC. The red coloration in rice is primarily regulated by *Rc* and *Rd* genes (Furukawa et al., 2006). Rice varieties with both *Rc* and *Rd* genes have red grains while rice varieties with *Rc* but without *Rd* produce brown grains. Rice varieties with either *rc* and *rd*, or *rc* and *Rd* produce white rice grains (Furukawa et al., 2006). Pigmented rice contains high phenolic compound and anthocyanin. It has been consumed as a functional rice, as the anthocyanin has been recognized as a health functional food due to its antioxidant activity, anticancer, hypoglycemia and its anti-inflammatory effects (Maulani et al., 2018). The studied accessions with pigmented rice *viz*., UNIMAS-07,

UNIMAS-08, UNIMAS-11, UNIMAS-13, UNIMAS-14 and UNIMAS-16 have the potential to be an alternative functional food.

5.1.2 Characterisation of Quantitative Traits

According to IRRI (2002), plant height is divided into three categories, namely short (< 110 cm), medium (110 cm-130 cm) and tall (> 130 cm). Based on these categories, only one accession in first generation (UNIMAS-09) was evaluated in the present study classified as short. Two out of 22 accessions were categorized having medium height, namely UNIMAS-01 (127.3 cm) and UNIMAS-19 (120.9 cm), respectively. The remaining accessions were classified as tall. Plant height of accessions in S_1 generation ranged from 133.87 cm to 210.73 cm, which were classified as tall. According to Arianti et al. (2022), genetic and local environmental conditions are the factors that contribute to the variation in plant heights such as nutrient intake. Variation observed in plant height might be due to soil nitrogenous content despite the fact same amount of fertiliser was applied.

Culm length (CL) ranged from 40.7-104.5 cm for accessions in first generation, while the range for mean value of CL at S_1 generation were ranging from 78.1 cm to 124.7 cm. Similar range was observed in Nascimento et al. (2011) in 146 accessions of Brazil upland rice. Surveys conducted in 2010 and 2012 of upland rice cultivated in Southeast Sulawesi, Indonesia by Kikuta et al. (2020) recorded much higher mean value of CL (124.0 cm to 54.0 cm) compared with the accessions in the present study.

The morphological traits such as size and number of leaves influence the photosynthetic rate, disease resistance and stress response. The leaf length (LL) of leaf below the flag leaf, ranged from 42.3 cm (UNIMAS-02) to 72.00 cm (UNIMAS-19) in first

generation. Based on IRRI (1996), The LL was classified as medium for UNIMAS-02 and long for the remaining accessions. In S_1 generation, all the accessions were classified as long (IRRI, 1996) based on the mean value. The length of leaf had been known to vary between rice genotypes as stated in Mehla and Kumar (2008). Short leaves are more erect than long ones. They are uniformly distributed throughout the canopy, therefore mutual shading is reduced, and light is used more efficiently, thus contributing to yield (Jennings *et al.,* 1979). The LL of all the leaves on each accession should be measured in future to have a good picture of the overall LL. Based on the width of leaf (LW) observed in first generation, the studied accessions in this study were grouped as medium (IRRI, 1996), except UNIMAS-03 which was grouped as broad. Similar group of LW was recorded in S_1 generation. Both LL and LW observed significant difference between the ten accessions in S_1 generation. Field observations of rice done by Fujino et al. (2008) suggested that leaves that are narrower are more beneficial, as they are presumed to contribute to higher yields since they are more evenly distributed than wide leaves which results in less shading within the canopy. Current observation on LL and LW of the accessions in the first and S_1 generation suggest that the distribution of leaves is probably unevenly, and mutual shading may happen which reduce the yield.

Heading days (HD) or emergence of the first panicle in an accession, range from 94-126 days. The timing of the panicle emergence is an important trait in rice, as it determines the flowering timing and the starting of the ripening phase (Maruyama et al., 2015). As a result, it directly affects the grain filling and yield depending on climatic conditions after flowering phase (Phapumma et al., 2020).

Flowering did not occur in one out of 22 accessions in the present study. The flowering days of the accessions ranged from 127 days to 155 days. The observation of

Sohrabi *et al*. (2012) on 50 upland rice varieties in Malaysia found that the days to flowering ranged from 76 days to112 days, which was shorter than the flowering days of the 22 accessions of the present study. Many factors affect the flowering phase of rice. Rice is a short-day monocotyledon plant (Ye et al., 2019). The pathway regulating rice flowering is mainly the photoperiod pathway (Zhang et al., 2012). Many hypotheses have been proposed to explain the regulation of rice flowering. For example, the nutrient diversion hypothesis indicates that appropriate induction treatment will lead to the accumulation of carbohydrate or energy in the apical meristem to induce flowering (Bernier, 1988). The multi-factor control model suggests that in addition to the accumulation of nutrients, a large number of inducers and inhibitors such as hormones and metabolites play a role in the flowering process of rice (Bernier, 2011). Shah *et al*. (1999) tested eight rice genotypes and found that days to flowering is a very important trait in classification of rice cultivars.

For number of panicles (NP), it is associated to the number of productive tillers where the number and proportion of tillers affect the number of panicles produced (Ashrafuzzaman *et al*., 2009).. Genotypes that produce higher number of productive tillers and grains per panicle also showed higher grain yield in rice (Kusutnani et al., 2000; Dutta et al., 2002). Accession with high number of tillers and panicles can be considered for Sarawak rice breeding program for the purpose of increasing rice production

The panicle length (PL) at main axis and NP per plant directly controls the yield of a particular variety. Morishima *et al*. (1962) reported that the average PL based on 106 lines of *O. sativa* was 20.0 cm in general, shorter than all the studied accessions in this study Sinton et al. (2019) reported an average PL of 22.72 -28.5 cm based on three upland rice accessions in Sabah, shorter than most of the studied accessions in this study. PL in

Kasim et al. (2020), ranging from 29.78 cm to 36.56 cm. Longer PL is more favourable as yield is higher among those varieties with shorter PL (Salam *et al*., 1990).

Grain size and shape are among the first quality criteria being considered in developing rice varieties for commercial production. They are primary quality factors in marketing, grading and processing (Husain, 1984). In Malaysia, whole kernel milled rice is classified as long (>6.2 mm), medium (5.2 mm to 6.2 mm) and short (<5.2mm) (Husain, 1984). According to the classification of Husain (1984), all the studied accessions were having long grain, ranging from 0.6-0.9 cm. The length observed for the 22 accessions in this study is longer than the 22 Sabah's traditional rice varieties observed by Chong et al. (2018), which ranges from 0.55 to 0.76 cm. Grain width (GW) observed in this study showed a range of 0.15 cm to 0.2 cm. The GW is narrower in comparison to the rice collected in Kasim et al. (2020), ranging from 0.23 cm to 0.33 cm. The findings suggested that the grain shape of the accessions resembling the shape of *indica* grain which exhibit long and narrow shape. Malaysian consumers mostly preferred rice that contained more head rice, lower amylose content and long shape characteristics (Husain, 1984; Wong et al., 1992, Hanis et al., 2012). Thus, the accessions studied in this study, which has long shape characteristics, can be considered for Sarawak rice breeding program to achieved rice grain quality that meet customer preferences.

5.2 Characterisation Based on Anatomical Traits

5.2.1 Epidermal Traits

The epidermal cell has shown that there was no difference between the accessions in terms of qualitative traits consists of epidermal characters on both abaxial and adaxial leaf surfaces. The leaf surface of the 22 accessions in the first generation and the ten

accessions in the S_1 generation, respectively, has been observed to have stomata with guard cell, long cell, short cell comprised of silica bodies and phellem cell. The long cell was observed dominating the various cells, correspond well with the report on epidermal features of rice leaf in Islam et al. (2009). The presence of those features was also in line with the rice cultivar observed by Luo et al. (2012). Rectangular shape and undulated cell outline in this study were also found similar in Luo et al. (2012). Like in other Poacea, the leaf of the studied accessions has costal and intercostal zones. Costal zone is the zone covers with veins and intercostal zone is located between the veins (Metcalfe, 1960). The observation of costal and intercostal zones was similar to the observation on leaf vascular system in rice by Weerasooriya et al. (2018).

In this study, silica body was dumbbell in shaped, similar result was found in Zhang et al. (2013). They described the silica cell in the leaf of rice plant as short and dumbbell in shaped. Silica body content may be correlated with resistance to fungal diseases (e.g., brown spot and blast) and insect pests (e.g., Asiatic stem borer and leaf roller) (Kim et al., 2002). The deposition of silica in the epidermal cells forms a mechanical barrier called the double silica-cuticle layer, which decreases transpiration and improves the water use efficiency (Fauteux et al., 2005; Liang et al., 2007; Rodrigues et al., 2017). The number of silica bodies and rows per cell may be diagnostic for a species, but there is always a range of values, and variations are due to the age of the leaf, and environmental conditions must be taken into account (Kim et al., 2002; Prychid et al., 2003). This trait can be investigated to understand the resistance of Sarawak rice against rice blast (Lai et al., 2019) and yellow stem borer (Cheok et al., 2019; Ling et al., 2020).

The range of epidermal cell length (LEPC) and width of epidermal cell (WEPC) of 22 accession in first generation and 10 accessions in second generation were longer and

wider than the range recorded in nine upland rice varieties from Thailand (Raumjit et al., 2019). The further detailed investigations of the molecular mechanism of stomatal and epidermal cells development in rice are still needed.

5.2.2 Diversity in Stomatal Complex

One of the important research areas in current rice science is stomatal study. Differences or any changes in stomatal features can alter the water and carbon flux, which may contribute significantly to create climate resilient rice. Stomata are the major organ which function to control water and gaseous exchange, hence making it the potential target for modification to increase and sustain rice yield with minimal water input (Franks et al., 2015; Flexas, 2016; Dittrich et al., 2018).

In the present study, the stomatal complex was observed having guard cell and subsidiary cell. The stomatal patterning of the studied rice accessions in first generation and second generation is consistent with the result in Chatterjee et al. (2020), who observed stomatal features in wild and cultivated *Oryza* species. A pair of stomatal complexes accompanied with guard cell, subsidiary cell and epidermal cell were also observed in the leaf of rice reported by Luo et al. (2012). The stomata in the present study were mostly arranged in 3-4 rows in the intercostal zones. The SuC shapes were observed triangular in shape, correspond well with the observation made by Sarwar and Ali (2002) on studies of the leaf epidermis of rice. In their study, SuC were generally triangular.

For stomata density (SD), in general, stomata were found abundance in abaxial surface compared with adaxial surface in most accessions. This corresponds well with the observations of Boonrueng et al. (2013) on Thailand jasmine rice, where the SD were also found abundance on abaxial surface when compared to adaxial surface. Chatterjee et al. (2020) also recorded high SD of Asian *Oryza sativa* on the abaxial surface when compared

to adaxial surface. Higher SD on the abaxial surface was also true for maize (*Zea mays*), barley (*Hordeum vulgare*) and soybean (*Glycine max*) (Laza et al., 2010). Rice plants with fewer stomata are better on maintaining stomatal conductance and survive longer under drought and high temperature (40 °C) (Caine et al., 2019). Low SD rice have achieved equivalent or even increased grain yields when compared with high SD rice, despite a reduced rate of photosynthesis in some stress conditions (Caine et al., 2019).

In the present study, the length of stomatal complex (LSC) was generally longer on the abaxial surface when compared with those in adaxial surface in both first and S_1 generation. Zhang et al. (2019) also observed the stomata were smaller on abaxial side when compared with adaxial surface. Previous reports stated that smaller stomata would respond faster and more efficiently in the uptake of CO_2 inside the leaf, and thus will increase the photosynthesis and water use efficiency (Giday et al., 2013; Lawson et al., 2014).

5.2.3 Midrib of 22 Upland Rice Accessions at First Generation

General anatomical features of the transverse section of midrib were found similar among the accessions in first generation. The midrib of the accessions has vascular bundle, schelerenchyma and parenchyma. Similar result was observed for Thai rice landrace by Taratima et al. (2020).

5.2.4 Root and Stele of 22 Upland Rice Accessions at First Generation

The root transverse section of the accessions in the first and S_1 generation were having the same general features. The root has a stele with differentiating proto- and metaxylem, a cortex with an endodermis, midcortex with thick cell walls in inner layers,

developing aerenchyma, and an enlarged outer ring of cells. Kundur et al. (2015) and Kadam et al. (2015) also reported similar general structures of the rice root.

Root diameter of the studied accessions in first generation ranging from 778.00 µm to 1427.47 µm, bigger than the rice root diameter that have been reported by Kondo et al. (2000). Lower root diameter of tropical upland and lowland rice varieties was recorded in Kondo et al. (2000). Kondo et al. (2000) had reported that the root diameter was higher in the submerged condition than aerobic condition due to larger cortex with higher level development of aerenchyma in the submerged conditions. According to Kadam et al. (2015), variation in root diameter is due to change in the number and size or width of cortical cells and in stele diameter.

Stele is the central part of the root system that contains vascular tissue (i.e. xylem and phloem). Stele diameter of the accessions in this study ranging from 213.55 (UNIMAS-22) µm to 399.91 µm (UNIMAS-13). Wider stele may lead to higher plant water status under water deficit due to higher hydraulic conductivity. Greater xylem diameter (which indicate larger stele diameters) is linked to better axial conductance, which improved rice's ability to absorb and hold more water during water limiting conditions due to thicker suberized endodermis (Yambao et al.,1992; Sibounheuang et al., 2006; Henry et al., 2012). Jeong et al. (2013) reported that two mutants of rice had greater root diameters and larger stele size were having higher grain yield during drought but without measuring plant physiological responses. Hence, accessions with large stele diameter can be considered for Sarawak rice breeding program to produce drought-tolerance rice as those traits are possibly advantageous under upland conditions for efficient water capture.

5.3 Characterisation Based on SSR Marker

The application of molecular markers in identifying plant varieties has obtained much recognition. Molecular markers offer numerous advantages over conventional markers in their ability to cover the whole genome. This include the ability for them to reveal polymorphism even in closely related varieties or population which are otherwise non-distinguishable in a specific region of DNA (Helentjaris et al., 1985; Sonnanteet al., 1994; Akkaya et al., 1999). Among various molecular markers that have been developed, SSR markers are more suitable and effectively used for a variety of applications in plant genetics and breeding. Although Single Nucleotide Polymorphic loci (SNPs) have been used in a variety of genetic studies recently, they still have not replaced SSRs completely (Wang et al., 2021).

5.3.1 Polymorphism of SSR Marker

In the present study, the 22 upland rice accessions collected from the North-Western region of Sarawak were genotyped with eight polymorphic SSR primers which produced repeatable and scoreable bands.

Three alleles were amplified by RM 1 and RM257. The remaining primer pairs RM489, RM166, RM164, RM481, RM444, RM552 amplified two alleles. In total, 18 alleles were amplified using the eight SSR markers with an average of 2.57 alleles per marker. Similarly, Supari et al. (2019) and Wong et al. (2009) reported an average of 2.26 and 2.6 alleles per SSR locus, respectively for their rice genotyping. Cho et al. (2000) stated that the average alleles per locus for various classes of SSR markes in rice germplasm is ranging from 2.0 to 5.5 alleles per locus. The number of alleles indicates the richness of the population (Sohrabi et al., 2013)

5.3.2 PIC Values and Genetic Diversity

Polymorphic alleles of each SSR primer are used to demonstrate the level polymorphic information content (PIC). PIC value acts as an indicator of a marker that reflected allele diversity and frequency (Shete et al., 2000).

The PIC value in this study ranged from 0.25 (RM552) to 0.88 (RM257), with an average of 0.67. Similar result was recorded by Sohrabi et al. (2013), where RM257 with PIC value of 0.88 was the highest PIC when genotyped on 50 upland rice accessions from two states of Malaysia. The average PIC values obtained in the present study also correspond well with the study on rice in Malaysia with the average PIC values ranging from 0.62 to 0.68 (Lee et al. 2011; Noorzuraini et al., 2013; Aljumaili et al., 2018). According to Yan et al. (2016), the PIC value higher than 0.5 indicates the locus has high diversity. PIC value ranging from 0.25 to 0.5 meaning a locus has intermediate diversity and PIC less than 0.25 indicates low diversity. In the present study, seven out of eight SSR markers showed that the PIC values higher than 0.5, except for marker RM552, indicating that the seven markers were considered as highly informative markers.

The effective number of alleles (Ne) among markers varied from 1.31 (RM1) to 2.00 (RM444) with an average of 1.639, higher than the value reported on aromatic rice accessions from Malaysia genotyped using SSR marker by Aljumaili et al. (2018). Sohrabi et al. (2013) revealed a higher value of *Ne* for the 50 upland rice accessions from Peninsular Malaysia and Sabah using ten SSR markers, with 2.89 and 2.98, respectively. Nei's gene diversity (h) is used to quantify the genetic variation and to evaluate genetic divergence and population relationship. Nei's gene diversity among the markers in the present study ranged from 0.219 (RM552) to 0.500 (RM444), with an average of 0.372. A study done by Sohrabi et al. (2013) showed a much higher value of *h* in the author's upland

rice populations originated from Peninsular Malaysia and Sabah, which was 0.62 and 0.58, respectively. According to Bobokashvili (2016), the Nei's gene diversity of value 0.95 to 1.00 indicates high divergence in a population. Nei's gene value ranging from 0.50 to 0.60 indicates two species were having similar features, however, unable to cross each other. Two subspecies having very similar features in range of one species were likely to have Nei's gene diversity value ranging from 0.17 to 0.22. This can conclude that the Nei's gene diversity in the present study was low in diversity.

In the present study, the Shannon's information index (*I*) among markers ranged from 0.360 (RM552) to 0.693 (RM444), with an average of 0.55. Sohrabi et al. (2013) reported much higher value of *I* with an average of 1.1653 for the author's upland rice populations from Peninsular Malaysia and 1.0754 for Sabah (Sohrabi et al., 2013). The result has concluded that the diversity among the 22 accessions of Sarawak upland rice is considered low compared to the upland rice accessions from Peninsular Malaysia and Sabah of Sohrabi et al. (2013).

5.3.3 Cluster Analysis based on SSR Markers

In the present study, the clustering analysis did not observe clear clustering of the 22 upland rice accessions according to their morphological traits nor geographical origin. In contrast, previous studies in China and Iraq observed the clustering of rice landraces according to their subspecies based on the SSR marker genotypes (Ma et al., 2016; Hassan & Hama-Ali, 2022). In addition, Zhang et al. (2010) and Aljumaili et al. (2018) revealed the SSR marker genotypes clustered their rice varieties according to geographical origin. For the unresolved clustering of the 22 upland rice accessions, neither according to their morphology nor geographical origin, may have been due to the number of SSR marker

used in the analysis. The numbers of SSR marker used in the present study is comparatively less compared to the previous studies (Zhang et al., 2010; Ma et al., 2016; Aljumaili et al., 2018; Hassan & Hama-Ali, 2022) which was around 32 to 140 pairs of SSR marker. It is known that the accuracy of population genetic and the genetic structure decreases with decreasing number of markers used (Wang et al. 2021).

According to Aljumaili et. al (2018), the accessions grouped in one cluster are considered to have high genetic similarity, and divergent when those accessions form distinct clades The highest genetic distance was recorded between accessions UNIMAS-07 and UNIMAS-13, it shows the two accessions has low genetic similarity although both coming from the same division, Kuching. The genetic diversity of rice might be attributed by cultural practice by the local farmers, where the rice seeds were obtained from relatives or friend from different villages (Tu et al., 2007; Yeo et al.,2018).

Meanwhile, accessions that shared the same name but collected from different villages were found to be genetically different based on SSR genotype as shown between UNIMAS-04, UNIMAS-05, UNIMAS-06, and between UNIMAS-07 and UNIMAS-22 (Table 5.1). According to Yeo et al. (2018), the registration of a variety based on the name given by farmers are unreliable. There is a possibility for a variety with the similar name, having different morphological characteristics. To support that, UNIMAS-08, UNIMAS-09, UNIMAS-10, UNIMAS-11, UNIMAS-12, UNIMAS-13, UNIMAS-14 in the present study exhibited different morphological traits *viz.*, seed colour and grain colour despite having same name given by the farmers.

Table 5.1: SSR Genotype of Five Upland Rice Accessions Based on Eight Polymorphic SSR Markers

Marker	Accessions				
	UNIMAS-04	**UNIMAS-05**	**UNIMAS-06**	**UNIMAS-07**	**UNIMAS-22**
RM1	1	2	1	3	4
RM489	1	1	1	1	2
RM552	1	1	2	1	3
RM444	1	1	1	1	1
RM257	1	1	1	1	2
RM481	1	2	2	1	2
RM166	1	1	1	1	2
RM164	1	1	1	1	2

* Number scored for each accession and SSR marker represents grouping made based on allele scoring

5.3.4 Phylogenetic Analysis Based on Chloroplast Gene Maturase-K

Molecular analysis using chloroplast gene *matK* showed that the 22 upland accessions were grouped in Cluster I with 100% bootstrap, together with 22 lowland accessions from UNIMAS collections along with 94 rice accessions, which mostly originated from India, followed by Australia (4 accessions), China (1 accession), and unknown origin (1 accession). Three accessions of *O. rufipogon* were clustered together with *O. sativa* in Cluster I. Accessions in Cluster I were seen not clustering with outgroup species, *O. punctata*, *O. granulata*, *O. australiensis* and *O. officinalis*.

The phylogenetic tree revealed that the entire *Oryza* species were grouped based on their genome when compared using chloroplast gene *matK*. *O. sativa* and *O. rufipogon* which have AA genome were clustered in one clade but separated from *Oryza* of different genomes, *O. punctata* (BB genome), *O. officinalis* (CC genome), *O. australiensis* (EE genome) and *O. granulata* (GG genome). Zodinpuii et al. (2013) showed similar result for

the phylogenetic relationships of genus *Oryza* from Eastern Himalayan based on *matK* gene. *O. sativa* and *O. rufipog*on in their study were also grouped in one clade with 100% bootstrap, supporting the previous hypothesis of an Asian origin of *O. sativa*, and *O. rufipogon* as the progenitor (Vaughan, 1994; Khush, 1997). This also indicating that *matK* marker was unable to distinguish closely related species.

Low variation of *matK* gene was also unable to distinguish closely-related species in species-rich genera groups such as Santiria, Ficus and Litsea (Amandita, 2015). A total of four species of Santiria (*S. tomentosa*, *S. oblongifolia*, *S. laevigata*, *S. rubiginosa*) shared a single haplotype of *matK*. This is due to chloroplast captures activity which occurs frequently in species with reproductive compatibility causing the sharing of DNA sequences between sister species. According to Rieseberg and Soltis (1991), chloroplast capture begins when the chloroplast genome of that specific species is replaced by that of another species through hybridization. As a result, these two different species would have identical chloroplast DNA. Hence, for the present study, a combination of barcoding markers from the plastid genome and intergenic spacers should be considered to improve the resolution of the phylogenetic tree. Such proposition is based on De Mattia et al. (2011), where the author manages to discriminate *Ocimum basilicum* from other *Ocimum* species up to cultivar level by using the combination of *matK* and *trnH-psbA* genes.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aims of this research study were to describe the morphological and anatomical characters as well as to establish the genetic relatedness using Simple Sequence Repeat markers and *matK* barcoding marker of 22 upland rice accessions collected from the North-Western region of Sarawak.

The 22 upland rice accessions were successfully characterised using morphological characterisation. Qualitative traits were varied among the accession in first generation except for ligule shape. The yield contributing traits in first generation such as heading days, flowering days, number of tillers and number of panicles were observed and recorded. Characterising the S_1 generation of ten accessions was achieved through studying four and seven of qualitative and quantitative traits, respectively. Traits such as blade colour, ligule colour, plant height, culm length and number of tillers were found varied between the accessions in S_1 generation. Anatomical study which presented epidermal cells, stomata and transverse sections of midrib and roots was successfully evaluated in first generation. In S_1 generation, epidermal cell and stomata traits were evaluated. There was no large variation in terms of general structure of plant tissue and arrangement of the cell in epidermal, stomatal complex, midrib and root in both generations. Leaf anatomy revealed that all the accessions were having silica body content, which may correlate with resistance to fungal diseases and insect pests. Relatedness among the 22 genotypes was obtained through a UPGMA dendrogram based on eight polymorphism SSR marker. Analysis using the *matK* genes using Maximum Likelihood method revealed the clustering

pattern was based on the genome. The characterisation of the accessions is of great significance in modern day agriculture as they play a major role in varietal improvement. This study confirmed the presence of variations among the genotypes for most of the studied traits, which will create an opportunity for breeders to improve the quality and quantity of rice production.

6.2 Recommendations

The characterisation of upland rice accessions based on morphological, anatomical as well as SSR genotyping and *matK* barcoding gene is the first to be examined from Sarawak. Results obtained from this study may be considered as primary steps to launch a national breeding program for the development of new rice varieties that have a high productivity and adaptability towards stress. More characterisation of more landraces from different regions needs to be done in future. In addition, more morphological traits observed is needed to record distinguishable traits that can differentiate the accessions. Ideally, more SSR markers is needed to give a better picture of the genetic structure of the upland rice accessions as the present study did not observe a clear grouping pattern due to few numbers of SSR markers used. Furthermore, continuous effort is needed to produce seeds from S_1 generation until S_5 to S_6 generation. The seeds can be used for other agronomical-traits characterisation related to pest and disease. Using *matK* gene alone in this present study did not resolve *O. sativa* from *O. rufipogon.* Combination of genes from the plastid genome and intergenic spacers can be done in future research to differentiate the Sarawak rice up to sub-species level.

REFERENCES

Adnan, N., & Nordin, S. (2021). How COVID-19 effect Malaysian paddy industry? Adoption of green fertilizer a potential resolution. *Environment, Development and Sustainability*, *23*, 1-41.

Agata, A., Ando, K., Ota, S., Kojima, M., Takehara, S., Matsuoka, M., & Hobo, T. (2020). Diverse panicle architecture results from various combinations of Prl5/GA20ox4 and Pbl6/APO1 alleles. *Communications Biology*, *3*(1), 1-17.

Ahmadi, N., Audebert, A., Bennett, M. J., Bishopp, A., de Oliveira, A. C., & Courtois, B. (2014). The roots of future rice harvests. *Rice, 7*(1), 1-9.

Aidy, E. A. N., Emery, E. M. I., Draz, A. E., & Badawi, A. T. (2000). Chemical and morphological characters of some local rice genotypes. *Egyptian Journal of Agricultural Research, 78*(2), 693-716.

Akkaya, M. S., Bhagwat, A. A., & Cregan, P. B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics*, *132*(4), 1131-1139.

Ali, M. A., Gyulai, G., & Al-Hemaid, F. (2007). *Plant DNA barcoding and phylogenetics*. Saarbrucken: LAP Lambert Academic Publishing.

Aljumaili, S. J., Rafii, M. Y., Latif, M. A., Sakimin, S. Z., Arolu, I. W., & Miah, G. (2018). Genetic diversity of aromatic rice germplasm revealed by SSR markers. *Biomed Research International, 11*, 1-11.

Amandita, F. Y. (2015). DNA barcoding of flowering plants in Jambi, Indonesia. (Doctoral Dissertation, GeorgAugust University of Göttingen).

Anderson, J. A., Churchill, G., Autrique, E., Tanksley, S., & Sorrells, M. E. (1993). Optimizing parental selection for genetic linkage maps. *Genome National Research Council Canada*, *36*(1), 181-186.

Anggraini, N. B., Sholihah, A., Khasna, E. N., Retnaningtyas, R. W., & Listyorini, D. (2020). *Genetic relationship between local rice varieties based on matK and rbcL genes*. AIP Conference Proceedings 2260, 020020. Retrieved from https://doi.org/10.1063/5.0015756.

Anonymous. (2012). *Making Sarawak a rice bowl of Malaysia*. Borneo Post Online. Retrieved from https://www.theborneopost.com/2012/02/12/making-sarawak-a-rice-bowl-of-malaysia/ on 21 January 2021.

Arianti, F. D., Pertiwi, M. D., Triastono, J., Purwaningsih, H., Minarsih, S., Hindarwati, Y., Jauhari, S., Sahara, D., & Nurwahyuni, E. (2022). Study of organic fertilizers and rice varieties on rice production and methane emissions in nutrient-poor irrigated rice fields. *Sustainability*, *14*(10), 5919-5936.

Ashrafuzzaman, M., Rafiqul Islam, M. D., Ismail, M. R., Shahidullah, S. M., & Hanafi, M. M. (2009). Evaluation of six aromatic rice varieties for yield and yield contributing characters. *International Journal of Agriculture and Biology*, *11*(5), 616-620.

Bell, A. D. (1991). *Plant form: An illustrated guide to flowering plant morphology*. New York: Oxford University Press.

Bernier, G. (2011). My favourite flowering image. *Journal of Experimental Botany*, *1*, 1-4.

Bernier, G. (1988). The control of floral evocation and morphogenesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, *39*, 175–219.

Bhattarai, G., Shi, A., Kandel, D. R., Solis-Gracia, N., da Silva, J. A., & Avila, C. A. (2021). Genome-wide simple sequence repeats (SSR) markers discovered from whole-genome sequence comparisons of multiple spinach accessions. *Scientific Report, 11*(1), 1-16.

Bhupenchandra, I., Athokpam, H. S., Singh, N., Soibam, H., Chongtham, S. K., Singh, L. K., Sinyorita, S., & Olivia, L. C. (2021). Leaf Color Chart (LCC): An instant tool for assessing nitrogen content in plant: A review. *The Pharma Innovation Journal, 10*(4), 1100-1104.

Blaxter, M., Elsworth, B., & Daub, J. (2004). DNA taxonomy of a neglected animal phylum: An unexpected diversity of tardigrades. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(4), 189-192.

Bobokashvili, Z. (2016). Re: How to interprete or infer Nei's phenotypic diversity index?. Retrieved from: https://www.researchgate.net/post/How_to_interprete_or_inferNeis_phenotypic_diversity_index/57ac7874615e27c611557908/citation/ download on 20 January 2022.

Boonrueng, N., Anuntalabhochai, S., & Jampeetong, A. (2013). Morphological and anatomical assessment of KDML 105 (*Oryza sativa* L. spp. *indica*) and its mutants induced by low-energy ion beam. *Rice Science, 20*(3), 213-219.

Bose, L. K., & Pradhan, S. K. (2005). Genetic divergence in deep water rice genotypes. *Journal of Central European Agriculture, 6*(4), 635-640.

Botstein, D., White, D. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics, 32*(3), 314–331.

Brown, C. P. (1973). Rice price stabilization and support in Malaysia. *The Developing Economies, 11*(2), 164-183.

Burgess, K. S., Fazekas, A. J., Kesanakurti, P. R., Graham, S. W., Husband, B. C., Newmaster, S. G., Percy, D. M., Hajibabaei, M., & Barrett, S. C. H. (2011). Discriminating plant species in a local temperate flora using the rbcL+matK DNA barcode. *Methods in Ecology and Evolution*, *2*(4), 333–340.

Caine, R. S., Yin, X., Sloan, J., Harrison, E. L., Mohammed, U., Fulton, T., & Gray, J. E. (2019). Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. *New Phytologist*, *221*(1), 371-384.

Chan, D. (2020, March 28). *"Malaysia's rice stock stable" New Straits Times*. NST Online. Retrieved from https://www.nst.com.my/news/nation/2020/03/579071/malaysias-rice-stock-stable on 24 May 2021.

Chang, T. T., & Bardenas, E. A. (1965). *The Morphology and Varietal Characteristics of The Rice Plant*. The International Rice Research Institute, Phillipines.

Chase, M. W., Salamin, N., Wilkinson, M., Dunwell, J. M., Kesanakurthi, R. P., Haider, N., & Savolainen, V. (2005). Land plants and DNA barcodes: short-term and long-term goals. *Biological Sciences*, *360*(1462), 1889–1895.

Chatterjee, J., Dionora, J., Elmido-Mabilangan, A., Wanchana, S., Thakur, V., Bandyopadhyay, A., & Quick, W. P. (2016). The evolutionary basis of naturally diverse rice leaves anatomy. *PloS ONE*, *11*(10), e0164532.

Chatterjee, J., Thakur, V., Nepomuceno, R., Coe, R. A., Dionora, J., Elmido-Mabilangan, A., & Quick, W. P. (2020). Natural diversity in stomatal features of cultivated and wild *Oryza* species. *Rice*, *13*(1), 1-20.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., & Leon, C. (2010). Validation of the ITS2 region as a novel DNA Barcode for identifying medicinal plant species. *PLoS ONE, 5*(1), e8613.

Cheok, Y. H., Yeo, F. K. S., & Chong, Y. L. (2019). Oviposition behavior *of Scirpophaga incertulas*, the yellow stemborer (Lepidoptera: Crambidae) in a non-choice study. *Pertanika Journal of Tropical Agricultural Science*, *42*(3), 1167-1172.

Chimezie, E., Alozie, O. C., & Ikechukwu, M. S. (2016). Importance of leaf, stem and flower stalk anatomical characters in the identification of Emilia Cass. *International Journal of Plant Soil Science, 12*(6), 1-12.

Cho, Y. G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., McCouch, S. R., & Cartinhour, S. (2000). Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, *100*(5), 713-722.

Chong, E. T. J., Goh, L. P. W., Wong, J. J., Aziz, Z. A., Surugau, N., Latip, M. A., & Lee, P. C. (2018). The genetic diversity and relationship of Sabah traditional rice varieties as revealed by RAPD markers. *Pertanika Journal of Tropical Agricultural Science*, *41*(1), 177-190.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*, 169–196.

Condit, R., & Hubbell, S. P. (1991). Abundance and DNA sequence of 2-base repeat regions in tropical tree genomes. *Genome, 34*, 66–71.

Cowan, R. S., & Fay, M. F. (2012). Challenges in the DNA barcoding of plant material. *Methods in Molecular Biology, 862*, 23–33.

Cutler, D. F. (1978). *Applied Plant Anatomy*. London and New York: Longman.

Dangi, G., Mendum, M., Prins, B., Walker, M., Meredith, C., & Simon, C. (2001). Simple sequence repeats analysis of a clonally propagated species: A tool for managing a grape germplasm collection. *Genome, 44*, 432–438.

De Mattia, F., Bruni, I., Galimberti, A., Cattaneo, F., Casiraghi, M., & Labra, M. (2011). A comparative study of different DNA barcoding markers for the identification of some members of Lamiacaea. *Food Research International, 44*(3), 693–702.

Department of Agriculture. (2005). *Agriculture Statistical Handbook*. Ministry of Agriculture and Agro-based Industry. Pp. 31.

Department of Irrigation and Drainage. (2015, January 22). *Pioneer hybrid rice project at Tanjung Suduru a success*. Did.sarawak.gov.my. Retrieved from https://did. sarawak.gov.my/modules/web/pages.php?lang=bm&mod=news&sub=news_ view&nid=141 on 28 July 2021.

Department of Statistics Malaysia (DOSM). (2021). Malaysia Trade Statistics Review: How Malaysia's Trade Perform in Agriculture Sector?. DOSM: Malaysia. Department of Statistics Malaysia (DOSM). (2022). *Malaysia Trade Statistics Review (MTSR) Volume 1 2021*. DOSM, Malaysia.

Dittrich, M., Mueller, H. M., Bauer, H., Peirats-Llobet, M., Rodriguez, P. L., Geilfus, C. M., & Hedrich, R. (2019). The role of Arabidopsis ABA receptors from the PYR/PYL/RCAR family in stomatal acclimation and closure signal integration. *Nature Plants, 5*(9), 1002-1011.

Dow, R. A. (2021). An annotated checklist of the odonata (Insecta) known from Sarawak with records to district level. *Sarawak Museum Journal*, *10*, 313-422.

Dutta, R. K., Baset Mia, M. A., & Khanam, S. (2002). Plant architecture and growth characteristics of fine grain and aromatic rices and their relation with grain yield. *International Rescue Committee Newsletter, 51,* 51-56.

Fauteux, F., Rémus-Borel, W., Menzies, J. G., & Bélanger, R. R. (2005). Silicon and plant disease resistance against pathogenic fungi. *Federal of European Microbiological Studies, 249*(1), 1-6.

Flexas, J. (2016). Genetic improvement of leaf photosynthesis and intrinsic water use efficiency in C3 plants: Why so much little success?. *Plant Science, 251,* 155-161.

Food and Agriculture Organization of the United Nations. (2021, September 28). *FAO GIEWS Country Brief on Malaysia*. Retrieved from https://www.fao.org/giews/countrybrief/country.jsp?code=MYS on 7 July 2021.

Franks, P. J. W., Doheny-Adams, T., Britton-Harper, Z. J., & Gray, J. E. (2015). Increasing water-use efficiency directly through genetic manipulation of stomatal density. *New Phytologist, 207*(1), 188-195.

Fuente, V., & Ortunez, E. (2001). Festuca sect. Eskia (Poaceae) in the Iberian Peninsula. *Folia Geobotanica, 36*, 385 –392.

Fujii, N., Tomaru, N., Okuyama, K., Koike, T., Mikami, T., & Ueda, K. (2002). Chloroplast DNA phylogeography of *Fagus crenata* (Fagaceae) in Japan. *Plant Systematics and Evolution, 232*(1-2), 21–33.

Fujino, K., Sekiguchi, H., Matsuda, Y., & Yano, M. (2008). Molecular identification of a major quantitative trait locus, Qltg3-1, controlling low-temperature germinability

in rice. *The Proceedings of the National Academy of Sciences, 105*(34), 12623-12628.

Fukugawa, N., & Ziska, L. (2019). Rice: Importance for global nutrition. *Journal of Nutritional Science and Vitaminology, 65,* 1-3.

Furukawa, T., Maekawa, M., Oki, T., Suda, I., Iida, S., & Shimada, H. (2006). The *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant Journal, 49*(1), 91-102.

Garcia, A. A. F., Benchimol, L. L., Barbosa, A. M. M., Geraldi, I. O., Souza, C. L., & de Souza, A. P. (2004). Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genetic and Molecular Biology, 27* (4), 579–588.

Ge, S., Li, A., Lu, B. R., Zhang, S. Z., & Hong, D. Y. (2002). A phylogeny of the rice tribe Oryzeae (Poaceae) based on matK sequence data. *American Journal of Botany, 89*(12), 1967–1972.

Ge, S., Sang, T., Lu, B. R., & Hong, D. Y. (1999). Phylogeny of rice genomes with emphasis on origins of allotetraploid species. *Proceedings of the National Academy of Sciences of the United States of America, 96*(25),14400-14005.

Geetha, S., Soundarraj, A. M. K., & Palnisamy, S. (1994). Grain characteristics of rice hybrids. *Crop Research, 7*(2), 303-305.

Giarrocco, L. E., Marassi, M. A., & Salerno, G. L. (2007). Assessment of the genetic diversity in Argentina rice cultivars with SSR Markers. *Crop Science, 47*(2), 853-858.

Giday, H., Kjaer, K. H., Fanourakis, D., & Ottosen, C. O. (2013). Smaller stomata require less severe leaf drying to close: a case study in Rosa hydrida. *Journal of Plant Physiology, 170*(15), 1309-1316.

Graham, R. J. D. (1913). Preliminary note of the classification of rice in the central provinces. *Agriculture India Botanical Survey, 6*(1), 209-229.

Gu, D., Zhen, F., Hannaway, D., Zhu, Y., Liu, L., Cao, W., & Tang, L. (2017). Quantitative classification of rice (*Oryza sativa* L.) root length and diameter using image analysis. *PLoS ONE, 12*, 12-18.

Gumma, M. K., Nelson, A., Thenkabail, P. S., & Singh, A. N. (2011). Mapping rice areas of South Asia using MODIS multitemporal data. *Journal of Applied Remote Sensing, 5*(1), 053547-053547.

Guttikonda, H., & Surapaneni, M., Balakrishnan, D., Swamy, M., Mangrauthia, S., & Neelamraju, S. (2018). *Oryza nivara* Sharma et Shastry. Switzerland: Springer Cham.

Halil, S., & Necmi, B. (2005). Selection for grain yield and its components in early generations in rice (*Oryza sativa* L.). *Trakya University Journal Science, 6*(1), 51-58.

Hanafi, M. M., Hartinie, A., Shukor, J., & Mahmud, T. M. M. (2009). Upland rice varieties in Malaysia: agronomic and soil physico-chemical characteristics. *Pertanika Journal of Tropical Agricultural Science, 32*(2), 1511-3701.

Hanis, A., Nasir, M., Shahrim, M., & Paddy, R. (2012). Consumers' demand and willingness to pay for rice attributes in Malaysia. *International Food Research Journal, 19*(1), 363-369.

Hassan, D. A., & Hama-Ali, E. O. (2022). Evaluation of gene flow and genetic diversity in rice accessions across Kurdistan region-Iraq using SSR Markers. *Molecular Biology Reports*, *49*(2),1007-1016.

Hayashi, K., & Kawano, S. (2000). Molecular systematics of Lilium and allied genera (Liliaceae): Phylogenetic relationships among Lilium and related genera based on the rbcL and matK gene sequence data. *Plant Species Biology*, *15*(1), 73-93.

Helentjaris, T., King, G., Slocum, M., Sidenstrang, G., & Wegman, S. (1985). Restriction fragment polymorphisms a probe for plant diversity and their development as tools for applied plant breeding. *Plant Molecular Biology*, *5*(2), 109-118.

Henry, A., Cal, A. J., Batoto, T. C., Torres, R. O., & Serraj, R. (2012). Root attributes affecting water uptake of rice (*Oryza sativa*) under drought. *Journal of Experimental Botany*, *63*(13), 4751-4763.

Herbert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings Biological Sciences*, *270*(1512), 313-321.

Herman, T., Murchie, E. H., & Warsi, A. A. (2015). Rice production and climate change: A case study of Malaysian rice. *Pertanika Journal of Tropical Agricultural Science*, *28*(3), 321-328.

Hilu, K. W., & Liang, G. (1997). The matK gene: sequence variation and application in plant systematics. *American Journal of Botany*, *84*(6), 830–839.

Hilu, K. W., Borsch, T., Müller, K., Soltis, D. E., Soltis, P. S., Savolainen, V., Chase, M. W., Powell, M. P., Alice, L. A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T. A. Rohwer, J. G., Campbell, C. S., & Chatrou, L. W. (2003). Angiosperm phylogeny

based on mat K sequence information. *American Journal of Botany*, *90*(12), 1758–1776.

Hilu, K. W., Alice, L. A., & Liang, H. (1999). Phylogeny of Poaceae inferred from matK Sequences. *Annals of the Missouri Botanical Garden, 86*(4), 835-851.

Ho, S. Y., & Wasli, M. E. (2015). *Soil Physicochemical Properties of Upland Rice Cultivation by Shifting Cultivation in Sarawak, Malaysia*. Conference: Taxanomy and Ecology Conference, Kuching.

Husain, A. N. (1984). Quality parameters for Malaysian rice varieties. *MARDI Research Bulletin*, *12*(3), 320-332.

Idio, E. R., & Jayeola, A. (2020). Comparative Anatomy and Systemeatics Investigation of *Oryza sativa* species in Nigeria. *International Journal of Innovative Science and Research Technology*, *5*(3), 768-777.

International Rice Research Institute (IRRI). 1980. *Standard Evaluation System for Rice, 2nd edition*. Philippines: International Rice Research Institutes.

IRRI. (n.d.). *Rice and the Environment*. Retrieved from http://www.knowledgebank. irri.org/ewatermgtt/courses/course1/resources/presentations1/RiceEnvironment.pdf on 18 July 2021.

IRRI. (1996). *Standard Evaluation System for Rice, 4th edition*. Philippines: International Rice Research Institutes.

IRRI. (2002). *Standard Evaluation System for Rice*. Philippines: International Rice Research Institutes.

IRRI. (2009). Morphology of rice. Retrieved fromhttps://irri.org/extension/morphology of the rice plant- leaf.html on 25/2/2021.

Islam, M. T., Sarwar, A. G., Begum, H. H., & Ito, T. (2009). Epidermal features of rice leaf CV. BRRI Dhan29. *Bangladesh Journal of Plant Taxonomy*, *16*(2), 177-180.

Jennings, P. R., Coffman, W. R., & Kauffman, H. E. (1979). *Breeding for agronomic and morphological characteristic. In: Rice improvement*. Philippines: International Rice Research Institutes.

Jeong, J. S., Kim, Y. S., Redillas, M. C., Jang, G., Jung, H., Bang, S. W., & Kim, J. K. (2013). OsNAC5 overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. *Plant Biotechnology Journal*, *11*(1), 101-114.

Jiajia, W., Jing, X., Qian, Q., & Zhang, G. (2020). Development of rice leaves: How histocytes modulate leaf polarity establishment. *Rice Science*, *27(6)*, 468-479.

Johnson, L. A., & Soltis, D. E. (1994). matK DNA sequences and phylogenetic reconstruction in saxifragaceae s. str. *Systematic Botany*, *19*(1), 143-156.

Kadam, N. N., Yin, X., Bindraban, P. S., Struik, P. C., & Jagadish, K. S. (2015). Does morphological and anatomical plasticity during the vegetative stage make wheat more tolerant of water deficit than rice. *Plant Physiology*, *167*, 1389-1401.

Kalaichelvan, C. (2009). Studies on identification of rice (*Oryza sativa* L.) cultivars using morphological and molecular markers [M.Sc thesis]. Acarya N G Ranga Agricultural University, Rajendranagar, Hyderaba.

Kano, M., Inukai, Y., Wade, L. J., Siopongco, J. D. L. C. & Yamauchi, A. (2011). Root development, water uptake, and shoot dry matter production under water deficit conditions in two cssls of rice: functional roles of root plasticity. *Plant Production Science*, *14*(4), 307–317.

Karubi, N. P., & Peter, T. S. (2021). The Shrinking Paddy Farms and the Bidayuh Women Rice Farmers: What Has/Have Changed?'. *Journal of Borneo-Kalimantan, 7*(2), 60-72.

Karuppaiyan, R., & Nandini, K. (2006). *Techniques in anatomy, cytology and histochemistry of plants.* Department of Plant Physiology College of Horticulture. Kerala, India.

Kasim, N., Widiayani, N., & Revaldi, A. (2020). Growth and production of sixth generation of brown rice mutants in a high-altitude location. *Institute of Physic Publishing*, *575*(1), 12149-12158.

Kato, S., Kosaka, H., & Hara, S. (1928). On the affinity of rice varieties as shown by the fertility of hybrid plants. *Science Bulletin College of Agriculture, 3*, 132-147.

Keramas, G., Bang, D. D., Lund, M., Madsen, M., Bunkenborg, H., Telleman, P., & Christensen, C. B. V. (2004). Use of culture, PCR analysis and DNA microarrays for detection of Campylobacter and jejuni *Campylobacter coli* from chicken faeces. *Journal of Clinical Microbiology, 47*, 3985–3991.

Khush, G. S. (1997). Origin, Dispersal, Cultivation and Variation of Rice. *Plant Molecular Biology*, *35*, 25-34.

Kikkawa, S. (1912) On the classification of cultivated rice. *Journal of College of Agriculture, 3*, 1-108.

Kikuta, M., Makihara, D., Pasolon, Y. B., Rembon, F. S., Miyazaki, A., & Yamamoto, Y. (2020). Agronomic traits and grain quality of upland rice cultivated in Southeast Sulawesi, Indonesia. *Journal of International Cooperation for Agricultural Development*, *18*, 41-47.

Kim, Y. L., Hussin, N. A., Mohamad, N. K., Hui, Y. T., Lee, S. L., & Yeo, F. K. S. (2019). Qualitative resistance of Sarawak rice landraces against *Pyriculariaoryzae*. *Borneo Journal of Resource Science and Technology*, *9*(2), 115-118.

Kim, S. G., Kim, K. W., Park, E. W., & Choi, D. (2002). Silicon induced cell wall fortification of rice leaves: A possible cellular mechanism of enhanced host resistance to blast. *Phytopathology*, *92*, 1095−1103.

Kleinman, P. J. A., Pimentel, D., & Bryant, R. B. (1995). The ecological sustainability of slash and burn agriculture. *Agriculture, Ecosystems & Environment*, *52*, 235-49.

Klomklao, N., Mekanawakul, M., & Ngamriabsakul, C. (2013). Phylogenetic relationship study of *Oryza sativa* L. cultivars in Southern Thailand based on matK gene sequence. *Journal of Agricultural Research and Extension*, *30*(2), 27-36.

Kooistra, E. (1964). Identification research on pulses. *International Seed Testing Association*, *29*(4), 937-947.

Kovach, M., Sweeney, M. T., & McCouch, S. (2007). New insights into the history of rice domestication. *Trends in Genetics, 23*(11), 578-87.

Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., & Bermingham, E. (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences*, *106*(44), 18621–18626.

Kundur, P. J., Vimarsha, H. S., Sanjay, R., Krishnamurthy, K. V., & Harish, B. G. (2015). Study of rice (*Oryza sativa* L.) root anatomy under aerobic and waterlogged conditions. *International Journal of Applied and Pure Science and Agriculture*, *1*(5), 18-26.

Kusutnani, A., Tovata, M., Asanuma, K., & Cu, J. (2000). Studies on the varietal differences of harvest index and morphological characteristics of rice. *Japanese Journal of Crop Science*, *69*, 359-362.

Kondo, M., Murty, M. V., & Aragones, D. V. (2000). Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. *Soil Science and Plant Nutrition*, *46*(3), 721-732.

Laborte, A. G., Paguirigan, N. C., Moya, P. F., Nelson, A., Sparks, A. H., & Gregorio, G. B. (2015). Farmers' preference for rice traits: insights from farm surveys in Central Luzon, Philippines, 1966-2012. *PLoS One*, *10*(8), e0136562.

Lahaye, R., van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T. G., & Savolainen, V. (2008). DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(8), 2923–2928.

Lambert, D. M., Baker, A., Huynen, L., Haddrath, O., Hebert, P. D. N., & Millar, C. D. (2005). Is a large-scale DNA-based inventory of ancient life possible? *Journal of Heredity*, *96*(3), 279–284.

Lawson, T., & Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology*, *164*(4), 1556-1570.

Laza, M., Rebecca, C., Kondo, M., Ideta, O., Barlaan, E., & Imbe, T. (2010). Quantitative trait loci for stomatal density and size in lowland rice. *Euphytica*, *172*(2), 149-158.

Lee, H. H., Neoh, P. P. N., Bong, W. S. T., Puvaneswaran, J., Wong, S. C., Yiu, P. H., & Rajan, A. (2011). Genotyping of Sarawak rice cultivars using microsatellite markers. *Pertanika Journal of Tropical Agriculture Science*, *34*, 123-136.

Lei, Q., Zhou, J., & Zhang, W. (2018). Morphological diversity of panicle traits in Kam fragrant glutinous rice (*Oryza sativa*). *Genetic Resources Crop Evolution*, *65*, 775–786.

Liang, Y., Sun, W., Zhu, Y. G., & Christie, P. (2007). Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environmental Pollution*, *147*(2), 422-428.

Ling, A. X. R., Yeo, F. K. S., Hamsein, N. N., Ting, H. M., Ismail, W. N. W., Taji, A. S., & Cheok, Y. H. (2020). Screening for Sarawak paddy landraces with resistance to yellow rice stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Crambidae). *Pertanika Journal of Science and Technology*, *43*(4), 491-501.

Lombardo, F., & Yoshida, H. (2015). Interpreting Lemma and Palea Homologies: A Point of view from rice floral mutants. *Frontiers in Plant Science, 6*(61), 61-67.

Luo, L., Zhou, W. Q., Liu, P., Li, C. X., & Hou, S. W. (2012). The development of stomata and other epidermal cells on the rice leaves. *Biologia Plantarum*, *56*(3), 521-527.

Ma, M., Liu, Y., Wang, T., & Lu, B. (2016). Cluster Analysis of Red Rice based on SSR markers from Hani's terraced fields in Yunnan Province. *Advances in Computer Science Research*, *63*, 159-162.

Maeda, H., Yamaguchi, T., Omoteno, M., Takarada, T., Fujita, K., Murata, K., & Iyama, Y., Kojima, Y., Morikawa, M., Ozaki, H., Mukaino, N., Kidani, Y., & Ebitani, T. (2014). Genetic dissection of black grain rice by the development of a near isogenic line. *Breeding Science, 64*(2), 134-141.

Magistrado, P. A., Garcia, M. M., & Raymundo, A. K. (2001). Isolation and polymerase chain reaction-based detection of *Campylobacter jejuni* and *Campylobacter coli*

from poultry in the Philippines. *International Journal of Food Microbiology, 70*(1-2), 197-206.

Mail, R. (2015, February 8). *Farmers reap golden harvest*. Borneo Post Online. Retrieved from https://www.theborneopost.com/2015/02/08/farmers-reap-golden-harvest/ on 5 May 2021.

Mankga, L. T., Yessoufou, K., Moteetee, A. M., Daru, B. H., & van der Bank, M. (2013). Efficacy of the core DNA barcodes in identifying processed and poorly conserved plant materials commonly used in South African traditional medicine. *ZooKeys, 365*, 215–233.

Mariam, A. L., Masahuling, B., & Jamilah, I. (1991). Hill paddy cultivation in Sabah. *Sabah Society Journal, 9*(3), 284-289.

Markmann, M., & Tautz, D. (2005). Reverse taxonomy: an approach towards determining the diversity of meiobenthic organisms based on ribosomal RNA signature sequences. *Biological Sciences, 360*(1462), 1917–1924.

Maruyama, A., Hamasaki, T., Sameshima, R., Nemoto, M., Ohno, H., Ozawa, K., & Wakiyama, Y. (2015). Panicle emergence pattern and grain yield of rice plants in response to high temperature stress. *Journal of Agricultural Meteorology*, *71*(4), 282-291.

Maulani, R. R., Sumardi, D., & Adi, P. (2018). Total flavonoids and anthocyanins content of pigmented rice. *Drug Invention Today, 12*, 369-373.

McCouch, S. R., Chen, X., Panaud, O., Temnykh, S., Xu, Y., Cho, Y. G., Huang, N., Ishii, T., & Blair, M. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology, 35*, 89–99.

McCouch, S. R., Kochert, G., & Yu, Z. H. (1988). Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics, 76,* 815–829.

Mehla, B. S., & Kumar, S. (2008). Use of morphological traits as descriptors for identification of rice genotypes. *Agricultural Science Digest, 28,* 101-104.

Metcalfe, C. R. (1960). *Anatomy of the monocotyledons*. New York: Oxford University Press.

Miah, G., Rafii, M. Y, Ismail, M. R., Puteh, A. B., Rahim, H. A., Islam, K. H., & Latif, M. A. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences, 14*, 22499-22528.

Mo, Y. J., Kim, K. Y., Park, H. S., Ko, J. C., Shin, W. C., Nam, J. K., Kim, B. K., & Ko, J. K. (2012). Changes in the panicle-related traits of different rice varieties under high temperature condition. *Australian Journal of Crop Science, 6,* 436-443.

Moldenhauer, K., & Gibbons J. H. (2003). Rice morphology and development. In *Rice Origin, History, Technology, and Production*. New Jersey: Wiley.

Moldenhauer, K., & Slaton, N. (2001). Rice growth and development. In *Rice Production Handbook,* pp. 7-14. Arkansas: Little Rock.

Mondini, L., Noorani, A., & Pagnotta, M. A. (2009). Assessing plant genetic diversity by molecular tools. *Diversity, 1,* 19-35.

Morishima, H., Hinata, K., & Oka, H. I. (1962). Comparison between two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud. *Japanese Journal of Breeding, 12*, 17-29.

Moriya, S., Iwanami, H., Okada, K., Yamamoto, T., & Abe, K. (2011). A Practical Method for apple cultivar identification and parent-offspring analysis using Simple Sequence Repeat Markers. *Euphytica, 177*, 135-150.

Muller, K. F., Borsch, T., & Hilu, K. W. (2006). Phylogenetic utility of rapidly evolving DNA at high taxonomical levels: Contrasting matK, trnT-F, and rbcL in basal angiosperms. *Molecular Phylogenetics and Evolution*, *41*(1), 99–117.

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., & Baloch, F. S. (2018). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology and Biotechnological Equipment, 32*(2), 261-285.

Naidu, B. S., Gouda, B. B. C., & Laxmi, A. B. (1986). Grain type classification of high yielding rice varieties in Karnataka. *Current Research*, *15*(7-8), 61-63.

Nascimento, W. F. D., Silva, E. F. D., & Veasey, E. A. (2011). Agro-Morphological characterization of upland rice accessions. *Science Agriculture*, *68*(6), 652-660.

Nayak, S., Rajpalsingh, J. K., Bastia, D. N., Samal, K. C., & Rout, G. R. (2014). Assessment of seventy-three rice germplasm by using simple sequence repeats markers. *Journal of Crop Science and Biotechnology*, *17*(4), 297-304.

Neelam, K., Malik, P., Kaur, K., Kumar, K., Jain, S., & Singh, K. (2018). In *The Wild Oryza Genomes*, pp. 277-294. Switzerland: Springer Cham.

Nelson, A., Setiyono, T., Rala, A. B., Quicho, E. D., Raviz, J. V., Abonete, P. J., Maunahan, A. A., Garcia, C. A., Bhatti, H. Z. M., Villano, L. S., Thongbai, P., Holecz, F., Barbieri, M., Collivignarelli, F., Gatti, L., Quilang, E., Mabalay, M., Mabalot, P., Barroga, M., & Ninh, N. H. (2014). Towards an

operational SAR-based rice monitoring system in asia: Examples from 13 Demonstration Sites across Asia in the RIICE Project. *Remote Sensing*, *14*, 10773-10812.

Neumann, H. (1938). ZurKenntnis der Anatomie und ersten Anlage der Graminenligula. *Beitra Wgezur Biologie der Pflanzen*, *25*, 1–22.

Noorzuraini, A. R. S., Borromeo, T. H., Altoveros, N. C., & Kumar, A. (2012). Growth performance of selected Malaysian rice germplasm under drought stress environment. *Journal of Tropical Agriculture and Food Science, 40*(2), 169–179.

Norimah Jr, A. K., Safiah, M., Jamal, K., Haslinda, S., Zuhaida, H., Rohida, S., & Azmi, M. Y. (2008). Food Consumption Patterns: Findings from the Malaysian Adult Nutrition Survey (MANS). *Malaysian Journal of Nutrition, 14*(1), 25.

Ogunbayo, S. A., Ojo, D. K., Guei, R. G., Oyelakin, O. O., & Sanni, K. A. (2005) Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPD techniques. *African Journal of Biotechnology*, *4*(11), 1234-1244.

Olmstead, R. G., & Palmer, J. D. (1994). Chloroplast DNA systematics: A review of methods and data analysis. *American Journal of Botany, 81*(9), 1205-1224.

Olufowote, J. O., Xu, Y., Chen, X., Goto, M., McCouch, S. R., Park, W. D., Beachell, H. M., & Dilday, R. H. (1997). Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. *Genome Volume, 40*(3), 370-378.

Omar, S. C., Shaharudin, A., & Tumin, S. A. (2019). *The status of the paddy and rice industry in Malaysia*. Kuala Lumpur: Khazanah Research Institute.

Pascual, V. M. J, Oritz, J. M., & Coorreal, E. (1993). Morphometric characterization of seeds of *Euphorbia lagascae*. *Seed Science and Technology*, *21*, 53-60.

Patil, R. G., Jadhao, K. R., Samal, K. C., & Rout, G. R. (2015). Molecular phylogeny of indian indigenous aromatic rice based on sequence diversity of the chloroplast-encoded *matK* Gene. *Rice Genomics and Genetics*, *6*(8), 1–8.

Paul, V., Sharma, L., Pandey, R., & Meena, R. C. (2017). Measurement of stomatal density and stomatal index on leaf/plant surfaces. *Indian Council of Agricultural Research, 27*, 27-31.

Peakall, R., & Smouse, P. E. (2012). GenAlex 6.5: Genetic Analysis in excel population genetic software for teaching and research an update. *Bioinformatics*, *28*, 2537.

Peterson, P. M. (2003). Poaceae (Gramineae). *Encyclopedia of Life Sciences* retrieved from https://doi.org/10.1038/npg.els.0003689 on 28 July 2022.

Phanchaisri, B., Samsang, N., Yu, L. D., Singkarat, S., & Anuntalabhochai, S. (2012). Expression of OsSPY and 14-3-3 genes involved in plant height variations of ion-beam-induced KDML 105 rice mutants. *Mutation Research*, *734*, 56–61.

Phapumma, A., Monkham, T., Chankaew, S., Kaewpradit, W., Harakotr, P., & Sanitchon, J. (2020). Characterization of indigenous upland rice varieties for high yield potential and grain quality characters under rainfed conditions in Thailand. *Annals of Agricultural Sciences*, *65*(2), 179-187.

Piazza, P., Jasinski, S., & Tsiantis, M. (2005). Evolution of leaf developmental mechanisms. *New Phytologist*, *167*, 693–710.

Prychid, C. J., Rudall, P. J., & Gregory, M. (2003). Systematics and biology of silica bodies in monocotyledons. *The Botanical Review*, *69*(4), 377-440.

Rahim, H., Jamal, M. S. Engku, E. E. A., & Suhaimee, S. (2021). The Economic Potential of first hybrid rice variety (KADARIA 1) adoption in Malaysia. *Economic and Technology Management Review, 16*, 21-32.

Rahman, M. M., Rasaul, M. G., Hossain, M. A., Khandakar, M. I., & Hasegawa, H. (2012). Molecular characterization and genetic diversity analysis of rice (*Oryza sativa* L.) using SSR markers. *Journal of Crop Improvement, 26*, 244-257.

Rajamoorthy, Y., Rahim, K. A., & Munusamy, S. (2015). Rice industry in Malaysia: Challenges policies and implications. *Procedia Economics and Finance, 31,* 861-867.

Ram, K., & Chetty, C. V. S. (1934). Classification of rice of Bihar and Orissa. *Indian Journal of Agricultural Sciences*, *4*, 537-541.

Raumjit, N., Siwadon, S., Sukanya, S., & Thirayut, W. (2019). Comparison of root system and stomata in nine upland rice varieties. *International Journal of Agricultural Technology*, *15*(5), 769-778.

Ray, A., Deb, D., Ray, R., & Chattopadhayay, B. (2013). Phenotypic characters of rice landraces reveal independent lineages of short-grain aromatic indica rice. *Annals of Botany Plants*, *5*, 32-41.

Rieseberg, L. H., & Soltis, D. E. (1991). Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolution Trends Plant, 5*, 65-84.

Rodrigues, F. Á., Rios, J. A., Debona, D., & Aucique-Pérez, C. E. (2017). *Pyricularia oryzae*-wheat interaction: physiological changes and disease management using mineral nutrition and fungicides. *Tropical Plant Pathology*, *42*(3), 223-229.

Rost, L. (1997). *Rice Anatomy*. California: University of California Davis.

Rosta. (1975). Variety Determination in rice (*Oryza sativa*). *Seed Science and Technology, 3*(1), 161-164.

Ruslan, R., Aznan, A. A., Azizan, F. A., & Roslan, N. (2018). Extraction of morphological features of Malaysian rice seed varieties using flatbed scanner. *International Journal on Advanced Science, Engineering and Information Technology, 8*(1), 93-98.

Salam, M. A., Khorshed, A., & Chowdhury, S. I. (1990). Morpho-Physiological Aspects of Binasail for its improved yield potential over the parent nizersail. Bangladesh *Journal of Nuclear Agriculture, 5*(5), 15-20.

Sang, A. J., Tay, K. M., Lim, C. P., & Saeid, N. (2018). Application of a genetic-fuzzy FMEA to rainfed lowland rice production in Sarawak: Environmental, health, and safety perspectives. *Institutes of Electrical and Electronics Engineers, 6*, 74628–74647.

Sang, T., & Ge, S. (2007). Genetics and phylogenetics of rice domestication. *Current Opinion in Genetics and Development, 17*, 533-538.

Sarao, N., Vikal, Y., Singh, K., & Joshi, M. A. (2009). SSR marker-based DNA fingerprinting and cultivar identification of rice (*Oryza sativa* L.) in Punjab state of India. *Plant Genetic Resources: Characterization and Utilization, 8*(1), 42–44.

Sarma, M. K., Richharia, A. K., & Agarwal, R. K. (2004). Characterisation of ahu rices of Assam for morphological and agronomic traits under transplanted conditions. *Oryza, 41*(1-2), 8-12.

Sarwar, A. K. M., & Ali, M. A. (2002). Studies on the leaf epidermis of rice (*Oryza sativa* L.). *Indian Journal of Agricultural Research, 36*(1), 24-28.

Schander, C., & Willassen, E. (2005). What can biological barcoding do to marine biology?. *Marine Biology Research, 1*(1), 79-83.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). 'NIH Image to ImageJ: 25 Years of image analysis. *Nature Methods, 9*(7), 671-675.

Semsang, N., Kawaree, R., Cutler, R. W., Chundet, R., Yu, L. D., & Anuntalabhochai, S. (2012). Improved antioxidant activity of BKOS Thai jasmine rice. *National Product Research, 26*(12), 1-7.

Sethi, R. L., & Saxena, B. P. (1930). Classification and study of characters of cultivated rice in the United Provinces. Memoirs of the Department of Agriculture in India. *Botanical, 18,* 140-209.

Shah, R., Sulemani, M. Z., Balah, M. S., & Hassan, G. (1999). Performance of coarse rice genotypes in plains of D.I. Khan, Pakistan. *Pakistan Journal of Biological Science, 2*, 507-509.

Shete, S., Tiwari, H., & Elston, R. C. (2000). On estimating the heterozygosity and polymorphism information content value. *Theoretical Population Biology, 57*(3), 265-271.

Sibounheuang, V., Basnayake, J., & Fukai, S. (2006). Genotypic consistency in the expression of leaf water potential in rice (*Oryza sativa* L.). *Field Crops Research, 97*(2-3), 142-154.

Siddiqui, S. U., Kumamaru, T., & Satoh, H. (2007). Pakistan rice genetic resources-II: Distribution of pattern of grain morphological diversity. *Pakistan Journal of Botany, 39*(5), 1533-1538.

Simpson, M. G. (2019). Plant Anatomy and Physiology. In *Plant Systematic 3rd Edition*, pp. 337-566. Cambridge: Academic Press.

Singh, B., Singh, Y., Ladha, J. K., Bronson, K. F., Balasubramanian, V., Singh, J., & Khind, C. S. (2002). Chlorophyll meter–and leaf color chart–based nitrogen management for rice and wheat in Northwestern India. *Agronomy Journal*, *94*(4), 821-829.

Singh, A. K., & Mishra, P. K. (2013). Agro-morphological characterization and morphology based genetic diversity analysis of landraces of rice variety (*Oryza sativa* L.) of Bankura district of West Bengal. *International Journal of Current Research, 5*(10), 2764-2769.

Singh, P. K., Mishra, M. N., Hore, D. K., & Verma, M. R. (2006). Genetic divergence in lowland rice of northeastern region of India. *Communications in Biometry and Crop Science, 1*(1), 35-40.

Sinton, F. V., Sam, L. M., Benedick, S., & Jalloh, M. B. (2019). Evaluation of the yield of upland rice varieties under open field trial. *Transactions on Science and Technology, 6*(2), 253-258.

Sohrabi, M., Rafii, M. Y., Hanafi, M. M., Abdullah, S. N. A., & Latif, M. A. (2012). Genetic diversity of upland rice germplasm in Malaysia Based on quantitative traits. *The Scientific World Journal, 2012*, 416291-416300.

Sohrabi, M., Rafii, M. Y., Hanafi, M. M., & Latif, M. A. (2013). Genetic divergence of Malaysian upland rices revealed by microsatellite markers. *Plant Omics Journal, 6*(3), 175-182.

Soltis, P. S., Soltis, D. E., & Doyle, J. J. (1998). *Molecular systematics of plants*. London: International Thomson Publishing.

Soltis, P. S., & Soltis, D. E. (2004). The origin and diversification of angiosperms. *American Journal of Botany*, *91*(10), 1614–1626.

Sonnante, G., Stockton, T., Nodari, R. O., Becerra Velásquez, V. L., & Gepts, P. (1994). Evolution of genetic diversity during the domestication of common-bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, *89*(5), 629-635.

Spada, A., Mantegazza, R., Biloni, M., Capoali, E., & Sala, F. (2004). Italian rice varieties: historical data, molecular markers, and pedigrees to reveal their genetic relationships. *Plant Breed, 123*, 105–111.

Steane, D. A. (2005). Complete Nucleotide sequence of the chloroplast genome from the tasmanian blue gum, *Eucalyptus globulus* (Myrtaceae). *DNA Research*, *12*(3), 215–220.

Stern, K. R., Jansky, S., & Bidlack, J. E. (2003). *Introductory plant biology*, *9th edition*. New York: McGraw-Hill Companies.

Stoeckle, M. Y., Gamble, C. C., Kirpekar, R., Young, G., Ahmed, S., & Little, D. P. (2011). Commercial teas highlight plant DNA barcode identification successes and obstacles. *Scientific Reports*, *1*(1), 42.

Sugita, M., Shinozakit, K., & Sugiuratf, M. (1985). Tobacco chloroplast tRNALYS (UUU) gene contains a 2.5-kilobase- pair intron: An open reading frame and a conserved boundary sequence in the intron (molecular cloning/DNAsequence/blot hybridization/precursor RNA/codon usage). *Proceedings of the National Academy of Sciences, 82*, 3557–3561.

Supari, N., Kaya, Y., Biroudian, M., & Javed, M. (2019). Molecular characterization of Malaysian rice cultivars using SSR markers. *American Institutes of Proceedings Conference Proceedings, 2155*(1), 020016-020021.

Suriyagoda, L. D. B., Thilakarathne, R. M. M. S., Nissanka, S. P., & Samita, S. (2011). Morphological variation in selected rice (*Oryza sativa* L.). *Journal of the National Science Foundation of Sri Lanka*, *39*(2), 129-137.

Tanaka, W., Pautler, M., Jackson, D., & Hirano, H. Y. (2013). Grass meristems II: inflorescence architecture, flower development and meristem fate. *Plant and Cell Physiology*, *54*(3), 313-324.

Taratima, W., Reanprayoon, P., & Raso, S. (2020). Physiological and anatomical changes in Thai rice landrace (*Oryza sativa* L.) CV pakaumpuel after colchicine treatment. *Pakistan Journal of Botany*, *52*(5), 1631-1638.

Tay, C. Y., & Othman, O. (1992). The importance of primary and secondary rachis with respect to total spikelet number in the rice panicle. *MARDI Research Journal*, *19*(2), 203–207.

Teo, G. K. (n.d). Rice Biodiversity. Retrieved from https://doa.sarawak.gov.my/page-0-0-158-Rice-Bioderversity.html. on 19 January 2021.

The Dayak Daily. (2022). 60 peratus SSL menjelang 2030- Dr Rundi. Dayak Daily Online. Retrieved from https://dayakdaily.com/60-peratus-ssl-padi-menjelang-2030-dr-rundi/ on 27 July 2022.

TrendEconomy. (2021). Rice Imports and Exports in 2020. Retrieved from https://trendeconomy.com/data/commodity_h2/1006 on 18 November 2021.

Tu, M., Lu, B. R., Zhu, Y., & Wang, Y. (2007). Abundant within-varietal genetic diversity in rice germplasm from Yunnan Province of China revealed by SSR Fingerprints. *Biochemical Genetics*, *45*(11), 789-801.

Ulukan, H. (2009). The evolution of cultivated plant species: classical plant breeding versus genetic engineering. *Plant System Evolution*, *280*, 133-142.

United States Department of Agriculture (USDA). (2006). *Plants Database.* Retrieved from http://plants.usda.gov/. on 11 October 2021.

Vaughan, D. A., Lu, B. R., & Tomooka, N. (2008). The evolving story of rice evolution. *Plant Science, 174,* 394-408.

Vaughan, D. A. (1994). The wild relatives of rice. A genetic resources handbook. Los Banos: IRRI.

Veasey, E. A., da Silva, E. F., Sahammass, E. A., Oliveira, G. C. X., & Ando, A. (2008). Morphoagronomic genetic diversity in American wild rice species Braz. *Archives of Biology and Technology, 51*(1), 95-104.

Wahab, A. G (2017). Malaysia: Grain and Feed Annual. *USDA Foreign Agricultural Service GAIN* Report. Retrieved from https://www.fas.usda.gov/data/malaysia-grain-and-feed-annual-0. on 4 January 2021.

Wang, H., Yang, B., & Wang, H. (2021). Impact of different numbers of microsatellite markers on population genetic results using SLAF-seq data for *Rhododendron* species. *Scientific Reports*, *11*, 8597.

Wang, Y. C. (2011). Carbon sequestration and foliar dust deposition by woody plants in the greenbelts along two major Taiwan highways. *Analysis of Applied Biology*, *159*, 244–251.

Wang, Z. Y., & Tanksley, S. D. (1989). Restriction fragment length polymorphisms in *Oryza sativa* L. *Genome, 32,* 1113-1118.

Ward, R. D., Hanner, R., & Hebert, P. D. N. (2009). The campaign to DNA barcodes all fishes, FISH-BOL. *Journal of Fish Biology*, *74*(2), 329–356.

Weerasooriya, H. A., Jayasekera, A., & Caldera, I. (2018). Tendencies towards a C4 leaf: quantitative studies on leaf anatomy of selected C3 and C4 grasses. *Journal of Rice Research and Developments*, *6*(2), 188-195.

Wei, C., Xie, P., Chen, Y., Yu, H., Su, Y., Gu, M., & Yan, C. J. (2011). Anatomical and chemical characteristics of culm of rice brittle mutant bc7(t). *Functional Plant Biology*, *38*, 227-235.

Wiangsamut, B., Lafarge, T., Mendoza, T., & Pasuquin, E. (2013). Agronomic Traits and Yield Components Associated with Broadcasted and Transplanted High-Yielding Rice Genotypes. *Journal of Crop Production*, *2*, 19-30.

Wong, S. C., Yiu, P. H., Bong, S. T. W., Lee, H. H., Neoh, P. N. P., & Rajan, A. (2009). Analysis of Sarawak Bario rice diversity using microsatellite markers. *American Journal of Agricultural and Biological Sciences*, *4*(4), 298-304.

Workman, D. (2021). Rice Exports by Country. Retrieved from https://www.worldstopexports.com/rice-exports-country/ on 18 November 2021.

Woyessa, G. (2006). *Morphological characterization and divergence analysis of Plectranthus edulis (Vatke) Agnew collection in Ethiopia*. [M.Sc. dissertation, Hawassa University].

Wu, J., Qi, Y., & Hu, G. (2017). Genetic architecture of flag leaf length and width in rice (*Oryza sativa* L.) revealed by association mapping. *Genes Genome, 39*, 341–352.

Yamaguchi, T., Nagasawa, N., Kawasaki, S., Matsuoka, M., Nagato, Y., & Hirano, H. Y. (2004). The YABBY gene drooping leaf regulates carpel specification and midrib development in *Oryza Sativa*. *Plant Cell*, *16*(2), 500-509.

Yambao, E. B., Ingram, K. T., & Real, J. G. (1992). Root xylem influence on the water relations and drought resistance of rice. *Journal of Experimental Botany*, *43*(7), 925-932.

Yancy, H. F., Zemlak, T. S., Mason, J. A., Washington, J. D., Tenge, B. J., Nguyen, N.-L. T., Barnett, J. D., Savary, W. E., Hill, W. E., Moore, M. M., Fry, F. S., Randolph, S. C., Rogers, P. L., & Hebert, P. D. N. (2008). Potential Use of DNA Barcodes in Regulatory Science: Applications of the Regulatory Fish Encyclopedia. *Journal of Food Protection*, *71*(1), 210–217.

Yang, Y., Zhu, K., Xia, H., Chen, L., & Chen, K. (2014). Comparative Proteomic Analysis of *Indica* and *Japonica* Rice Varieties. *Genetic Molecular Biology*, *37*(4), 652-661.

Ye, T., Li, Y., Zhang, J., Hou, W., Zhou, W., Lu, J., ... & Li, X. (2019). Nitrogen, phosphorus, and potassium fertilization affects the flowering time of rice (*Oryza sativa* L.). *Global Ecology and Conservation*, *20*, 753-759.

Yeo, F. K. S., Kalu, M., Shabdin, Z., Mohamad, N. K., Hussin, N. A., & Chung, H. H. (2018). Diversity of Sarawak rice in Kampung Lebor, Serian–A first insight. *Glimpses of Bornean Biodiversity*, *1*, 155-167.

Yusop, K., Aziz, A. S. A., Saili, A. R., Nor, R. C. M., & Sahmat, S. S. (2021). Constraints of Paddy Production Among Smallholding Farmers. *International Journal of Service Management and Sustainability*, *6*(1), 107-119.

Zafar, N., Aziz, S., & Masood, S. (2004). Phenotypic divergence for agro-morphological traits among landrace genotypes of rice (*Oryza sativa* L.) from Pakistan. *International Journal of Agriculture and Biology*, *6*(2), 335-339.

Zeng, Y., Zhang, H., Li, Z., Shen, S., Sun, J., Wang, M., Liao, D., Liu, X., Wang, X., Xiao F., & Wen, G. (2007). Evaluation of genetic diversity of rice landraces (*Oryza sativa* L.) in Yunnan, China. *Breeding Science, 57*(2), 91-99.

Zhang, P., Li, J., Li, X., Liu, X., Zhao, X., & Lu, Y. (2011). Population structure and genetic diversity in a rice core collection (*Oryza sativa* L.) investigated with SSR markers. *PLoS ONE, 6*(12), 27565-27571.

Zhang, Q., Peng, S., & Li, Y. (2019). Increase rate of light-induced stomatal conductance is related to stomatal size in the genus *Oryza*. *Journal of Experimental Botany*, *70*, 5259–5269.

Zhang, C. H., Li, J. Z., Zhu, Z., Zhang, Y. D., Zhao, L., & Wang, C. L. (2010). Cluster analysis on *Japonica* rice (Oryza sativa L.) with good eating quality based on SSR markers and phenotypic traits. *Rice Science*, *17*, 111-121.

Zhang, C., Wang, L., Zhang, W., & Zhang, F. (2013). Do lignification and silicification of the cell wall precede silicon deposition in the silica cell of the rice (*Oryza sativa* L.) leaf epidermis?. *Plant and Soil, 372*(1), 137-149.

Zhang, Z. H., Wang, K., Guo, L., Zhu, Y. J., Fan, Y. Y., Cheng, S. H., & Zhuang, J. Y. (2012). Pleiotropism of the photoperiod-insensitive allele of Hd1 on heading date, plant height and yield traits in rice. *PloS ONE*, *7*(12), 52538-52544.

Zhu, S., Bai, Y., Oya, M., Tanaka, K., Komatsu, K., Maruyama, T., Goda, Y., Kawasaki, T., Fujita, M., & Shibata, T. (2011). Genetic and chemical diversity of *Eleutherococcus senticosus* and molecular identification of Siberian ginseng by PCR-RFLP analysis based on chloroplast trnK intron sequence. *Food Chemistry*, *129*(4), 1844-1850.

Zhu, Y., Chang, L., Tang, L., Jiang, H., Zhang, H., & Cao, W. (2009). The modelling leaf shape dynamics in rice. *NJAS-Wageningen Journal of Life Sciences*, *57*, 73-81.

Zhu, Y., Hu, J., Han, R., Wang, Y., & Zhu, S. (2011). Fingerprinting and identification of closely related wheat (*Triticumaestivum* L.) cultivars using ISSR and fluorescence-labeled TP-M13-SSR markers. *American Journal of Crop Science* *5*(7), 846-850.

Zhu, Y. F., Qin, G.C., Yang, W., Wang, J. C., & Zhu, S. J. (2012). Fingerprinting and variety identification of rice (*Oryza sativa* L.) based on Simple Sequence Repeat Markers. *Plant Omics Journal*, *5*(4), 421-426.

Zodinpuii, D., Ghatak, S., Mukherjee, S., & Kumar, N. S. (2013). Genetic relatedness of genus *Oryza* from Eastern Himalayan region as revealed by chloroplast matK gene. *Asian Journal of Conservation Biology*, *2*(2), 144-151.

Zuloaga, F. O., Morrone, O., Vega, A. S., & Giussani, L. M. (1998). Revision and cladistic analysis of Steinchisma (Poaceae: Panicoideae, Paniceae). *Annals of the Missouri Botanical Garden*, *85*(4), 631-665.

APPENDICES

Appendix 1: PHOTOGRAPHS OF THE STUDIED RICE ACCESSIONS

Figure 1: A Part of Different Blade Colours Observed at the 22 Upland rice accessions in first generation. A-L: UNIMAS-01-UNIMAS-12.

Appendix 2: PHOTOGRAPHS OF THE STUDIED RICE ACCESSIONS

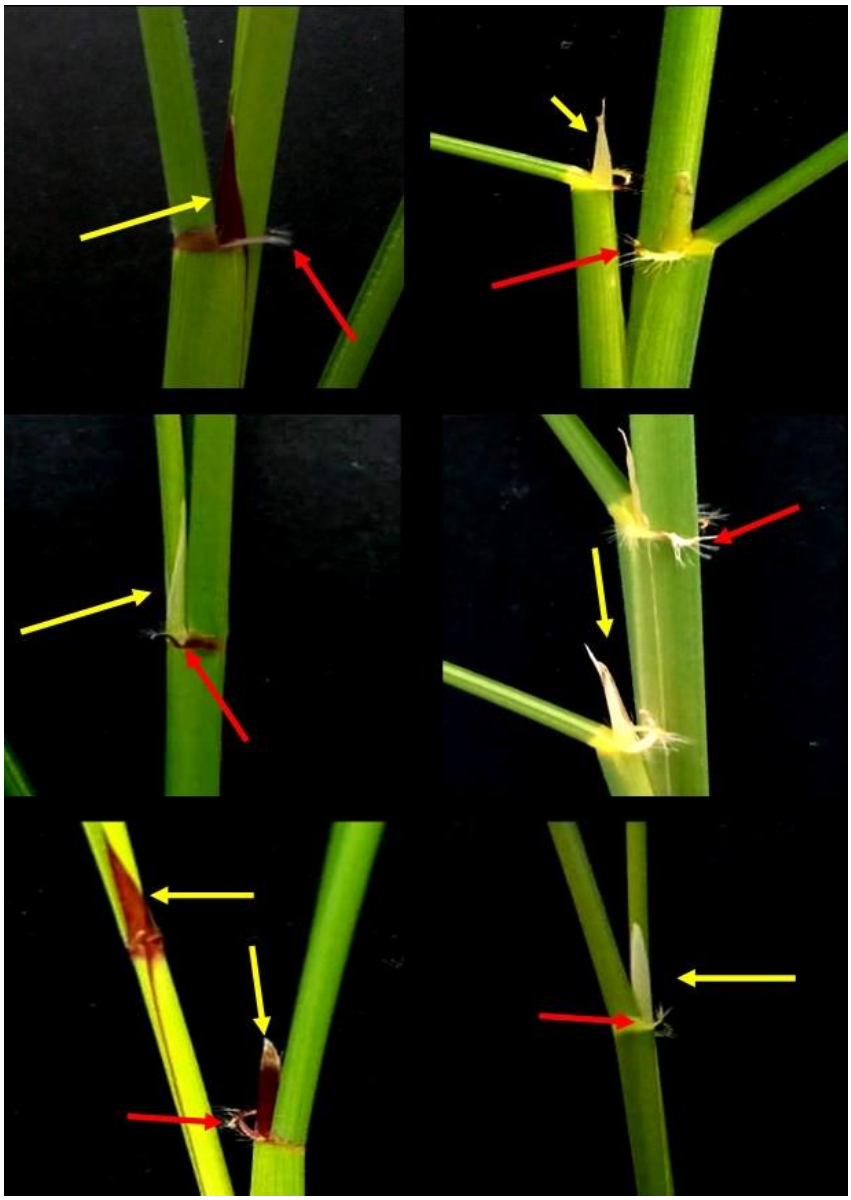

Figure 1: A Part of Ligule and Auricle Observed in this Study. Red Arrow: Auricle, Yellow Arrow: Ligule.

Appendix 3: PHOTOGRAPHS OF THE STUDIED RICE ACCESSION

Figure 1: The Panicle of First Generation. A-D represents accession UNIMAS-01 to UNIMAS-04. White Scale Bar Represents 1 cm.

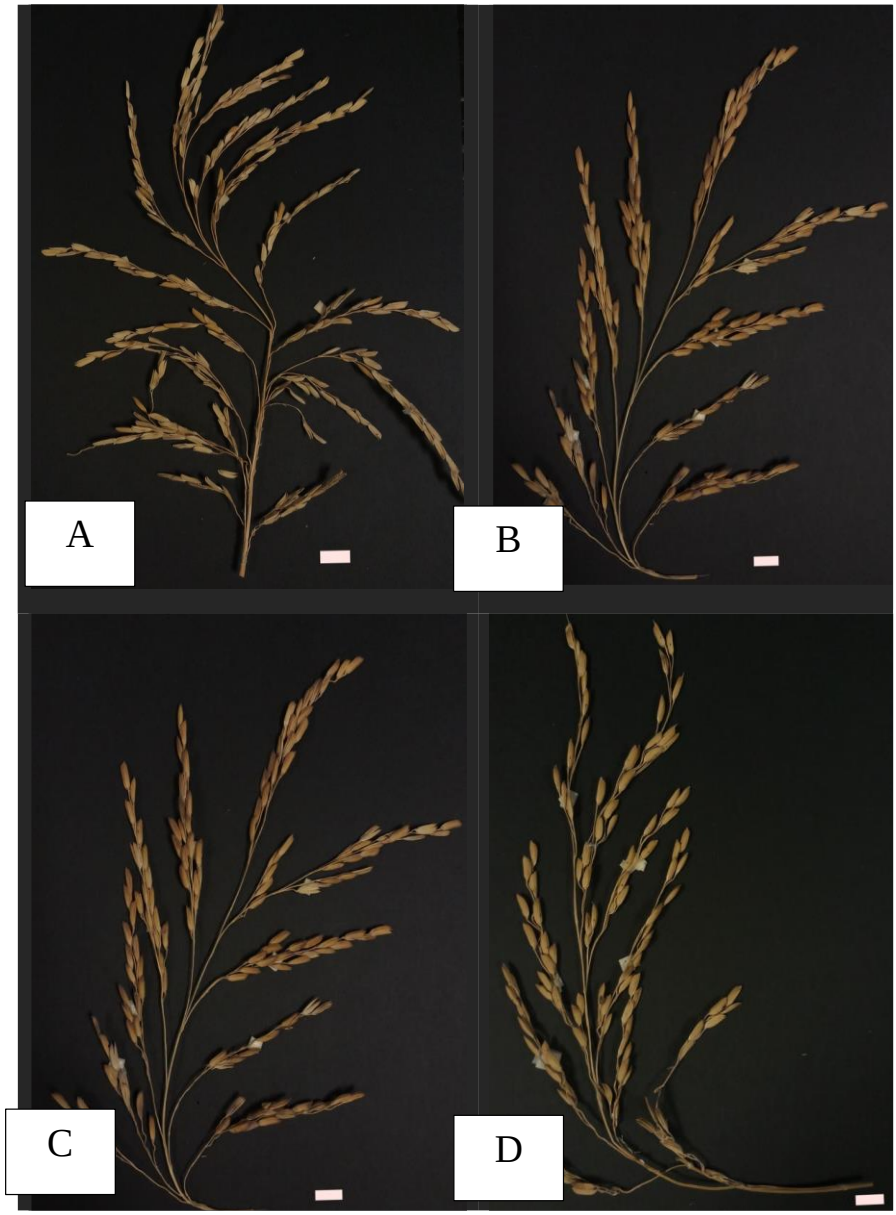

Figure 2: The Panicle of First Generation. A-D represents accession UNIMAS-05 to UNIMAS-08. White Scale Bar Represents 1 cm.

Figure 3: The Panicle of First Generation. A-D represents accession UNIMAS-09 to UNIMAS-12. White Scale Bar Represents 1 cm.

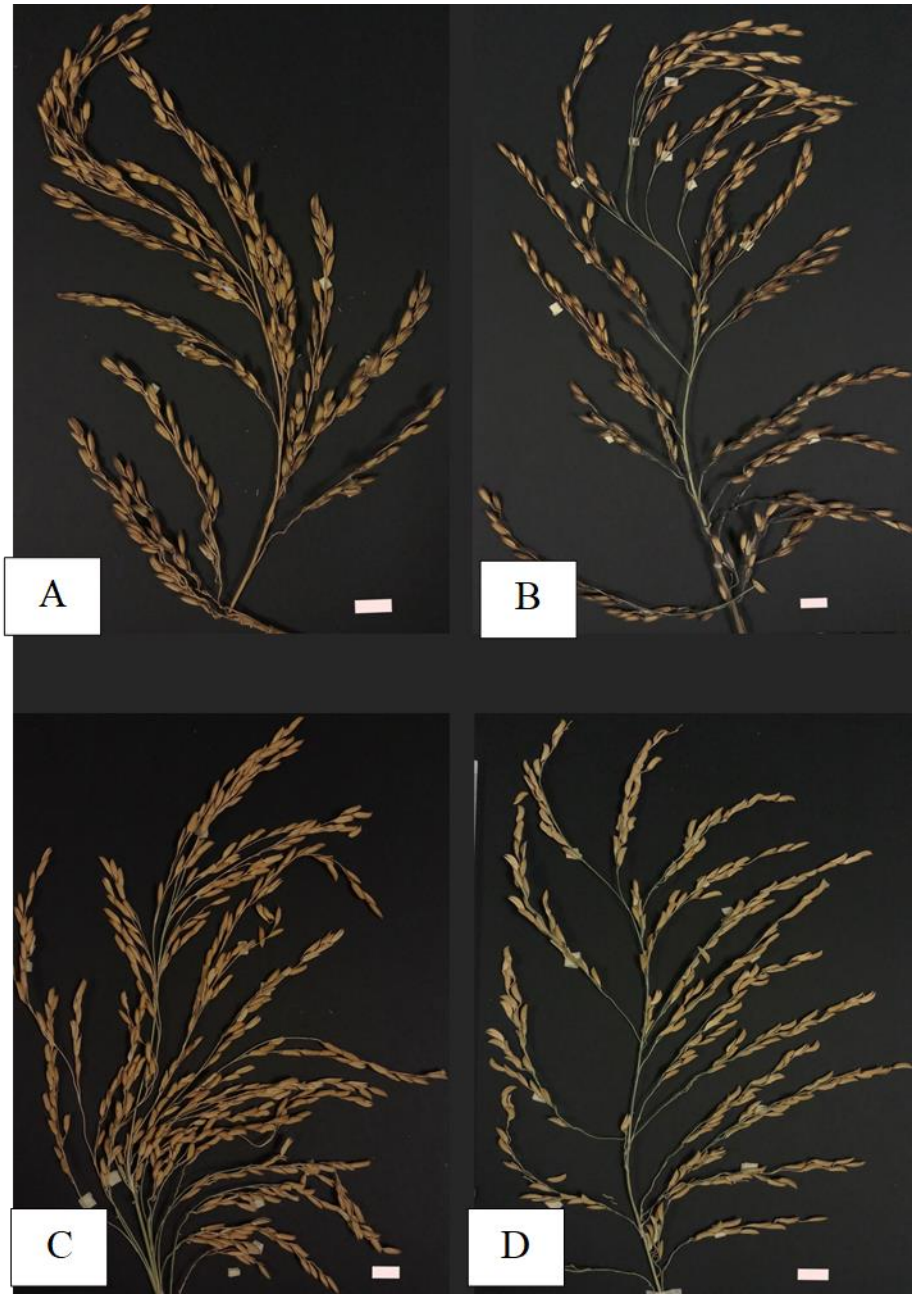

Figure 4: The Panicle of First Generation. A-D represents accession UNIMAS-13 to UNIMAS-16. White Scale Bar Represents 1 cm.

Figure 5: The Panicle of First Generation. **A-E** represents accession UNIMAS-17 to UNIMAS-21. White Scale Bar represents 1 cm.

Appendix 4: PHOTOGRAPHS OF THE STUDIED RICE ACCESSIONS

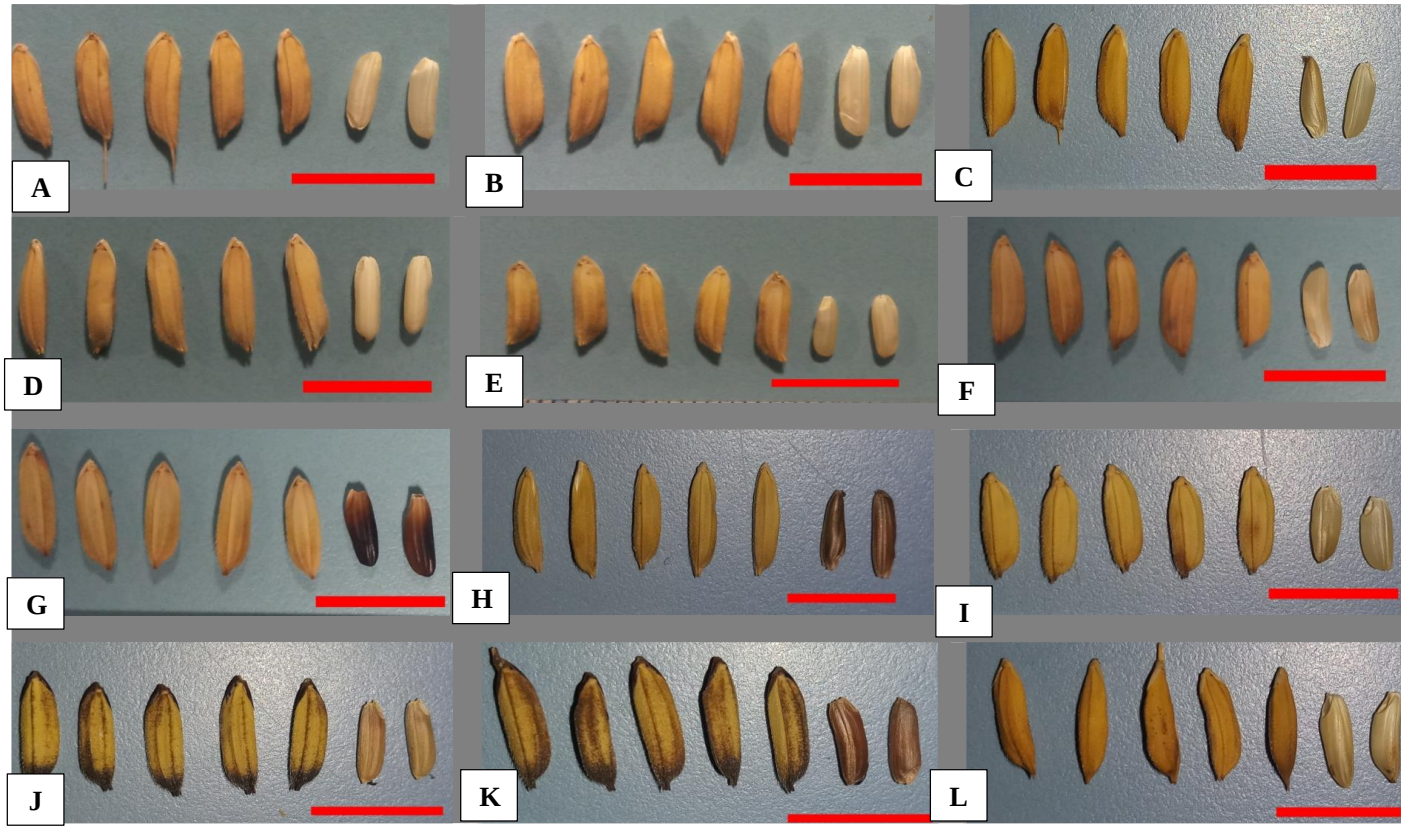

Figure 1: Seeds of Upland Rice Accessions studied. Alphabet A-L represents UNIMAS-01 to UNIMAS-12. Red Scale Bar represents 1 cm.

Figure 2: Seeds of Upland Rice Accessions studied. Alphabet **A-I** represents UNIMAS-13 to UNIMAS-21. Red Scale Bar represents 1 cm.

Appendix 5: PHOTOGRAPHS OF THE STUDIED RICE ACCESSIONS

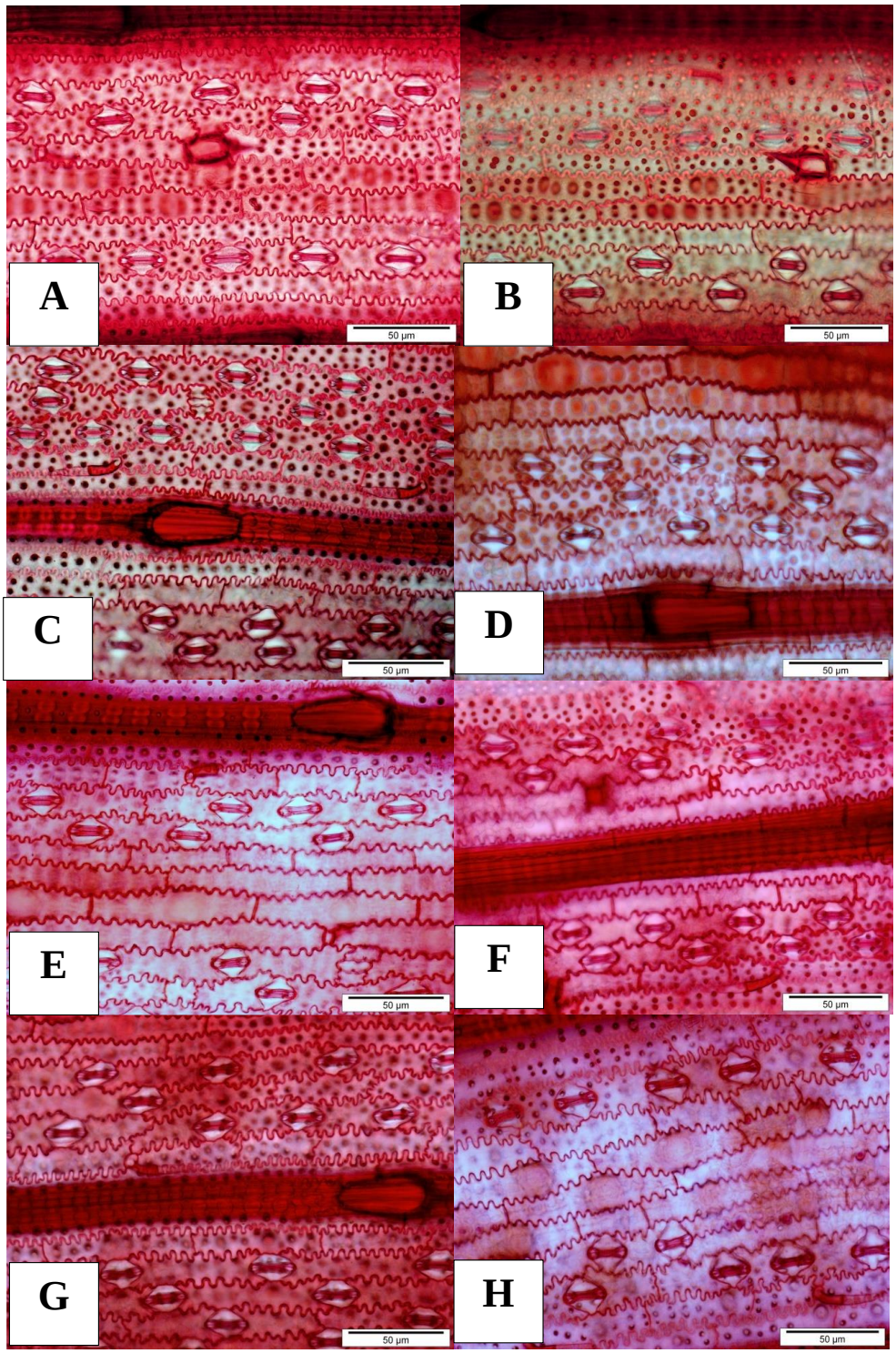

Figure 1: General Structure of Epidermal Cell. A, C, E, G represents UNIMAS-01-S1, UNIMAS-02-S1, UNIMAS-03-S1, UNIMAS-04-S1 on abaxial surface. B, D, F, H represents UNIMAS-01-S1, UNIMAS-02-S1, UNIMAS-03-S1, UNIMAS-04-S1 on adaxial surface. Magnification: 40x. Scale Bar: 50 µm

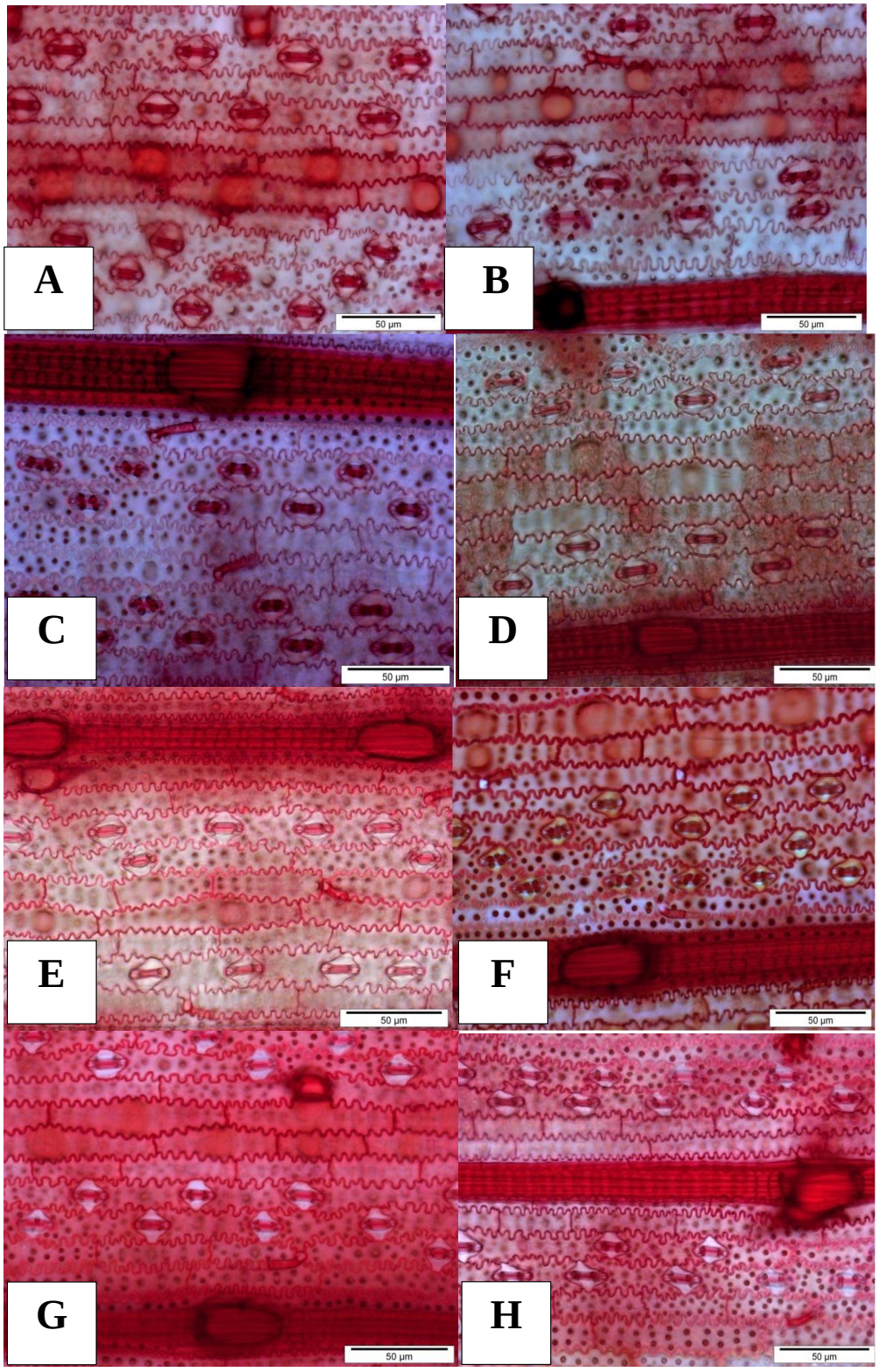

Figure 2: General Structure of Epidermal Cell. A, C, E, G represents UNIMAS-07-S1, UNIMAS-08-S1, UNIMAS-09-S1, UNIMAS-12-S1 on abaxial surface. B, D, F, H represents UNIMAS-07-S1, UNIMAS-08-S1, UNIMAS-09-S1, UNIMAS-12-S1 on adaxial surface. Magnification: 40x. Scale Bar: 50 µm

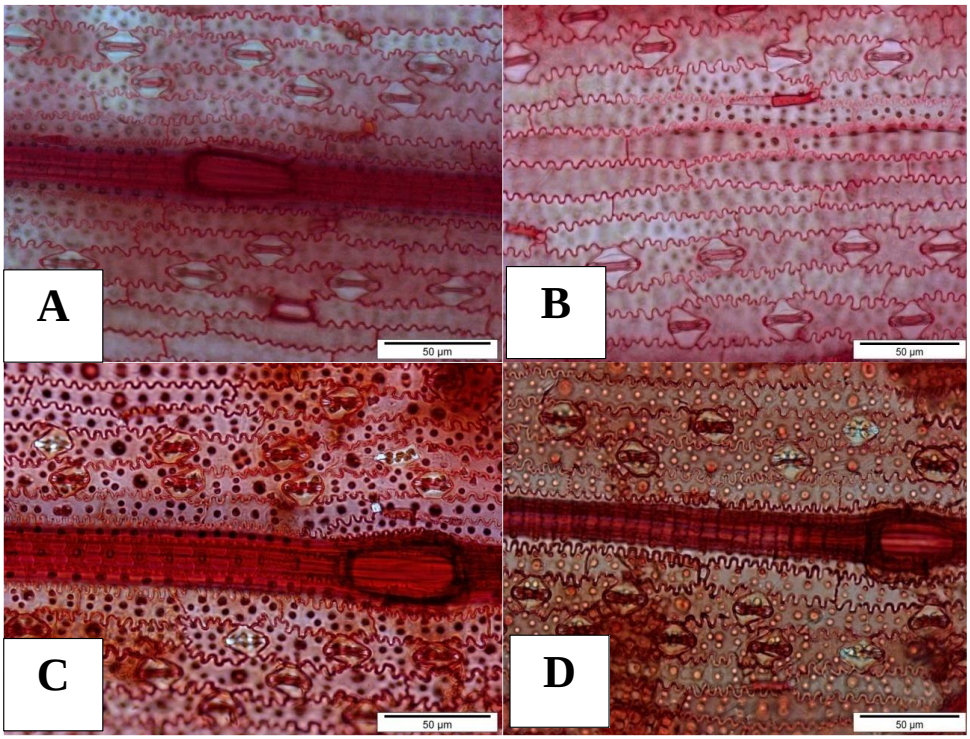

Figure 3: General Structure of Epidermal Cell. A and C represent UNIMAS-17-S1 and UNIMAS-18-S1 on abaxial surface. B and D represent UNIMAS-17-S1 and UNIMAS-18-S1 on adaxial surface.Magnification: 40x. Scale Bar: 50 µm

Appendix 6: PHOTOGRAPHS OF THE STUDIED RICE ACCESSIONS

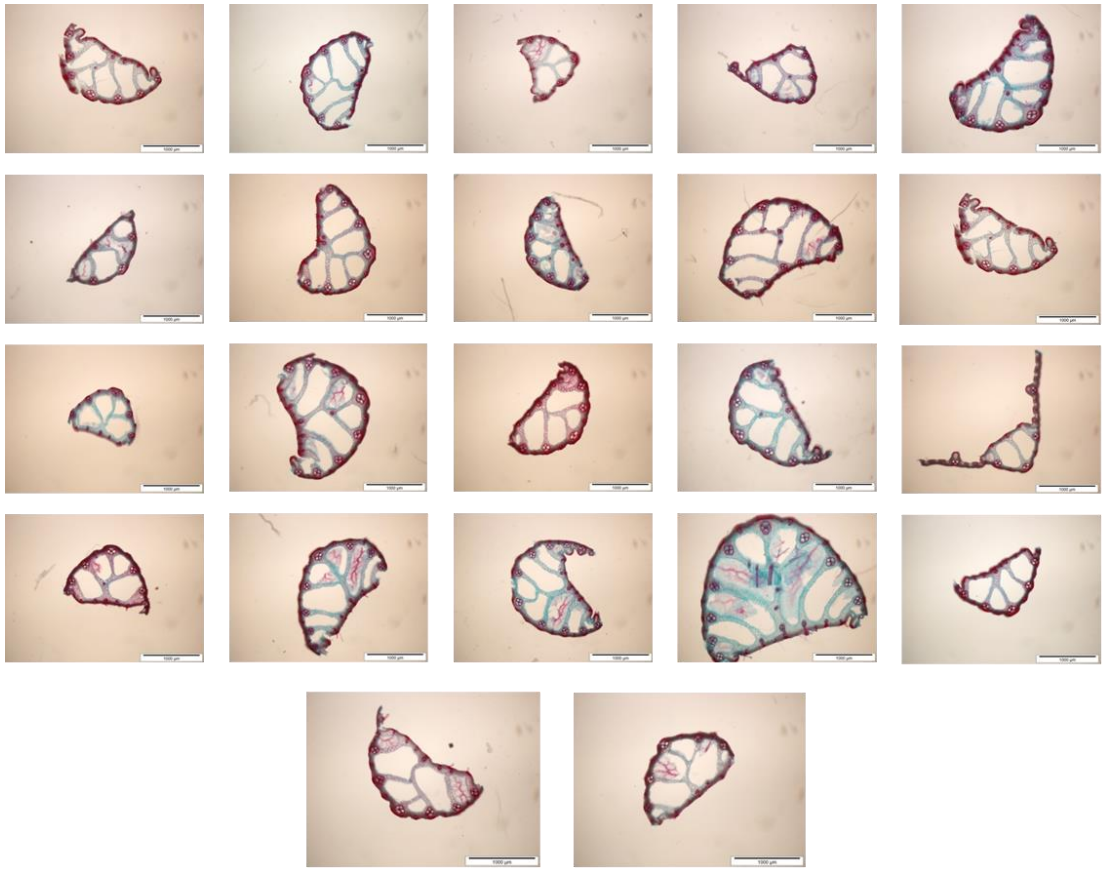

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Figure 1: Transverse Section Showing General Shape of Midrib in 22 Upland Accessions in First Generation. Magnification: 10x. Scale Bar: 1000 µm

Appendix 7: PHOTOGRAPHS OF THE STUDIED RICE ACCESSIONS

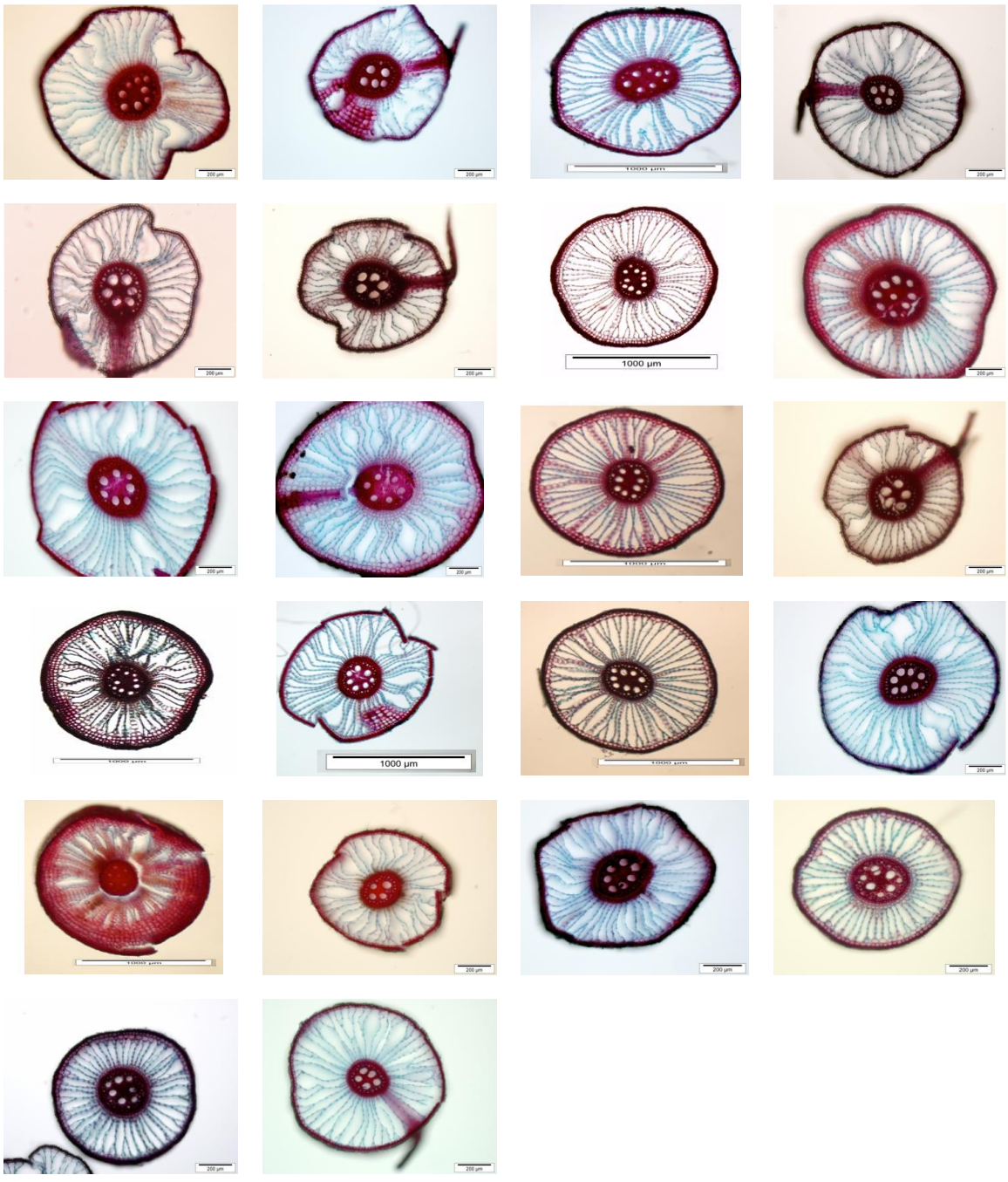

Figure 1: Transverse Section of Root showing the General Structure of 22 Upland Accessions in First Generation. Magnification: 10x and 20x. Scale Bar: 1000 µm and 200 µm.

Appendix 8: Table of Genetic Distance between 22 Upland Rice Accessions Based on Eight Polymorphic Simple Sequence Repeat Markers

01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	
0																						**01**
5	0																					**02**
4	1	0																				**03**
11	10	11	0																			**04**
9	12	13	4	0																		**05**
9	14	13	4	4	0																	**06**
12	11	12	1	3	5	0																**07**
6	3	2	9	11	11	10	0															**08**
4	5	4	9	11	11	10	6	0														**09**
6	3	2	9	11	11	10	0	6	0													**10**
6	3	2	9	11	11	10	0	6	0	0												**11**
6	3	2	11	13	13	12	4	2	4	4	0											**12**
6	3	2	9	11	11	10	0	6	0	0	4	0										**13**
6	3	2	9	11	11	10	0	6	0	0	4	0	0									**14**
6	11	10	11	9	9	12	8	6	8	8	8	8	8	0								**15**
7	6	7	4	6	8	5	5	5	5	5	7	5	5	7	0							**16**
6	3	2	13	15	15	14	4	6	4	4	4	4	4	8	9	0						**17**
7	4	3	12	14	14	13	5	5	5	5	3	5	5	7	8	1	0					**18**
8	5	4	11	13	13	12	2	8	2	2	6	2	2	6	7	2	3	0				**19**
2	7	6	11	9	9	12	8	2	8	8	4	8	8	4	7	8	7	10	0			**20**
4	9	8	13	11	11	14	10	4	10	10	6	10	10	2	9	6	5	8	2	0		**21**
2	7	6	13	11	11	14	8	6	8	8	8	8	8	4	9	4	5	6	4	2	0	**22**

*Number 01-22 indicates UNIMAS-01 to UNIMAS-22

Appendix 9: THE BANDING PATTERN OF THE EIGHT POLYMORPHIC SSR MARKER

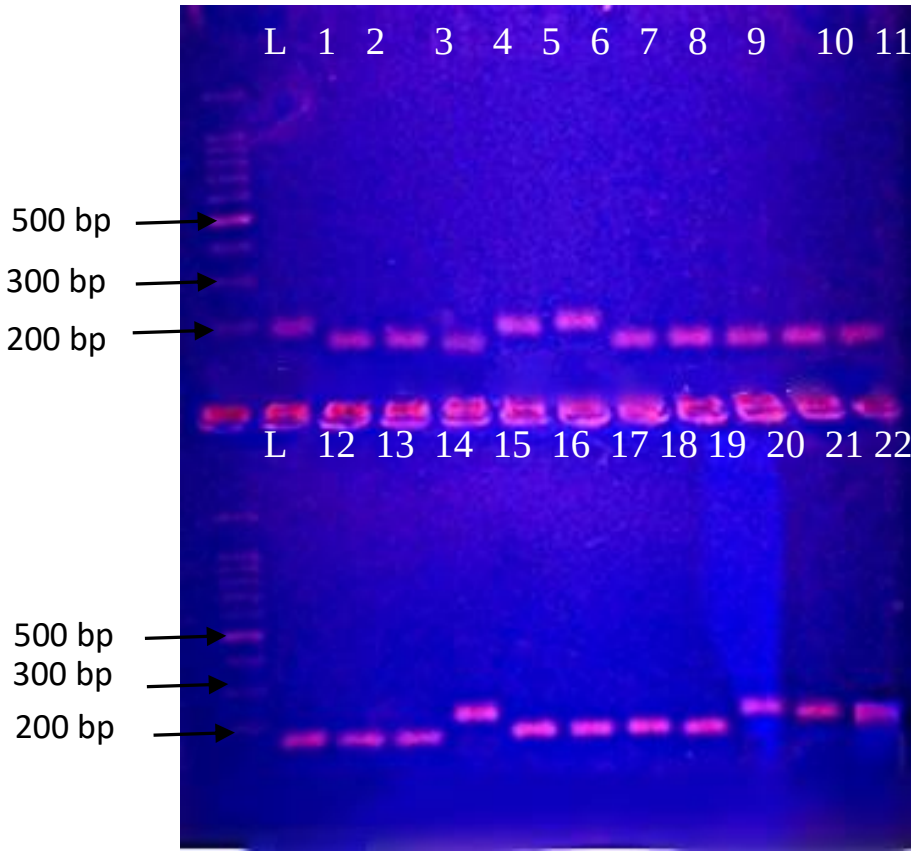

Figure 1: SSR Banding Pattern of the 22 Upland Rice Accessions Amplified Using Primer RM481. Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22.

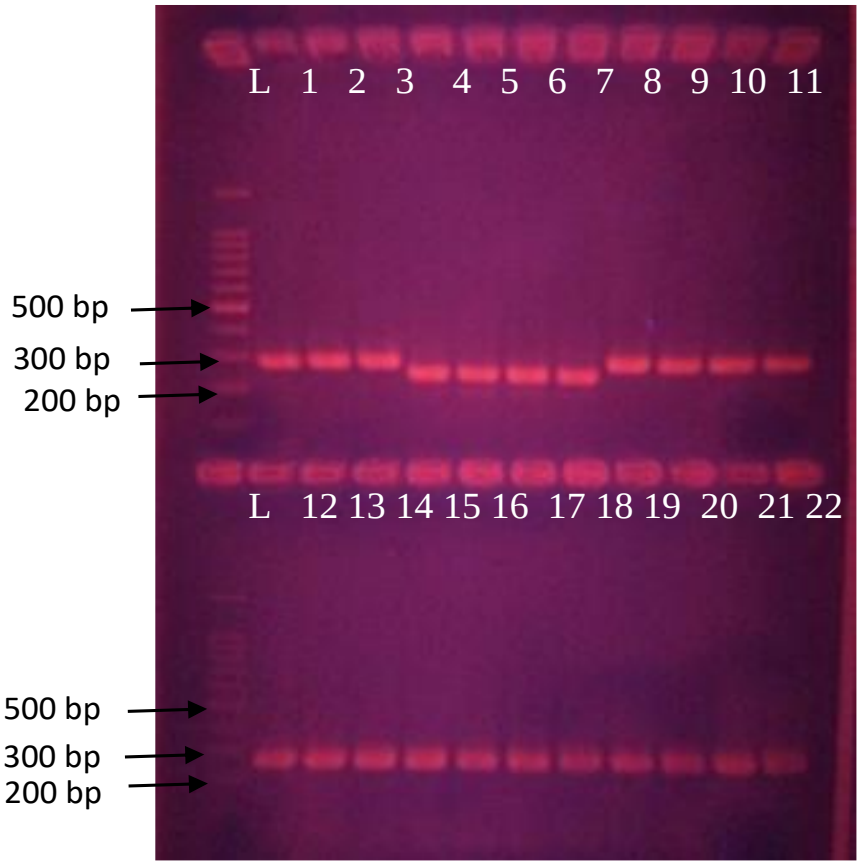

Figure 2: SSR Banding Pattern of the 22 Upland Rice Accessions Amplified Using Primer RM489. Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22.

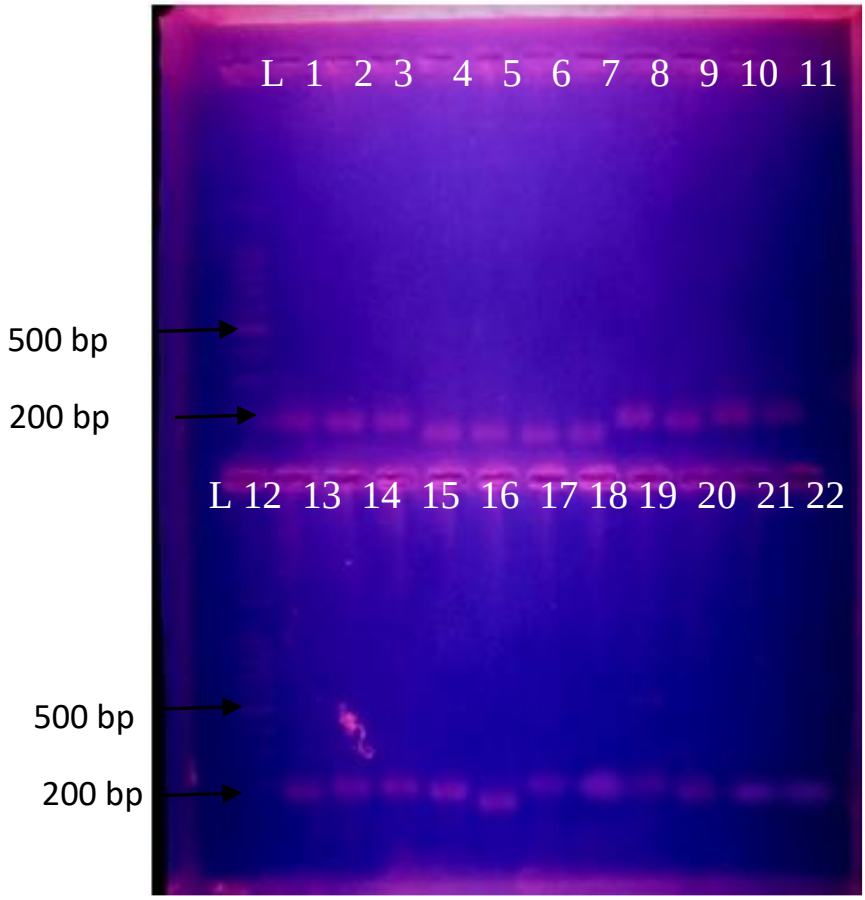

Figure 3: SSR Banding Pattern of the 22 Upland Rice Accessions Amplified Using Primer RM257. Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22.

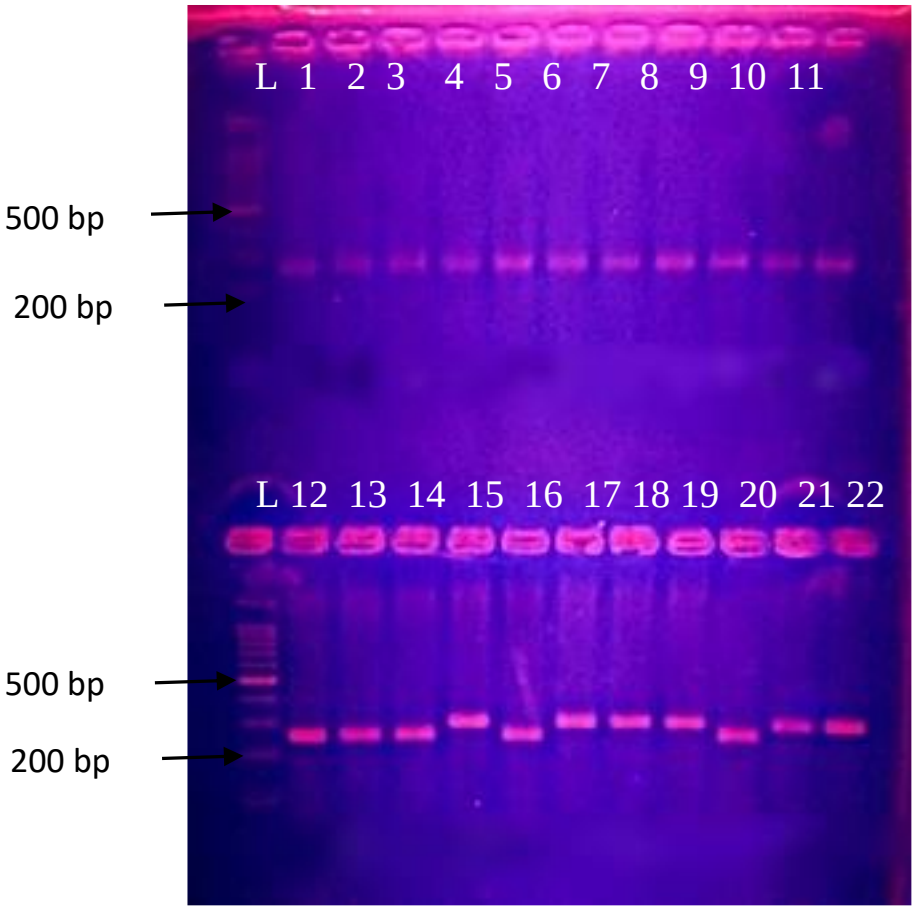

Figure 4: SSR Banding Pattern of the 22 Upland Rice Accessions Amplified Using Primer RM164. Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22.

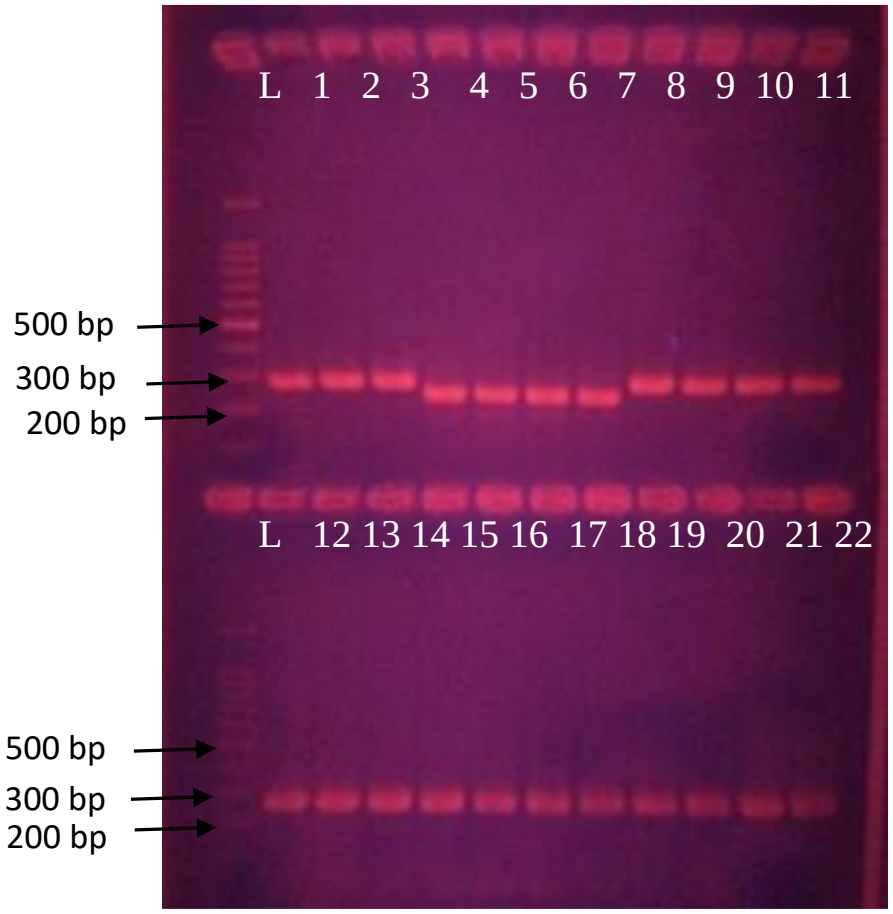

Figure 5: SSR Banding Pattern of the 22 Upland Rice Accessions Amplified Using Primer RM481.Note, =M represents Marker Ladder 100 bp, Numbers 1-22 represents accessions UNIMAS-01 to UNIMAS-22.