



Faculty of Resource Science and Technology

**Characterisation of Lowland Rice Landraces From the North-Western
Region of Sarawak, Malaysia**

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Characterisation of Lowland Rice Landraces From the North-Western Region of Sarawak, Malaysia

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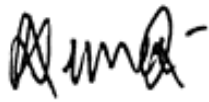
Faculty of Resource Science and Technology

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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 rice is Malaysia's primary staple food and food crop. In Malaysia, rice is cultivated either as lowland type or upland type. Lowland rice is mostly cultivated in Malaysia due to high yield potential. Sarawak is known for its richness in rice diversity making the rice valuable for breeding material. However breeding using Sarawak rice are challenging due to little documentation has been made on varietal characterisation. The objectives of the current work were to find distinct morphological traits, anatomical traits and Simple Sequence Repeat (SSR) markers to delineate lowland rice landraces collected across four divisions in North-Western of Sarawak. Utilisation of *Maturase-K* (*matK*) was to assist in varietal discrimination. A total of 22 rice accessions was chosen. A set of 25 qualitative (QL) and quantitative (QT) morphological traits together with anatomical observation were used for characterisation. Further observation was conducted on S₁ generation derived from 10 accessions, by observing the 25 morphological traits and anatomy observation on leaf samples with triplicates. Accessions in first generation showed genetic variations in all QL traits except for ligule and auricle colour. The S₁ generation exhibited variations in QL traits, except for blade colour. The QT traits were helpful in characterising the accessions in both generations due to great variations they provide. Present study showed QL yield-related traits such as compact panicle type with presence of secondary branching have positive impact on number of filled grains. Accessions in present study recorded the same fundamental cell structure in leaf, midrib, root and stele. No variation observed in anatomical cell arrangement. Stomata density in S₁ generation was found lower compared to first generation present study. Out of the 39 SSR markers, seven were polymorphic and cluster analysis was performed using Unweighted Pair Group Method with Arithmetic Mean. The number of alleles recorded was 18 with an average of 2.57 and

mean Polymorphic Information Content recorded 0.67, suggesting the markers used were highly informative. Mean number of effective alleles was 1.61. The recorded Nei's gene diversity (0.36) and Shannon's Information Index (0.53) indicate low diversity in present study. The cluster analysis using seven SSR markers did not show clear clustering based on morphological traits nor origins. Phylogenetic analysis based on *matK* grouped all accessions into one cluster together with other accessions of *O. sativa* and *O. rufipogon* accessions. The phylogenetic tree based on *matK* showed clustering based on the genome type. Information collected from morphological and anatomical characterisation along with molecular analysis will provide basic information on Sarawak lowland rice. The information obtained are important for conservation of Sarawak rice germplasm and can be used in criteria selection for breeding attempt using Sarawak rice to ensure food security of the country.

Keywords: Lowland rice, Sarawak, characterisation, morphological, anatomical, SSR, *matK*

Pencirian Padi Sawah Tradisional Dari Wilayah Barat-Laut Sarawak, Malaysia

ABSTRAK

Oryza sativa L. atau padi merupakan makanan ruji dan tanaman makanan di Malaysia. Di Malaysia, padi diusahakan sebagai padi sawah atau padi bukit. Kebanyakan padi sawah yang ditanam di Malaysia memiliki potensi menghasilkan pengeluaran yang tinggi. Sarawak terkenal dengan kekayaan dalam kepelbagaian padi menjadikan padi berharga untuk bahan pembiakan. Walau bagaimanapun, pembiakan menggunakan padi Sarawak adalah mencabar kerana kurang dokumentasi telah dibuat mengenai pencirian varieti. Objektif kajian semasa adalah untuk mencari ciri-ciri morfologi dan anatomi yang berbeza, selain penanda Simple Sequence Repeat (SSR) untuk menggambarkan padi sawah yang dikumpul dari empat bahagian di Utara-Barat Sarawak. Penggunaan Maturase-K (matK) adalah untuk membantu dalam membezakan varieti. Sebanyak 22 aksesori beras telah dipilih. Satu set 25 ciri morfologi kualitatif (QL) dan kuantitatif (QT) bersama dengan pemerhatian anatomi digunakan untuk pencirian. Pemerhatian lanjut dijalankan ke atas generasi S_1 yang diperoleh daripada 10 aksesori, dengan merekod 25 ciri morfologi dan ciri anatomi pada sampel daun dengan tiga kali ganda. Aksesori dalam generasi pertama menunjukkan variasi genetik dalam semua sifat QL kecuali untuk warna ligul dan aurikel. Generasi S_1 mempamerkan variasi dalam ciri QL, kecuali untuk warna bilah. Ciri-ciri QT membantu dalam mencirikan aksesori dalam kedua-dua generasi kerana variasi yang ditunjukkan. Kajian sekarang menunjukkan ciri-ciri berkaitan hasil QL seperti jenis malai padat dengan kehadiran cawangan sekunder mempunyai kesan positif ke atas bilangan bijirin yang terisi. Aksesori dalam kajian ini merekodkan struktur sel asas yang sama dalam daun, pelepah, akar dan stele. Tiada variasi diperhatikan dalam susunan sel anatomi. Ketumpatan stomata didapati kurang daripada generasi pertama dalam kajian ini. Daripada 39 penanda SSR,

tujuh adalah polimorfik dan analisis kelompok dilakukan menggunakan Kaedah Kumpulan Pasangan Tidak Berwajaran dengan Min Aritmetik. Bilangan alel yang direkodkan ialah 18 dengan purata 2.57 dan min Kandungan Maklumat Polimorfik mencatatkan 0.67, menunjukkan penanda yang digunakan adalah sangat bermaklumat. Purata bilangan alel berkesan ialah 1.61. Kepelbagaian gen Nei yang direkodkan (0.36) dan Indeks Maklumat Shannon (0.53) menunjukkan kepelbagaian yang rendah dalam kajian ini. Analisis kelompok menggunakan tujuh penanda SSR tidak menunjukkan pengelompokan yang jelas berdasarkan ciri morfologi mahupun asal usul. Analisis filogenetik berdasarkan matK mengumpulkan semua aksesori ke dalam satu kelompok bersama dengan aksesori O. sativa dan O. rufipogon yang lain. Pokok filogenetik berdasarkan matK menunjukkan pengelompokan berdasarkan jenis genom. Maklumat yang dikumpul daripada pencirian morfologi dan anatomi bersama dengan analisis molekul akan memberikan maklumat asas tentang padi Sarawak. Maklumat yang diperolehi adalah penting untuk pemuliharaan padi Sarawak disamping digunakan untuk pemilihan kriteria percubaan pembiakan menggunakan padi Sarawak dalam memperkukuh sekuriti makanan dalam negara.

Kata kunci: *Padi sawah, Sarawak, pencirian, morfologi, anatomi, SSR, matK*

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
ABSTRAK	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvi
CHAPTER 1 INTRODUCTION	1
1.1 Study Background	1
1.2 Problem Statement	3
1.3 Objectives	5
CHAPTER 2 LITERATURE REVIEWS	7
2.1 <i>Oryza sativa</i> Linnaeus	7
2.1.1 Lowland Rice Cultivation	8
2.2 Rice as Global Staple Food	8
2.3 Sarawak as Major Rice Producer in Malaysia	10

2.4	Breeding of High Yielding Rice Varieties	11
2.4.1	Diversity in Sarawak Rice Varieties	12
2.5	Approaches Towards Variety Characterisation	14
2.6	Morphological Characteristics of <i>Oryza sativa</i>	15
2.7	Anatomical Study of <i>Oryza sativa</i>	19
2.8	Molecular Characterisation	21
2.8.1	Simple Sequence Repeat	22
2.8.2	Maturase-K	24
CHAPTER 3 MATERIALS AND METHODS		26
3.1	Samples Collection	26
3.1.1	Planting Rice Landraces	29
3.1.2	Deoxyribonucleic Acid Extraction	30
3.1.3	Simple Sequence Repeat Marker	30
3.1.4	Pre-selection of Rice Accessions	33
3.2	Morphological Characterization	34
3.3	Anatomical Characterization	36
3.3.1	Leaf Sample Preparation	37
3.3.2	Stomatal Complex Observation	37
3.3.3	Midrib Sample Preparation	38
3.3.4	Root Sample Preparation	38

3.3.5	Observation on S1 Generation	38
3.4	Simple Sequence Repeat Genotyping	39
3.4.1	Polymorphic Information Content Values and Genetic Diversity	39
3.4.2	Cluster Analysis Based on SSR	40
3.5	<i>Maturase-K</i> as Barcoding Marker	40
3.5.1	Cluster Analysis Based on matK	41
CHAPTER 4 RESULTS		45
4.1	Pre-selection of Accession	45
4.2	Characterisation Based on Morphological Traits	47
4.2.1	Qualitative Traits	47
4.2.2	Quantitative Traits	55
4.2.3	Morphological Traits in Distinguishing Accessions	63
4.3	Characterisation Based on Anatomical Traits	64
4.3.1	Epidermal Cell	64
4.3.2	Diversity of Stomatal Complex	68
4.3.3	Midrib	70
4.3.4	Root and Stele	73
4.3.5	Anatomical Traits in Distinguishing Accessions	73
4.4	Simple Sequence Repeat Genotyping	73
4.4.1	Polymorphism Information Content Values and Genetic Diversity	76

4.4.2	Cluster Analysis Based on SSR Markers	77
4.5	Maturase-K Amplification	80
4.5.1	Phylogenetic Analysis of Maturase-K	81
CHAPTER 5 DISCUSSION		83
5.1	Morphological Characterisation	83
5.1.1	Qualitative Traits Study	83
5.1.2	Quantitative Traits Study	85
5.1.3	Morphological Traits in Distinguishing Accessions	90
5.2	Anatomical Study of Accessions	90
5.2.1	Epidermal cell	91
5.2.2	Diversity in stomatal complex	91
5.2.3	Midrib Architecture	92
5.2.4	Root Architecture	92
5.3	Simple Sequence Repeat Analysis	94
5.3.1	Assessment of Simple Sequence Repeat allelic diversity	94
5.3.2	The Simple Sequence Repeat Cluster Analysis	96
5.4	Genetic relatedness based on chloroplast <i>Maturase-K</i>	98
CHAPTER 6 CONCLUSION AND RECOMMENDATION		100
6.1	Conclusion	100
6.2	Recommendation	101

REFERENCES	102
APPENDICES	129

LIST OF TABLES

	Page
Table 3.1 List Landraces with Its Locality and Global Positioning System.	27
Table 3.2 The Germination Date of 11 Lowland Landraces.	29
Table 3.3 The 39 Sets of SSR Markers with Sequences and Details.	31
Table 3.4 The 25 Morphological Traits Selected Based on IRRI Guidelines Including 10 Additional Traits.	34
Table 3.5 The Accession Number for <i>matK</i> Sequences Downloaded From NCBI.	41
Table 4.1 The SSR genotype of 22 Selected Lowland Rice Accessions Based on Four SSR Markers.	45
Table 4.2 List of 11 Collected Landraces, the 22 Chosen Accessions and 10 Selected S ₁ Accessions.	46
Table 4.3 The Nine Qualitative Traits of 22 Lowland Rice Accession in First Generation.	48
Table 4.4 The Nine Qualitative Traits of 10 Lowland Rice Accession in S ₁ Generation.	49
Table 4.5 The 16 Quantitative Traits of 22 Lowland Rice Accession in First Generation.	56
Table 4.6 The Nine Quantitative Traits of 10 Lowland Rice Accession in S ₁ Generation Based on Analysis of Variance.	58

Table 4.7	The Seven Quantitative Traits in S ₁ Generation Based on Kruskal-Wallis Test.	61
Table 4.8	Epidermal Traits of 22 Accessions in First Generation on Both Abaxial and Adaxial Surfaces.	66
Table 4.9	Epidermal Traits of 10 Accessions in S ₁ Generation on Both Abaxial and Adaxial Surfaces.	67
Table 4.10	The Recorded Measurement of Midrib, Root and Stele in 22 Lowland Accessions.	72
Table 4.11	The Seven SSR Markers with High Yield Amplification.	74
Table 4.12	The Details of Seven SSR Markers with Polymorphic Information Content Values.	76
Table 4.13	Allelic Diversity Revealed by Seven Polymorphic SSR Markers.	77
Table 4.14	Cluster Group of 22 Lowland Rice Accessions Based on Seven SSR Markers.	79
Table 4.15	Cluster Group Based on Chloroplast <i>Maturase-K</i> .	82
Table 5.1	The Genotype of Three Accessions selected from the Same Landrace Based on Seven SSR Markers.	97

LIST OF FIGURES

	Page
Figure 2.1	General Morphology of <i>Oryza sativa</i> . 16
Figure 2.2	The Rice Plant Growth Phases. 17
Figure 2.3	The General Root Structure of <i>Oryza sativa</i> . 21
Figure 3.1	The Flowchart of The Experiment. 26
Figure 3.2	The Distribution of the 11 Collected Lowland Rice Landraces. 28
Figure 4.1	The Three Groups of Blade Colour. 50
Figure 4.2	Representative of Ligule and Auricle. 51
Figure 4.3	The Three Recorded Panicle Types. 52
Figure 4.4	The Three Types of Recorded Secondary Branching. 53
Figure 4.5	Seed Morphology Recorded in Both First and S ₁ Generation. 54
Figure 4.6	Grain Colours Observed in Both First Generation and S ₁ Generation. 55
Figure 4.7	The Abaxial Surface of Leaf Anatomy of <i>O. sativa</i> . 65
Figure 4.8	The General Structure of <i>Oryza sativa</i> Stomatal Complex. 69
Figure 4.9	The Representative General Structure of Midrib, Root and Stele of <i>Oryza sativa</i> Observed in 22 Lowland Rice Accessions in First Generation. 71

Figure 4.10	A Representative Figure for The Banding Pattern of The Seven Polymorphic SSR markers.	75
Figure 4.11	The Cluster Analysis of 22 Lowland Rice Accessions Collected from North-Western Region of Sarawak Based on Seven SSR Markers.	78
Figure 4.12	The Amplification of <i>matk</i> on 22 Lowland Rice Accessions.	80
Figure 4.13	Phylogenetic Tree of <i>Maturase-K</i> on 22 Lowland Rice Accessions Using Maximum Likelihood Analysis.	81

LIST OF ABBREVIATIONS

AC	Auricle Colour
ANOVA	Analysis of Variance
BC	Blade Colour
bp	Base pair
CD	Culm Diameter
Ch	Chromosome
CL	Culm Length
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleoside triphosphate
DOA	Department of Agriculture
EPS	Expected Product Size
EtBr	Ethidium bromide
FD	Flowering Days
GC	Grain Colour
GL	Grain Length
GPS	Global Positioning System

GuC	Guard cell
GW	Grain Width
<i>h</i>	Nei's gene diversity
HCl	Hydrochloric acid
HD	Heading Days
<i>I</i>	Shannon's diversity index
IRRI	International Rice Research Institute
LC	Ligule Colour
LEPC	Length of long cell
LL	Leaf Length
LP	Length of Panicles
LS	Ligule Shape
LSC	Length of stomatal complex
LW	Leaf Width
MARDI	Malaysian Agricultural Research and Development Institute
<i>matK</i>	<i>Maturase-K</i>
MEGA	Molecular Evolutionary Genetic Analysis
<i>Ne</i>	Number of effective alleles
NFG	Number of Filled Grains

NP	Number of Panicle
NT	Number of Tillers
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
PH	Plant Height
PT	Panicle Type
SB	Secondary Branching
SC	Seed Colour
SCS	Subsidiary cell shape
SD	Stomata Density
SH	Seedling Height
SL	Seed Length
SPSS	Statistical Package for the Social Sciences
SS	Seed Shape
SSR	Simple Sequence Repeat
SuC	Subsidiary cell
SW	Seed Width
Ta	Annealing Temperature
TAE	Tris Acetate EDTA

TRIS	Trisaminomethane
UNIMAS	Universiti Malaysia Sarawak
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
unit/mm ²	Unit per millimetres square
WEPC	Width of long cell
WSC	Width of stomatal complex
%	Percentage
°C	Degree celcius

CHAPTER 1

INTRODUCTION

1.1 Study Background

Oryza sativa Linnaeus, or rice plant is one of the three leading grain crops in the world besides wheat and maize, and is critical to daily survival of human population with Asia having the largest number of consumers and producer (Awika, 2011; Rajamoothy et al., 2015). China led other countries in year 2019 in global rice production with an average production of 211.41 million tonnes, followed by India (177.65 million tonnes) and Indonesia (54.60 million tonnes) (Food and Agriculture Organization of the United Nations [FAO], 2021a). Our country Malaysia, however, managed to come out with 2.5 million tonnes in average in year 2016 to 2020 (FAO, 2021b), which is still considered to be insufficient to meet domestic requirements with its growing number of its population every year. From year 1990 to 2020, the nation's production of its staple foods climbed from 1.9 million tonnes to 2.6 million tonnes (Omar et al., 2019; Food and Agriculture Organisation, 2021c).

The large rice consumption with the growing number of residents in Malaysia may contribute to food insecurity in a prolonged decade to meet the demand (Norimah et al., 2008). Due to insufficient domestic rice output, there will be a future need for imported rice to meet the nation's rising rice demand (Rajamoorthy et al., 2015). The Ministry of Agriculture and Food Industry specified that the nation's dependency on imported rice in 2020 was between 30% to 40% to meet its population rice demand as the rice consumption is not up to the mark with only 72.85% self-sufficient level (SSL) (Chan, 2020). Department

of Statistics Malaysia stated that the rice imports in 2020 was valued at RM 2.5 billion, rose 32.1% compared to the previous year with its main rice suppliers were from Vietnam (42.0%) and India (27.5%), followed by Pakistan (10.9%), Myanmar (6.9%), Thailand (6.6%) and Cambodia (5.5%) (Department of Statistics Malaysia, 2021).

In Malaysia, rice is cultivated either as lowland type or upland type; lowland rice in which grown in field that is either rain-fed or irrigated; and upland rice is a rice type normally grown on hillsides or naturally well-drained field without surface water accumulation (International Rice Research Institute, n.d.a; Hanafi, 2009; Herman et al., 2015). Due to a vast amount of swamp field in Peninsular Malaysia (503 184 ha), farmers have their favours in cultivating lowland rice type, whilst upland rice type is mostly cultivated by farmers in Sabah and Sarawak (165 888 ha) due to the presence of hilly areas (Sohrabi et al., 2012). Farmers prefer cultivating lowland rice varieties as they offer a higher yield and the Malaysian Agricultural Research and Development Institute (MARDI) has created several high yielding lowland rice varieties including MRQ76, MR219, MR220, MR253, MR263 and MR269 (Farahin & Abiddin, 2015). The lowland varieties such as MR219, MR220 and MR269 can yield up to 10 tonnes per ha and high yielding varieties are crucial in increasing rice production in Malaysia (Ismail, 2001; Ismail, 2005; Yusob et al., 2014).

Sarawak is a state with diverse types of rice in comparison with other states in the country, making its rice landraces valuable for rice breeding (Hanafi, 2009). The success of breeding strategies relies heavily on the diversity of the crop and this requires research and development on rice plant breeding in order to produce new rice varieties that contain features desired by the industry (Omar et al., 2019). An increase in grain yield potential is

always one of the objectives of any crop breeding program including rice (Khan et al., 2015a). In Sarawak, efforts were initiated since year 1991 between Agriculture Research Centre (ARC) of Sarawak, Malaysian Agriculture Research and Development Institute (MARDI) and International Research Rice Institute (IRRI) to collect landraces around Sarawak to compile information on Sarawak rice diversity and throughout the cooperation, 1652 accession from ten different divisions around Sarawak were successfully collected (Yeo et al., 2018). To this date, a total of 2011 accessions of Sarawak rice landraces which exhibit a very large amount of genetic diversity present in the landraces from local collections, imported collections from MARDI and IRRI are maintained in the germplasm bank of ARC (Department of Agriculture Sarawak, 2020).

Sarawak landraces are valuable for rice breeding and breeding of high yielding is one of the procedures to boost the state and national rice production. Sarawak, a state rich in rice biodiversity, could soon turn into Malaysia's rice producer with the government genuinely examining the capability of the nation's biggest state in handling the issue of low yields (The Borneo Post, 2012). Rice breeding will help the change in rice productivity in Sarawak which is a task under the National Key Economic Areas, expected to enhance the efficiency of rice cultivation in Sarawak (Oxford Business Group, 2015; Adnan & Nordin, 2021).

1.2 Problem Statement

Sarawak is rich in biodiversity of cultivated traditional rice landraces making its rice landraces valuable for rice breeding. Rice diversity is essential since breeders are interested in variety improvement by stacking desirable plant traits which can meet the desires of both farmers and consumers, for example in terms of taste and aroma. However, it is challenging

to use traditional landrace collected from farmers for breeding due to the problem with landrace identification. This leads to difficulty in documenting the traits and varieties in rice collections. The problem arises when the farmers planted rice seeds of different variety, which they inherited from generations to generations, separately either in one plot without clear boundary or in different plots but close to each other. Worse comes to worst when different varieties are mixed up in one plot due to common practice by the farmers. Purity of rice seeds may not be important to farmers who intended to cultivate the rice for their own consumption. For breeders, however, the impurity will give difficulty in their attempt to use the materials for breeding purpose. Characterising different traits of a landrace using clusters of impure seed may observe incredible variations. In the meantime, this will obstruct the examinations of characteristics between landraces or varieties. Another issue is that the local name of the landraces depends on the name given by the farmers themselves for the time being. Issues emerge when landraces having the same morphological character were given different names or landraces having different morphological attributes were given a similar name by the farmers. This will create confusion among the farmers and breeders in recognizing a landrace. The breeders are interested to evaluate genetic diversity based on morphological traits; both qualitative and quantitative characteristics, because they are inexpensive, rapid, and simple to score. Until today, scientific classification of plant is still based on morphological traits. Study by Sohrabi et al. (2012) showed variations can be observed in vegetative traits such as plant height and leaf length. Morphological descriptions, however, were strongly influenced by the environmental factors (Liu et al., 2012). Anatomical data may assist in providing data to support morphological data in establishing a varietal identity. Moreover, study on plant anatomy plays an important role in the understanding of a plant biology (Susanti & Anwar, 2018). The DNA analysis may

contribute in-depth information on rice germplasm over morphological and biochemical markers in exhibiting polymorphisms and serves as powerful tools to detect genetic variation and genetic relationship within and among species (Al-Musawi et al., 2019). Fingerprinting using molecular markers provide a more precise and rapid cultivar identification, which has been proved to be an efficient tool for crop germplasm characterisation, collection and management (Zhu et al., 2012). Molecular markers based on differences in DNA sequence between individuals can generally detect more polymorphism than morphological traits (Jalaluddin et al., 2007; Filiz et al., 2018). Simple Sequence Repeats (SSR) are usually genetic markers of choice in genotyping approaches because it is polymorphic and evenly distributed throughout the plant genome (Mahalingam et al., 2013). Barcoding marker such as *Maturase-K* (*matK*) is among marker suggested by plant taxonomists that may resolve closely related plant species (Saddhe & Kumar, 2018). Varietal identification that utilizes DNA could overcome the limitations associated with morphological and anatomical characterisation methods which influenced by the growth stages and various environmental factors (Eagles et al., 2001; Kalaichelvan, 2009). Hence, characterisation of rice landraces based on morphological traits and anatomical traits supported with molecular fingerprinting are expected to help in future breeding attempts using Sarawak rice and to assess the genetic diversity of lowland rice landraces in Sarawak.

1.3 Objectives

This study aimed to document the characteristics of lowland rice landraces in Sarawak. This can be achieved by the following sets of objectives:

- i. To describe the morphological and anatomical characteristics of lowland rice landraces collected from the North-West region of Sarawak.

- ii. To genotype and analyse the genetic diversity of the Sarawak lowland rice landraces collected from the North-West region of Sarawak using Simple Sequence Repeat markers and barcoding marker (*Maturase-K*).

CHAPTER 2

LITERATURE REVIEWS

2.1 *Oryza sativa* Linnaeus

Oryza sativa L. or known as the rice plant came as one of the top three grain crops in the world along with wheat and maize (Awika, 2011). Genus *Oryza* presumably started around 130 million years back in Gondwanaland (composed of the major continental blocks of South America, Africa, Arabia, Madagascar, Sri Lanka, India, Antarctica, and Australia) and the *Oryza* species were distributed among various landmasses when Gondwanaland split up. Since then it has evolved, diversified and dispersed into wild *Oryza* species and distributed throughout the tropics. As of today, there are a total of more than 40 000 varieties of *O. sativa* worldwide (Rathna, 2019). Genus *Oryza* has two cultivated species, which are *O. sativa* and *O. glaberrima* (Linares, 2002). Meanwhile, two major cultivated rice subspecies are *O. sativa indica* and *O. sativa japonica* (Sweeney & McCouch, 2007).

Rice is grown in over 100 countries today with people in Asian nations being its largest consumer and Asia is accounted for 90% of global rice production (Omar et al., 2019). According to the International Rice Research Institute, rice typically grows in four ecosystems: rain-fed upland or dryland, rain-fed lowland (wet rice), irrigated lowland (flooded), and deep-water (floating) (IRRI, n.d.a). In Sarawak however, rice is grown in all of those ecologies except deep-water (Sang et al., 2018).

2.1.1 Lowland Rice Cultivation

In Malaysia, irrigated and lowland farming environment predominate in Peninsular Malaysia while upland farming is more familiar in East Malaysia Sabah and Sarawak due to mountainous topography (Sohrabi et al., 2012). Farmers, however, prefer lowland rice as it could grow well and furnish higher yield than the upland rice (Liu et al., 2019). Because of high grain yields, lowland rice are cultivated for the farmer's own consumption, and the excess produce will be sold at a premium price for their income (Khazanah Research Institute, 2022).

2.2 Rice as Global Staple Food

There are two types of *Oryza* species considered as significant grains in human nourishment: *O. sativa* or known as Asian rice, and *O. glaberrima* or called African rice, which commonly cultivated in parts of West Africa (Stein et al, 2018). According to Feng et al. (2017), *O. sativa* has supported a large number of people for a longer period of time compared to any other crop since it was domesticated about 8000 to 10 000 years ago. Rice is the second most significant harvest on the planet after wheat as rice accounts for dietary energy requirements for the body by a large portion of the world's population (Food and Agriculture Organization of the United Nations, n.d.). In addition to providing minerals, nutrients, and fibre, rice supplies 21% of the calories and 15% of the protein a person needs (IRRI, n.d.b). Rice contains the highest digestible protein and has relatively a good balance of amino acids even though having approximately lower protein content compared to other cereals (Liyanaarachchi et al., 2021). Apart from contributing food to the human population, rice farming too provides employment and livelihood to the rural families (Zhang et al., 2022a).

Norimah et al. (2008) conducted a survey on Malaysian adult rice diet demonstrated an adult individual consumes an average of two and half plates of rice per day. With the large rice consumption with the growing number of residents in the country, rice production in Malaysia is not self-sufficient. This matter may contribute to food insecure in a prolonged decade to meet the demand. The Ministry of Agriculture and Food Security targeted the nation to achieved 75% self-sufficiency level (SSL) in rice production by 2025 and 80% by 2030 with the government currently implementing the Large-Scale Smart Paddy Field to develop paddy cultivation with a target of 150,000 hectares per season to produce an average yield of seven metric tonnes per hectare in order to achieve the targeted SSL (Malay Mail, 2023).

Food and Agriculture Organization (2021d) stated that, the global rice production in 2020 was 756.7 million tonnes, where China produced approximately 213.6 million tonnes which is about 28.2% of global rice production, while Malaysia on the other hand came with approximately 2.3 million tonnes, which is about only 0.3% of global rice production. The rice production in Malaysia for year 2020 was 6.4% less than the average rice production from year 2014 to 2019 (Food and Agriculture Organization, 2021a). With the rice production shows a decreasing trend, and the growing number of residents in Malaysia annually, the production of rice in the country will be insufficient to meet the domestic demand. Currently, Malaysia is still reliant on import at around 30% of aggregate utilization from other nations such as Vietnam, Thailand, and Pakistan (Harun et al., 2015; Omar et al., 2019). Malaysia's total rice imports is around 700 000 to 900 000 million tonnes per year in average (Kari, 2018). In respond to this matter, the Malaysian Government initiated various efforts to reduce the dependency on imported rice and improve farmers' welfare, including allocating a total of RM 1.7 billion in Malaysian government's Budget 2022 for subsidies,

assistance and incentives that will benefit paddy farmer all over Malaysia (Department of Statistics Malaysia, 2021). Among the efforts initiated includes improving the implementation of rice farming through Paddy Industry Development programs by Muda Agricultural Development Authority. Rice sector is one of the most assisted and subsidized industries within the country with the government actively providing agricultural subsidies in terms of seeds, herbicides and fertilizers to local farmers and breeders to ensure the production of rice yields can be increased from time to time (Siwar et al., 2014). The rice industry has always received special attention from the government and regarded the most important food crop for ensuring the nation's food security. With all the efforts given, national food supply is hoped to have an adequate amount to meet the fundamental needs of Malaysian residents and reducing its dependency on imported rice.

2.3 Sarawak as Major Rice Producer in Malaysia

Malaysia produced 1.68 million metric tonnes of rice in 2021, with Peninsula Malaysia accounting for the majority of that amount (90.5%), Sarawak coming in second (5.3%), and Sabah contributing the least at 4.2% (Mail, 2023). Rich ecosystem of paddy and vast amount of land in Sarawak are the few reasons rice has a great potential to be cultivated here and made Sarawak the country's fifth largest paddy producer after Kedah, Perak, Kelantan and Perlis (Masni & Wasli, 2019). Sarawak is implementing measures to improve paddy production by rehabilitating and opening up new areas for cultivation for instance in Tanjung Purun Scheme and Kampung Pueh Scheme, both in Kuching Division, Mid-Sadong Scheme in Samarahan, Kampung Lebor and Kampung Mujat in Serian Division, an area in Abok, Pantu along with another new area for large scale paddy cultivation at Selepong-Panggil in Sri Aman Division, which are all under the 12th Malaysia Plan (Tawie, 2022). A

double crop of 5400 metric tonnes of paddy annually might be produced by this project once it is finished.

Sarawak is a home for a diversity of rice landraces that are important for rice genetics conservation which makes them valuable for breeding purpose (Yeo et al., 2018). Rich and diverse landraces contain large number of important genetic traits, making them crucial for breeders who are working to create rice varieties with high yield and high-quality grains (Rabara et al., 2018). An increase in productivity is always one of the major goals of any crop breeding program including rice and rice breeding is sure a good step to intensify rice production in Sarawak.

2.4 Breeding of High Yielding Rice Varieties

The main focus in research and development in rice is always the development of the new varieties that could increase the rice production apart from other priority research areas such as development of resistant varieties to pests and diseases, production of quality seeds, agronomic package, weed control, mechanization, improving the aroma and enhancing the nutrition (IRRI, n.d.c; Harun & Engku, 2017). Sarawak, being a state rich in rice diversity makes its rice valuable in the eyes of breeders. Sarawak local cultivars are of different sizes and shapes, interesting colours and shades and having an excellent taste and texture (Teo, 2015). Diversity in plant genetic resources provides opportunity for plant breeders to improved cultivars with desirable characteristics, which include both farmer-preferred traits including higher yield potential with improved taste and aroma and breeders-preferred traits such as pest and disease resistance, lodging resistance and shorter maturation days to name a few (Louwaars, 2018). To date, MARDI has successfully developed new rice varieties

which are MR315 and MRQ104 that might be able to boost rice yields in Malaysia, where these two rice varieties are recommended in most granaries because they are more resistance to disease such as blast disease and panicle blast caused by the fungus, more tolerant to environmental changes and Malaysia's soil condition, (MARDI, 2021).

2.4.1 Diversity in Sarawak Rice Varieties

According to Teo (n.d.a), rice conservation in Sarawak only began in 1963 where only 305 accessions were gathered and later in year 1966, 184 accessions were managed to be collected. There was no effort to gather rice germplasm in Sarawak until year 1991 where a systematic and organized efforts were made mutually with MARDI Bertam and International Rice Research Institute (IRRI). At present, a total of 2011 accessions of Sarawak rice landraces which exhibit a very large amount of genetic diversity present in the landraces from local collections, imported collections from MARDI and IRRI are maintained in the germplasm bank of ARC (Department of Agriculture Sarawak, 2020).

Sarawak traditional varieties are unique in every aspect. Khazanah Research Institute (2018) stated the traditional varieties have superior qualities in terms of texture, fragrance and excellent in taste. Those unique characteristics the rice landraces possess make these materials valuable for rice breeding. Former Sarawak Deputy Chief Minister Tan Sri Alfred Jabu Numpang mentioned there were over 100 local varieties grown by farmers and sold in the market throughout the state and among the most popular varieties sold in the markets are Bario, Biris, Bajong, Bali, Mamut, Selasih, Katek Merah, Lemak and Kenawit and revealed three high quality traditional rice varieties in Sarawak namely Bario, Bajong and Biris had been conceded the Geographical Indications (GI) by the Malaysian Intellectual Property

Organization and another local variety named Padi Wai, or some called as Padi Rotan, is in the process of being registered to get the GI certificate (Saai, 2011). The GI certification adds value to the products and provide entrepreneurs as well as consumers the assurance of authenticity of a product. A myriad of interesting traditional landraces is now sold in market costing ranging RM2.80 to RM9.50 per kilogram according to Teo (n.d.b). The prices are different depending on the characteristic it has for example the taste, texture, grain length and quality. The Sarawak consumers consider their choice for local rice varieties over imported rice for the value they contain.

Sadly, there is a possibility the seeds from the farmers are impure due to the common practices they applied including planting rice seeds of different variety either in one plot without clear boundary or in different plots but closely to each other (Bajracharya et al., 2006). Some of the varieties has lost due to total crop failure which is a very common problem among shifting cultivators. The traditional varieties gradually being replaced in numerous areas with the introduction of modern varieties by government to the farmers in Sarawak. It is consequently important to gather and preserve the traditional varieties before they become wiped out in years to come (Teo, n.d.a). A mix of agricultural practices such as direct seeding and shared utilization of machinery has contributed to the rapid proliferation of weedy rice across paddy fields in recent decades (Centre for Agriculture and Bioscience International, n.d.). These common practices made by farmers has cause great morphological variation in the genus *Oryza* leading to taxonomic difficulties as characterizing different traits of a landrace using cluster of impure seeds will watch incredible variations. As breeders today are in their attempt to create unique rice varieties, plant breeders need to come with methods to identify and describe cultivated varieties for a better seed improvement and seed production programmes.

2.5 Approaches Towards Variety Characterisation

Plant characterisation is one of the most important aspects in agricultural systems. Plant characterisation is referred to as providing information of accessions for heritable characters which involves morphological and anatomical features including molecular markers (Lutatenekwa et al., 2020). Characterisation of a crop is important for registration process, pure seed production and especially crucial in plant breeding program to provide essential information which can assure maximum utilization of the germplasm. Breeders are now in their effort to improve the current variety with desirable characteristics of not only for the farmers but also for consumers (Qaim, 2020). The conventional way in variety characterisation includes observation and recording of morphological traits throughout all growth stages. Evaluating genetic diversity based on morphological traits; both qualitative and quantitative characteristics, are preferable back then because they are inexpensive, rapid, and simple to score. Describing a variety morphological characteristic alone, however, did not provide enough information in establishing an identity of a variety. To mitigate this, the scientist has come up with a more precise tools which give informative data aside from less time consuming by developing molecular markers, statistical tests and software to identify and characterize the required plant varieties for cultivation, breeding programs as well as for cultivar-right-protection (Korir et al., 2013). DNA-based species identification or molecular identification, makes it possible to identify species precisely in the clarification of hereditary connections without being influenced by environmental factor, as conventional way of identification does (De Boer et al., 2022; Dorice et al., 2020). To obtain a more accurate data, there is a need to study the rice diversity and the first step towards varietal identification is through evaluation and characterization of available rice germplasm at both

morphological, anatomical and molecular levels. A thorough characterisation is necessary to establish the identity of a variety.

2.6 Morphological Characteristics of *Oryza sativa*

Morphological taxonomy through various plant parts is a common method used in species identification (Friedheim, 2016). Morphological characters of various plant parts can be used as to describe the growth stages of plants, monitor the development of plants, provide selection criteria in crop development plans and provide indicators for crop management (Smith et al., 2002). The study on morphological traits needs neither sophisticated methods nor complicated equipment to score. Further, this conventional method is simple to score, results can be obtained rapidly, inexpensive and can be used directly on plant population (Sholikhah et al., 2019). Morphological characteristic has been long used in classification of plants, delineating rice landraces or varieties (Suriyagoda et al., 2011; Ray et al., 2013). Morphological study on *Oryza sativa* has been done by many researchers because this plant shows interesting morphological variations, especially in vegetative traits such as plant height, leaf length, culm length, number of tillers, number of panicles and panicle length (Ishak et al., 2022).

Rice plants usually take around three to six months to develop from seeds into develop plants, depending on the type of varieties and ecological conditions. The vegetative parts of rice plant according to Duan et al., (2012) consists of root, culm and leaves, while the reproductive plant part includes the floral organs comprises of panicle, spikelets and flower. Figure 2.1 shows the general morphology of *O. sativa*.

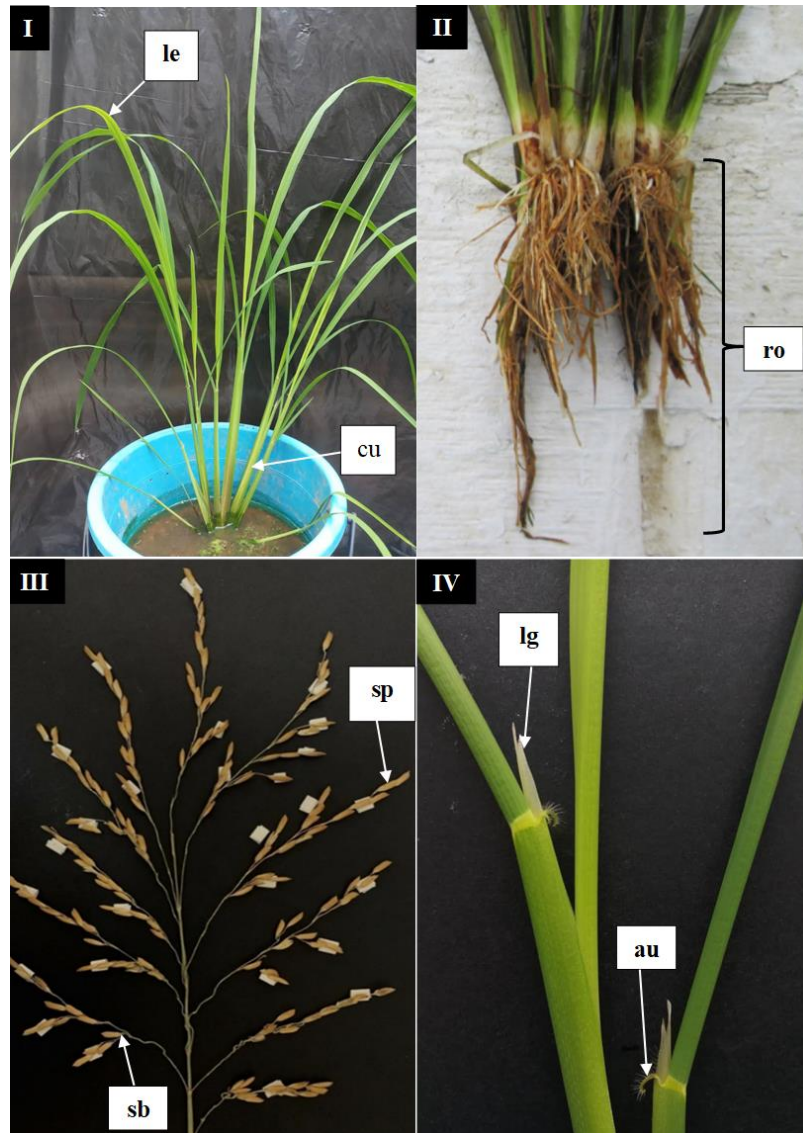


Figure 2.1: General Morphology of *Oryza sativa*. **I:** The rice plant, **II:** Root, **III:** Panicle, **IV:** Ligule and auricle. **le:** Leaf, **cu:** Culm, **ro:** Root, **sb:** Secondary branching, **sp:** Spikelet; **lg:** Ligule, **au:** Auricle.

Generally, there are three important growth phases of rice plant according to Moldenhauer and Slaton (2001), after the seeds are germinated, where the process of observation of plant morphology will be done (Figure 2.2); Phase 1: The vegetative phase which runs from germination to panicle initiation; Phase 2: The reproductive phase which runs from panicle initiation to flowering; Phase 3: The ripening phase which runs from flowering to maturity.

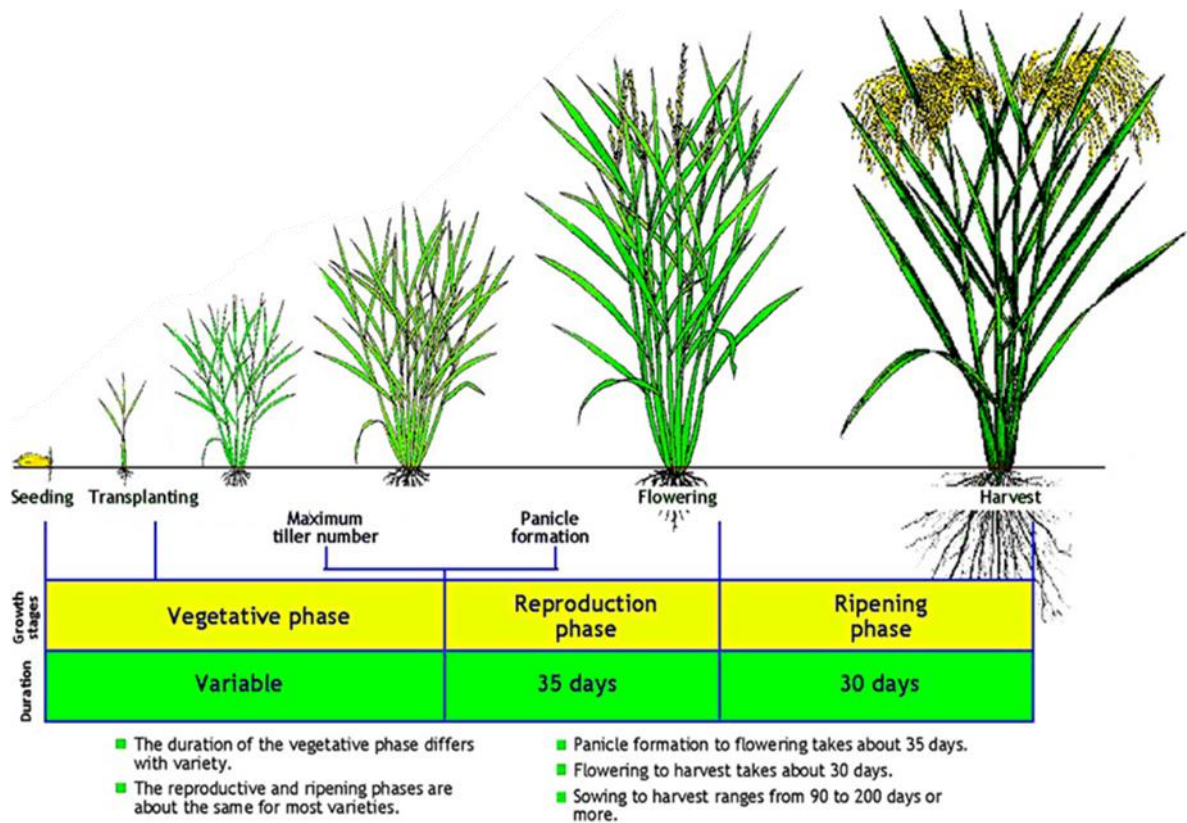


Figure 2.2: The Rice Plant Growth Phases (IRRI, 2015).

Every plant morphological characters will be scored according to their respective phase starting from vegetative phase to reproduction phase and will continue until ripening phase. Description of a plant morphology, however, does not end after the plant has reached its maturity. According to Kalaichelvan (2009) the grain characteristics are too, valuable in seed certification process in controlling field and seed standards hence needs to be described as well to add an additional data in plant variation.

Morphological characterization can be divided into two categories; qualitative and quantitative traits (Boumis, n.d.). Qualitative traits are referred to as traits controlled by a single gene, making the traits heritable (Walsh, 2019). Qualitative traits can fit into discrete categories for example the shape of seed and ligule, and also in colouration of leaves, grains,

ligule and auricle. Evaluation on grain shape and size done by Khan et al. (2015b) revealed the morphology varies among the 15 observed rice accessions, where two types of grain shapes (medium and slender) and three grain sizes (short, medium and long) were recorded. Meanwhile, type of compact panicle and presence of dense secondary branching are admirable characteristics to be observed as they significantly affect the possibility of a larger yield (Das et al., 2018; Mohd Kasim et al., 2020).

Conversely, quantitative traits are traits with phenotype that can be quantified. Quantitative traits are controlled by multiple genes and measurements may be unstable due to environmental factors (Hittalmani et al., 2003). Some traits that marked as quantitative traits such as the height of plant, the length of the grains and leaf and culm diameter. Hussain et al. (2010), Ashfaq and Khan (2012) shared their observation on their rice accessions where an incredible variation in several characters between the accessions specifically in their plant height, number of tillers, number of panicles, panicle lengths, culm height, leaf length and width, flag leaf area, seed per panicle, seed weight per panicle as well as in seed length and width. Variations can be useful for future rice breeding plan and be used in breeding or genetic engineering to create rice that produces higher quality and yield.

Morphological characters may contribute adequate polymorphic characters to portray the different landraces. However, limitation of using this method as an approach towards variety identification is that it is time consuming and requires manpower when a large number of samples needed to be characterized. Further, according to Korir (2013), a large number of varieties has made it difficult to be identified and characterized based on morphological traits alone because they are unstable as they are being influenced by environmental factors, ergo the phenotypic plasticity is an outcome of the adaptation.

2.7 Anatomical Study of *Oryza sativa*

Plant anatomy is the study of the tissue and cell structure of plant organ and anatomical characters have been used traditionally in characterising rice landraces or varieties (Kondo et al., 2000; Simpson, 2019). Study of plant anatomy have wide range of application and benefits not only taxonomically but also in modern botanical research, medicinal plant, food adulterants, contaminants, plant archaeology, palaeobotany and also in forensic application. The anatomical data providing an advance understanding of the plant interrelationships and confirming evidence of plant families natural relationships in combined analysis (Cutler et al., 2007).

Anatomically, leaf in rice plant consist of mesophyll-l cells, bundle sheath cells, epidermal cells, bulliform cells, stone cells, and vascular bundles that composed with xylem and phloem and their associated companion cells (Chatterjee et al., 2016). Bertolino et al. (2019) in their study on leaves stated that stomata density (number of stomata per unit of area) is responsible in the rate of water loss and carbon dioxide uptake in plant, suggesting difference in stomata density and size may arise due to growth under various environmental circumstances or genetic factors of the plant. Furthermore, high stomata density has the advantage of high carbon dioxide uptake, hence increasing the photosynthesis in plant while the disadvantage is high in amount of water loss, so it is not suitable for drought condition. On the contrary, advantage of low stomata density is less amount of water loss thus increasing the plant drought tolerance while the disadvantage is low in carbon dioxide uptake therefore decreasing the rate of photosynthesis in plant. Chatterjee et al. (2020) studies also support this statement where they hypothesize that anatomically increasing size of stomata rather than the stomata number is the better option for breeding program to reduce water loss

through stomata in rice. Thus, stomatal characters are among the interesting character to look for in leaf anatomy.

Leaf midrib anatomically consist of vascular bundles (phloem and xylem), epidermis and associated fundamental tissues parenchyma, collenchyma and also sclerenchyma, in which the midrib main function is as support for leaf structure and also assisting in nutrient transport of the plant (Da Silva et al., 2015). Although midrib can provide us with many important information in the plant species identification, the study on rice midrib is still lacking and does not receive much of attention in the present (Taratima et al., 2020).

Root not only function as an anchor for the stabilization of shoot development and growth but also uptake water and nutrients from the soil to the plant (Crang et al., 2018). In the study conducted by Kondo et al. (2000), they stated that the water capture ability of roots of 12 lowland and upland *O. sativa* depends on the anatomical differences of stele proportion, structure of the xylem and development of sclerenchyma. The central part of a root, called the stele, is made up of vascular tissue, consisted of phloem and xylem, which are crucial in nutrient and water uptake (Phoura et al., 2020). According to Rebouillat et al. (2009), the rice radicle root typical radial anatomy consists of epidermis, exodermis sclerenchyma layer, mesodermis, endodermis, pericycle, fibers, phloem and metaxylem as shown in Figure 2.3. Root anatomical characters had been successfully used in identification and discrimination of *O. glaberrima*, *O. barthii* and *O. longistaminata* from each other (Idio & Jayeola, 2020).

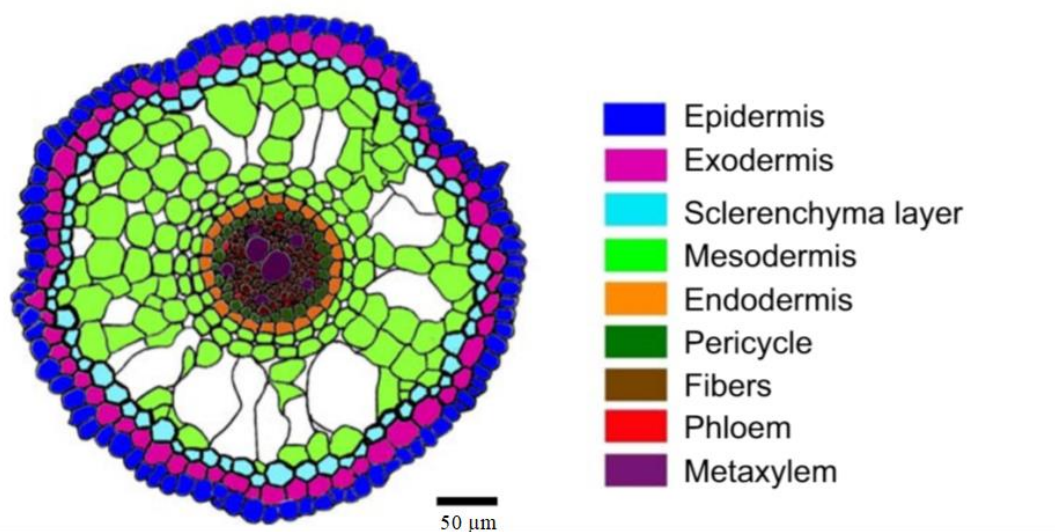


Figure 2.3: The General Root Structure of *Oryza sativa* (Rebouillat et al., 2009).

2.8 Molecular Characterisation

Over the decades, molecular methods have been extensively used in the study of species diversity for it provides a closer assessment of genetic variation with limited ecological impacts, as opposed to the morphological characterization (Kalaichelvan, 2009). The advents of several techniques in DNA analysis has extended the potential outcomes for applying such techniques in the issue of varietal identification and characterisation, phylogenetic studies, analysis of recombination frequencies between genotypes and identification of genes for desired agricultural traits and even proven as efficient tool in crop management (Magistrado et al., 2001; Keramas et al., 2004; Sonah et al., 2011). Utilization of DNA in analysis could overcome the constraints related with morphological characterization. Scientists prefer using molecular analysis in delineating plant species because the assessment of genetic variations can be obtained more precisely without effects

from environmental factor such as temperature, water availability, soil nutrient uptake and light intensity which could influence the chemical diversity of plant (Pacheco-Hernández, 2021). Plus, by applying molecular genetic techniques, a large number of varieties can be characterized in shorter period of time.

Molecular markers offer various points of interest in showing uniqueness compared to morphological characters. Nybom et al. (2014) reviewed DNA fingerprinting has been increasingly being used in identification of varieties, especially when encountering problems in recognizing different varieties that goes by a same name or the same variety but goes by several names which later creates confusion to the farmers and breeders. Molecular characterization of rice germplasm offers several advantages over conventional method and surely will upgrade their utilization in the improvement of rice worldwide.

2.8.1 Simple Sequence Repeat

Microsatellites or Simple Sequence Repeat (SSR) markers have been long known as powerful tools in DNA fingerprinting as it is polymorphic in plant genomes as to why it is DNA marker of choice in genetic analysis of rice germplasm (Bhattarai et al., 2021). Microsatellites can be found abundantly in rice and has been extensively used to study the genetic diversity of rice as well as fingerprinting and aids in cultivar identification of rice (Sarao et al., 2009; Zhang et al., 2011; Miah et al., 2013). McCouch et al. (2002) pointed SSR markers are considered as the most versatile markers in genetic diversity studies due to their multiallelic nature, high reproducibility, codominant inheritance, abundance and broad genome coverage. SSR markers can detect high level of polymorphisms at multiple loci enabling it to categorize landraces, varieties and wild relatives of rice germplasm which can be used for future rice improvement purpose.

In our country Malaysia, many studies have been conducted on rice germplasm by utilising SSR markers. Sohrabi et al. (2013) conducted a study on 50 Malaysian upland rice (24 accessions from Peninsular Malaysia, 26 accessions from Sabah) using ten sets of SSR markers to study the genetic divergence of upland rice in Malaysia and the outcome of the study has identified a few accessions chosen as possible parents for a hybridization breeding programme in the Malaysian environment.

Study by Aljumaili et al. (2018) on 53 aromatic rice accessions from Peninsular Malaysia, Sabah and Sarawak including three cultivars (MR219, MRQ74 and MR253) collected from MARDI has revealed 32 out of 147 SSR markers are polymorphic and suitable for diversity analysis after showing a high level of diversity among the 53 observed accessions which proved utilisation of SSR in rice can provide an accurate assessment and display a detailed information on genetic divergence. In the study, a total of seven accessions were found to be diverse accessions that would make good parents for the breeding programme for aromatic rice.

Recently, a total of 21 rice cultivars collected from MARDI were studied using 27 pairs of SSR markers and unveiled the potential of the SSR markers in determining the closely and distantly related rice cultivars (Supari et al., 2019). A total of 53 Sarawak rice landraces were genotyped using 54 sets of SSR markers by Lee et al. (2011) but the small number of markers used are considered as inadequate to uncover the genetic diversity of the 53 Sarawak rice landraces after it unable to recognise the variations between the Sarawak rice landraces.

While many studies have been reported using microsatellite markers in Malaysia, less effort has been made using Sarawak local lowland rice landraces, which is known to have high genetic diversity.

2.8.2 Maturase-K

Another conceivable way to provide identification of Sarawak rice landraces is by utilising DNA barcoding. Chloroplast *Maturase-K* (*matK*) has been suggested as the barcode for land plants and said to be the most versatile coding gene of angiosperms due to its high phylogenetic signal compared to other genes used so far (Patil et al., 2015; Müller et al., 2006). The 1500 bp *matK* gene had been used effectively in the study of plant systematic for having such a distinct mode of evolution, where *matK* has a relatively high substitution rate as high as three times at nucleotide level and six times higher at amino acid level compared to other coding gene which is a prove that *matK* as a rapidly evolving gene (Barthet & Hilu, 2007; Soltis & Soltis, 2004; Ismail et al., 2020).

Over the decades, many scientists have been studying the evolution of plants at taxonomic levels using chloroplast gene. Genus *Oryza*, which includes *O. sativa* and its closely related wild relatives, is among the most popular genus studied by the scientist by utilising *matK* concerning the understanding of major evolutionary relationship of aromatic rice under the family, genus and up to species level (Patil et al., 2015). Other study using *matK* gene was reported in Song et al. (2002) emphasized on the phylogenetic analyses between 26 species representing 11 genera of the tribe Oryzeae and indicated the sequence data formed a strongly supported monophyletic group. Zodinpuui et al. (2013) has reported their success in using chloroplast *matK* gene in revealing the phylogenetic relationship

between *Oryza* species from the Eastern Himalayan range (Mizoram) and *Oryza* from other countries such as from Africa, Brazil, China, Indonesia, India, Philippines and Malaysia. While many studies using *matK* in phylogenetic analyses has been reported, Sarawak lowland rice landraces have not yet been the subject of any studies employing *matK* barcoding marker.

CHAPTER 3

MATERIALS AND METHODS

3.1 Samples Collection

The flowchart of designated experiment is illustrated in Figure 3.1. A total of 11 lowland rice landraces were collected from eight different localities in four divisions in Sarawak. The name of the landraces was based on the name given by farmers (Table 3.1). Figure 3.2 on the other hand displays a map of the sampling region.

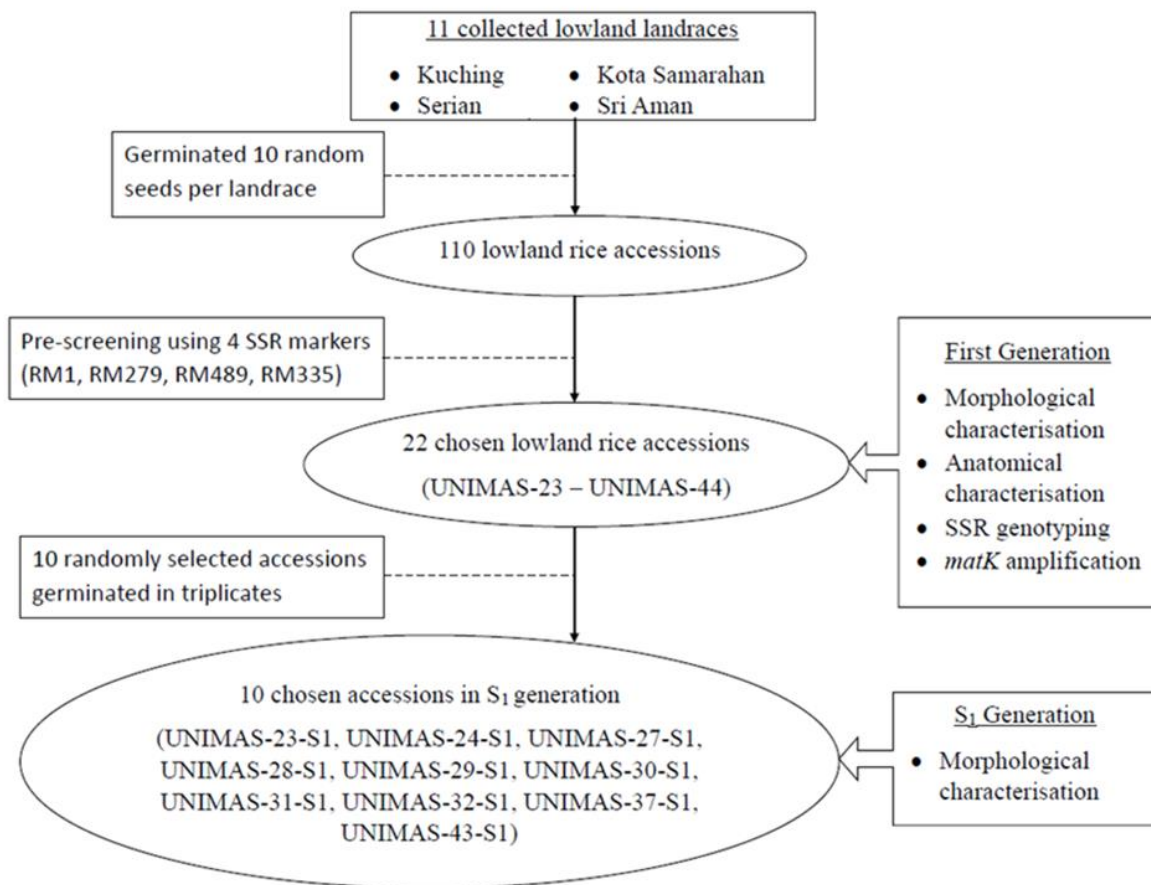


Figure 3.1: The Flowchart of The Experiment.

Table 3.1: List Landraces with Its Localities and Coordinates.

Name	Location	GPS	Division
UNISR2018-02 Padi Pandan	Kg. Paon Gahat	0° 57' 0" North, 110° 39' 0" East	Serian
UNIPDW 2018-18 Padi Merah	Kg. Pesak	1° 45' 0" North, 110° 46' 0" East	Kuching
UNISMT 2018-21 Padi Hitam	Kg. Pueh	1° 51' 0" North, 109° 41' 0" East	Kuching
UNIPDW2018- 48 Padi Kihuai	Kg. Mambong	1° 22' 0" North, 110° 21' 0" East	Kuching
UNISAMA 2018- 44 Padi Bario	Kg. Baru	1° 33' 28" North, 110° 22' 22" East	Kota Samarahan
UNISAMA 2018- 45 Padi Sabak Hitam	Kg. Baru	1° 33' 28" North, 110° 22' 22" East	Kota Samarahan
UNISAMA 2018- 47 Padi Sabak Angin	Kg. Baru	1° 33' 28" North, 110° 22' 22" East	Kota Samarahan
UNISRA 2018-24 Padi Bajong	Kg. Panggil	1°05' 48" North, 111°23' 23" East	Sri Aman
UNISRA 2018-26 Padi Kanowit	Kg. Melugu Skim	1° 07' 10" North, 111° 25' 33" East	Sri Aman
UNISRA 2018-38 Padi Arang	Kg. Sg. Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman
UNISRA 2018-40 Padi Selasih	Kg. Melugu Skim	1°07' 10" North, 111°25' 33" East	Sri Aman

* **Kg.:** Kampung, **Sg.:** Sungai.

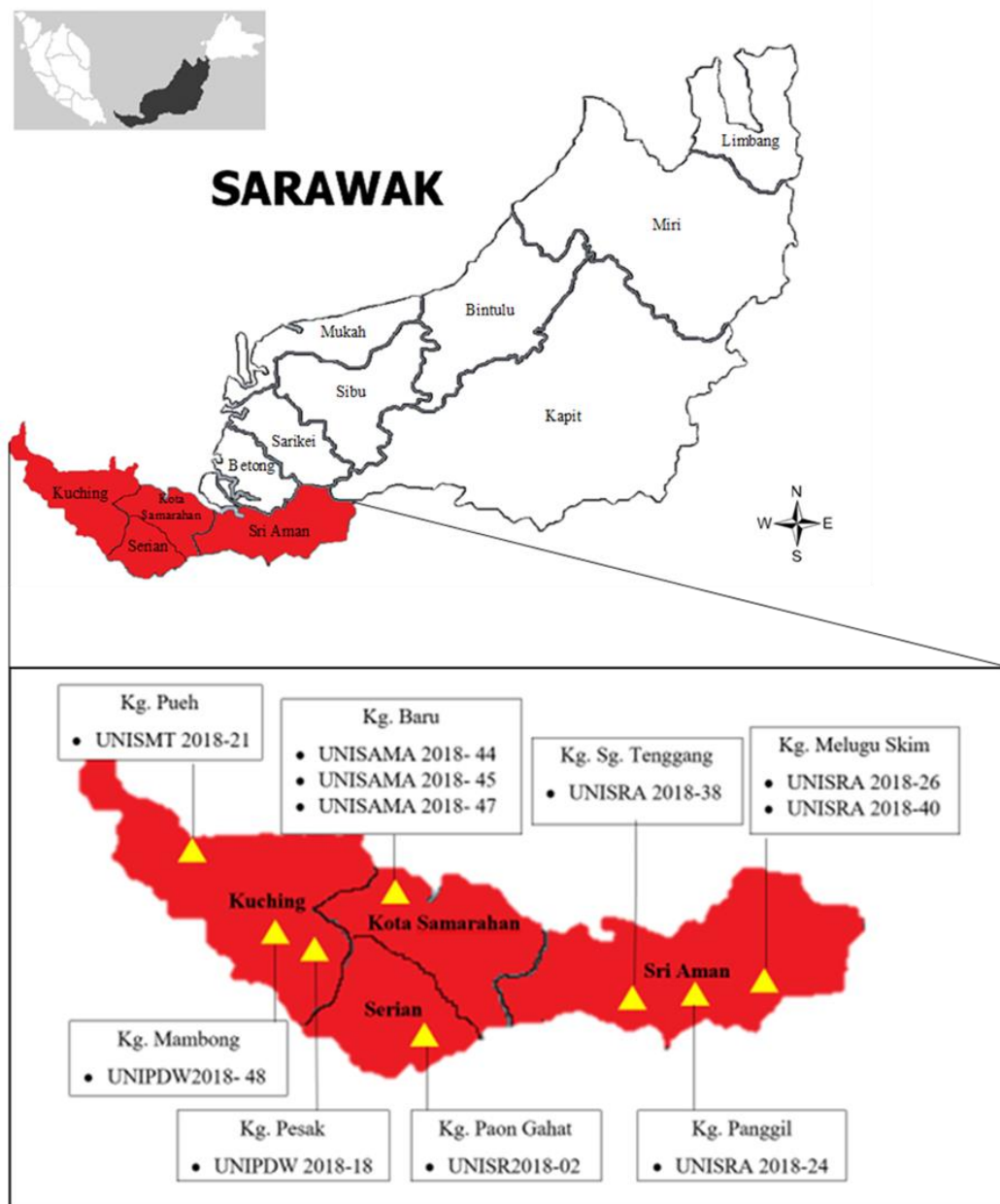


Figure 3.2: The Distribution of the 11 Collected Lowland Rice Landraces.

3.1.1 Planting Rice Landraces

A total of ten random seeds per landrace, making a total of 110 individual accessions with unique characteristic in terms of seed shape, size or husk colour, were germinated by soaking in distilled water for five days in the laboratory. The germination date for every landrace is as tabulated in Table 3.2.

Upon germination, the 110 accessions were planted into pot (size: 5 gallon; diameter: 33 cm; height: 31 cm) filled with same amount of soil mixture consisted of topsoil, compost and sand at 3:2:1 ratio. The accessions were watered ones a day and first fertiliser application (NPK granular fertiliser with ratio 17.5: 15.5: 10) was made on the day of transplanting the accessions into pots in a net house located in Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. Further fertilizer application and watering of plant are described in Section 3.1.4.

Table 3.2: The Germination Date of 11 Lowland Landraces.

Landrace	Date of germination
UNISR2018-02 (Padi Pandan)	26 th Oct 2018
UNIPDW 2018-18 (Padi Merah)	1 st Nov 2018
UNISMT 2018-21 (Padi Hitam)	26 th Oct 2018
UNIPDW2018- 48 (Padi Kihuai)	30 th Nov 2018
UNISAMA 2018- 44 (Padi Bario)	1 st Nov 2018
UNISAMA 2018- 45 (Padi Sabak Hitam)	1 st Nov 2018
UNISAMA 2018- 47 (Padi Sabak Angin)	30 th Nov 2018
UNISRA 2018-24 (Padi Bajong)	1 st Nov 2018
UNISRA 2018-26 (Padi Kanowit)	30 th Nov 2018
UNISRA 2018-38 (Padi Arang)	1 st Nov 2018
UNISRA 2018-40 (Padi Selasih)	30 th Nov 2018
* Oct: October; Nov: November.	

3.1.2 Deoxyribonucleic Acid Extraction

Young leaf samples, about 4-5 cm were collected from every accession. Total deoxyribonucleic acid (DNA) was extracted from the collected leaf samples using cetyltrimethylammonium bromide (CTAB) protocol by Doyle and Doyle (1987). The quality and concentration of extracted DNA was measured using Nano-drop machine (ND-2800-ODJ Nano DOT Nucleic Acid Analyser; Hercuvan). An amount of 3 µl of DNA sample was loaded into 0.8% 1X-TAE (Tris base, glacial acetic acid and 0.5M EDTA, pH 8.0) agarose gel and electrophoresed at a voltage of 90 V. The agarose gel was visualized using an ultraviolet transilluminator after staining with ethidium bromide (EtBr).

3.1.3 Simple Sequence Repeat Marker

A set of 12 Simple Sequence Repeat (SSR) markers were randomly selected from Gramene database (<https://archive.gramene.org/markers/microsat/all-ssr.html>) representing the 12 rice chromosomes (Table 3.3). Polymerase Chain Reaction (PCR) was performed using the 12 SSR markers on two randomly selected accessions following the protocol by Zhu et al. (2012).

The PCR amplification was performed in a 20 µL volume containing 5 ng of genomic DNA, 0.2 µM of forward and reverse primers, respectively, 2.5 mM MgCl₂, 0.2 mM dNTPs, 1X PCR buffer (750 mM Tris, pH 8.8, 200 mM KCl and 50 mM (NH₄)₂SO₄) and 1 unit of Taq DNA polymerase (Apical Laboratories Sdn. Bhd.). The PCR started with DNA initial 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 45 seconds, the annealing temperature was set according to Table 3.3 for each primer for

45 seconds, primer extension at 72°C for 1 minute with a final extension at 72°C for 10 minutes. The amplified PCR products were electrophoresed on 2% agarose gel prepared in 1X TAE buffer. The gel was run in 1X TAE buffer at 90 V and later stained with EtBr before visualizing it under UV transilluminator. Polymorphic SSR markers were used for genotyping of the rice accessions (Section 4.4).

Table 3.3: The 39 Sets of SSR Markers with Sequences and Details.

SSR	Primer sequence	Ch	EPS (bp)	Ta (°C)	Source
¹ RM1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	1	90-120	55	Gramene (https://archive.gramene.org/markers/microsat/all-ssr.html)
RM226	F: GAAGCTAAGGTCTGGGAGAAACC R: AATGGCCTTAACCAAGTAGGATGG	1	280-350	55	
¹ RM279	F: GCGGGAGAGGGATCTCCT R: GGCTAGGAGTTAACCTCGCG	2	170-190	55	
RM207	F: CCATTTCGTGAGAAGATCTGA R: CACCTCATCCTCGTAACGCC	2	N/A	55	
¹ RM489	F: ACTTGAGACGATCGGACACC R: TCACCCATGGATGTTGTCAG	3	250-270	55	
RM514	F: AGATTGATCTCCCATTCCCC R: CACGAGCATATTACTAGTGG	3	N/A	55	
¹ RM335	F: GTACACACCCACATCGAGAAG R: GCTCTATGCGAGTATCCATGG	4	100-200	55	
RM567	F: ATCAGGGAAATCCTGAAGGG R: GGAAGGAGCAATCACCCTG	4	280-400	55	
RM317	F: CATACTTACCAGTTCACCGCC R: CTGGAGAGTGTCAGCTAGTTGA	4	160	55	
RM229	F: CACTCACACGAACGACTGAC R: CGCAGGTTCTTGTGAAATGT	4	100-120	55	
RM166	F: GGTCCTGGGTCAATAATTGGGTTACC R: TTGCTGCATGATCCTAAACCGG	4	321-410	61	
RM108	F: TCTCTTGCGCGCACACTGGCAC R: CGTGCACCACCACCACCAC	4	N/A	67	
RM413	F: GGCGATTCTTGGATGAAGAG R: TCCCCACCAATCTTGTCTTC	5	N/A	53	
RM480	F: GCTCAAGCATTCTGCAGTTG R: GCGCTTCTGCTTATTGGAAG	5	N/A	58	

Table 3.3 continued

RM164	F: TCTTGCCCGTCACTGCAGATATCC R: GCAGCCCTAATGCTACAATTCTTC	5	246-300	58
RM250	F: GGTTCAAACCAAGCTGATCA R: GATGAAGGCCTTCCACGCAG	5	170-190	57
RM469	F: AGCTGAACAAGCCCTGAAAG R: GACTTGGGCAGTGTGACATG	6	100-120	55
RM454	F: CTCAAGCTTAGCTGCTGCTG R: GTGATCAGTGCACCATAGCG	6	N/A	55
RM276	F: CTCAACGTTGACACCTCGTG R: TCCTCCATCGAGCAGTATCA	6	100-180	59
RM125	F: ATCAGCAGCCATGGCAGCGACC R: AGGGGATCATGTGCCGAAGGCC	7	130-600	63
RM455	F: AACCAACCCACCACCTGTCTC R: AGAAGGAAAAGGGCTCGATC	7	140	55
RM481	F: TAGCTAGCCGATTGAATGGC R: CTCCACCTCCTATGTTGTTG	7	190-300	59
RM47	F: ACTCCACTCCACTCCCCAC R: GTCAGCAGGTCGGACGTC	7	N/A	59
RM152	F: GAAACCACCACACCTCACCG R: CCGTAGACCTTCTTGAAGTAG	8	140	53
RM447	F: CCCTTGTGCTGTCTCCTCTC R: ACGGGCTTCTTCTCCTTCTC	8	100-600	53
RM210	F: TCACATTTCGGTGGCATTG R: CGAGGATGGTTGTTCACTTG	8	120-180	57
RM444	F: GCTCCACCTGCTTAAGCATC R: TGAAGACCATGTTCTGCAGG	9	190-280	55
RM215	F: CAAAATGGAGCAGCAAGAGC R: TGAGCACCTCCTTCTCTGTAG	9	150	56.7
RM244	F: CCGACTGTTCGTCCTTATCA R: CTGCTCTCGGGTGAACGT	10	N/A	55
RM228	F: CTGGCCATTAGTCCTTGG R: GCTTGCGGCTCTGCTTAC	10	N/A	55
RM552	F: CGCAGTTGTGGATTTCAAGTG R: TGCTCAACGTTTGACTGTCC	11	200-210	55
RM206	F: CCCATGCGTTTAACTATTCT R: CGTTCCATCGATCCGTATGG	11	N/A	58.1
RM257	F: CAGTTCCGAGCAAGAGTACTC R: GGATCGGACGTGGCATATG	11	150-200	55

Gramene (<https://archive.gramene.org/markers/microsat/all-ssr.html>)

Table 3.3 continued

RM224	F: ATCGATCGATCTTCACGAGG R: TGCTATAAAAGGCATTCGGG	11	150- 200	55
RM21	F: ACAGTATTCCGTAGGCACGG R: GCTCCATGAGGGTGGTAGAG	11	140- 180	51
RM247	F: TAGTGCCGATCGATGTAACG R: CATATGGTTTTGACAAAGCG	12	140- 190	55
RM17	F: TGCCCTGTTATTTTCTTCTCTC R: GGTGATCCTTTCCCATTTCA	12	190- 220	55
RM519	F: AGAGAGCCCCTAAATTTCCG R: AGGTACGCTCACCTGTGGAC	12	120- 140	55
RM561	F: GAGCTGTTTTGGACTACGGC R: GAGTAGCTTTCTCCACCCC	N/ A	N/A	55

* **Ch**: Chromosome; **EPS**: Expected product size in base pair; **Ta**: Annealing temperature in degree Celsius; **F**: Forward primer; **R**: Reverse primer; "I": Four chosen SSR primers used for pre-selection. N/A indicates data not available.

3.1.4 Pre-selection of Rice Accessions

From the 12 randomly selected SSR markers, four markers (RM1, RM279, RM489 and RM335), which showed polymorphisms between the two randomly chosen accessions, were used for pre-selection to reduce the number of accessions for further characterization. Individuals with polymorphic genotype (Table 4.1) from every landrace based on the four SSR markers were chosen for further evaluation. For a landrace with 10 seedlings of monomorphic genotype, only one individual was selected randomly. The chosen accessions were transplanted into pots. with drainage holes drilled on the side at about 8 cm from soil surface to allow water flow. The pots were watered once a day in the evening until the water level ran out of the drainage holes. Second fertilizer application were done 30-40 days from the first application. Third application later took place during flowering stage. The pots were arranged randomly in a net house located in Faculty of Resource Science and Technology, Universiti Malaysia Sarawak.

3.2 Morphological Characterization

A total of 15 morphological traits selected from the International Rice Research Institute guidelines (IRRI, 1980) and ten additional traits upon observation (Total = 25 morphological traits), were used to score the chosen accessions grown in the net house. The scoring methods and criteria of the 25 traits are as given in Table 3.4.

Table 3.4: The 25 Morphological Traits Selected Based on IRRI Guidelines Including 10 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 characterized by the emergence of the flag leaf.
*Flowering days	FD	Flowering day is recorded when the flower opens and that 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 mid-portion 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.

Table 3.4 continued

Blade colour	BC	Six broad classes of blade colour were recognized following (IRRI, 1980) guidelines: (1) pale green; (2) green; (3) dark green; (4) purple tips; (5) purple margins and (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.
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 spikelets were classified as: (0) absent; (1) light and (2) heavy.
*Number of filled grains	NFG	Measured by counting the number of fully filled grains of the main panicle from main tiller.
*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.

Table 3.4 continued

Grain width	GW	The widest portion of grain middle section 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.

* Symbol “*” indicated the ten additional traits added upon observation.

3.3 Anatomical Characterization

Anatomical samples such as leaves, midribs and roots were prepared based on Cutler et al. (1978). For the preparation of leaf sample, the middle portion of the fifth leaf was selected and hand-sectioned by using a scalpel. The leaf sections were soaked in 70% Clorox (bleach) solution, to remove the chlorophyll. The leaf sections were rinsed with distilled water and stained using Safranin O. The red-stained leaf sections were dehydrated by soaking in 50% ethanol for 3-5 minutes and later rinsed with distilled water. The dehydration process was repeated with 60%, 70%, 80%, 90% and finally 95% ethanol.

The midrib of the fifth leaf was carefully hand-sectioned using a microtome blade. The midrib sections were processed using the same protocol as described for leaf sample preparation. The midrib was stained using Alcian Blue (Rios et al., 2016). Dehydration was performed using 70% ethanol added with a drop of 1% hydrochloric acid (HCl). For root sample preparation, the mid-section of root was selected randomly and hand-sectioned using microtome blade and the process of staining and dehydration was following the protocol for midrib sample preparation.

Microscopic slides were prepared using wet mount techniques by 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 drew out with paper towel. For leaf samples, at least three slides were observed and two slides each for midrib and root samples. Clear cross-sectioned of leaf, midrib and root tissues were photographed using Olympus DP72 camera attached to Olympus BX151 light microscope. A Java-based image processing program called ImageJ was used to process all anatomical measurements (Schneider et al., 2012) in epidermal, midrib and root.

3.3.1 Leaf Sample Preparation

Epidermal characters of leaves, specifically the size of the long cell (length and width), the cell shape, cell arrangement and cell outline were studied under light microscope (Olympus BX15).

3.3.2 Stomatal Complex Observation

For stomatal analysis, characters such as the size (length and width), shape of subsidiary cell and number of stomata were measured and recorded. The Stomata Density (SD) were calculated (Equation 3.1) based on Paul et al. (2017).

Stomata Density is the number of stomata per unit area:

$$\text{Stomata Density (SD)} = \frac{\text{Total number of stomata}}{A=\pi r^2} \quad \text{Equation 3.1}$$

Where A= Area (mm²)

$\pi= 3.142$

r= Radius (mm²)

3.3.3 Midrib Sample Preparation

The midrib of the fifth leaf was carefully hand-sectioned using a microtome blade. The midrib sections were processed using the same protocol as described for leaf sample preparation. The midrib was stained using Alcian Blue. Dehydration was performed using 70% ethanol added with a drop of 1% HCl. As for midrib, the length and width were measured and shape were described.

3.3.4 Root Sample Preparation

For root sample preparation, the mid-section of root was selected randomly and hand-sectioned using microtome blade and the process of staining and dehydration was following the protocol for midrib sample preparation (Section 3.3.3). Diameter of the root and stele were recorded and the shape were described.

3.3.5 Observation on S1 Generation

Upon completed characterizing first generation, S_1 seeds from 10 accessions (Table 4.2) were planted in triplicates (three plants per accession). A total of 30 plants were planted in pots and placed in a net house. The plants were fertilized and watered following the same conditions as the first generation. Morphological observations as in Table 3.4 were recorded. Meanwhile, only leaves were observed under anatomical observation as midrib and root sample were not available for observation. Normality test was conducted and One-way Analysis of Variance (ANOVA) followed by Tukey Honestly Significant Difference (HSD) post hoc test was performed using Statistical Package for the Social Sciences (IBM SPSS) Statistics Version 20 for nine quantitative characters; seedling height, plant height, culm

length, culm diameter, number of tillers, leaf length, leaf width, heading days and flowering days. Kruskal-Wallis test followed by multiple comparisons was done on seven quantitative traits; number of panicles, length of panicle, number of filled grains, seed length, seed width, grain length and grain width.

3.4 Simple Sequence Repeat Genotyping

The DNA of chosen accessions from 11 lowland landraces were further genotyped with 39 SSR markers (Table 3.3) following similar PCR amplification in Section 3.1.3. Amplified products were electrophoresed, agarose gel was stained with EtBr and viewed under UV transilluminator. Amplified bands were observed and only clear bands were scored (1) as present and (0) as absent.

3.4.1 Polymorphic Information Content Values and Genetic Diversity

The scored bands were pooled to create a binary data matrix. The Polymorphic Information Content (PIC) were calculated (Equation 3.2) following Anderson et al. (1993).

$$PIC_i = 1 - \sum P_{ij}^2 \quad \text{Equation 3.2}$$

Where i is the total number of alleles detected for SSR marker and P_{ij} is the frequency of the j^{th} allele for marker i .

The genetic distances as well as Analysis of Molecular Variation where number of different alleles (N_a), number of effective alleles (N_e), Nei's gene diversity (h) and Shannon's Information Index (I) were calculated in Genetic Analysis in Excel (GenAEx) version 6.51 (Smouse et al., 2017).

3.4.2 Cluster Analysis Based on SSR

A dendrogram was constructed based on genetic distances matrices using Unweighted Pair Group Method with Arithmetic Means (UPGMA), performed using Molecular Evolutionary Genetics Analysis version 10.1 (MEGA X v. 10.1) (Tamura et al., 2011).

3.5 *Maturase-K* as Barcoding Marker

The DNA of chosen accessions from 11 lowland landraces were further amplified with barcoding marker, *Maturase-K* (*matK*). The *matK* region was amplified by using forward primer: 5'-TAA TTA AGA GGA TTC ACC AG-3' and reverse primer: 5'-ATG CAA CAC CCT GTT CTG AC -3'. The PCR protocols were based on Patil et al. (2015) with expected product range size 1500 bp.

The total reaction volume was 25 µL volume containing 3-5 ng of genomic DNA, 0.5 µM each of forward and reverse primers, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1X PCR buffer, 2.5 unit of Taq DNA polymerase (Apical Scientific Sdn. Bhd.). PCR amplification of *matK* consisted of initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57.8°C for 2 min, extension at 72°C for 2 min and final extension at 72°C for 15 min.

The PCR products were electrophoresed in a 1.5% agarose gel, stained with EtBr and viewed under UV transilluminator. A single bright amplicon was eluted using QIAquick Gel Extraction Kit (QIAGEN) following the provided protocol. The PCR amplified DNA fragments were subjected for two-way sequencing by Apical Scientific Sdn. Bhd.

3.5.1 Cluster Analysis Based on *matK*

The *matk* sequences from the chosen accessions were manually edited in BioEdit software. Additional 94 *matk* sequences of *Oryza sativa* were downloaded from GenBank database at National Centre for Biotechnology Information (NCBI) for phylogenetic analysis (Table 3.5). One to three *matk* sequences of *O. rufipogon*, *O. punctata*, *O. granulata*, *O. australiensis* and *O. officinalis* were also downloaded (Table 3.5). Using MEGA X v. 10.1, *matk* sequences of the 22 chosen lowland rice accessions, along with 22 unpublished *matk* sequences of upland rice accessions from UNIMAS collection and the sequences from NCBI were aligned (Table 3.5). Phylogenetic tree was constructed using Maximum Likelihood with Tamura 3-parameter model evolutionary rates among sites with discrete gamma distribution (T92+G) at 1000 bootstrap value (Tamura et al, 2011).

Table 3.5: The Accession Number for *matK* Sequences Downloaded From NCBI.

Species	Identification Code	Country of Origin	GenBank Accession Number
<i>O. sativa</i>	OS India 1	India	KT894765.1
<i>O. sativa</i>	OS India 2	India	KT894764.1
<i>O. sativa</i>	OS India 3	India	KT894763.1
<i>O. sativa</i>	OS India 4	India	KT894762.1
<i>O. sativa</i>	OS India 5	India	KT894761.1
<i>O. sativa</i>	OS India 6	India	KT894760.1
<i>O. sativa</i>	OS India 7	India	KT894759.1
<i>O. sativa</i>	OS India 8	India	KT894758.1
<i>O. sativa</i>	OS India 10	India	KT894756.1
<i>O. sativa</i>	OS India 11	India	KF731111.1
<i>O. sativa</i>	OS India 12	India	KF731110.1
<i>O. sativa</i>	OS India 13	India	KF731109.1
<i>O. sativa</i>	OS India 14	India	KF731108.1
<i>O. sativa</i>	OS India 15	India	KF731107.1
<i>O. sativa</i>	OS India 16	India	KF731106.1
<i>O. sativa</i>	OS India 17	India	KF731105.1
<i>O. sativa</i>	OS India 18	India	KF731104.1
<i>O. sativa</i>	OS India 19	India	KF731103.1
<i>O. sativa</i>	OS India 20	India	KF731102.1
<i>O. sativa</i>	OS India 21	India	KF731101.1

Table 3.5 continued

<i>O. sativa</i>	OS India 22	India	KF731100.1
<i>O. sativa</i>	OS India 23	India	KF731099.1
<i>O. sativa</i>	OS India 24	India	KF731098.1
<i>O. sativa</i>	OS India 25	India	KF731097.1
<i>O. sativa</i>	OS India 26	India	KF731096.1
<i>O. sativa</i>	OS India 27	India	KF731095.1
<i>O. sativa</i>	OS India 28	India	KF731094.1
<i>O. sativa</i>	OS India 29	India	KF731093.1
<i>O. sativa</i>	OS India 30	India	KF731092.1
<i>O. sativa</i>	OS India 31	India	KF731091.1
<i>O. sativa</i>	OS India 32	India	KF731090.1
<i>O. sativa</i>	OS India 33	India	KF731089.1
<i>O. sativa</i>	OS India 34	India	KF731088.1
<i>O. sativa</i>	OS India 35	India	KF731087.1
<i>O. sativa</i>	OS India 36	India	KF731086.1
<i>O. sativa</i>	OS India 37	India	KF731085.1
<i>O. sativa</i>	OS India 38	India	KF731084.1
<i>O. sativa</i>	OS India 39	India	KF731083.1
<i>O. sativa</i>	OS India 40	India	KF731082.1
<i>O. sativa</i>	OS India 41	India	KF731081.1
<i>O. sativa</i>	OS India 42	India	KF731080.1
<i>O. sativa</i>	OS India 43	India	KF731079.1
<i>O. sativa</i>	OS India 44	India	KF731078.1
<i>O. sativa</i>	OS India 45	India	KF731077.1
<i>O. sativa</i>	OS India 46	India	KF731074.1
<i>O. sativa</i>	OS India 47	India	KF731073.1
<i>O. sativa</i>	OS India 48	India	KF731072.1
<i>O. sativa</i>	OS India 49	India	KF731071.1
<i>O. sativa</i>	OS India 50	India	KF731070.1
<i>O. sativa</i>	OS India 51	India	KF731069.1
<i>O. sativa</i>	OS India 52	India	KF731068.1
<i>O. sativa</i>	OS India 53	India	KF731067.1
<i>O. sativa</i>	OS India 54	India	KF731066.1
<i>O. sativa</i>	OS India 55	India	KF731065.1
<i>O. sativa</i>	OS India 56	India	KF731064.1
<i>O. sativa</i>	OS India 57	India	KF731063.1
<i>O. sativa</i>	OS India 58	India	KF731062.1
<i>O. sativa</i>	OS India 59	India	KF731061.1
<i>O. sativa</i>	OS India 60	India	KF731060.1
<i>O. sativa</i>	OS India 61	India	KF731059.1
<i>O. sativa</i>	OS India 62	India	KF731058.1
<i>O. sativa</i>	OS India 63	India	KF731057.1
<i>O. sativa</i>	OS India 64	India	KF731056.1
<i>O. sativa</i>	OS India 65	India	KF731055.1
<i>O. sativa</i>	OS India 66	India	KF731054.1
<i>O. sativa</i>	OS India 67	India	KF731053.1
<i>O. sativa</i>	OS India 68	India	KF731052.1

Table 3.5 continued

<i>O. sativa</i>	OS India 69	India	KF731051.1
<i>O. sativa</i>	OS India 70	India	KF731050.1
<i>O. sativa</i>	OS India 71	India	KF731049.1
<i>O. sativa</i>	OS India 72	India	KF731048.1
<i>O. sativa</i>	OS India 73	India	KF731047.1
<i>O. sativa</i>	OS India 74	India	KF731046.1
<i>O. sativa</i>	OS India 75	India	KF731045.1
<i>O. sativa</i>	OS India 76	India	KF731044.1
<i>O. sativa</i>	OS India 77	India	KF731043.1
<i>O. sativa</i>	OS India 78	India	KF731042.1
<i>O. sativa</i>	OS India 79	India	KF731041.1
<i>O. sativa</i>	OS India 80	India	KF731040.1
<i>O. sativa</i>	OS India 81	India	KF731039.1
<i>O. sativa</i>	OS India 82	India	KF731038.1
<i>O. sativa</i>	OS India 83	India	KF731037.1
<i>O. sativa</i>	OS India 84	India	KF731036.1
<i>O. sativa</i>	OS India 85	India	KF731035.1
<i>O. sativa</i>	OS India 86	India	KF731034.1
<i>O. sativa</i>	OS India 87	India	KF731033.1
<i>O. sativa</i>	OS India 88	India	KF731032.1
<i>O. sativa</i>	OS India 89	India	HG794000.1
<i>O. sativa</i>	OS Australia 1	Australia	KU923990.1
<i>O. sativa</i>	OS Australia 2	Australia	KU923989.1
<i>O. sativa</i>	OS Australia 3	Australia	KU923988.1
<i>O. sativa</i>	OS Australia 4	Australia	KU923987.1
<i>O. sativa</i>	OS China 1	China	AF148650.1
<i>O. sativa</i>	OS Unknown	Unknown	EU434287.1
<i>O. rufipogon</i>	<i>O. rufipogon</i> 1	Unknown	EU434286.1
<i>O. rufipogon</i>	<i>O. rufipogon</i> 2	India	FJ908261.1
<i>O. rufipogon</i>	<i>O. rufipogon</i> 3	China	AF148651.1
<i>O. punctata</i>	<i>O. punctata</i> 1	Unknown	KP864529.1
<i>O. punctata</i>	<i>O. punctata</i> 2	Unknown	KP864528.1
<i>O. punctata</i>	<i>O. punctata</i> 3	Unknown	KP864527.1
<i>O. officinalis</i>	<i>O. officinalis</i> 1	Philippines	AF148658.1
<i>O. officinalis</i>	<i>O. officinalis</i> 2	China	KP121859.1
<i>O. officinalis</i>	<i>O. officinalis</i> 3	Papua New Guinea	KP121858.1
<i>O. australiensis</i>	<i>O. australiensis</i> 1	Australia	AF148667
<i>O. granulata</i>	<i>O. granulata</i> 1	China	AF148674.1
<i>O. granulata</i>	<i>O. granulata</i> 2	Vietnam	KP121857.1
<i>O. sativa</i>	UNIMAS-01	Malaysia	-
<i>O. sativa</i>	UNIMAS-02	Malaysia	-
<i>O. sativa</i>	UNIMAS-03	Malaysia	-
<i>O. sativa</i>	UNIMAS-04	Malaysia	-
<i>O. sativa</i>	UNIMAS-05	Malaysia	-
<i>O. sativa</i>	UNIMAS-06	Malaysia	-

Table 3.5 continued

<i>O. sativa</i>	UNIMAS-07	Malaysia	-
<i>O. sativa</i>	UNIMAS-08	Malaysia	-
<i>O. sativa</i>	UNIMAS-09	Malaysia	-
<i>O. sativa</i>	UNIMAS-10	Malaysia	-
<i>O. sativa</i>	UNIMAS-11	Malaysia	-
<i>O. sativa</i>	UNIMAS-12	Malaysia	-
<i>O. sativa</i>	UNIMAS-13	Malaysia	-
<i>O. sativa</i>	UNIMAS-14	Malaysia	-
<i>O. sativa</i>	UNIMAS-15	Malaysia	-
<i>O. sativa</i>	UNIMAS-16	Malaysia	-
<i>O. sativa</i>	UNIMAS-17	Malaysia	-
<i>O. sativa</i>	UNIMAS-18	Malaysia	-
<i>O. sativa</i>	UNIMAS-19	Malaysia	-
<i>O. sativa</i>	UNIMAS-20	Malaysia	-
<i>O. sativa</i>	UNIMAS-21	Malaysia	-
<i>O. sativa</i>	UNIMAS-22	Malaysia	-

CHAPTER 4

RESULTS

4.1 Pre-selection of Accession

From a total of 110 accessions germinated, 22 accessions representing each of the 11 lowland landraces collected were selected based on four Simple Sequence Repeat (SSR) markers (RM1, RM279, RM489 and RM335) (Table 4.1) for further observation. The SSR marker RM279 and RM335 were not used for pre-selection in few landraces and noted as not available (Table 4.1) due to poor amplification and unclear banding.

The 22 selected lowland accessions were designated as UNIMAS-23 to UNIMAS-44, called as first generation accessions (Table 4.2). Also characterised were 10 accessions in S_1 generation (Table 4.2).

Table 4.1: The SSR Genotype of 22 Selected Lowland Rice Accessions Based on Four SSR Markers.

Landrace	Selected Accession	SSR Marker*			
		RM1	RM279	RM335	RM489
UNISR2018-02	UNIMAS-23	0	1	1	N/A
	UNIMAS-24	1	1	1	1
UNISMT 2018-21	UNIMAS-25	1	1	1	1
	UNIMAS-26	3	3	3	2
UNIPDW 2018-18	UNIMAS-27	1	N/A	N/A	1
UNISRA 2018-24	UNIMAS-28	0	N/A	N/A	1
UNISRA 2018-38	UNIMAS-29	1	N/A	N/A	1
UNISAMA 2018- 44	UNIMAS-30	1	N/A	N/A	1

Table 4.1 continued

UNISAMA 2018- 45	UNIMAS-31	3	N/A	N/A	0
	UNIMAS-32	3	N/A	N/A	1
UNISRA 2018-40	UNIMAS-33	1	1	N/A	1
	UNIMAS-34	1	1	N/A	0
UNISAMA 2018- 47	UNIMAS-35	2	1	N/A	0
	UNIMAS-36	3	1	N/A	1
	UNIMAS-37	5	1	N/A	1
	UNIMAS-38	4	1	N/A	2
	UNIMAS-39	0	0	N/A	1
	UNIMAS-40	1	1	N/A	1
UNIPDW2018- 48	UNIMAS-41	1	0	N/A	1
	UNIMAS-42	1	0	N/A	1
UNISRA 2018-26	UNIMAS-43	1	2	N/A	1
	UNIMAS-44	1	1	N/A	1

* Scoring number indicates grouping based on allele scoring. **N/A** indicates data not available.

Table 4.2: List of 11 Collected Landraces, the 22 Chosen Accessions and 10 Selected S₁ Accessions.

Landrace	First generation accession	Selected S ₁ generation
UNISR2018-02 Padi Pandan	UNIMAS-23	UNIMAS-23-S1
UNISMT 2018-21 Padi Hitam	UNIMAS-24	UNIMAS-24-S1
	UNIMAS-25	N/A
	UNIMAS-26	N/A
UNIPDW 2018-18 Padi Merah	UNIMAS-27	UNIMAS-27-S1
UNIPDW2018- 48 Padi Kihuai	UNIMAS-40	N/A
	UNIMAS-41	N/A
UNISAMA 2018- 44 Padi Bario	UNIMAS-30	UNIMAS-30-S1

Table 4.2 continued

UNISAMA 2018- 45	UNIMAS-31	UNIMAS-31-S1
Padi Sabak Hitam	UNIMAS-32	UNIMAS-32-S1
UNISAMA 2018- 47	UNIMAS-35	N/A
	UNIMAS-36	N/A
	UNIMAS-37	UNIMAS-37-S1
	UNIMAS-38	N/A
	UNIMAS-39	N/A
UNISRA 2018-38	UNIMAS-29	UNIMAS-29-S1
Padi Arang		
UNISRA 2018-40	UNIMAS-33	N/A
Padi Selasih	UNIMAS-34	N/A
UNISRA 2018-26	UNIMAS-42	N/A
	UNIMAS-43	UNIMAS-43-S1
	UNIMAS-44	N/A
UNISRA 2018-24	UNIMAS-28	UNIMAS-28-S1
Padi Bajong		

* N/A indicates data not available.

4.2 Characterisation Based on Morphological Traits

A total of 25 morphological traits, which comprised of nine qualitative and 16 quantitative traits were characterised. Morphological observation was made on the 22 rice accessions in first generation and later made on 10 accessions at S₁ generation based on qualitative and quantitative traits.

4.2.1 Qualitative Traits

The nine qualitative observed for accessions in first generation and S₁ generation are tabulated in Table 4.3 and Table 4.4, respectively.

Table 4.3: The Nine Qualitative Traits of 22 Lowland Rice Accession in First Generation.

Accession	BC	LS	LC	AC	PT	SB	SS	SC	GC
UNIMAS-23	1	1	1	1	2	0	2	4	1
UNIMAS-24	3	1	1	1	2	0	2	2	4
UNIMAS-25	1	1	1	1	2	1	2	2	3
UNIMAS-26	1	1	1	1	2	1	2	2	4
UNIMAS-27	1	1	1	1	2	2	2	3	1
UNIMAS-28	1	1	1	1	2	0	4	2	3
UNIMAS-29	3	2	1	1	2	1	2	3	4
UNIMAS-30	1	2	1	1	2	1	4	1	1
UNIMAS-31	1	1	1	1	3	1	4	2	2
UNIMAS-32	2	2	1	1	2	1	4	1	2
UNIMAS-33	1	2	1	1	N/A	N/A	N/A	N/A	N/A
UNIMAS-34	1	1	1	1	N/A	N/A	N/A	N/A	N/A
UNIMAS-35	3	1	1	1	2	1	4	2	2
UNIMAS-36	3	1	1	1	2	1	4	2	2
UNIMAS-37	3	1	1	1	1	2	2	2	2
UNIMAS-38	3	1	1	1	1	1	4	2	6
UNIMAS-39	3	1	1	1	3	1	4	2	2
UNIMAS-40	3	1	1	1	N/A	N/A	N/A	N/A	N/A
UNIMAS-41	2	1	1	1	N/A	N/A	N/A	N/A	N/A
UNIMAS-42	1	1	1	1	3	1	4	1	1
UNIMAS-43	1	2	1	1	2	1	4	1	1
UNIMAS-44	1	1	1	1	N/A	N/A	N/A	N/A	N/A

* **BC:** Blade colour (1= pale green, 2= green, 3= dark green, 4= purple tips, 5= purple margins, 6= purple blotch); **LS:** Ligule shape (1= acute to acuminate, 2= 2-cleft, 3; truncate); **LC:** Ligule colour (1= white, 2= purple lines, 3= purple); **AC:** Auricle colour (1= white, 2= purple, 3= pale green); **PT:** Panicle type (1= compact, 2= intermediate, 3= open); **SB:** Secondary Branching (0= absent, 1= light, 2= heavy); **SS:** Seed shape (1= round, 2= oblong, 3= elliptic, 4= linear); **SC:** Seed colour (1= yellow, 2= pale yellow, 3= blackish yellow, 4= golden brown); **GC:** Grain colour (1= Milky white, 2= reddish brown, 3= dark brown, 4= black, 5= greenish white, 6= light reddish brown). **N/A:** Not Available.

Table 4.4: The Nine Qualitative Traits of 10 Lowland Rice Accession in S₁ Generation.

Accession	BC	LS	LC	AC	PT	SB	SS	SC	GC
UNIMAS-23-S1	3	1	1	1	N/A	N/A	N/A	N/A	N/A
UNIMAS-24-S1	3*	1	1	1*	N/A	N/A	N/A	N/A	N/A
UNIMAS-27-S1	3	1	1	1	2*	2	2	3	1
UNIMAS-28-S1	3	1	1*	2^	2^^	0^^	4^^	2^^	3^^
UNIMAS-29-S1	3	2	1	1	2	1	2	3	4
UNIMAS-30-S1	3	2	1	1	2	1*	4	1	1
UNIMAS-31-S1	3	1	1	1	3 [!]	1*	4	2	2
UNIMAS-32-S1	3	2^	1	1	2	2	4	1	2
UNIMAS-37-S1	3	1	2	1	1^	2^	2^	2^	2^
UNIMAS-43-S1	3	2^	1	1	2	1	4	1	1

* **BC:** Blade colour (1= pale green, 2= green, 3= dark green, 4= purple tips, 5= purple margins, 6= purple blotch); **LS:** Ligule shape (1= acute to acuminate, 2= 2-cleft, 3; truncate); **LC:** Ligule colour (1= white, 2= purple lines, 3= purple); **AC:** Auricle colour (1= white, 2= purple, 3= pale green); **PT:** Panicle type (1= compact, 2= intermediate, 3= open); **SB:** Secondary Branching (0= absent, 1= light, 2= heavy); **SS:** Seed shape (1= round, 2= oblong, 3= elliptic, 4= linear); **SC:** Seed colour (1= yellow, 2= pale yellow, 3= blackish yellow, 4= golden brown); **GC:** Grain colour (1= Milky white, 2= reddish brown, 3= dark brown, 4= black, 5= greenish white, 6= light reddish brown). **N/A:** Not Available.

* in BC indicates one individual out of three replicates scored pale green.

^ in LS indicates one individual out of three replicates scored acute to acuminate.

* in LC indicates one individual out of three replicates scored purple lines.

* in AC indicates one individual out of three replicates scored purple.

^ in AC indicates one individual out of three replicates scored white.

* in PT indicates one individual out of three replicates scored open.

[!] in PT indicates one individual out of three replicates scored compact.

* in SB indicates one individual out of three replicates scored heavy.

^ in PT, SB, SS, SC and GC indicates one individual out of three replicates was not available. ^^ indicates two individuals unavailable.

There were three groups of BC can be observed among the accessions, which were (1) pale green, (2) green and (3) dark green (Figure 4.1). From the observed 22 rice accessions in the first generation, 12 accessions had pale green BC, eight recorded dark green and only two accessions (UNIMAS-32 and UNIMAS-41) recorded green BC. In S_1 generation, only dark green BC was recorded. Seven accessions in S_1 generation recorded different BC from first generation namely UNIMAS-23-S1, UNIMAS-27-S1, UNIMAS-28-S1 UNIMAS-30-S1, UNIMAS-31-S1, UNIMAS-32-S1 and UNIMAS-43-S1.

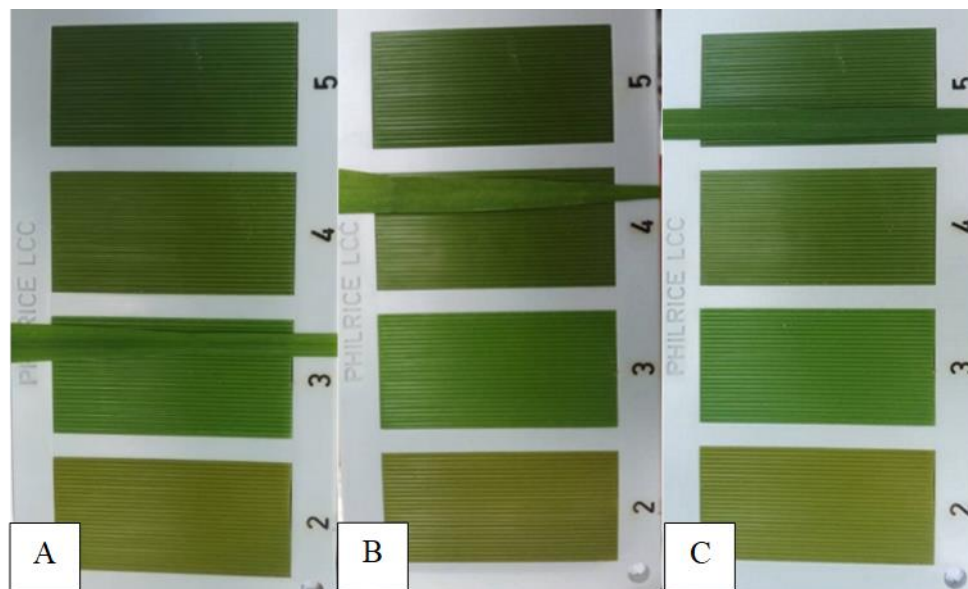


Figure 4.1: The Three Groups of Blade Colour. **A:** Pale green (UNIMAS-33); **B:** Green (UNIMAS-41); **C:** Dark green (UNIMAS-36).

The ligule shape (LS) varied for the observed rice accessions in first generation where two types of LS recorded: (1) acute to acuminate and (2) 2-cleft (Table 4.3). A total of 17 lowland rice accessions recorded as having acute to acuminate LS while five accessions had 2-cleft LS (Table 4.3). Ligule colour (LC) and auricle colour (AC), were not

polymorphic among the observed 22 accessions with only white-coloured LC and AC can be observed (Table 4.3, Figure 4.2).

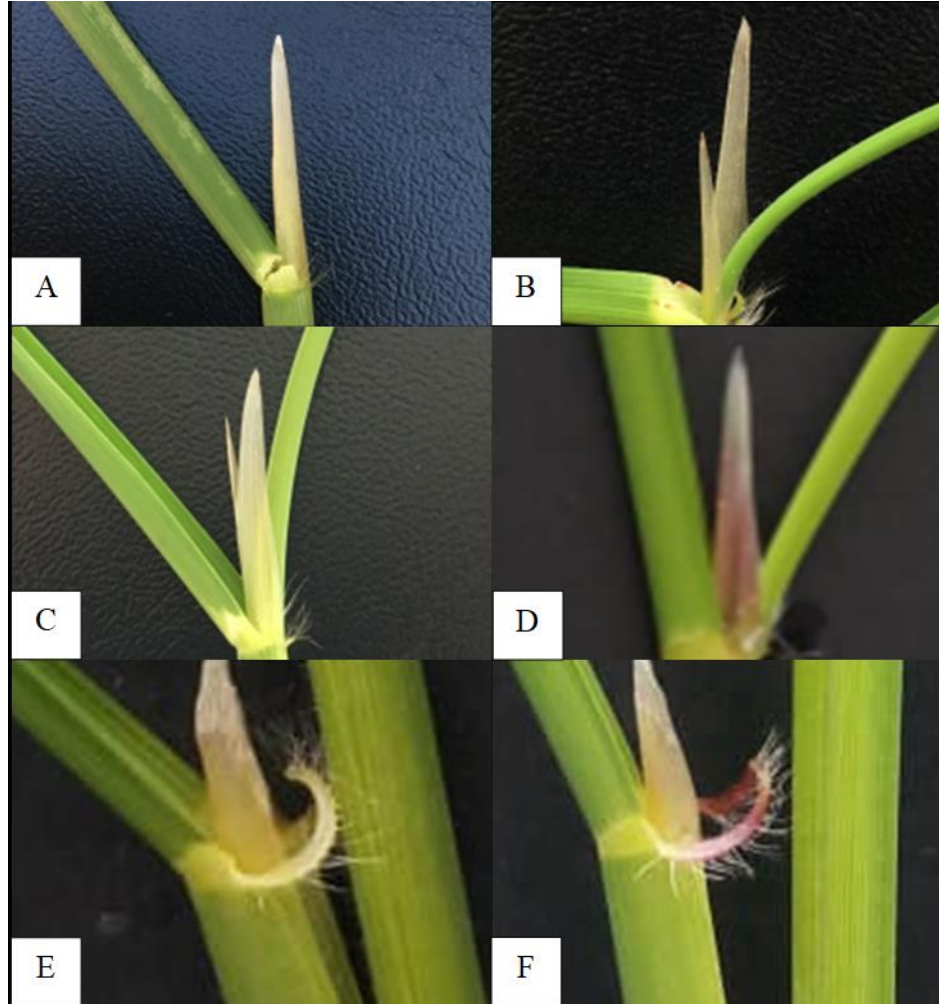


Figure 4.2: Representative of Ligule And Auricle. **A:** Acute to acuminate ligule shape (UNIMAS-31); **B:** 2-cleft ligule shape (UNIMAS-29); **C:** White ligule colour (UNIMAS-30); **D:** Purple lines ligule colour (UNIMAS-28-S1-A); **E:** White auricle (UNIMAS-23-S1-B); **F:** Purple auricle (UNIMAS-24-S1-C).

Accessions in S_1 generation also recorded the same two LS categories as in the first generation, with six accessions had acute to acuminate LS and the other four accessions had 2-cleft LS (Table 4.4). There was no difference in LS observed in first generation and S_1

generation. LC for the 10 accessions in S_1 generation observed two different colour which were, (1) white and (2) purple lines (Figure 4.2C and Figure 4.2D). There was no difference in LC in first generation and S_1 generation except for in UNIMAS-37- S_1 (Table 4.4), where purple line was exhibited (Figure 4.2D). No difference in AC recorded in nine accessions in S_1 generation except for UNIMAS-28- S_1 where purple AC (Figure 4.2F) can be observed.

The types of panicle (PT) separated the 22 accessions in first generation into three different groups: (1) compact, (2) intermediate and (3) open (Table 4.3, Figure 4.3). A total of 12 accessions had intermediate PT, three accessions (UNIMAS-31, UNIMAS-39 and UNIMAS-42) showed an open PT and two accessions (UNIMAS-37 and UNIMAS-38) with compact PT. Five other accessions, did not produce any panicle during the period of observation.

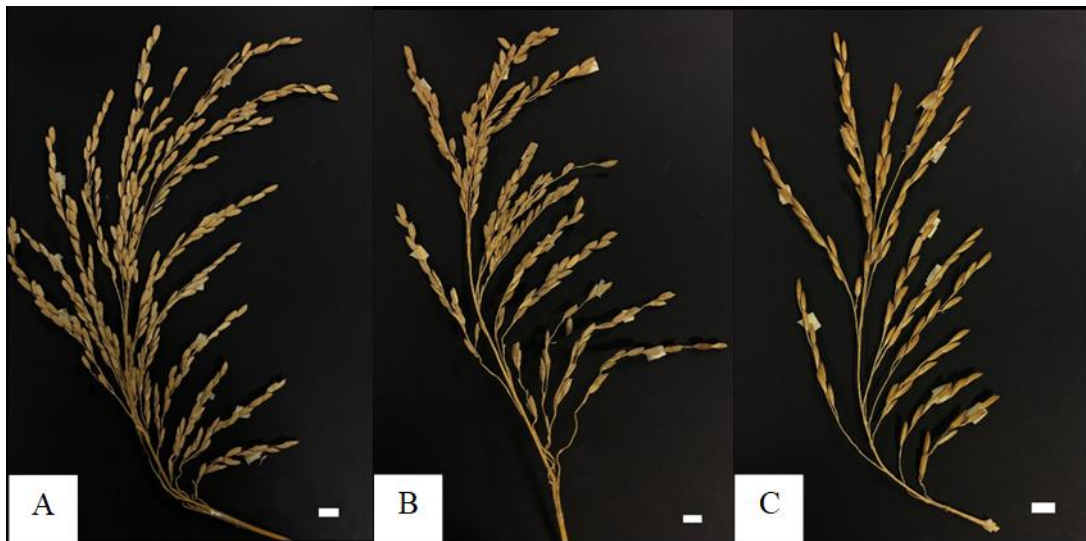


Figure 4.3: The Three Recorded Panicle Types. **A:** Compact (UNIMAS-38); **B:** Intermediate (UNIMAS-29); **C:** Open (UNIMAS-31). White bar indicates scale= 1 cm.

For the 10 accessions at S_1 generation, the three PT types were also observed as illustrated in Figure 4.3. There was no difference in PT between accessions in first and S_1 generation (Table 4.4). Nine out of 30 individuals in S_1 generation failed to produce panicle.

Secondary branches (SB) of panicles for accessions in the first generation was grouped into three types; (0) absent, (1) light and (3) heavy (Figure 4.4). The SB was absent, in three accessions (UNIMAS-23, UNIMAS-24 and UNIMAS-28), 12 accessions recorded light SB and remaining two accessions with heavy SB (UNIMAS-27 and UNIMAS-37) (Table 4.3). The remaining lowland rice accessions had light SB. Accessions in S_1 generation did not record any difference in SB from accessions in first generation (Table 4.4).



Figure 4.4: The Three Types of Recorded Secondary Branching. **A:** Absent (UNIMAS-28-S1-C); **B:** Light (UNIMAS-31-S1-B); **C:** Heavy (UNIMAS-30-S1-B). White bar indicates scale= 1cm.

First generation and S_1 generation both recorded two classes of seed shape (SS); (2) oblong and (4) linear (Figure 4.5). Seven accessions had oblong SS and ten accessions

yielded seeds with a linear SS in the first generation (Table 4.3). There was no variation observed in S_1 generation (Table 4.4)



Figure 4.5: Seed Morphology Recorded in Both First and S_1 Generation. **A:** Oblong (UNIMAS-37); **B:** Linear (UNIMAS-43); **C:** Yellow (UNIMAS-30); **D:** Pale yellow (UNIMAS-36); **E:** Blackish yellow (UNIMAS-27-S1-A); **F:** Golden brown (UNIMAS-23). White bar indicates scale= 1cm.

The 22 accessions in first generation have shown great variation in seed colour (SC) and grain colour (GC) (Table 4.3). The accessions were grouped into four categories of SC; (1) yellow, (2) pale yellow, (3) blackish yellow and (4) golden-brown husk colour (Figure 4.5). Ten out of 17 accessions exhibited pale yellow SC; four accessions with yellow SC, two accessions had blackish yellow SC and one accession with golden brown SC (Table 4.3). The same observation was recorded in S_1 generation (Table 4.4).

The accessions in first generation were grouped into five GC groups with five accessions had milky white GC, six accessions had reddish brown GC, two accessions exhibited dark brown grain, three accessions with black coloured grain and one accession observed with light reddish-brown GC (Table 4.3, Figure 4.6). Same GC result was obtained in S₁ generation (Table 4.4).



Figure 4.6: Grain Colours Observed in Both First Generation and S₁ Generation. **A:** Milky white (UNIMAS-43-S1-B); **B:** Reddish brown (UNIMAS-31); **C:** Dark brown (UNIMAS-28-S1-C); **D:** Black (UNIMAS-29); **E:** Light reddish brown (UNIMAS-38). White bar indicates scale = 1cm.

4.2.2 Quantitative Traits

The 16 quantitative traits in first generation and S₁ generation were recorded in Table 4.5 and 4.6, respectively.

Table 4.5: The 16 Quantitative Traits of 22 Lowland Rice Accession in First Generation.

Accession	SH (cm)	PH (cm)	CL (cm)	CD (cm)	NT	LL (cm)	LW (cm)	HD (days)	FD (days)	NP	LP (cm)	NFG	SL (cm)	SW (cm)	GL (cm)	GW (cm)
UNIMAS-23	51.4	168.2	113.5	3.5	4	53.2	1.5	125	154	3	35.6	320	1.0	0.3	0.5	0.2
UNIMAS-24	44.6	172.3	116.6	3.5	5	57.5	1.3	134	158	2	25.2	300	0.9	0.2	0.7	0.2
UNIMAS-25	40.3	172.6	108.3	3.2	4	62.8	1.1	134	158	1	24.5	250	0.7	0.3	0.6	0.2
UNIMAS-26	47.0	153.4	81.5	3.5	7	71.4	1.2	134	158	1	26.4	200	0.8	0.3	0.6	0.2
UNIMAS-27	46.8	174.0	101.5	3.0	5	70.9	1.6	119	145	2	30.1	159	0.8	0.3	0.5	0.2
UNIMAS-28	44.3	199.2	130.9	3.1	7	68.5	2.1	114	147	1	32.7	120	0.9	0.2	0.6	0.2
UNIMAS-29	54.5	187.5	114.6	3.4	6	74.8	1.9	130	159	6	28.3	660	1.0	0.4	0.6	0.2
UNIMAS-30	50.1	165.1	89.4	3.2	6	75.2	2.1	119	143	5	29.9	391	1.0	0.3	0.7	0.2
UNIMAS-31	48.9	173.6	121.6	3.4	6	54.1	2.2	112	140	8	24.6	690	1.1	0.2	0.7	0.2
UNIMAS-32	45.7	174.4	120.5	3.2	5	51.0	1.8	113	138	10	27.5	1091	1.0	0.2	0.7	0.2
UNIMAS-33	38.8	210.0	132.8	3.2	4	78.1	1.7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 4.5 continued

UNIMAS-34	42.4	225.3	143.4	3.3	6	80.3	1.6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
UNIMAS-35	47.2	176.0	94.50	3.1	5	80.5	1.8	103	138	2	32.6	118	1.0	0.3	0.7	0.2
UNIMAS-36	40.8	189.4	118.0	3.2	8	71.4	1.7	89	117	2	34.5	274	1.0	0.3	0.8	0.2
UNIMAS-37	46.5	180.1	119.2	3.0	5	59.3	1.5	86	115	5	31.4	1055	0.8	0.3	0.6	0.3
UNIMAS-38	43.3	175.2	116.7	3.2	6	60.7	2.1	83	116	3	30.3	493	0.8	0.3	0.6	0.3
UNIMAS-39	39.5	170.8	103.2	3.0	6	65.8	1.9	89	118	2	28.2	696	0.9	0.3	0.6	0.2
UNIMAS-40	40.2	164.3	111.0	3.4	5	55.5	1.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
UNIMAS-41	43.4	159.5	105.2	3.6	4	57.0	1.7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
UNIMAS-42	46.0	170.1	99.40	3.1	7	72.1	1.3	120	151	2	25.5	84	0.8	0.2	0.6	0.2
UNIMAS-43	48.2	173.0	106.1	3.0	8	70.7	1.3	111	138	7	29.6	630	0.9	0.2	0.6	0.2
UNIMAS-44	49.8	179.0	107.5	3.3	4	69.4	1.1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

***SH**: Seedling height in centimetres; **PH**: Plant height in centimetres; **CL**: Culm length in centimetres; **CD**: Culm diameter in centimetres; **NT**: Number of tillers; **LL**: Leaf length in centimetres; **LW**: Leaf width in centimetres; **HD**: Heading days; **FD**: Flowering days; **NP**: Number of panicles; **LP**: Length of panicle in centimetres; **NFG**: Number of filled grains; **SL**: Seed length; **SW**: Seed width; **GL**: Grain length; **GW**: Grain width. **N/A**: Not Available.

Table 4.6: The Nine Quantitative Traits of 10 Lowland Rice Accession in S₁ Generation Based on Analysis of Variance.

Quantitative traits	Accession									
	UNIMAS-23-S1	UNIMAS-24-S1	UNIMAS-27-S1	UNIMAS-28-S1	UNIMAS-29-S1	UNIMAS-30-S1	UNIMAS-31-S1	UNIMAS-32-S1	UNIMAS-37-S1	UNIMAS-43-S1
SH (cm)	52.23 ^b	41.63 ^{ab}	45.50 ^{ab}	39.73 ^a	50.20 ^{ab}	47.87 ^{ab}	46.83 ^{ab}	41.83 ^{ab}	44.53 ^{ab}	47.97 ^{ab}
PH (days)	148.43 ^a	153.53 ^{ab}	188.30 ^{bcd}	194.60 ^{cd}	200.70 ^d	216.40 ^d	181.03 ^{abcd}	160.60 ^{abc}	177.40 ^{abcd}	190.80 ^{bcd}
CL (cm)	79.67 ^a	80.33 ^a	116.73 ^{ab}	114.00 ^{ab}	131.17 ^b	132.27 ^b	123.43 ^{ab}	101.03 ^{ab}	105.37 ^{ab}	122.00 ^{ab}
CD (cm)	3.77 ^a	3.87 ^a	3.77 ^a	3.00 ^a	3.87 ^a	3.60 ^a	3.20 ^a	3.33 ^a	3.17 ^a	2.60 ^a
NT	5.67 ^a	4.67 ^a	4.67 ^a	5.67 ^a	5.33 ^a	6.33 ^a	5.00 ^a	6.33 ^a	6.00 ^a	5.33 ^a
LL (cm)	68.67 ^a	72.47 ^a	71.17 ^a	80.50 ^a	69.57 ^a	83.70 ^a	57.67 ^a	58.97 ^a	69.63 ^a	68.23 ^a
LW (cm)	1.87 ^a	1.73 ^a	2.20 ^a	2.10 ^a	2.30 ^a	2.10 ^a	2.03 ^a	2.47 ^a	1.77 ^a	1.73 ^a
HD (days)	139.00 ^b	48.33 ^a	94.33 ^{ab}	108.00 ^{ab}	119.00 ^{ab}	101.67 ^{ab}	100.33 ^{ab}	98.67 ^{ab}	98.67 ^{ab}	99.67 ^{ab}
FD (days)	168.00 ^b	58.67 ^a	125.33 ^{ab}	137.33 ^{ab}	148.67 ^{ab}	134.33 ^{ab}	130.00 ^{ab}	127.00 ^{ab}	131.00 ^{ab}	127.00 ^{ab}

***SH:** Seedling height in centimetres; **PH:** Plant height in centimetres; **CL:** Culm length in centimetres; **CD:** Culm diameter in centimetre; **NT:** Number of tillers; **LL:** Leaf length in centimetres; **LW:** Leaf width in centimetres; **HD:** Heading days; **FD:** Flowering days. Difference in morphological traits between accession were compared using Variance Analysis (ANOVA) followed by Tukey HSD Post Hoc Test. Numbers followed by different lowercase letters indicate significant different ($p < 0.05$).

Plant height at five leaves stage or seedling height (SH) of the 22 rice accessions in the first generation ranged from 38.80 cm (UNIMAS-33) to 54.50 cm (UNIMAS-29) (Table 4.5). Accessions in S₁ generation has mean SH ranging from 39.73 cm (UNIMAS-28-S1) to 52.23 cm (UNIMAS-23-S1) and was significantly different between accessions (Table 4.6).

At mature stage, the plant height (PH) ranged from 153.40 cm (UNIMAS-26) to 225.30 cm (UNIMAS-34) (Table 4.5). Meanwhile in S₁ PH mean value ranging from 148.43 cm (UNIMAS-23-S1) to 216.40 cm (UNIMAS-30-S1) (Table 4.6). Significant difference was observed for SH and PH between the accession accessions in S₁ generation (Table 4.6).

In the first generation, culm length (CL) measurements ranged from 81.50 cm (UNIMAS-26) to 143.40 cm (UNIMAS-34) (Table 4.5). Accessions in S₁ generation had a CL mean ranging from 79.67 cm (UNIMAS-23-S1) to 132.27 cm (UNIMAS-30-S1) and was significantly different between the accessions (Table 4.6).

Culm diameter (CD), ranged from 3.00 cm (UNIMAS-27, UNIMAS-37, UNIMAS-39, UNIMAS-43) to 3.60 cm (UNIMAS-41) (Table 4.5). Meanwhile CD in S₁ generation recorded mean ranged from 2.60 cm (UNIMAS-43-S1) to 3.87 cm (UNIMAS-24-S1 and UNIMAS-29-S1) (Table 4.6). CD was not significantly different in S₁ generation.

The number of tillers (NT) were counted with minimum of four tillers to eight tillers at most (Table 4.5), with 68.18% (15 accessions) from the 22 accessions in first generation were recorded having five to seven tillers. The NT for accessions in S₁ generation had a range mean from 4.67 to 6.33. There was no significant difference between accessions for NT (Table 4.6).

The leaf length (LL) of accessions in the first generation ranged from 51.00 cm (UNIMAS-32) to 80.50 cm (UNIMAS-35) (Table 4.5). The range mean of LL for accessions in S₁ generation was from 57.67 (UNIMAS-31-S1) to 83.70 cm (UNIMAS-30-S1) and no significant different was recorded (Table 4.6).

The leaf width (LW) measured in first generation ranging from 1.10 cm (UNIMAS-25 and UNIMAS-44) to 2.20 cm (UNIMAS-31) (Table 4.5). The LW in S₁ generation recorded range mean from 1.73 cm (UNIMAS-24-S1 and UNIMAS-43-S1) to 2.30 cm (UNIMAS-29-S1) (Table 4.6). The LW was not significantly different between the accessions in S₁ generation (Table 4.6).

The heading days (HD) of rice accessions in the first generation ranged from 83 (UNIMAS-38) to 134 days (UNIMAS-24, UNIMAS-25 and UNIMAS-26) (Table 4.5). Accessions in S₁ generation recorded mean HD ranging from 48.33 (UNIMAS-24-S1) to 139 days (UNIMAS-23-S1) (Table 4.6). The HD was statistically different between accessions in S₁ generation (Table 4.6).

Flowering days (FD) in first generation took around 115 days (UNIMAS-37) to 159 days (UNIMAS-29) after germination (Table 4.5). There were, however, five accessions (UNIMAS-33, UNIMAS-34, UNIMAS-40, UNIMAS-41 and UNIMAS-44) did not flower at the time of data collection. Meanwhile, FD mean ranged from 58 days (UNIMAS-24-S1) to 168 days (UNIMAS-23-S1) in S₁ generation (Table 4.6). FD in S₁ generation were statistically different between accessions (Table 4.6).

UNIMAS-32 had the highest number of panicles (NP) among the accessions in the first generation, which was 10 panicles per plant (Table 4.5). The least number of panicles produced per plant was one, which was recorded in few accessions namely UNIMAS-25,

UNIMAS-26, UNIMAS-28 and UNIMAS-41. In S₁ generation, there were however, two accessions (UNIMAS-23-S1 and UNIMAS-24-S1) and three individuals (UNIMAS-28-S1-A, UNIMAS-28-S1-B and UNIMAS-37-S1-C) failed to produce a healthy panicle for observation due to attack by rice bugs. The mean range for NP in S₁ generation was from 3.33 to 7.00 (Table 4.7). The NP was not statistically significant across accessions in S₁ generation.

Table 4.7: The Seven Quantitative Traits in S₁ Generation Based on Kruskal-Wallis Test.

Accession	NP	LP	NFG	SL	SW	GL	GW
UNIMAS-27-S1	4.33 ^a	25.83 ^a	389.33 ^a	0.77 ^a	0.20 ^a	0.60 ^a	0.20 ^a
UNIMAS-28-S1	6.00 ^a	20.40 ^a	180.00 ^a	0.80 ^a	0.20 ^{ab}	0.60 ^a	0.20 ^a
UNIMAS-29-S1	3.33 ^a	22.87 ^a	206.33 ^a	0.87 ^a	0.30 ^b	0.60 ^a	0.20 ^a
UNIMAS-30-S1	5.67 ^a	28.13 ^a	437.33 ^a	1.00 ^a	0.20 ^a	0.77 ^a	0.20 ^a
UNIMAS-31-S1	5.33 ^a	23.93 ^a	366.33 ^a	1.00 ^a	0.20 ^a	0.70 ^a	0.20 ^a
UNIMAS-32-S1	3.33 ^a	22.97 ^a	359.67 ^a	1.00 ^a	0.20 ^a	0.73 ^a	0.20 ^a
UNIMAS-37-S1	6.00 ^a	27.95 ^a	818.50 ^a	0.85 ^a	0.20 ^{ab}	0.60 ^a	0.20 ^a
UNIMAS-43-S1	7.00 ^a	26.80 ^a	573.67 ^a	0.93 ^a	0.20 ^a	0.67 ^a	0.20 ^a

* **NP:** Number of panicles; **LP:** Length of panicle in centimetres; **NFG:** Number of filled grains; **SL:** Seed length in centimetres; **SW:** Seed width in centimetres; **GL:** Grain length in centimetres; **GW:** Grain width in centimetres. Difference in morphological traits between accession were compared using Kruskal-Wallis One Way ANOVA followed by multiple comparisons. Numbers followed by different superscript lowercase letters indicate significant different ($p < 0.05$).

The length of panicle (LP) for accessions in the first generation ranged from 24.50 cm (UNIMAS-25) to 35.60 cm (UNIMAS-23) (Table 4.5). In S_1 generation, mean of LP ranged from 20.40 cm to 28.13 cm (Table 4.7). The LP was not statistically different between accessions in S_1 generation.

The numbers of filled grain (NFG) for accessions in the first generation (Table 4.5) ranged from 84 grains (UNIMAS-42) to 1091 grains (UNIMAS-32). Meanwhile the mean NFG for the eight accessions in S_1 generation ranged from 180.00 to 818.50 and was not statistically different between accessions (Table 4.7).

The seed length (SL) of accessions in the first generation ranged from 0.70 cm (UNIMAS-25) to 1.10 cm (UNIMAS-31) (Table 4.5). The mean SL in S_1 generation ranged from 0.77 cm to 1.00 cm and was not statistically different across accessions in S_1 generation (Table 4.7).

Seed width (SW) in first generation recorded measurement ranging from 0.20 cm (UNIMAS-24, UNIMAS-28, UNIMAS-31, UNIMAS-32, UNIMAS-42 and UNIMAS-43) to 0.40 cm (UNIMAS-29) (Table 4.5). The mean SW of accessions in S_1 generation ranged from 0.20 cm to 0.30 and was significantly different between accession (Table 4.7).

The grain length (GL) of the 22 rice accessions in the first generation ranged from 0.50 cm (UNIMAS-23 and UNIMAS-27) to 0.80 cm (UNIMAS-36) (Table 4.5). In S_1 generation, mean of GL ranged from 0.60 cm to 0.77 cm (Table 4.7). GL was not significantly different in S_1 generation.

A total of 15 accessions in first generation were recorded with range grain width (GW) from 0.20 cm to 0.30 cm (Table 4.5), recorded in accessions UNIMAS-37 and

UNIMAS-38. Meanwhile, mean GW in S₁ generation was 0.20 cm and was not significantly different between accessions (Table 4.7).

4.2.3 Morphological Traits in Distinguishing Accessions

Observation on the nine qualitative traits across 22 accessions in first generation have shown great variations in BC, LS, PT, SB, SS, SC and GC and were able to distinguish between accessions and groups of accessions. The LC and AC, however, did not manage to distinguish the accessions in first generation.

Comparison on qualitative traits between accessions in first generation and S₁ generation, however did not show variation except for in two respective traits. The LC and AC have watched variations in accessions between the two generations.

Quantitative traits had shown variation in all 16 traits used in characterising accessions in present study such as in SH, PH, CL, CD, NT, LL, LW, HD, FD, NT, LP, NFG, SL, SW, GL and GW.

There are informative characters which can be used for breeding purpose such as PT and SB which directly correlated with NFG. Current observation showed a plant having a compact PT and/or heavy SB produced high NFG. Thus, accessions with the said informative characters (UNIMAS-27, UNIMAS-37 and UNIMAS-38) can be considered for Sarawak rice breeding program for the purpose of increasing rice production.

4.3 Characterisation Based on Anatomical Traits

Anatomical observation was made on leaves on both sides (abaxial and adaxial), the epidermal cell and stomata of the accessions in both first and S₁ generations. The cross section of leaf midrib and root were only observed on the 22 rice accessions in first generation and not in S₁ generation because anatomical sample was not available for S₁ generation.

4.3.1 Epidermal Cell

Figure 4.7 illustrates the cell structures of leaf observed under light microscope. There was no difference in epidermal characters observed in all accessions for both generations, be in abaxial or adaxial epidermis. The long cells were rectangular in shape. Figure 7 illustrate the linear and elongate cell arrangement, with alternating long and short cell. Undulated cell outlines can be observed and papillae were found present in all leaves samples.

The length of long cell (LEPC) on abaxial (Ab) epidermis of accessions in first generation ranged from 53.20 µm (UNIMAS-32) to 79.66 µm (UNIMAS-29) (Table 4.8). The LEPC of adaxial (Ad) surface were from 47.94 µm (UNIMAS-37) to 104.94 µm (UNIMAS-28) (Table 4.8).

Meanwhile in S₁ generation, the mean LEPC of Ab epidermis ranged from 68.58 µm (UNIMAS-24-S1) to 97.53 µm (UNIMAS-23-S1) (Table 4.9). The mean LEPC of Ad surface in S₁ generation ranged from 71.22 µm (UNIMAS-29-S1) to 97.62 µm (UNIMAS-

23-S1) (Table 4.9). The LEPC on both Ab and Ad surfaces in S₁ generation were not statistically different between accessions.

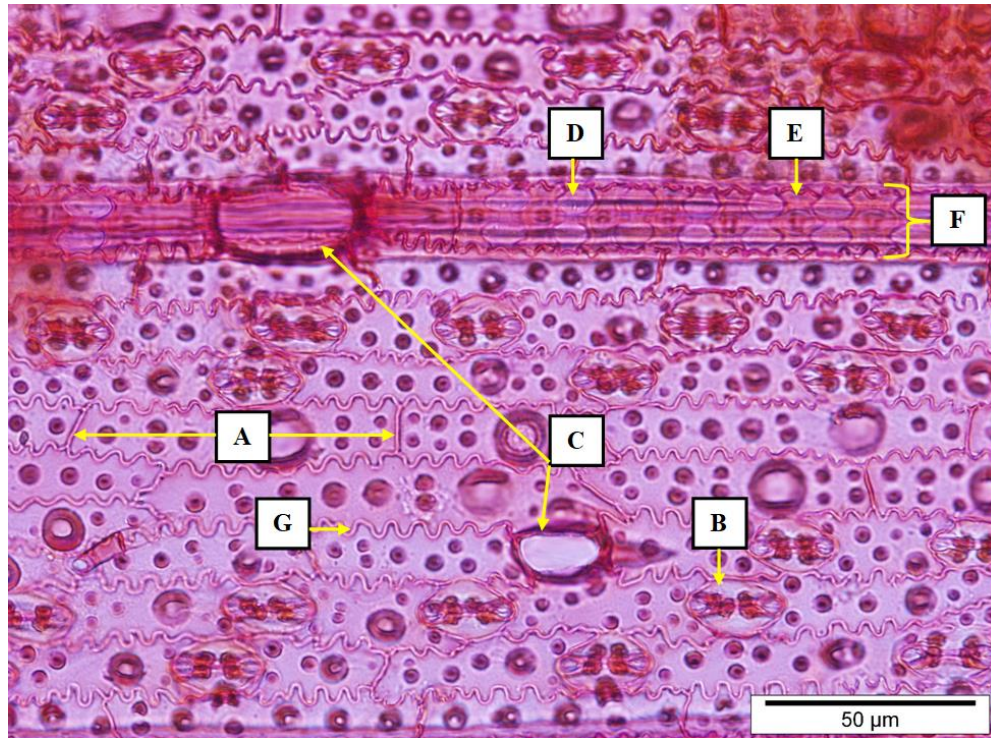


Figure 4.7: The Abaxial Surface of Leaf Anatomy of *O. sativa*. A: Rectangular long cell; B: Stomatal complex; C: Trichome; D: Silica cell; E: Phellem cell; F: Short cell row; G: Undulated cell outline. Magnification= 60x. Scale bar= 50 μm.

Table 4.8: Epidermal Traits of 22 Accessions in First Generation on Both Abaxial and Adaxial Surfaces.

Accession	Abaxial						Adaxial					
	LEPC (μm)	WEPC (μm)	SCS	LSC (μm)	WSC (μm)	SD (mm^2)	LEPC (μm)	WEPC (μm)	SCS	LSC (μm)	WSC (μm)	SD (mm^2)
UNIMAS-23	59.26	13.91	2	18.63	14.60	323	63.67	15.29	2	18.23	14.66	346
UNIMAS-24	64.17	15.19	2	18.91	14.59	500	80.57	12.83	2	21.48	15.93	269
UNIMAS-25	69.31	16.75	2	17.55	12.42	323	97.26	15.98	2	17.81	13.18	169
UNIMAS-26	76.95	13.21	2	18.40	14.52	385	58.02	11.90	2	19.64	13.20	315
UNIMAS-27	74.00	12.05	2	17.87	12.71	292	77.72	13.79	2	18.44	12.98	285
UNIMAS-28	65.78	17.55	2	21.64	19.03	262	104.94	12.95	2	20.09	16.74	169
UNIMAS-29	79.66	12.88	2	22.38	14.52	315	76.48	16.15	2	22.56	13.45	262
UNIMAS-30	65.08	15.30	2	19.09	15.53	454	69.71	13.36	2	21.24	15.80	254
UNIMAS-31	67.13	16.34	2	19.32	14.89	346	73.20	12.46	2	18.64	13.32	162
UNIMAS-32	53.20	17.95	2	18.31	14.68	346	77.80	14.52	2	21.85	14.52	231
UNIMAS-33	58.84	15.97	2	15.49	14.50	546	78.22	12.01	2	20.42	12.93	315
UNIMAS-34	66.22	15.75	2	16.63	14.29	292	84.55	13.04	2	16.78	13.67	308
UNIMAS-35	67.62	13.75	2	23.13	17.16	338	62.92	12.56	2	22.16	17.26	362
UNIMAS-36	69.66	14.49	2	20.46	13.01	300	92.60	14.17	2	26.20	16.57	162
UNIMAS-37	65.88	15.93	2	19.40	16.29	300	47.94	15.41	2	17.68	17.68	338
UNIMAS-38	62.29	12.98	2	18.08	15.57	415	48.38	14.01	2	19.55	15.58	185
UNIMAS-39	57.57	14.34	2	19.29	15.34	369	56.07	13.96	2	17.82	14.61	277
UNIMAS-40	70.18	13.87	2	19.64	15.46	415	55.48	14.96	2	17.96	13.77	385
UNIMAS-41	72.18	13.31	2	20.39	14.00	192	54.10	11.82	2	18.18	14.71	254
UNIMAS-42	66.82	12.46	2	19.04	12.62	469	60.57	11.49	2	19.02	13.94	215
UNIMAS-43	75.79	16.41	2	18.29	15.32	415	57.19	12.76	2	19.37	14.43	338
UNIMAS-44	68.64	13.16	2	17.31	15.04	377	53.82	10.46	2	17.39	13.54	246

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

Table 4.9: Epidermal Traits of 10 Accessions in S₁ Generation on Both Abaxial and Adaxial Surfaces.

Surface	Accession	LEPC (µm)	WEPC (µm)	SCS	LSC (µm)	WSC (µm)	SD (units/mm ²)
Abaxial	UNIMAS-23-S1	97.53 ^a	15.31 ^a	2	20.67 ^a	15.90 ^a	233.33 ^b
	UNIMAS-24-S1	68.58 ^a	15.31 ^a	2	23.46 ^a	20.02 ^{abc}	197.33 ^{ab}
	UNIMAS-27-S1	74.70 ^a	13.03 ^a	2	21.59 ^a	16.62 ^{ab}	225.67 ^b
	UNIMAS-28-S1	72.75 ^a	13.79 ^a	2	21.93 ^a	17.18 ^{abc}	210.33 ^b
	UNIMAS-29-S1	87.47 ^a	17.81 ^a	2	23.41 ^a	21.22 ^c	172.00 ^{ab}
	UNIMAS-30-S1	86.36 ^a	15.64 ^a	2	21.59 ^a	17.23 ^{abc}	192.33 ^{ab}
	UNIMAS-31-S1	75.18 ^a	15.91 ^a	2	24.52 ^a	17.73 ^{abc}	110.33 ^a
	UNIMAS-32-S1	69.07 ^a	15.93 ^a	2	20.93 ^a	17.09 ^{abc}	243.67 ^b
	UNIMAS-37-S1	72.71 ^a	16.84 ^a	2	22.88 ^a	20.28 ^{bc}	210.33 ^b
	UNIMAS-43-S1	69.59 ^a	15.10 ^a	2	21.84 ^a	19.03 ^{abc}	217.67 ^b
Adaxial	UNIMAS-23-S1	97.62 ^a	14.99 ^a	2	19.10 ^a	13.57 ^a	225.67 ^b
	UNIMAS-24-S1	73.02 ^a	14.49 ^a	2	24.06 ^a	18.83 ^c	174.33 ^{ab}
	UNIMAS-27-S1	72.77 ^a	14.26 ^a	2	21.39 ^a	16.54 ^{bc}	194.67 ^{ab}
	UNIMAS-28-S1	74.76 ^a	13.98 ^a	2	21.29 ^a	15.09 ^{ab}	194.67 ^{ab}
	UNIMAS-29-S1	71.22 ^a	16.73 ^a	2	24.52 ^a	18.80 ^c	156.33 ^{ab}
	UNIMAS-30-S1	80.75 ^a	14.12 ^a	2	20.88 ^a	17.02 ^{bc}	174.33 ^{ab}
	UNIMAS-31-S1	86.41 ^a	13.98 ^a	2	24.03 ^a	17.06 ^{bc}	125.33 ^a
	UNIMAS-32-S1	77.83 ^a	16.81 ^a	2	22.90 ^a	16.49 ^{bc}	164.00 ^{ab}

Table 4.9 continued

UNIMAS-37-S1	73.33 ^a	14.09 ^a	2	23.14 ^a	16.63 ^{bc}	123.00 ^a
UNIMAS-43-S1	73.61 ^a	15.55 ^a	2	22.03 ^a	16.37 ^{abc}	156.33 ^{ab}

***LEPC**: Length of long cell in micrometres; **WEPC**: Width of long cell in micrometres; **SCS**: Subsidiary cell shape (1=ellipsoid, 2=triangular); **LSC**: Length of stomatal complex in micrometres; **WSC**: Width of stomatal complex in micrometres; **SD**: Stomata density in millimetres per square. Difference in morphological traits between accession were compared using Variance Analysis (ANOVA) followed by Tukey HSD Post Hoc Test. Numbers followed by different superscript lowercase letters indicate significant different ($p < 0.05$).

The width of long cell (WEPC) on Ab epidermis of accessions in first generation recorded a range of 12.05 μm (UNIMAS-27) to 17.55 μm (UNIMAS-28) (Table 4.8). The WEPC on Ad epidermis ranging from 10.46 μm (UNIMAS-44) to 16.15 μm (UNIMAS-29) (Table 4.8).

In S_1 generation, the mean of WEPC on Ab epidermis ranged from 13.03 μm in UNIMAS-27-S1 to 17.81 μm in UNIMAS-29-S1 (Table 4.9). The mean WEPC on Ad epidermis ranged from 13.98 μm (UNIMAS-28-S1 and UNIMAS-31-S1) to 16.81 μm (UNIMAS-32-S1) (Table 4.9). There is no significant difference between accessions based on WEPC in both Ab and Ad surfaces.

4.3.2 Diversity of Stomatal Complex

Figure 4.8 illustrated the general structure of stomatal complex. The stomatal complex are composed of two adjacent subsidiary cells (SCS) and a pair of dumbbell-shaped guard cells. There was only one type of subsidiary cell shape (SCS), which is triangular-shaped SCS, recognized among accessions in both generations (Figure 4.8).

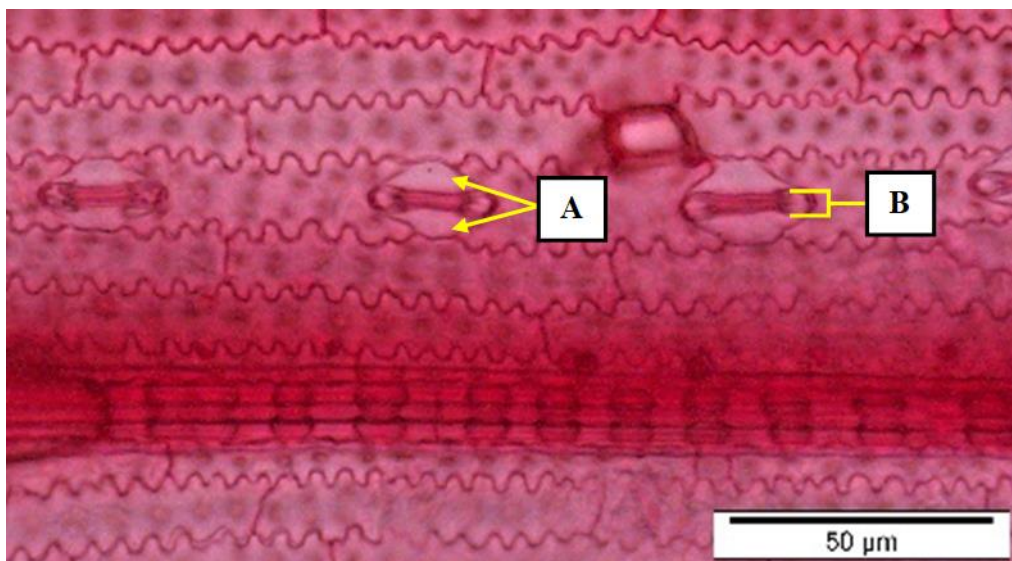


Figure 4.8: The General Structure of *Oryza sativa* Stomatal Complex. **A:** A pair of triangular-shaped subsidiary cells. **B:** A pair of dumbbell-shaped guard cells. Magnification= 60x. Scale bar= 50 μm .

The length of stomatal complex (LSC) on Ab surface of accessions in first generation ranged from 15.49 μm (UNIMAS-33) to 23.13 μm (UNIMAS-35) and LSC on Ad surface ranging from 16.78 μm (UNIMAS-34) to 26.20 μm (UNIMAS-36) (Table 4.8).

In S_1 generation, the mean of LSC on Ab surface ranged from 20.67 μm (UNIMAS-23-S1) to 24.52 μm (UNIMAS-31-S1) and mean of LSC on Ad surface ranged from 19.10 μm (UNIMAS-23-S1) to 24.52 μm (UNIMAS-29-S1) (Table 4.9). Statistically, the LSC in S_1 generation are not significantly different between accessions on both Ab and Ad surfaces.

In first generation, the width of stomatal complex (WSC) on Ab surface ranged from 12.42 μm (UNIMAS-25) to 19.03 μm (UNIMAS-28) (Table 4.8). Meanwhile WSC on Ad surface ranged from 12.93 μm (UNIMAS-33) to 17.68 μm (UNIMAS-37) (Table 4.8).

The mean range of WSC in S_1 generation on Ab surface ranged from 15.90 μm (UNIMAS-23-S1) to 21.22 μm (UNIMAS-29-S1) (Table 4.9). The mean WSC on Ad epidermis ranged from 13.57 μm (UNIMAS-23-S1) to 18.83 μm (UNIMAS-24-S1) (Table

4.9). The WSC are significantly different between accessions in S₁ generation on both Ab and Ad surfaces.

The stomata density (SD) in Ab epidermis of accessions in first generation recorded a range of 192.00 units/mm² for UNIMAS-41 to 546.00 mm² for UNIMAS-33 (Table 4.8). SD on Ad epidermis ranging from 162.00 mm² (UNIMAS-31 and UNIMAS-36) to 385.00 units/mm² (UNIMAS-40) (Table 4.8).

Meanwhile in S₁ generation, a mean SD ranging from 110.33 units/mm² in UNIMAS-31-S1 to 243.67 units/mm² in UNIMAS-32-S1 was observed on Ab epidermis (Table 4.9). The mean SD on Ad epidermis ranged from 123.00 units/mm² (UNIMAS-37-S1) to 225.67 units/mm² (UNIMAS-23-S1) (Table 4.9). Between the accessions in S₁ generation, significant difference was detected for SD on both Ab and Ad surfaces.

4.3.3 Midrib

The general structure of midrib comprises of vascular bundle, sclerenchyma strand, parenchyma and air space (Figure 4.9I). All midrib structures can be observed in 22 accessions. The shape of midrib is the same for all 22 accessions, which was concave-shaped for abaxial and flat-shaped for adaxial epidermis. The midrib width for 22 accessions ranged from 321.00 µm (UNIMAS-37) to 1422.22 µm (UNIMAS-41) (Table 4.10).

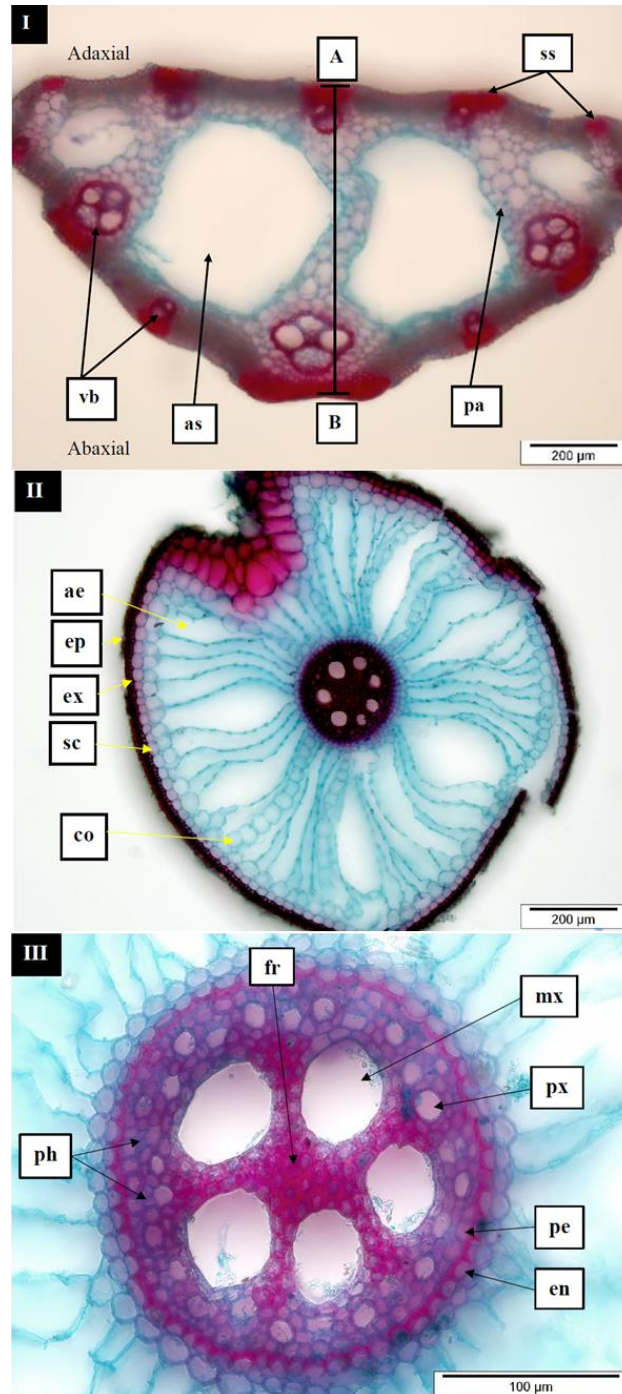


Figure 4.9: The Representative General Structure of Midrib, Root and Stele of *Oryza sativa* Observed in 22 Lowland Rice Accessions in First Generation. **I:** The general structure of midrib; **A** and **B:** Midrib width point of measurement using ImageJ software; **ss:** Sclerenchyma strand; **pa:** Parenchyma; **as:** Air space; **vb:** Vascular bundle. **II:** The general structure of root; **ae:** Aerenchyma; **ep:** Epidermis; **ex:** Exodermis; **sc:** Sclerenchyma layer; **co:** Cortex. **III:** The stele general structure; **fr:** Fibers; **ph:** Phloem; **mx:** Metaxylem; **px:** Protoxylem; **pe:** Pericycle; **en:** Endodermis. Magnification: **I** and **II**= 10x, **III**= 40x. Scale bar: **I** and **II**= 200 µm, **III**= 100 µm.

Table 4.10: The Recorded Measurement of Midrib, Root and Stele in 22 Lowland Accessions.

Accession	Midrib Width (μm)	Root Diameter (μm)	Stele Diameter (μm)
UNIMAS-23	949.02	888.74	293.54
UNIMAS-24	582.28	946.59	226.88
UNIMAS-25	652.45	988.98	204.43
UNIMAS-26	751.15	869.91	223.75
UNIMAS-27	835.36	580.70	196.06
UNIMAS-28	728.02	938.31	252.90
UNIMAS-29	552.43	810.01	208.22
UNIMAS-30	331.81	710.11	176.37
UNIMAS-31	762.24	955.57	281.54
UNIMAS-32	522.26	705.83	211.92
UNIMAS-33	719.12	1084.64	247.79
UNIMAS-34	890.86	930.41	214.16
UNIMAS-35	566.16	741.24	255.96
UNIMAS-36	780.93	1058.72	246.41
UNIMAS-37	321.00	1123.56	220.28
UNIMAS-38	672.73	1104.77	246.75
UNIMAS-39	629.27	967.52	244.30
UNIMAS-40	1150.56	587.44	185.19
UNIMAS-41	1422.22	618.23	179.66
UNIMAS-42	654.68	878.34	221.99
UNIMAS-43	900.82	778.61	196.23
UNIMAS-44	1145.35	785.59	197.62

4.3.4 Root and Stele

Root is composed of exodermis, sclerenchyma layer; aerenchyma, cortex, epidermis and stele (Figure 4.9II). All root structures can be found in the 22 lowland rice accessions. The root diameter ranged from 580.70 μm for UNIMAS-27 to 1123.56 μm for UNIMAS-37 (Table 4.10).

Stele, which is the central part of the root is made up of fibers, phloem, metaxylem, protoxylem, pericycle and endodermis (Figure 4.9III) and all structures can be observed in all 22 accessions. The range of stele diameter was from 176.37 μm (UNIMAS-30) to 293.54 μm (UNIMAS-23) (Table 4.10).

4.3.5 Anatomical Traits in Distinguishing Accessions

There was no variation in terms of anatomical structures that can distinguish the accessions in current study. All of the accessions have recorded the same epidermal, midrib, root and stele characters. However, it is noted that the SD was recorded lower in adaxial surface compared to abaxial surface which is a good character to look into for maintaining stomatal conductance and survive longer under drought and high temperature. Another important character that can be considered for Sarawak rice breeding program is the large size of stele as recorded in the observed accessions. Greater stele diameter is linked to a better hydraulic conductance and associated with improved drought tolerance.

4.4 Simple Sequence Repeat Genotyping

Out of 39 tested Simple Sequence Repeat (SSR) primer pairs, seven primer pairs (17.9%) displayed polymorphic banding patterns between the accessions, while another 32

of the SSR primer pairs (82.1%) displayed either monomorphic banding patterns or unclear banding. The seven SSR markers with good amplification were RM1, RM489, RM552, RM444, RM257, RM481 and RM166 (Table 4.11, Figure 4.10). The primers with poor amplification and not polymorphic were excluded from the present study.

Table 4.11: The Seven SSR Markers with High Yield Amplification.

SSR marker	Primer sequence	Ta (°C)	Product size (bp)
RM1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	55	90-120
RM489	F: ACTTGAGACGATCGGACACC R: TCACCCATGGATGTTGTCAG	55	250-300
RM552	F: CGCAGTTGTGGATTTTCAGTG R: TGCTCAACGTTTGA CTGTCC	55	200-250
RM444	F: GCTCCACCTGCTTAAGCATC R: TGAAGACCATGTTCTGCA	55	150-300
RM257	F: CAGTTCCGAGCAAGAGTACTC R: GGATCGGACGTGGCATATG	55	150-200
RM481	F: TAGCTAGCCGATTGAATGGC R: CTCCACCTCCTATGTTGTTG	59	150-300
RM166	F: GGTCCCTGGGTCAATAATTGGGTTACC R: TTGCTGCATGATCCTAAAC	61	300-410

***Ta**: Annealing temperature in degree Celsius; **F**: Forward primer; **R**: Reverse primer; **bp**: Base pair

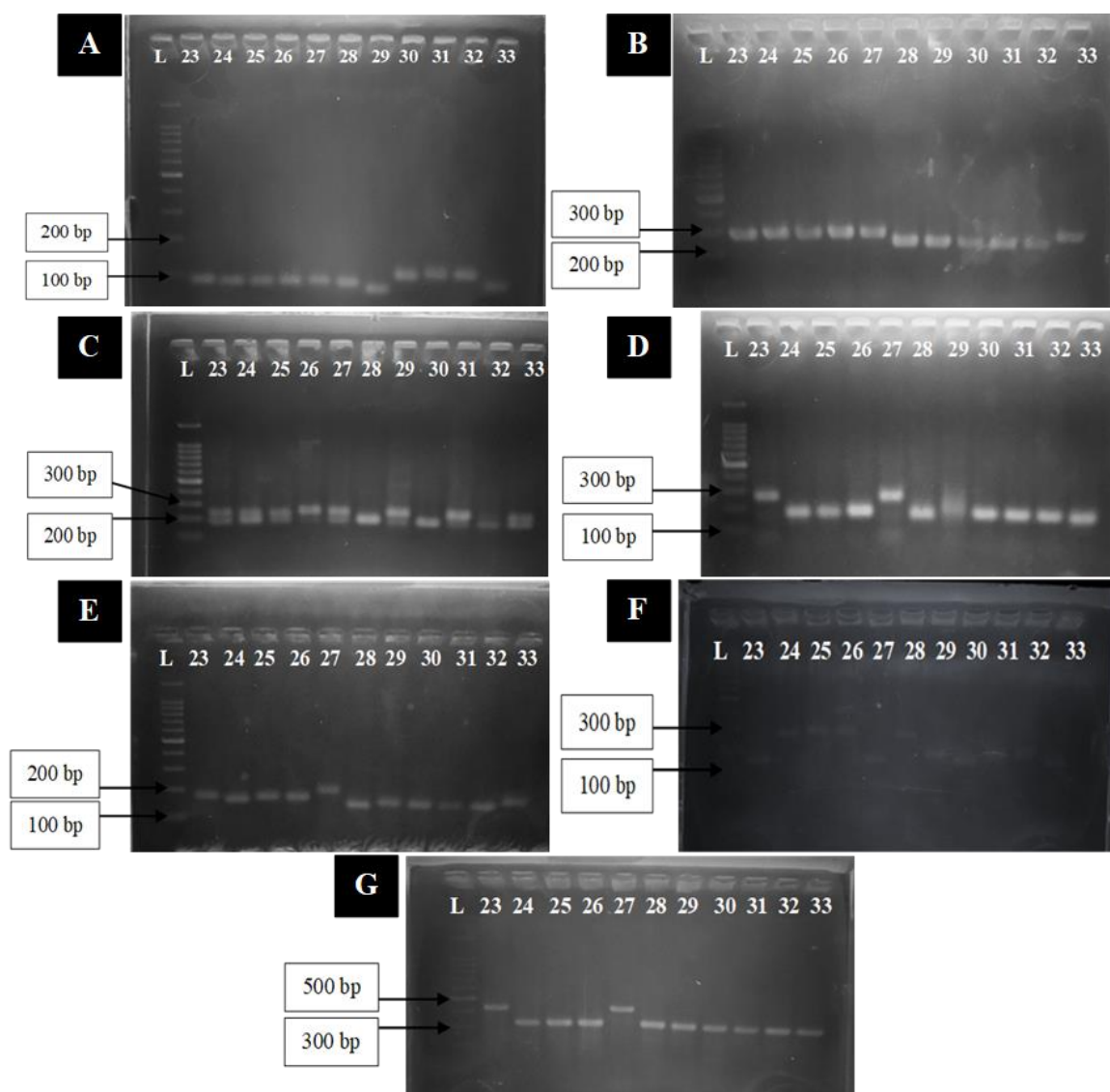


Figure 4.10: A Representative Figure for The Banding Pattern of The Seven Polymorphic SSR markers. **A:** RM1, **B:** RM489, **C:** RM552, **D:** RM444, **E:** RM257, **F:** RM481, **G:** RM166. L= 100 bp ladder. The number represents the 11 UNIMAS collections from UNIMAS-23 to UNIMAS-33.

4.4.1 Polymorphism Information Content Values and Genetic Diversity

The number of alleles and Polymorphism Information Content (PIC) values of the seven SSR markers are tabulated in Table 4.12. A total of 18 alleles were recorded and the number of alleles per locus ranged from two (RM489, RM552, RM444 and RM166) to four alleles in RM257 with an average of 2.57 alleles (Table 4.12). PIC greater than 0.5 was observed in six SSR markers and one SSR marker (RM166) had PIC value below 0.5 (Table 4.12). The highest PIC value (0.91) was recorded for RM257 and RM166 had the lowest PIC value (0.18) (Table 4.12). The average PIC value was 0.67 (Table 4.12).

Table 4.12: The Details of Seven SSR Markers with Polymorphic Information Content Values.

SSR marker	Ch.	Number of alleles	PIC
RM1	1	3	0.83
RM489	3	2	0.71
RM552	11	2	0.53
RM444	9	2	0.72
RM257	11	4	0.91
RM481	7	3	0.84
RM166	4	2	0.18
Mean		2.57	0.67

* **Ch.:** Chromosome number, **PIC:** Polymorphic Information Content.

The allelic diversity among markers recorded a mean of 1.61 number of effective allele (N_e), Nei's gene diversity (h) was 0.36 and Shannon's Information Index (I) was 0.53 (Table 4.13). The SSR marker RM489 has recorded the highest allelic diversity with mean 1.90 (N_e), 0.47 (h) and 0.67 (I) (Table 4.13). The lowest allelic diversity was in primer RM166 with mean 1.20 (N_e), 0.17 (h) and 0.31 (I) (Table 4.13).

Table 4.13: Allelic Diversity Revealed by Seven Polymorphic SSR Markers.

SSR markers	<i>Ne</i>	<i>h</i>	<i>I</i>
RM1	1.78	0.43	0.61
RM489	1.90	0.47	0.67
RM552	1.74	0.42	0.61
RM444	1.54	0.35	0.53
RM257	1.52	0.31	0.47
RM481	1.57	0.35	0.53
RM166	1.20	0.17	0.31
Mean	1.61	0.36	0.53

***Ne**: Number of effective alleles; **h**: Nei's gene diversity; **I**: Shannon's Information Index.

4.4.2 Cluster Analysis Based on SSR Markers

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis was performed and a dendrogram was constructed using seven SSR markers that amplified clear and repeatable polymorphic bands. The cluster analysis based on UPGMA method had the 22 accessions grouped into two major clusters and four sub-clusters (Figure 4.11). The clustering of accessions in both clusters was random and not dependent on either morphology nor place of origin except for in sub-cluster BI, where the accessions shared the same blade colour (pale green) and ligule shape (acute to acuminate). White ligules and auricles are found present in all the 22 accessions.

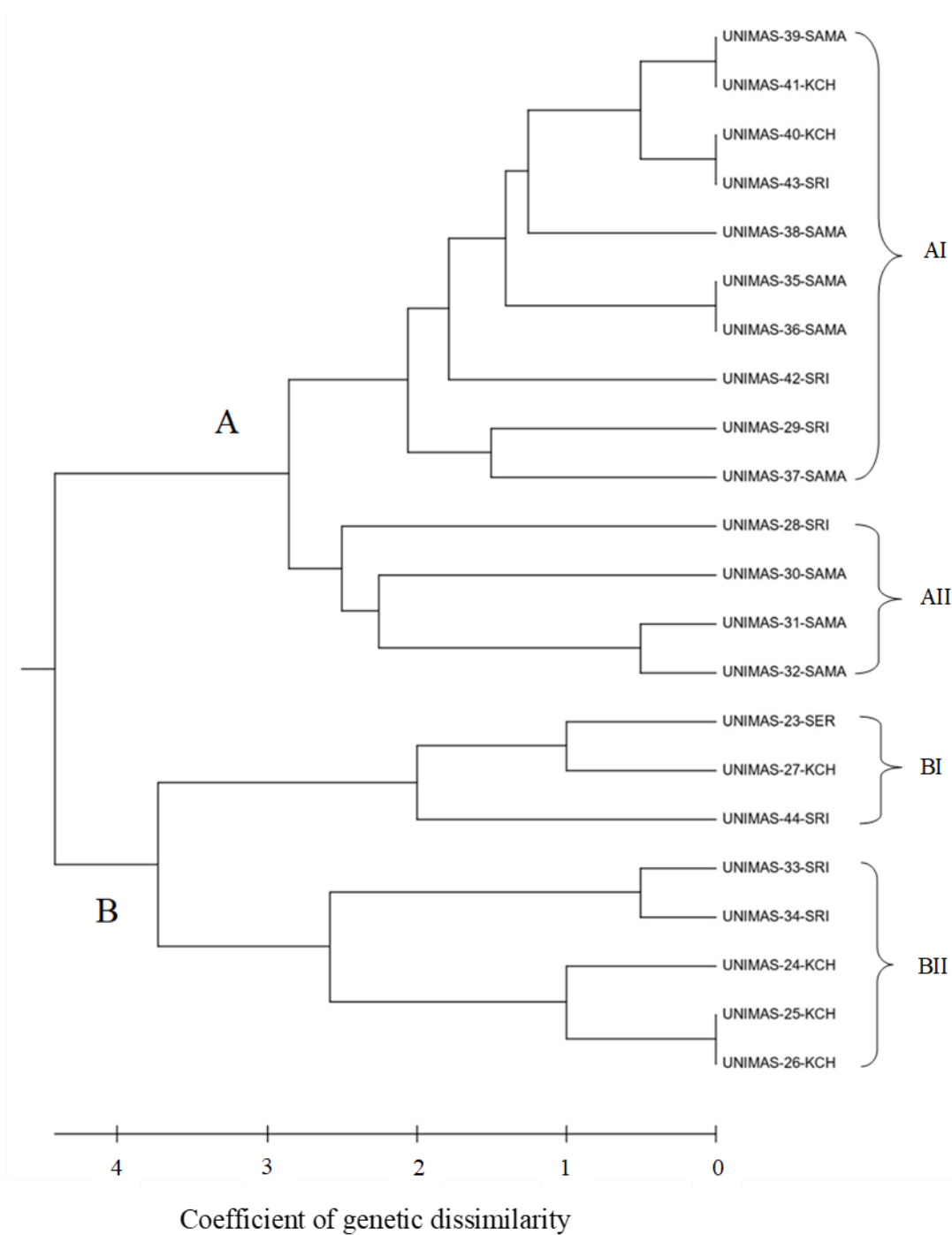


Figure 4.11: The Cluster Analysis of 22 Lowland Rice Accessions Collected From North-Western Region of Sarawak Based on Seven SSR Markers. **A:** Cluster A; **AI:** Sub-cluster AI; **AII:** Sub-cluster AII; **B:** Cluster B; **BI:** Sub-cluster BI; **BII:** Sub-cluster BII. The uppercase abbreviation after the accession number indicates the location of the collection. **KCH:** Kuching; **SER:** Serian; **SAMA:** Kota Samarahan; **SRI:** Sri Aman.

As tabulated in Table 4.14, Cluster A consisted of the highest number of accessions with a total of 14 accessions, with sub-cluster AI comprised of ten accessions and sub-cluster AII with four accessions. Cluster B comprised a total of eight accessions, split into two sub-cluster with sub-cluster BI consisted of three accessions and five accessions were in sub-cluster BII (Table 4.14).

Table 4.14: Cluster Group of 22 Lowland Rice Accessions Based on Seven SSR Markers.

Cluster	Sub-cluster	Number of accessions	Accession
A	AI	10	UNIMAS-39-SAMA UNIMAS-41-KCH UNIMAS-40-KCH UNIMAS-43-SRI UNIMAS-38-SAMA UNIMAS-35-SAMA UNIMAS-36-SAMA UNIMAS-42-SRI UNIMAS-29-SRI UNIMAS-37-SAMA
	AII	4	UNIMAS-28-SRI UNIMAS-30-SAMA UNIMAS-31-SAMA UNIMAS-32-SAMA
B	BI	3	UNIMAS-23-SER UNIMAS-27-KCH UNIMAS-44-SRI
	BII	5	UNIMAS-33-SRI UNIMAS-34-SRI UNIMAS-24-KCH UNIMAS-25-KCH UNIMAS-26-KCH

* The uppercase abbreviation after the accession number indicates the location of the collection. **KCH:** Kuching; **SER:** Serian; **SAMA:** Kota Samarahan; **SRI:** Sri Aman.

Three lowland rice accessions namely UNIMAS-42, UNIMAS-43 and UNIMAS-44 were from the same landrace (UNISRA 2018-26, Padi Kanowit), but they were found in different sub-clusters. UNIMAS-42 and UNIMAS-43 were grouped together in Cluster AI but UNIMAS-44 was separated into Cluster BI (Table 4.14).

4.5 Maturase-K Amplification

Figure 4.12 shows the approximately 1500 bp PCR product of *matK* gene amplified for 22 accessions from the first generation. The alignment of the *matK* gene sequence from the 22 rice accessions had 1470 bp. There were 140 segregating sites.

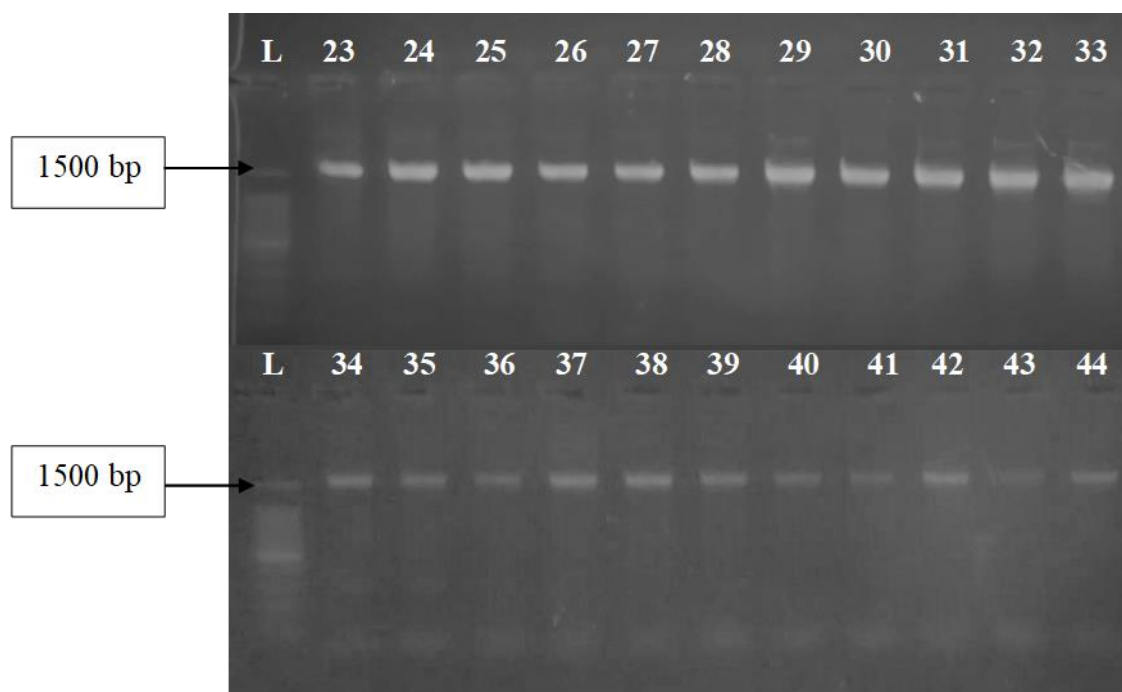


Figure 4.12: The Amplification of *matk* on 22 Lowland Rice Accessions. **L:** 100 bp DNA ladder. The number represents the 22 rice accessions (UNIMAS-23-UNIMAS-44).

4.5.1 Phylogenetic Analysis of Maturase-K

Figure 4.13 illustrated the phylogenetic tree constructed using Maximum Likelihood by MEGA X v.10.1 with Tamura 3-parameter model evolutionary rates among sites with discrete gamma distribution (T92+G) at 1000 bootstrap values.

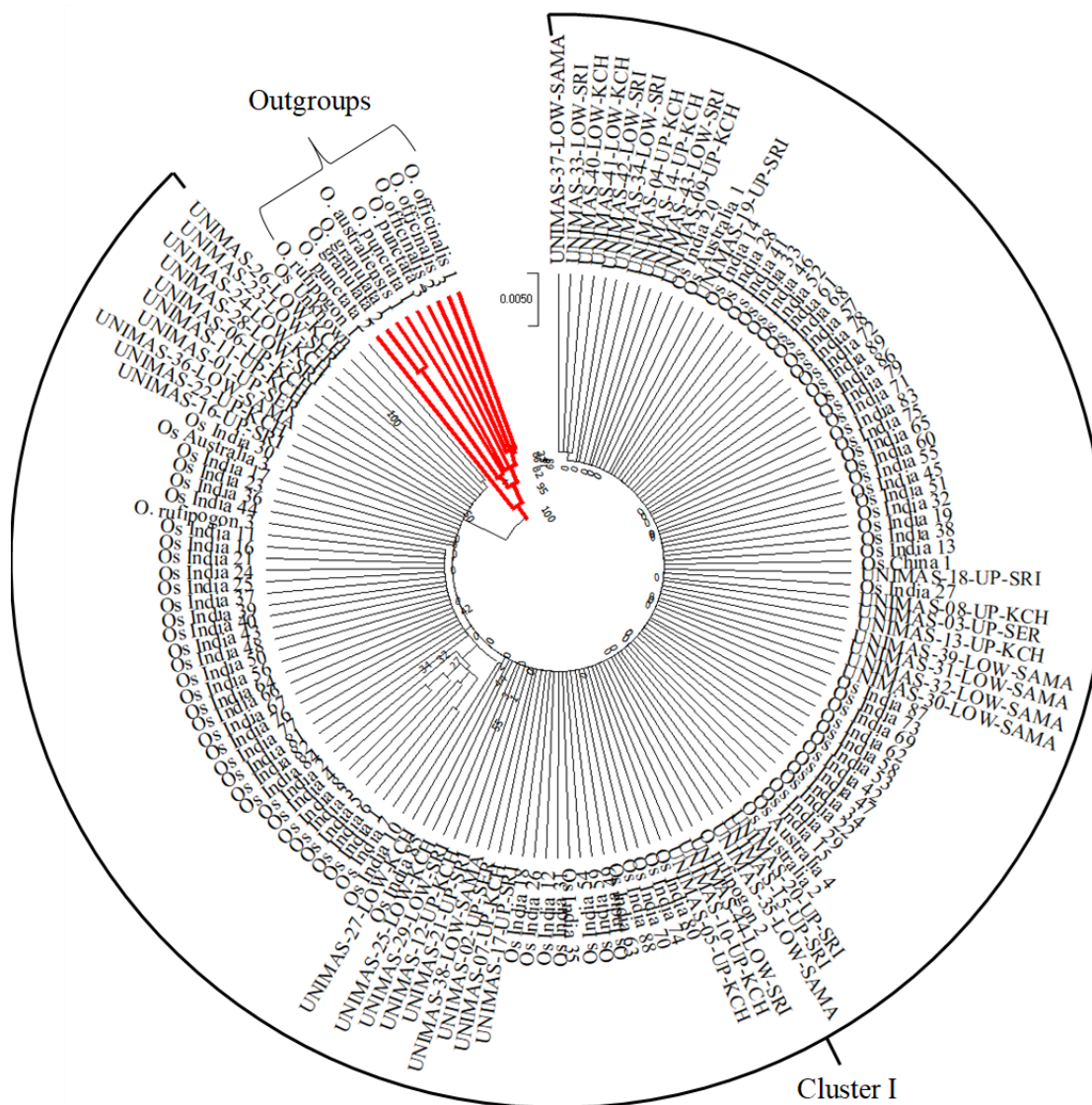


Figure 4.13: Phylogenetic Tree of *Maturase-K* on 22 Lowland Rice Accessions Using Maximum Likelihood Analysis. Best-fit Substitution Models: Tamura 3-Parameter Model with Discrete Gamma Distribution Evolutionary Rates Among Sites (T92+G) at 1000 Replicates Bootstrap Values in MEGA X v. 10.1.

In this study, the relationship between the 22 lowland accessions and their relationships with other *O. sativa* accessions could not be clarified by utilising *matK*. Only one major cluster can be observed which is Cluster I with 100% bootstrap support. Cluster I has clustered all *Oryza sativa* species which comprises of 22 closely related lowland rice accessions together with 22 upland rice accessions from UNIMAS collections and 88 *O. sativa* from India, four *O. sativa* from Australia, one *O. sativa* from China and one *O. sativa* was from an unknown origin (Table 4.15). Cluster I also included three accessions of *O. rufipogon*. The outgroups consists of *O. punctata*, *O. granulata*, *O. officinalis* and *O. australiensis*.

Table 4.15: Cluster Group Based on Chloroplast *Maturase-K*.

Cluster	Species	Country of origin	Number of accession
Cluster I	UNIMAS lowland rice collection	Malaysia	22
	UNIMAS upland rice collection	Malaysia	22
	<i>O. sativa</i>	India	88
	<i>O. sativa</i>	Australia	4
	<i>O. sativa</i>	China	1
	<i>O. sativa</i>	Unknown	1
	<i>O. rufipogon</i>	Unknown	1
	<i>O. rufipogon</i>	India	1
	<i>O. rufipogon</i>	China	1
	<i>O. punctata</i>	Unknown	1
Outgroups	<i>O. punctata</i>	Unknown	1
	<i>O. punctata</i>	Unknown	1
	<i>O. granulata</i>	China	1
	<i>O. granulata</i>	Vietnam	1
	<i>O. australiensis</i>	Australia	1
	<i>O. officinalis</i>	Philippines	1
	<i>O. officinalis</i>	China	1
	<i>O. officinalis</i>	Papua New Guinea	1

CHAPTER 5

DISCUSSION

5.1 Morphological Characterisation

A total of 25 morphological traits were used to characterise the accessions in this study. The morphological traits were divided into two categories, qualitative traits and quantitative traits.

5.1.1 Qualitative Traits Study

Leaves are one of the most important part of rice because they involve in the process of photosynthesis and transpiration which directly influence the yield performance (Huang et al., 2012). In present study, three classes of blade colour (BC) can be observed in first and S₁ generation. According to Singh et al. (2002) and Gealy (2005), BC is a trait related to leaf nitrogen (N) status. Bhupenchandra et al. (2021) has used the same Leaf Colour Chart as in present study in assessing nitrogen status in rice plant where they concluded the green colour intensity of rice plant leaves can determine N deficiency of a plant. The BC ranging from pale green to dark green is normal according to Deng et al. (2014) and Bhupenchandra et al. (2021), indicating the plant has a sufficient rate of nitrogenous fertilizer and can be considered as healthy. On the other hand, leaves with yellowish green colour may require nitrogen fertilization. In present study, three BC are recorded; pale green, green and dark green and none was recorded with yellowish green which indicates the accessions had been supplied with adequate nitrogenous fertilizer. Despite the fact that the same amount of fertiliser was provided, the BC of this study may indicate that there are variances in the amount of

nitrogen in the soil. Although BC varied between accessions in the study, this feature will not be a good indicator of identity because it is influenced by environmental factors.

Ligule shape (LS) was able to help in characterizing and distinguishing the accessions in both first and S₁ generation into two separate groups in this study; the accessions with an acute to acuminate LS and accessions with 2-cleft LS. Ligule colour (LC) and auricle colour (AC), however, did not manage to distinguish accessions in the first generation with only white coloured LC and AC were recorded. In S₁ generation, (LC) and (AC) was able to separate accessions into two groups of colouration, white and purple. There was one accession (UNIMAS-37-S1) detected for having different LC and one accession (UNIMAS-28-S1) recorded different AC from their parent in first generation. Since LC and AC are heritable trait, the variation in coloration may be due to anthocyanin pigmentation. Presence of anthocyanin pigmentation in rice plant ligule and auricle in some rice cultivars has been studied by Oshima et al. (2019). The author reported most of the field-grown rice cultivars have green leaves, some of them may accumulate anthocyanins in a variety of plant tissues such as leaf blades, sheaths, pericarps, stems and also ligule and auricle as well. The purple pigmentation might imply that environmental factors could contributed to the difference. A second opinion is that the traits segregates, but the current observation in triplicates were insufficient to show prove of segregation. Thus, this character cannot be relied on for classification of varieties.

In this study, panicle type (PT) has shown to influence over the number of filled grains (NFG). In first generation, UNIMAS-37 and UNIMAS-38 with compact PT had recorded high number of NFG ranging from 493 to 1055. This value contrast with NFG recorded for open PT observed in UNIMAS-39 and UNIMAS-42 which ranged from 84 to 696. There was no variation in PT between the first and S₁ generation. Cultivars with a compact PT bear more seeds with high grain

yield according to Das et al. (2018). The PT is a good criterion to be considered for breeding purpose in developing varieties with high yield potential.

The amount of primary and secondary branches, which directly affect the overall number of grains, governs panicle branching patterns thus the stronger the branching pattern, the greater the yield (Agata et al., 2020). The absence or light secondary branch (SB) was observed to have low NFG while accession with a heavy SB produces high NFG in present study. Accessions with heavy SB (UNIMAS-27 and UNIMAS-37) recorded NFG ranged from 159 to 1055. NFG can be seen lower (120 to 320) in accessions with absent of SB. Heavy SB contains great number of seeds per panicle have a benefit for high yielding and this is an example of great character preferred by crop breeder as it can be used for the purpose of maximizing crop grain production. Mohd Kasim et al. (2020). Rice breeding could consider the NFG as a selection criterion.

Present study showed that there were variations in three other qualitative traits, seed shape (SS), seed colour (SC) and grain colour (GC). UNIMAS-23 was the only accession with golden brown SC and UNIMAS-38 was the only accession having a light reddish brown (GC). This indicated that SC and GC may be the unique identification character for the respective accession. According to Kalaichelvan (2009) and Das et al (2018), qualitative trait such as GC is a heritable character and is a good trait used by many researchers in discriminating rice varieties.

5.1.2 Quantitative Traits Study

Seedling height (SH) in first generation varied between accessions and separated them into three groups of height with majority of the accessions (17 accessions) were considered as having intermediate height (<50.00 cm), three accessions with tall SH (≥ 50.00 cm) and two

accessions recorded short SH (≤ 40.00 cm). Present study showed that seedling height (SH) did not dictate the plant height (PH) for example UNIMAS-33 recorded the short SH (38.80 cm) but its PH was amongst the tall accessions at 210.00 cm. In contrast, UNIMAS-29 which recorded SH at 54.50 cm recorded PH at 187.50 cm. PH of lowland rice is taller than upland rice according to the International Rice Research Institute (IRRI, 2002) and can be grouped into three classes namely short (<110.00 cm), intermediate height (110.00 cm to 130.00 cm) and tall (>130.00 cm). PH of accessions in current study ranged from 153.40 to 225.30 cm in first generation. Based on the given PH categories, all accessions observed in the first generation were tall (>130.00 cm). In S_1 generation, the accessions were statistically different ranging from 148.43 cm to 216.40 cm. A total of five accessions (UNIMAS-23- S_1 , UNIMAS-24- S_1 , UNIMAS-28- S_1 , UNIMAS-32- S_1 and UNIMAS-37- S_1) in S_1 generation seem to be taller compared to its corresponding parent in first generation. This observation may be due to environmental factors. A tall PH, however may cause lodging in rice plant (Ünan et al., 2013; Shah et al. 2019).

According to Li et al. (2019), a positive correlation between PH and grain yield was demonstrated. For instance in current study, UNIMAS-26 with PH 153.40 cm produced 200 NFG, but a taller accession UNIMAS-32 with PH 174.40 cm produced 1091 NFG. In Malaysia, Malaysian Agricultural Research and Development Institute (MARDI) has introduced a high-yielding MR269 rice cultivar to the farmer due to its good property of having a tall PH and having a short maturation period (Malaysian Agricultural Research and Development Institute, n.d.). This shows PH is an excellent trait used in characterisation and can be included as criteria of selection for rice breeding.

Culm length (CL) in this study ranged from 79.67 cm to 143.40 cm. Meanwhile, CL reported by Elixon et al. (2017) on MARDI popular varieties MR253, MR263 and MR84 were 97.10 cm, 105.00 cm and 105.50 cm, respectively. The CL in present study can be grouped into

three categories; short (< 100.00 cm), medium (< 130.00 cm) and long (≥ 130.00 cm). In first generation, four accessions can be considered as having short CL, three accessions with a long CL and the other 15 were categorized as medium CL (Table 4.5). A shorter CL have an advantage of avoiding lodging issues but rice plant with short CL however, might negatively affect ripening process, reduce the crop yield, production of poor grain quality and might create problems for harvesting machinery to operate (Elixon et al. 2017). Range of culm diameter (CD) in present study was from 2.60 cm to 3.87 cm. All accessions in first and S_1 generation were having wide CD (≥ 3.00 cm) except for short CD recorded in UNIMAS-43-S1 with only 2.60 cm.

Number of tillers (NT) in present study ranged from four to six tillers meanwhile NT of Padi Bajong reported by DOA (2010) produces 12 to 15 tillers and ranging from 13 to 26 tillers in lowland rice varieties reported by Mohd Kasim et al. (2020). The NT in present study is considered as few in comparison to the other reported varieties. The number of productive tillers produced by rice plant is associated with crop yield, where the more tillers produced will resulting in a better yield (Dutta et al., 2002).

The leaf length (LL) varies in the accessions of present study ranging from 51.00 cm to 83.70 cm. This separated the LL into two groups; short LL (≤ 60.00 cm) and long LL (> 60.00 cm). The range of LW in present study was ranging from 1.10 cm to 2.47 cm. The leaf width (LW) in present study can be classified into narrow (< 2.00 cm) and wide (≥ 2.00 cm). Most of the accessions in present study are classified as having narrow LW.

Heading days (HD) in present study ranged from 83 days to 139 days at most. Study by Rohayu et al. (2020) on HD of MR263, a lowland variety by MARDI was 86 days which is

considered as short. Only four accessions in first generation and five accessions in S₁ generation have a short HD while the other accessions are categorised as having a long HD. Flowering days (FD) observed on present study was ranging from 115 days to 168 days. Padi Bajong, a local Sarawak landrace awarded with Geographical Indication, had FD around 130 days as described by Department of Agriculture Sarawak (DOA, 2010). In reference to IRRI (1996), FD can be classified into very early (<105 days), early (105 to 120 days), intermediate (121 to 135 days), late (136 to 160 days) and very late (above 160 days). Rice producers strongly prefer early FD since it allows them to plant for at least two seasons in a year, boosting their profits for the year (Zhang et al. 2022b). FD in rice can also be regulated by photoperiod response (Andrés et al. 2009).

One crucial attribute to be considered in MARDI rice breeding programs to increase rice production is yield potential. Number of panicle (NP) and panicle length (LP) are among agronomical traits that often determine rice crop yield and been utilized as criteria for genotype selection for high-yielding performance (Chiangmai et al., 2019). Lowland varieties in a study by Mohd Kasim et al. (2020) on Malaysian lowland varieties recorded NP ranging from 12 to 24 panicles while LP ranged from 21.98 cm to 27.08 cm. The NP in present study recorded one to 14 panicles with LP from 24.50 cm (UNIMAS-25) to 35.60 cm (UNIMAS-23). LP was positively correlated with yield produced. In current study, UNIMAS-25 with short LP produced 250 NFG while UNIMAS-25 recorded with long LP produced 320 NFG. Panicle size is a critical determinant of yield in rice, the longer LP is more favourable as yield is higher than those with a shorter LP (Heng et al., 2018).

The seed length (SL) can separate the accessions in present study into three groups; short (≤ 0.70 cm), intermediate (0.80 cm to 0.90 cm) and long (≥ 1.00 cm). Present study has documented nine accessions with medium SL, seven with long SL and one accession (UNIMAS-25) identified with short SL in first generation. Seed width (SW) has grouped the accessions into two groups where most of the accessions were grouped as having narrow SW and only one accession (UNIMAS-29) with a broad SW. Meanwhile both SL and SW in S_1 generation is statistically different between the observed ten accessions. Rajanna et al. (2011) discovered that shorter grains create broader grains whereas longer grains yield narrower grains. The same idea was upheld by Rafii et al. (2014) stating that grain shape was influenced by grain length (GL) and grain width (GW) where in their study on 12 F_1 progenies and five MARDI varieties (MR84, MR263, MR267, MRQ74 and Q76) revealed low GW and high GL resulted in a longer grain shape. This can be seen in grains of several accessions in current study for example UNIMAS-36 marked as having long GL of 0.80 cm and GW of 0.20 cm while a shorter and broad grain can be found in UNIMAS-37 with GL of 0.60 cm and GW of 0.30 cm. Nevertheless, there were few accessions recorded having the same GW though the GL were different as can be observed in UNIMAS-23 (GL: 0.50 cm, GW: 0.20 cm) and UNIMAS-24 (GL= 0.70 cm, GW= 0.20 cm). The GL in this study has grouped the accessions in present study into three classes namely short (0.50 cm), intermediate (0.60 cm to 0.70 cm) and long (0.80 cm). In first generation, two accessions were considered having a short GL, 14 recorded an intermediate GL and only one accession with long GL. Meanwhile, GW only grouped the accessions into two classes which are narrow (0.2 cm) and broad (0.30 cm). Broad GW can only be observed in two accession (UNIMAS-37 and UNIMAS-38) and all other accessions were recorded having narrow GW.

5.1.3 Morphological Traits in Distinguishing Accessions

The findings of this study indicated that plant qualitative morphological characteristics such BC, LS, PT, SB, SS, SC and GC are useful in characterization of varieties and help in distinguishing between the accessions. Two qualitative traits, LC and AC, however unable to discriminate the 22 rice accessions in first generation but variations in LC and AC can be observed in accessions of S_1 generation. The SC was able to characterise UNIMAS-23 as the only accession with golden brown SC and UNIMAS-38 was the only accession having a light reddish brown GC.

The results of 16 quantitative traits used in characterizing accessions in present study such as SH, PH, CL, CD, NT, LL, LW, HD, FD, NT, LP, NFG, SL, SW, GL and GW suggested these traits are useful in characterising the accessions due to the variations they offer which enable the accessions to be grouped accordingly.

5.2 Anatomical Study of Accessions

Anatomical characters have been used in identifying rice landraces or varieties (Kondo et al., 2000; Hoagland & Paul, 1978). The study of a plant anatomy, which include leaves and root are among the most fundamental plant organ for descriptive approach (Susetyarini et al., 2020). However at present, anatomical research on *O. sativa* has received little attention in Malaysia.

5.2.1 Epidermal cell

Basic structure of rice leaf anatomy can be seen under light microscope includes long cell, short cell row, stomatal complex, trichome, silica cell, phellem cell and cell outline. Present study showed no difference in terms of cell shape, cell arrangement and cell outlines be on the adaxial or abaxial surfaces, in both generations. The long cells were rectangular in shape. The cell arrangement were linearly elongate, with long and short cell alternated. All of the leaf samples had papillae and showed undulated cell outlines. Variations in long cell can only be observed in long cell length and width.

5.2.2 Diversity in stomatal complex

From the result, there is no variation in subsidiary cell shape (SCS) for accessions in both generation, on both abaxial and adaxial sides of the leave epidermis. All accessions were having a triangular-shaped SCS. Dumbbell-shaped guard cell were observed in all leaf samples. The result obtained in current study correspond well with the observation by Sarwar and Ali (2002) where they recorded triangular SCS and dumbbell-shaped guard cell in the epidermis of rice leaves.

The shape, sizes and numbers of stomata vary greatly between plant species and these variation in stomatal morphology according to Bertolino et al. (2019), is a way of the stomata adapt to the different environmental factors, thus ensuring optimum conditions for photosynthesis. This view is shared by Chen et al. (2017) and Muller et al. (2017) stating the diversity in stomatal morphology is correlated with the stomata adaption to certain conditions; for instance rice plants with fewer stomata perform better at maintaining stomatal conductance

and survive drought and high temperatures. From the current observation, stomata density can be found abundantly in abaxial surface compared to adaxial surface. This corresponds to Boonrueng et al. (2013) and Chatterjee et al. (2020) where stomata density was found abundantly in abaxial surface of leaf across the rice varieties they observed. Bertolino et al. (2019) suggested that a smaller number of stomata may help to reduce loss of water which allow the plant to save water.

5.2.3 Midrib Architecture

Midrib structures such as vascular bundle, sclerenchyma strand parenchyma and air space can be observed in all 22 accessions in first generation. Same structures of rice leaf midrib were recorded by Taratima et al. (2020) in their study on Thai rice landraces indicating that accessions in current study are not anatomically different that of Thai rice landraces although planted in different ecosystem, thus suggesting Malaysian and Thai rice are closely related (Sathongpan et al., 2021). In present study, the midrib width ranged from 321.00 μm to 1422.22 μm whilst Taratima et al. (2020) recorded an average of 761.50 μm for 20 Thai rice landraces. There is, however, not much research has been done on rice midrib.

5.2.4 Root Architecture

Rice plant root is made up of dense mass of adventitious root called the fibrous root system (Meng et al., 2019). In addition, for nutrient and water uptake, a plant's root serves a variety of purposes, including providing structural support (Rebouillat et al., 2009). The central

part of a root, called the stele, is made up of vascular tissue, consisted of phloem and xylem, which are crucial in nutrient and water uptake (Phoura et al., 2020). The root samples are very fragile and manual sectioning may rupture the cell structures. Higher quality sections can be achieved by using a vibrating microtome according to (Atkinson & Wells, 2017). General structures of root such as exodermis, sclerenchyma layer, aerenchyma, cortex, and epidermis can be observed in transverse section of the root of the 22 accessions in first generation. Stele consisted of fibers, phloem, metaxylem, protoxylem, pericycle and endodermis. Similar root and stele structures were recorded in Rebouillat et al. (2009) in the authors rice varieties. Study on upland accessions from UNIMAS collection by Frank (unpublished) had shown identical root cell structures to the present study.

The recorded root diameter in present study ranged from 580.70 μm to 1123.56 μm (Table 4.10). The recorded root diameter is considered high compared to four lowland rice varieties studied by Kondo et al. (2000) which ranges from 515 μm to 691 μm . Variation in root diameter was explained by Kadam et al. (2015) stating that changes in width of cortical cells and stele diameter can contribute to root diameter.

Stele diameter in present study ranged from 176.37 μm (UNIMAS-30) to 293.54 μm (UNIMAS-23) (Table 4.10) and considered as small. A smaller stele size according to Sandar et al. (2022) in their lowland rice samples, have smaller xylem vessels which then help to minimize water loss especially during drought and assist in conserving water during grain filling to maximize grain production. Hence, accession with small stele diameter can be considered for Sarawak breeding program to produce drought tolerance rice.

5.3 Simple Sequence Repeat Analysis

Varietal identification by molecular approach can eliminate the limitation associated with morphological characterization. Characterizing rice cultivar using a highly polymorphic microsatellite markers may provide in-depth information on rice germplasm and serves as powerful tools to detect genetic variation and genetic relationship within and among species (Al-Musawi et al., 2019). The SSR are markers of choice for studies because SSR demonstrate a higher per-locus information content (García et al., 2018). Hodel et al. (2016) stated the utilisation of a number of SSR markers are inexpensive and can deliver good results.

5.3.1 Assessment of Simple Sequence Repeat allelic diversity

The number of alleles of marker reveals the genetic diversity of a population. A total of 18 alleles, ranging from two (RM489, RM552, RM444 and RM166) to four (RM257) alleles per primer with an average of 2.57 alleles were successfully amplified by seven polymorphic SSR markers (RM1, RM489, RM552, RM444, RM257, RM481 and RM166). Ashfaq et al. (2020) also recorded two alleles for RM166 for 50 rice varieties from different countries of origin. For marker RM257, 11 alleles were recorded by Sohrabi et al. (2013) for 50 accessions from Peninsular Malaysia and Sabah. The number of alleles per SSR marker observed in the present study (2.57 alleles per marker) is similar to Supari et al. (2019) where the author recorded an average of 2.26 alleles per marker based on 27 SSR markers used to genotype 12 Malaysian rice accessions and nine Malaysian Agricultural Research and Development Institute varieties. The number of alleles amplified in the present study is, however, relatively lower

compared to Sohrabi et al. (2013) and Aljumaili et al. (2018) which reported two to eleven alleles from the SSR markers used for genotyping their rice samples.

The SSR markers in the current study showed PIC value ranged from 0.18 (RM166) to 0.91 (RM257) with an average of 0.67. From the seven polymorphic markers observed, only one marker (RM166) revealed PIC value less than 0.5. Similarly, Ashfaq et al. (2020) also reported RM166 has low PIC value with only 0.10 in 50 rice varieties. RM257 is also the SSR marker recorded the highest PIC value (0.88) in Sohrabi et al. (2013) in the author's 50 Malaysia rice accessions. The high PIC value of RM257 indicates that this marker is good to assess genetic diversity of rice.

The study of Dalimunthe et al. (2020) in polymorphism levels of SSR markers has divided PIC values into three classes, where PIC values greater than 0.5 ($PIC > 0.50$) as highly informative, $0.25 < PIC < 0.50$ as moderately informative and SSR markers with $PIC < 0.25$ is indicative of poor reliability. The PIC value is the reflection of allelic diversity and frequency among the genotype, hence SSR markers with PIC value above 0.50 are regarded as highly polymorphic and useful for genetic studies (Melaku et al., 2018). Meanwhile PIC value of 0.00 is an indicator that the marker is monomorphic and unable to distinguish polymorphism rate of a marker at a specific locus (Dalimunthe et al. 2020). The SSR markers used in the study has PIC value of more than 0.5, which were highly informative and capable of distinguishing between genotypes.

The number of effective alleles (N_e) ranged from 1.20 to 1.90 with an average 1.61. Nei's gene diversity (h) defined by Kosman (2003) as the measurement of average genetic diversity per locus. The h as explained by Bobokashvili (2016), when the value near 0.95-1.00

it indicates that there is little to no relationship between two species (high divergence) and value ranging from 0.50 - 0.60 is when two species with comparable morphological characteristics, however, unable to cross each other. On the other hand, h value ranging from 0.17 to 0.22 according to Bobokashvili (2016) occurs when two sub-species share common morphological characteristics in range of one species, thus demonstrating a low diversity. The h for markers in the present study ranged from 0.17 (RM166) to 0.47 (RM489) with an average of 0.36, thus concluded low level of divergence in current study. Similar result was obtained for h in ten Sarawak rice accessions assessed by Aljumaili et al. (2018), where low h value (0.37) was reported.

The I based on SSR markers of this study ranged from 0.31 (RM166) to 0.67 (RM489) with a mean value 0.53. The I in this study is low in comparison with I value reported by Sohrabi et al. (2013), where I value for the author's 24 rice accessions from Peninsular Malaysia was 1.17 and I for 26 accessions from Sabah was 1.08. The recorded I value in current study is indicating a low diversity between the 22 UNIMAS lowland rice collections.

5.3.2 The Simple Sequence Repeat Cluster Analysis

Cluster analysis based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) has grouped the 22 lowland accessions into two main clusters, Cluster A and Cluster B. Cluster A was further divided into two sub-clusters, sub-cluster AI and sub-cluster AII. Cluster B too were broken into two sub-clusters namely sub-cluster BI and sub-cluster BII.

The clustering of accession was random in both clusters and was not based on morphological or geographical origin. The random clustering might be related to the number of

SSR markers used in present study. This is further explained by Wang et al. (2021) in their study on the effect of population genetic analysis using various numbers of microsatellite markers where the consequences of using fewer SSR markers were verified by a decline in population genetic structure of *Rhododendron* species.

From the dendrogram obtained from UPGMA cluster analysis of SSR (Figure 4.11), three lowland rice accessions namely UNIMAS-42, UNIMAS-43 and UNIMAS-44, which derived from a same landrace named Padi Kanowit, were separated in different cluster. UNIMAS-42 and UNIMAS-43 were grouped together in Cluster AI but UNIMAS-44 was separated into Cluster BI. UNIMAS-44 was genetically different in comparison with UNIMAS-42 and UNIMAS-43 (Table 5.1, Appendix 11). This might be a result from seed impurity or mixture of seed provided by the farmers which contribute to diversity in the landrace (Yeo et al., 2018). The purity of farmer's seed is questionable due to common practice by farmers planting several rice one field or planting them close to each other without a clear boundary (Bajracharya et al., 2006; Vernoooy et al., 2022).

Table 5.1: The Genotype of Three Accessions selected from the Same Landrace Based On Seven SSR Markers.

Marker	Accession		
	UNIMAS-42	UNIMAS-43	UNIMAS-44
RM1	1	2	3
RM489	1	1	2
RM552	1	1	2
RM444	1	1	2
RM257	1	1	2
RM481	1	2	2
RM166	1	1	1

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

The impurity of the seed due to common practices by farmers may observe variations in morphological traits. Accessions derived from same landraces (having similar name) were clustered together (Figure 4.11, Table 4.14) but was having variation in morphological characters. Variation in panicle type (PT), ligule shape (LS) and seed colour (SC) can be observed in Padi Sabak Hitam (UNIMAS-31 and UNIMAS-32). Padi Sabak Angin (UNIMAS-35, UNIMAS-36, UNIMAS-37, UNIMAS-38 and UNIMAS-39) had variations in PT, secondary branching (SB), seed shape and grain colour (GC). Differences in PT and LS were recorded in Padi Kanowit (UNIMAS-42, UNIMAS-43 and UNIMAS-44). Padi Hitam (UNIMAS-24, UNIMAS-25, UNIMAS-26) on the other hand exhibits variations in SB and GC.

5.4 Genetic relatedness based on chloroplast *Maturase-K*

Phylogenetic analysis based on chloroplast *Maturase-K* (*matK*) has clustered the 22 lowland accessions together with the 22 upland accessions from UNIMAS collections, 94 *O. sativa* accessions from different origins and three *O. rufipogon* accessions into one major clusters. This result corresponds to result by Zodinpuui et al. (2013) where *O. sativa* species (from China) cannot be separated from *O. rufipogon* (from Thailand and India) using *matK* chloroplast. Same result was obtained in Patil et al. (2015) where *O. sativa* species were clustered in the same cluster with *O. rufipogon* in their study using *matK* chloroplast genome. According to Zodinpuui et al. (2013), the link found in the AA genome of wild species *O. rufipogon* is the reason it is closely related to the AA genome *O. sativa*.

The clustering of the 22 lowland rice accessions was random. The clustering by utilizing *matK* in current work was based on genome rather than geographical or morphological

characteristics. The usage of *matK* alone in phylogenetic analysis for this present study is probably insufficient to resolve the relationship among of the 22 lowland rice accessions and the relationship between them with other accessions of *O. sativa*. Similar result was reported by Chandrasekara et al. (2021) where *Cinnamomum* species is unable to be resolved using *matK* alone. Research by Ho et al. (2021) on the phylogenetic analysis of jewel orchid accessions in Vietnam by using *matK* and ribulose 1,5-biphosphate carboxylase (*rbcL*) sequences revealed, the analysis by combining the two barcoding markers have higher discrimination power in comparison to analysis based on *matK* alone. De Mattia et al. (2011) also reported a combination of barcoding markers (*matK* and *trnH-psbA*) can successfully distinguish *Ocimum basilicum* from two other *Ocimum* species and up to cultivar level.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

A total of 22 lowland rice accessions were collected from North-Western region of Sarawak in this study. The selected accessions were successfully characterised using 25 morphological traits. Variations among the 22 accessions can be observed using the assessed 25 morphological traits proving that they are a good traits which can be used in distinguishing rice accessions. There was no variation observed in morphological characteristics between accessions in the first generation and S_1 generation except in ligule and auricle colour. In this study, accessions that exhibited a compact panicle type and presence of secondary branching in its panicle has showed positive correlation with the number of filled grains. This shows the two morphological traits can be used as criteria for breeding purpose to produce varieties with high yield production. There was no large variation seen in first and S_1 generations in terms of general structure of plant tissue and arrangement of the cell of epidermal, stomatal complex, midrib and root. Stomata density was found lower in S_1 generation in present study. Stomata density is an important trait can be used in selection criteria for rice breeding as a smaller stomata density may help the plant to adapt well in dry condition by reducing water loss during transpiration.

Utilisation of Simple Sequence Repeat has revealed a low diversity in the assessed 22 lowland rice accessions. This is a consequence from a small number of SSR markers used in the study. The phylogenetic analysis based on *Maturase-K* (*matK*) was based on genome type. Phylogenetic analysis based on *matK* has grouped all accessions into one cluster together with other accessions of *O. sativa* and *O. rufipogon* accessions. The clustering by utilizing *matK* in

current work was based on genome. Utilization of *matK* alone in present study was insufficient to resolve the relationship among of the 22 lowland rice accessions and the relationship between them with other accessions of *O. sativa*.

6.2 Recommendation

Collection and characterisation of more landraces from different divisions in Sarawak will provide a better picture of the diversity of lowland rice landraces in Sarawak. More morphological traits are required to characterise the accession such as observation of blade pubescence, observation on flag leaf attitude and panicle exertion which will add more information on the accession. For quantitative traits, the 100-grain weight can be taken for a better estimation of yield produce. Continuous effort is needed to produce seeds from S₁ generation until S₅ to S₆ generation, where the seeds collected can be used for agronomical traits characterisation for study in pest and disease resistance. For anatomical, more cell structures and arrangement needed to be observed to provide better information of an accession. Further, anatomical traits may also be expanded to other parts of the rice plant, for instance the stem anatomical structures. Increasing the number of microsatellite markers can help to assess a better genetic diversity of the lowland rice accessions. Consideration of combining *matK* with different barcoding markers such as trnL-trnF and ribulose 1,5-biphosphate carboxylase can be used to resolve the relationship among the accessions for higher discrimination power in comparison to analysis based on *matK* alone and for future research to discriminate Sarawak lowland rice up to sub-species level.

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APPENDICES

APPENDIX 1: BLADE COLOUR OF ACCESSIONS IN FIRST GENERATION

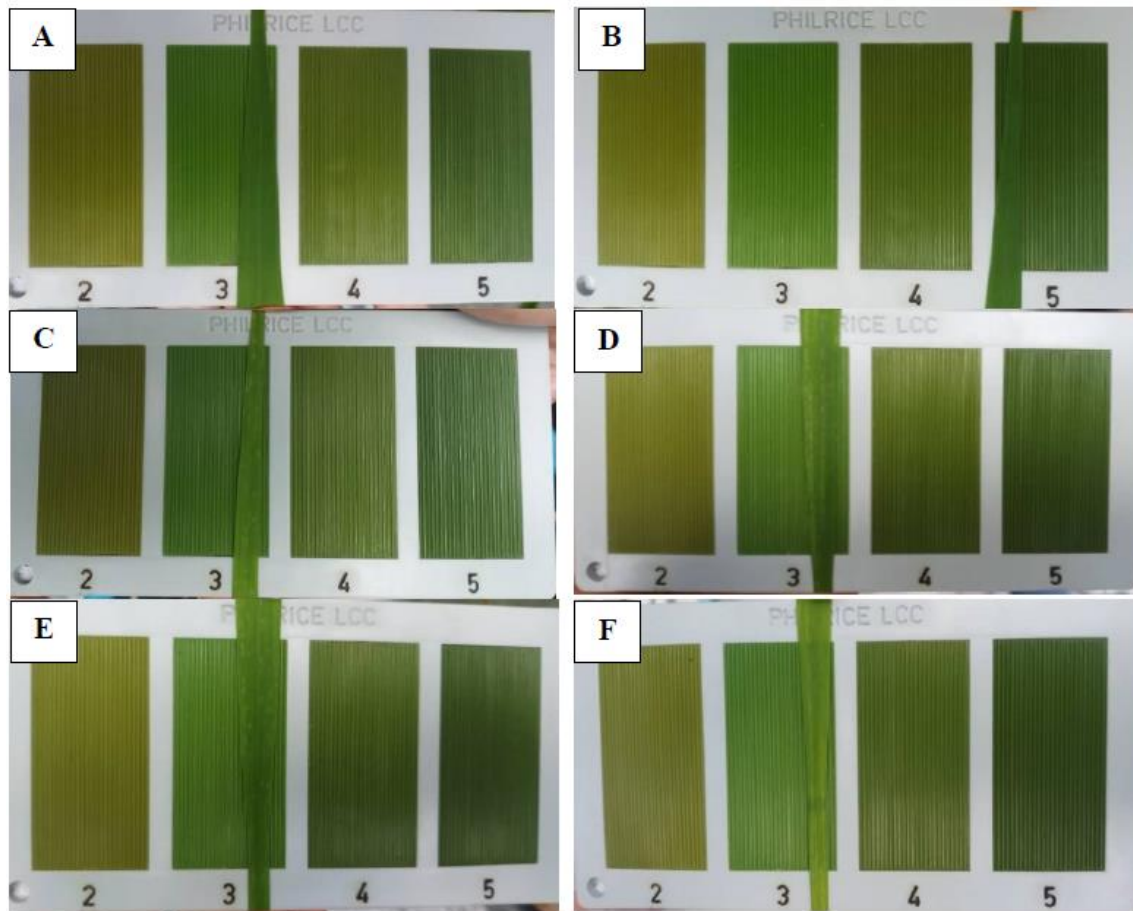


Figure 1: The Blade Colour of Rice Accessions in First Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A-F** represent UNIMAS-23 – UNIMAS-28.

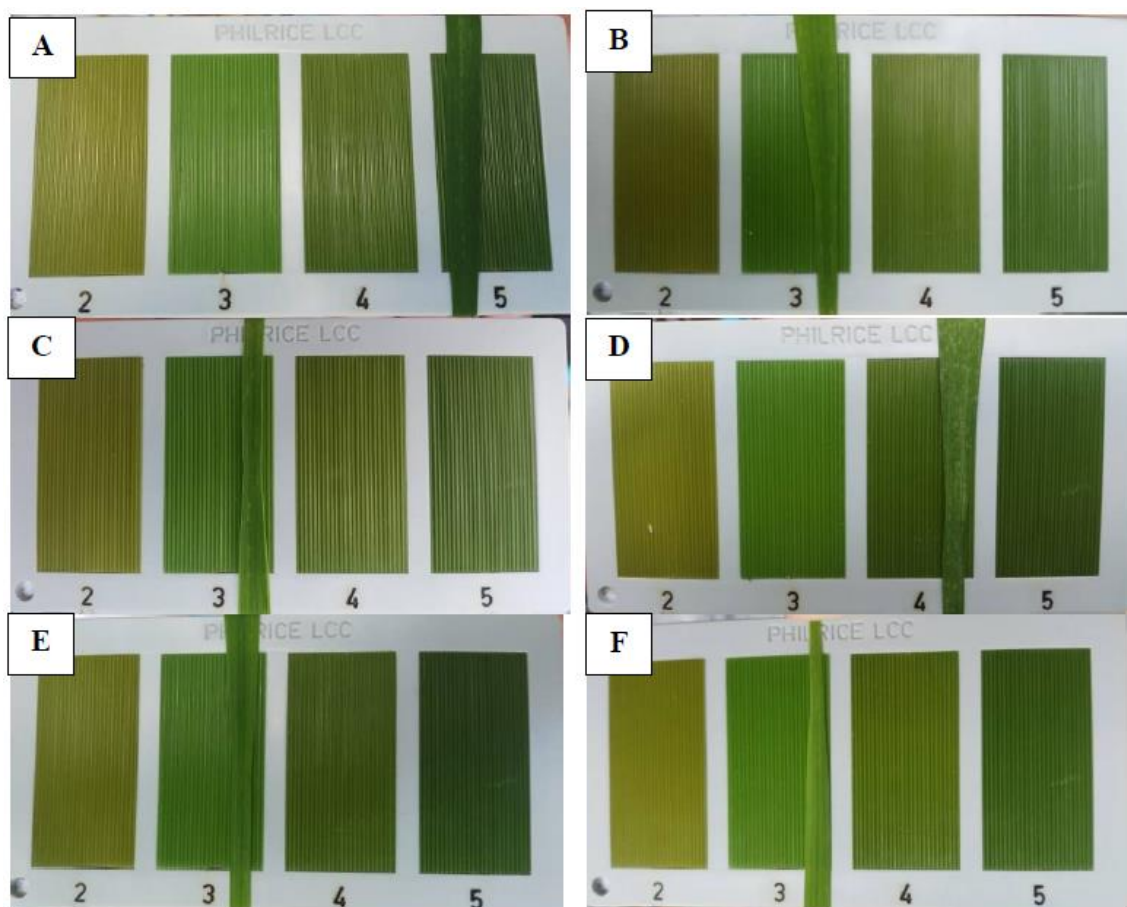


Figure 2: The Blade Colour of Rice Accessions in First Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A-F** represent UNIMAS-29 – UNIMAS-34.

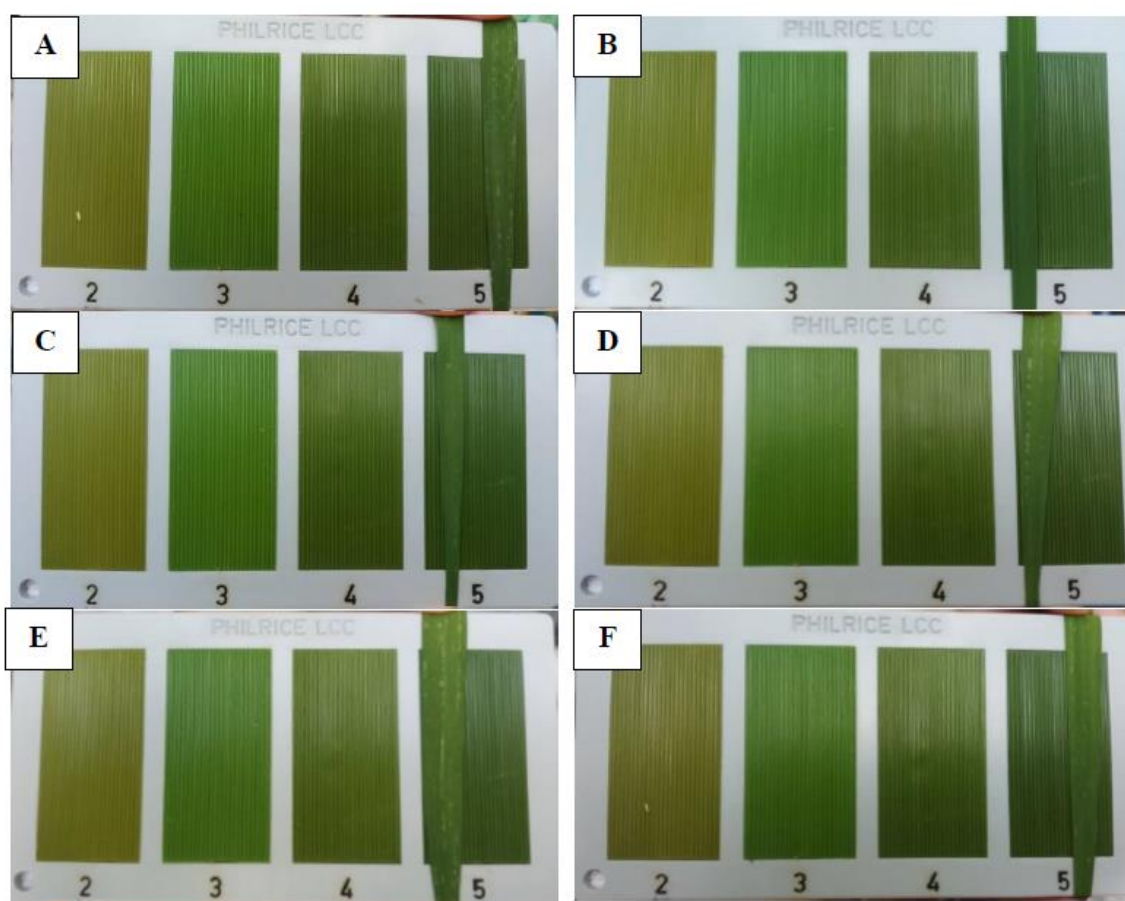


Figure 3: The Blade Colour of Rice Accessions in First Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A-F** represent UNIMAS-35 – UNIMAS-40.

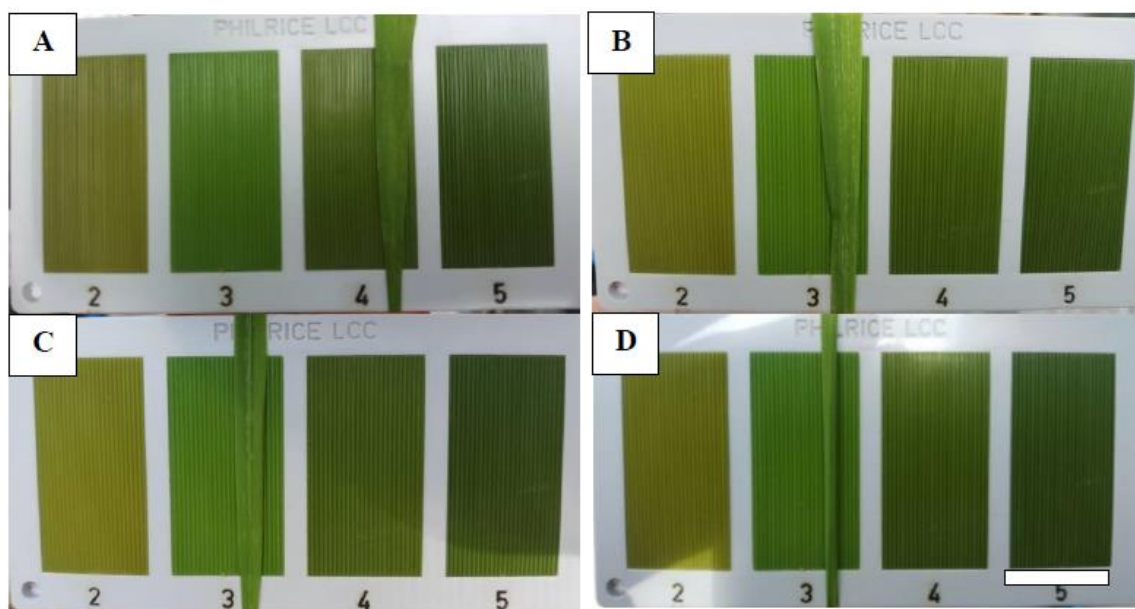


Figure 4: The Blade Colour of Rice Accessions in First Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A-D** represent UNIMAS-41 – UNIMAS-44.

APPENDIX 2: BLADE COLOUR OF ACCESSIONS IN S₁ GENERATION

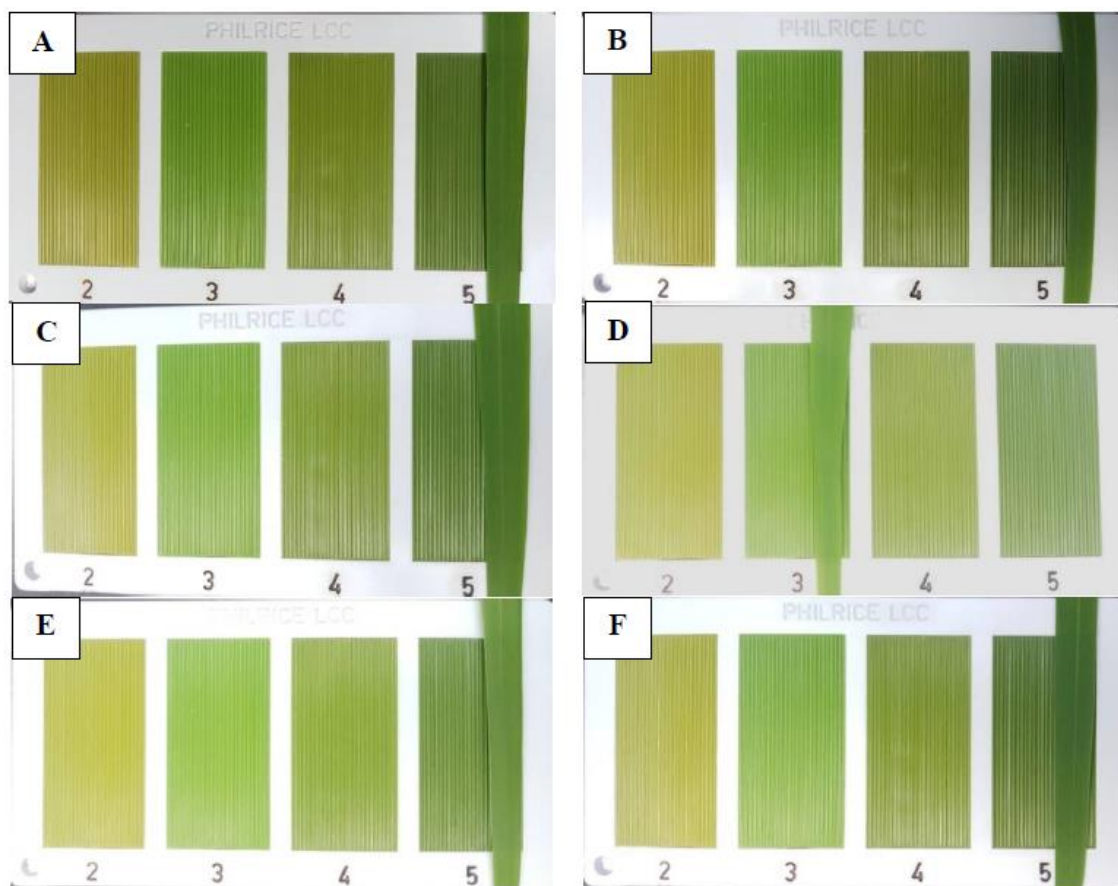


Figure 1: The Blade Colour of Rice Accessions in S₁ Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A, C and E** represent UNIMAS-23-S1. **B, D and F** represents UNIMAS-24-S1.

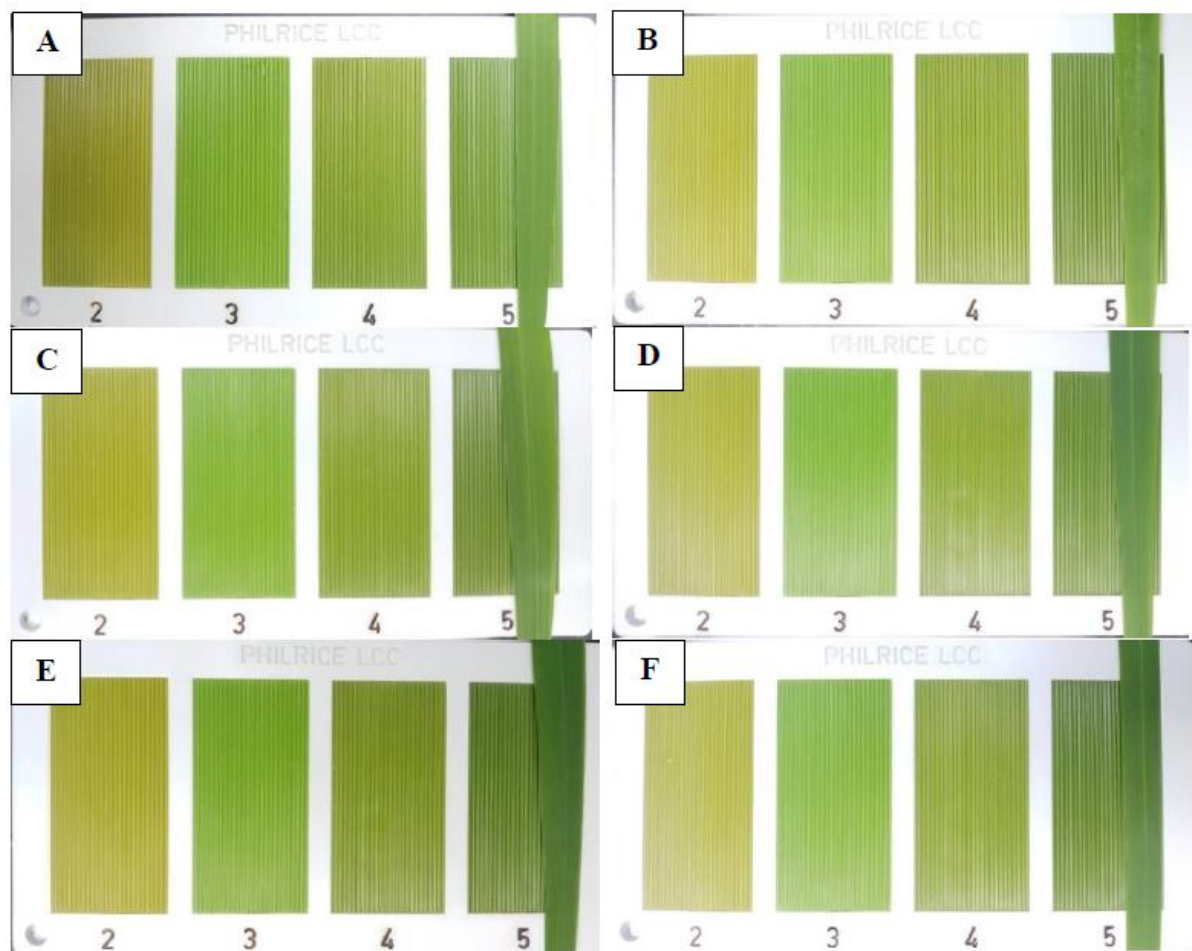


Figure 2: The Blade Colour of Rice Accessions in S_1 Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A, C and E** represent UNIMAS-27-S1. **B, D and F** represent UNIMAS-28-S1.

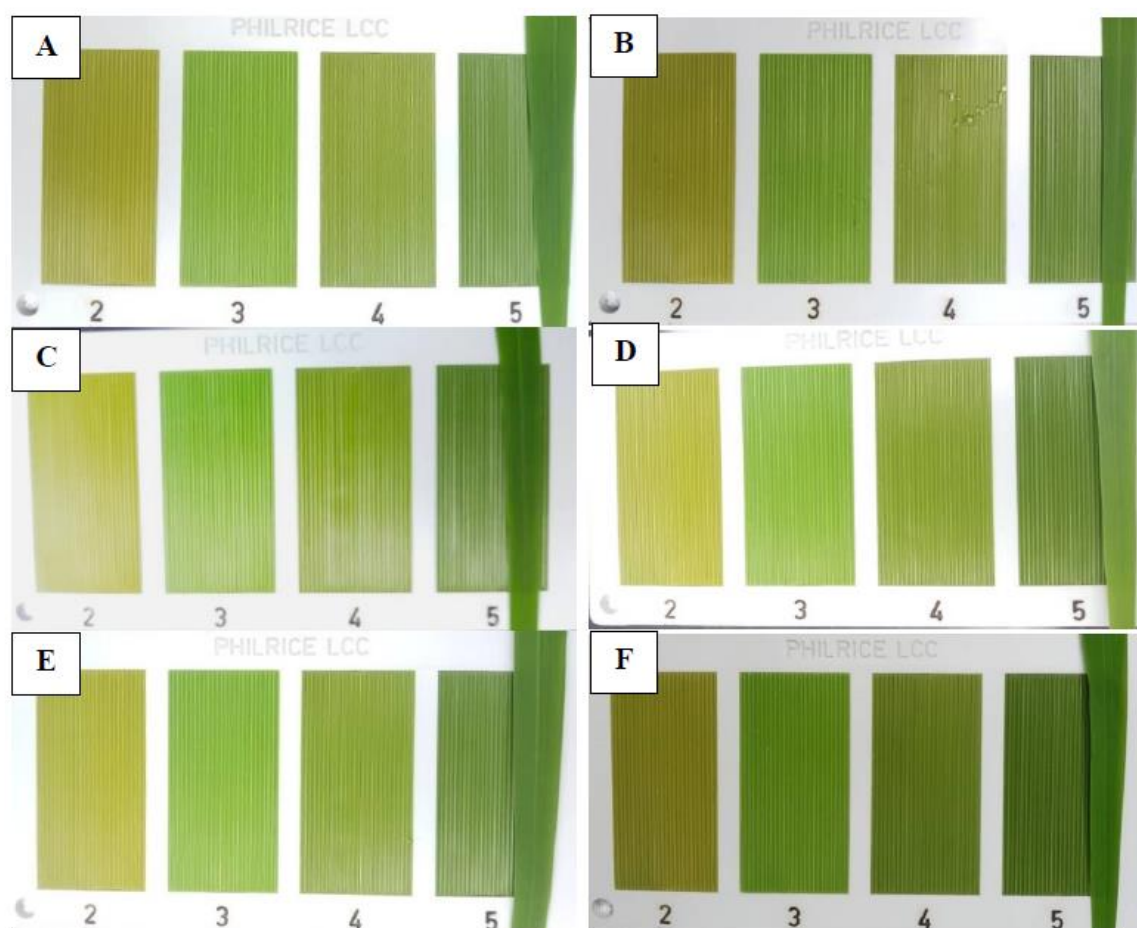


Figure 3: The Blade Colour of Rice Accessions in S₁ Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A, C and E** represent UNIMAS-29-S1. **B, D and F** represent UNIMAS-30-S1.

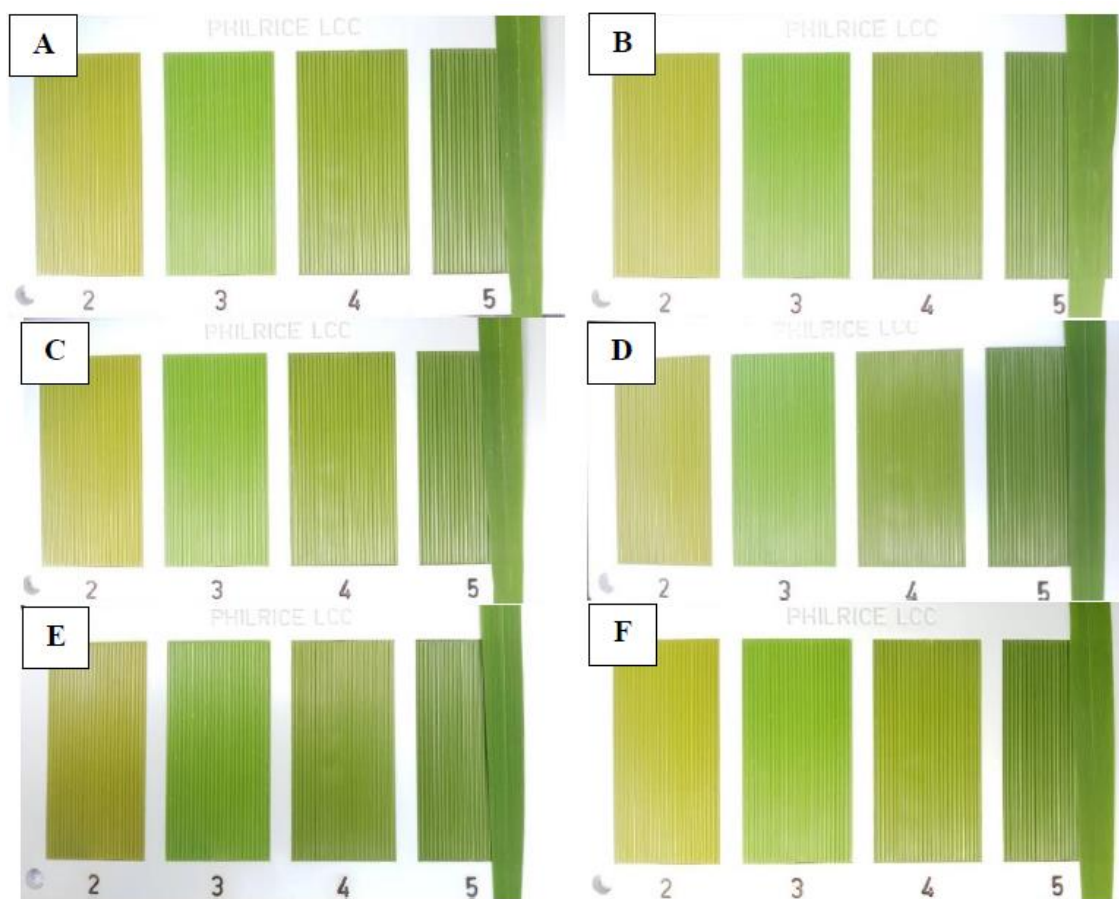


Figure 4: The Blade Colour of Rice Accessions in S₁ Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A, C and E** represent UNIMAS-31-S1. **B, D and F** represent UNIMAS-32-S1.

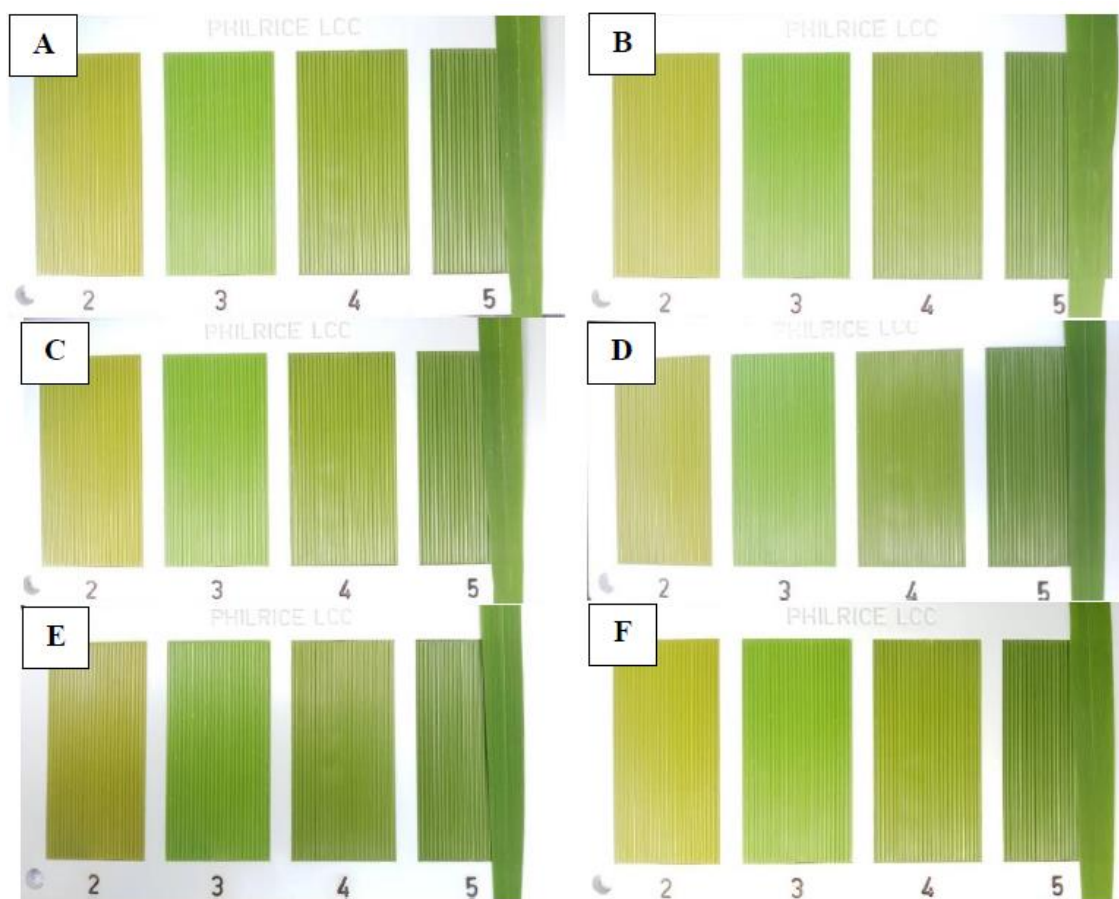


Figure 5: The Blade Colour of Rice Accessions in S_1 Generation. Scoring 3: Pale green; 4; Green; 5: Dark green. **A, C and E** represent UNIMAS-37-S1. **B, D and F** represent UNIMAS-43-S1.

APPENDIX 3: THE LIGULE AND AURICLE



Figure 1: The Ligule and Auricle. Vertical yellow arrows show the ligule. Horizontal white arrow represent auricle.

APPENDIX 4: THE PANICLES IN FIRST GENERATION

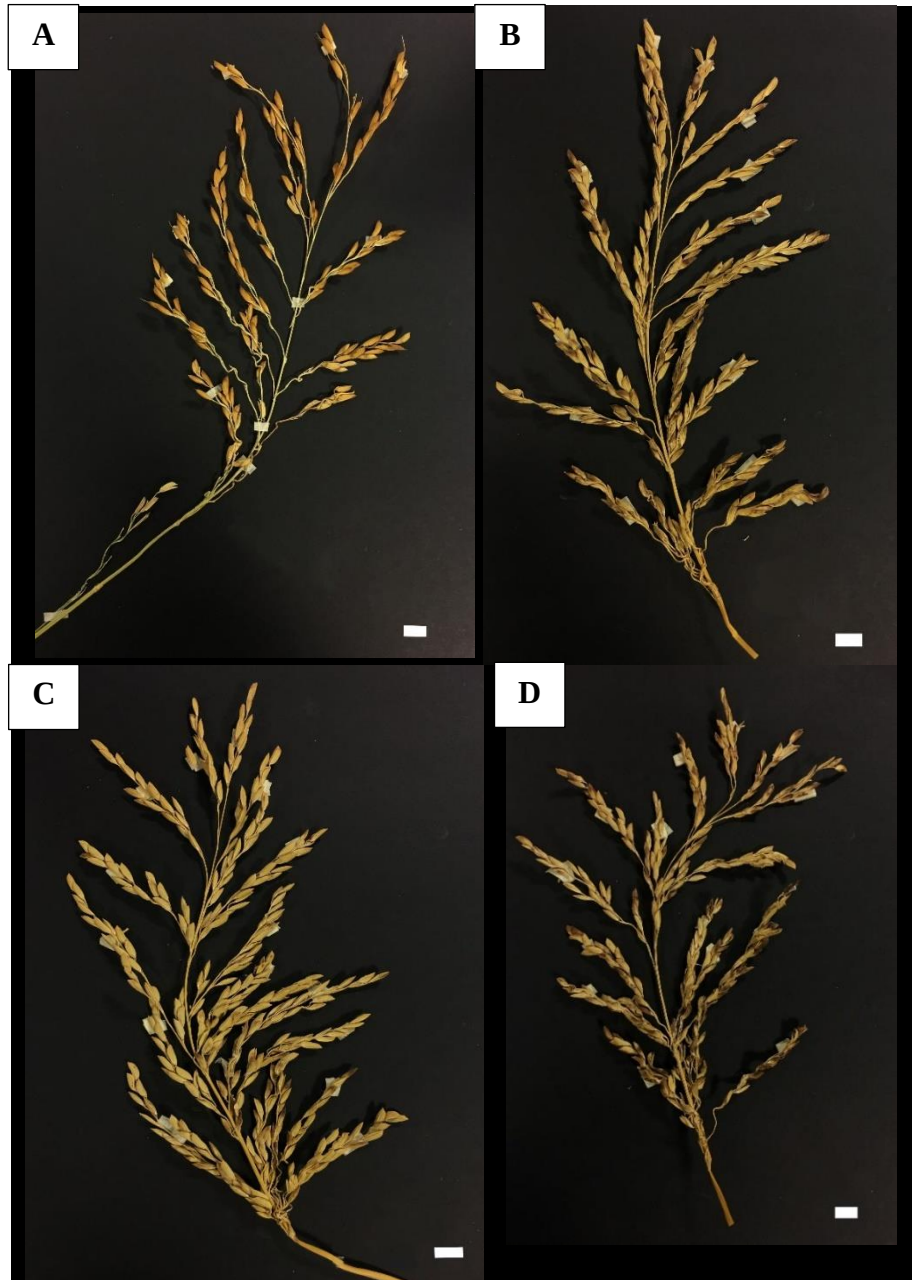


Figure 1: The Panicles of Rice Accessions in First Generation. Remarks: White bar indicates scale= 1 cm. **A-D** represent UNIMAS-23 – UNIMAS-26.

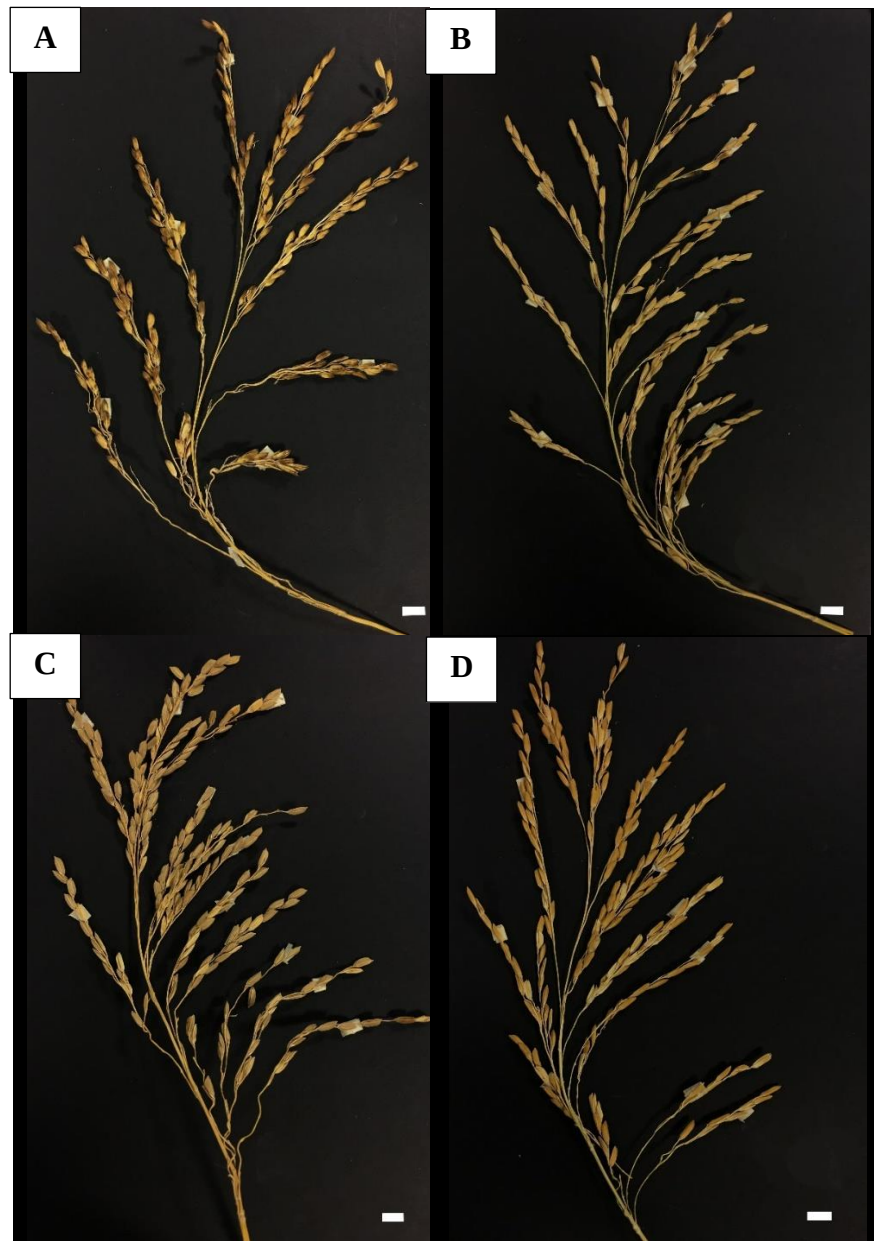


Figure 2: The Panicles of Rice Accessions in First Generation. Remarks: White bar indicates scale= 1 cm. **A-D** represent UNIMAS-27 – UNIMAS-30.



Figure 3: The Panicles of Rice Accessions in First Generation. Remarks: White bar indicates scale= 1 cm. **A-B** represent UNIMAS-31 – UNIMAS-32. **C-D** represent UNIMAS-35-36.

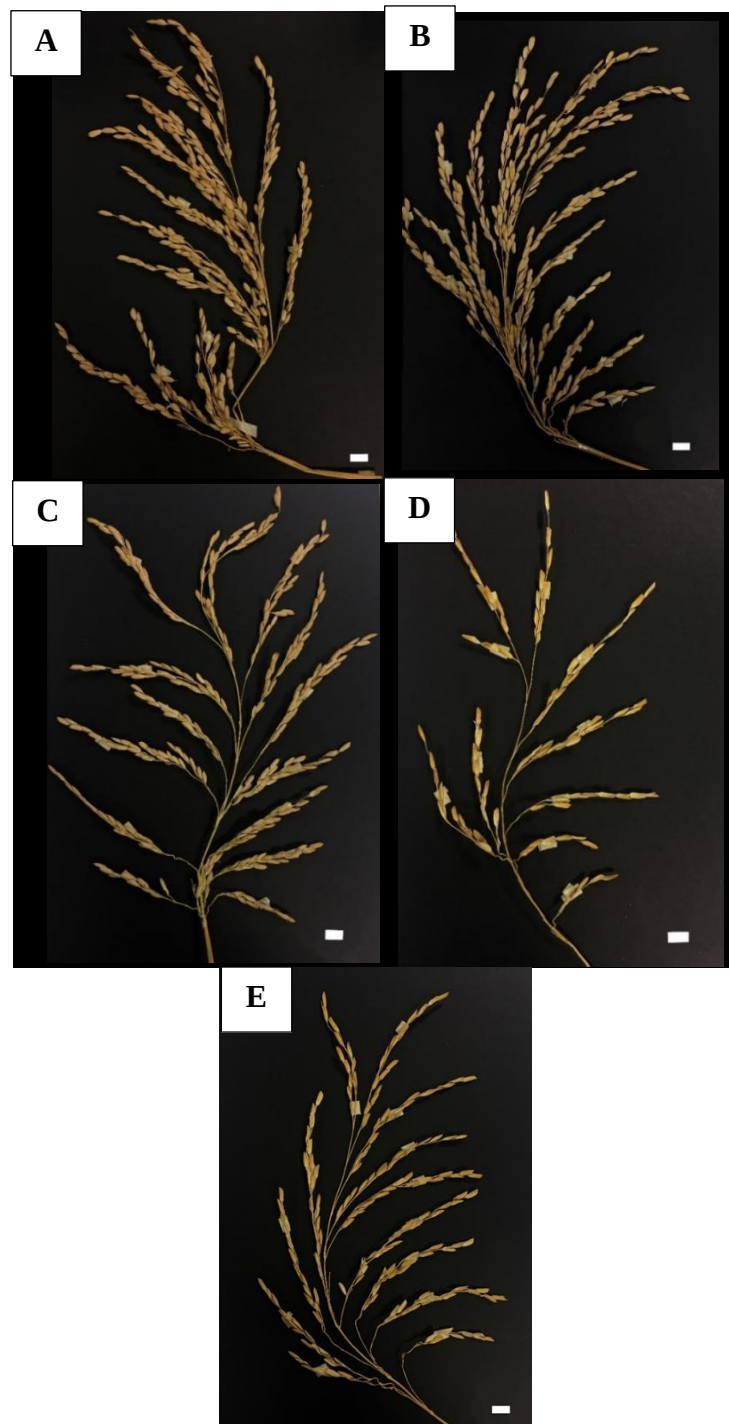


Figure 4: The Panicles of Rice Accessions in First Generation. Remarks: White bar indicates scale= 1 cm. **A-C** represent UNIMAS-37 – UNIMAS-39. **D-E** represents UNIMAS-42 – UNIMAS-43.

APPENDIX 5: THE PANICLES IN S₁ GENERATION



Figure 1: The Panicles of Triplicates in UNIMAS-27-S1. Remarks: White bar indicates scale= 1 cm.

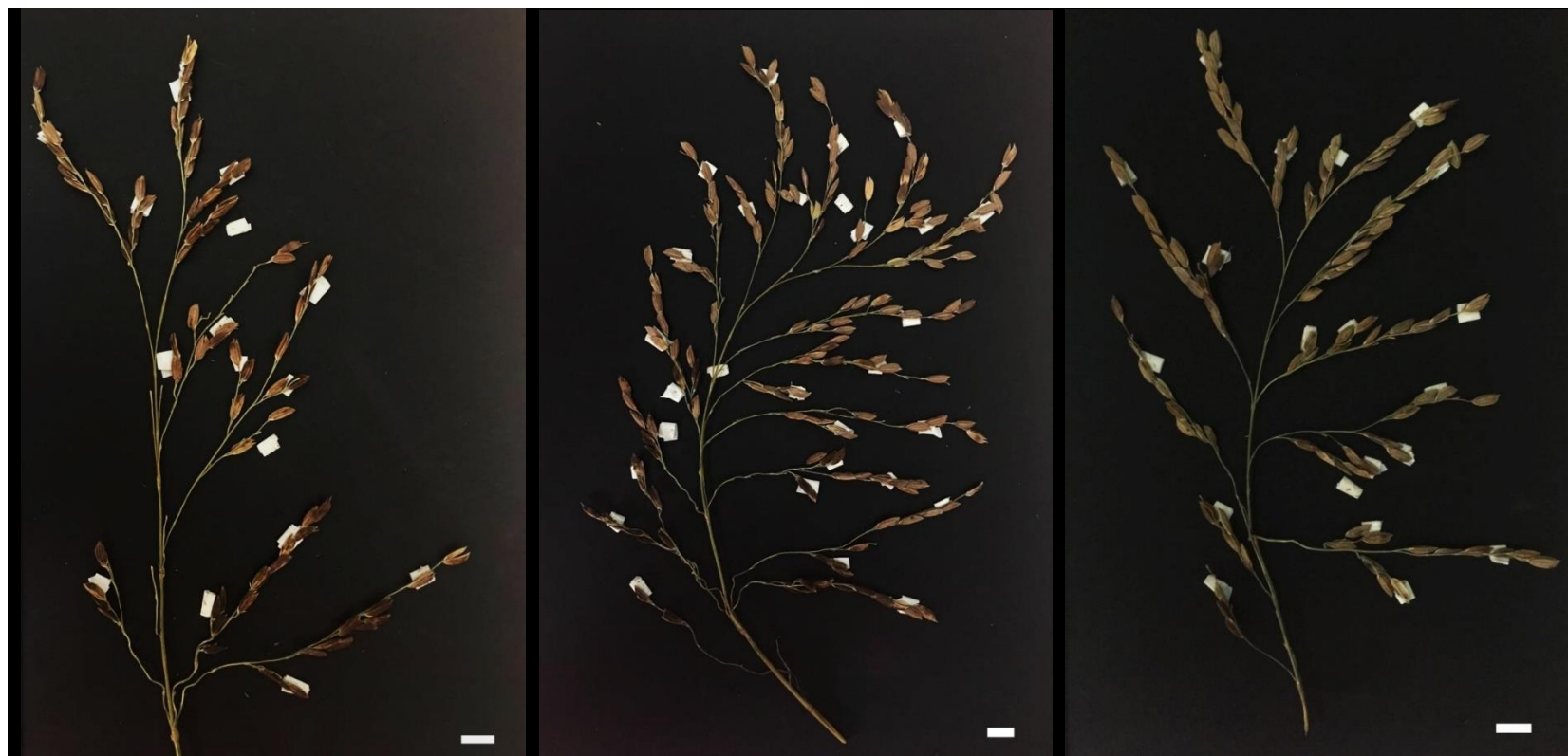


Figure 2: The Panicles of Triplicates in UNIMAS-29-S1. Remarks: White bar indicates scale= 1 cm.



Figure 3: The Panicles of Triplicates in UNIMAS-30-S1. Remarks: White bar indicates scale= 1 cm.



Figure 4: The Panicles of Triplicates in UNIMAS-31-S1. Remarks: White bar indicates scale= 1 cm.



Figure 5: The Panicles of Triplicates in UNIMAS-32-S1. Remarks: White bar indicates scale= 1 cm.



Figure 6: The Panicles of Triplicates in UNIMAS-43-S1. Remarks: White bar indicates scale= 1 cm.



Figure 7: The Panicles of Three Accessions in S₁ generation. **A:** UNIMAS-28-S1-C; **B:** UNIMAS-37-S1-A; **C:** UNIMAS-37-S1-B. Remarks: White bar indicates scale= 1 cm.

APPENDIX 6: THE SEEDS AND GRAINS MORPHOLOGY



Figure 1: The Morphology of Lowland Seeds and Grains From First Generation. **A-C** represent UNIMAS-23 – UNIMAS-25. Remarks: White bar indicates scale= 1 cm.



Figure 2: The Morphology of Lowland Seeds and Grains From First Generation. **A-C** represent UNIMAS-26 – UNIMAS-28. Remarks: White bar indicates scale= 1 cm.



Figure 3: The Morphology of Lowland Seeds and Grains From First Generation. **A-C** represent UNIMAS-29 – UNIMAS-31. Remarks: White bar indicates scale= 1 cm. * UNIMAS-31 was scored as having pale yellow seed colour upon observation on field.



Figure 4: The Morphology of Lowland Seeds and Grains From First Generation. **A:** UNIMAS-32; **B:** UNIMAS-35; **C:** UNIMAS-36. Remarks: White bar indicates scale= 1 cm.



Figure 5: The Morphology of Lowland Seeds and Grains From First Generation. **A-C** represent UNIMAS-37 – UNIMAS-39. Remarks: White bar indicates scale= 1 cm.



Figure 6: The Morphology of Lowland Seeds and Grains From First Generation. **A:** UNIMAS-42; **B:** UNIMAS-43. Remarks: White bar indicates scale= 1 cm.

APPENDIX 7: THE LEAVES ANATOMY

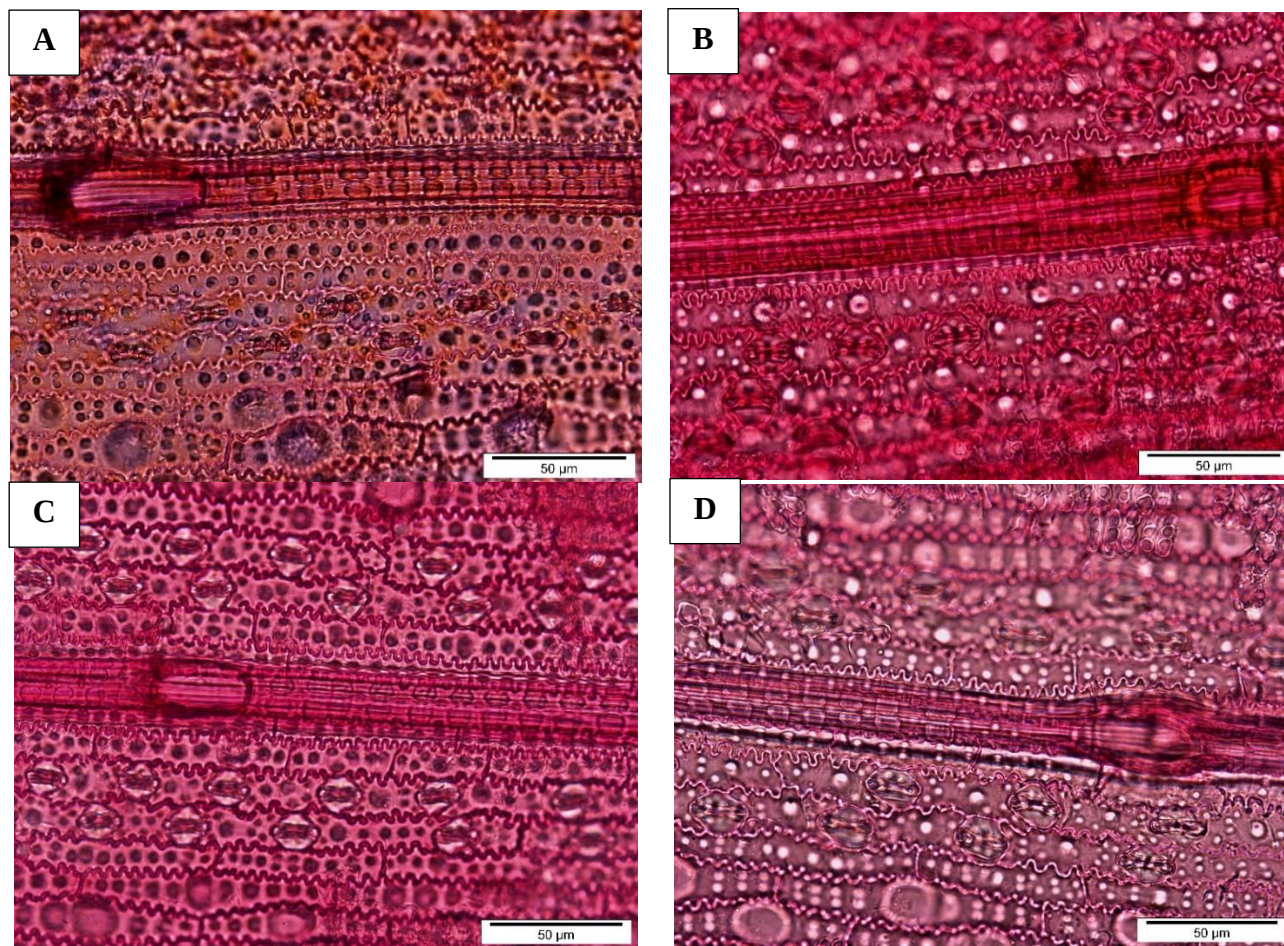


Figure 1: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation.
Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-23 abaxial and adaxial surfaces. **C-D:** UNIMAS-24 abaxial and adaxial surfaces

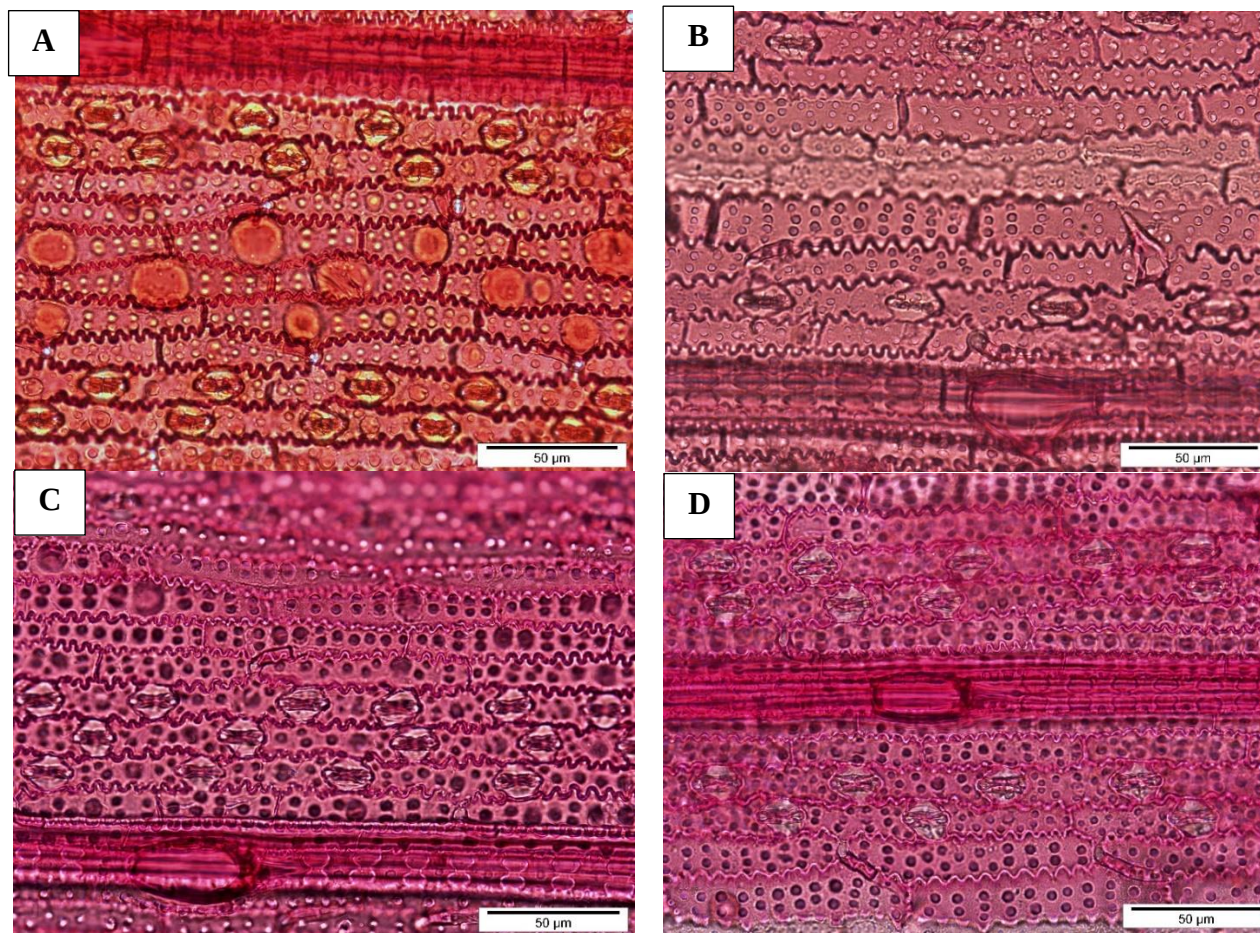


Figure 2: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-25 abaxial and adaxial surfaces. **C-D:** UNIMAS-26 abaxial and adaxial surfaces.

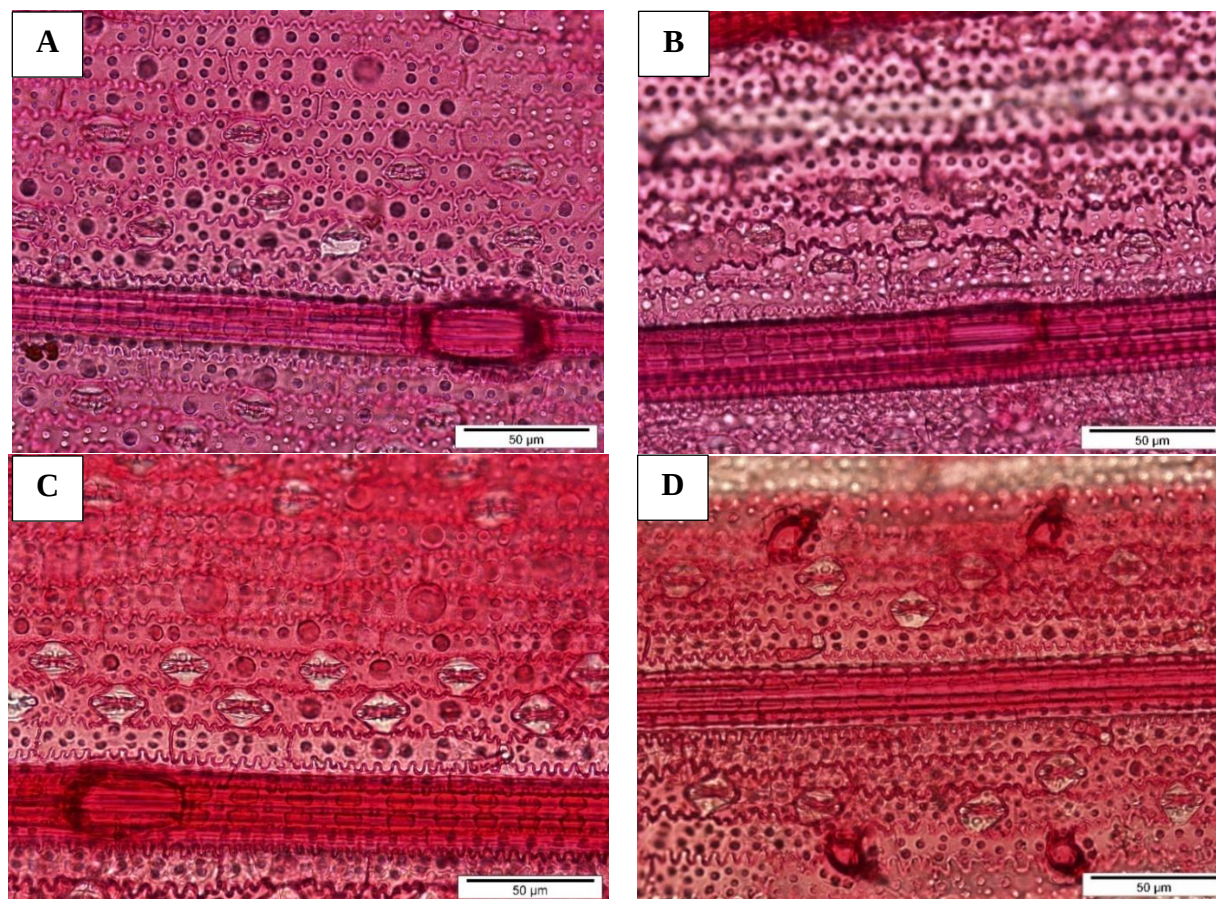


Figure 3: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-27 abaxial and adaxial surfaces. **C-D:** UNIMAS-28 abaxial and adaxial surfaces.

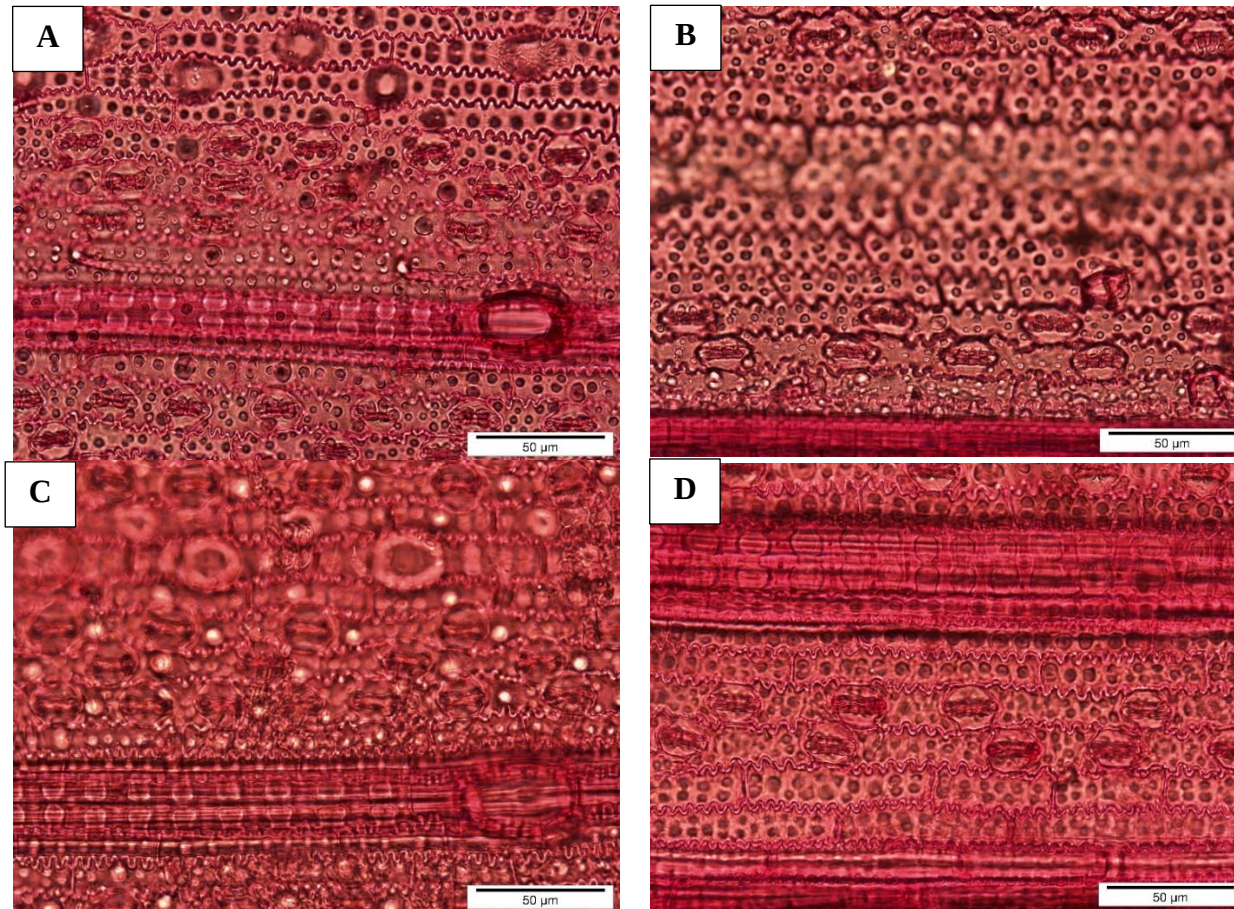


Figure 4: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-29 abaxial and adaxial surfaces. **C-D:** UNIMAS-30 abaxial and adaxial surfaces.

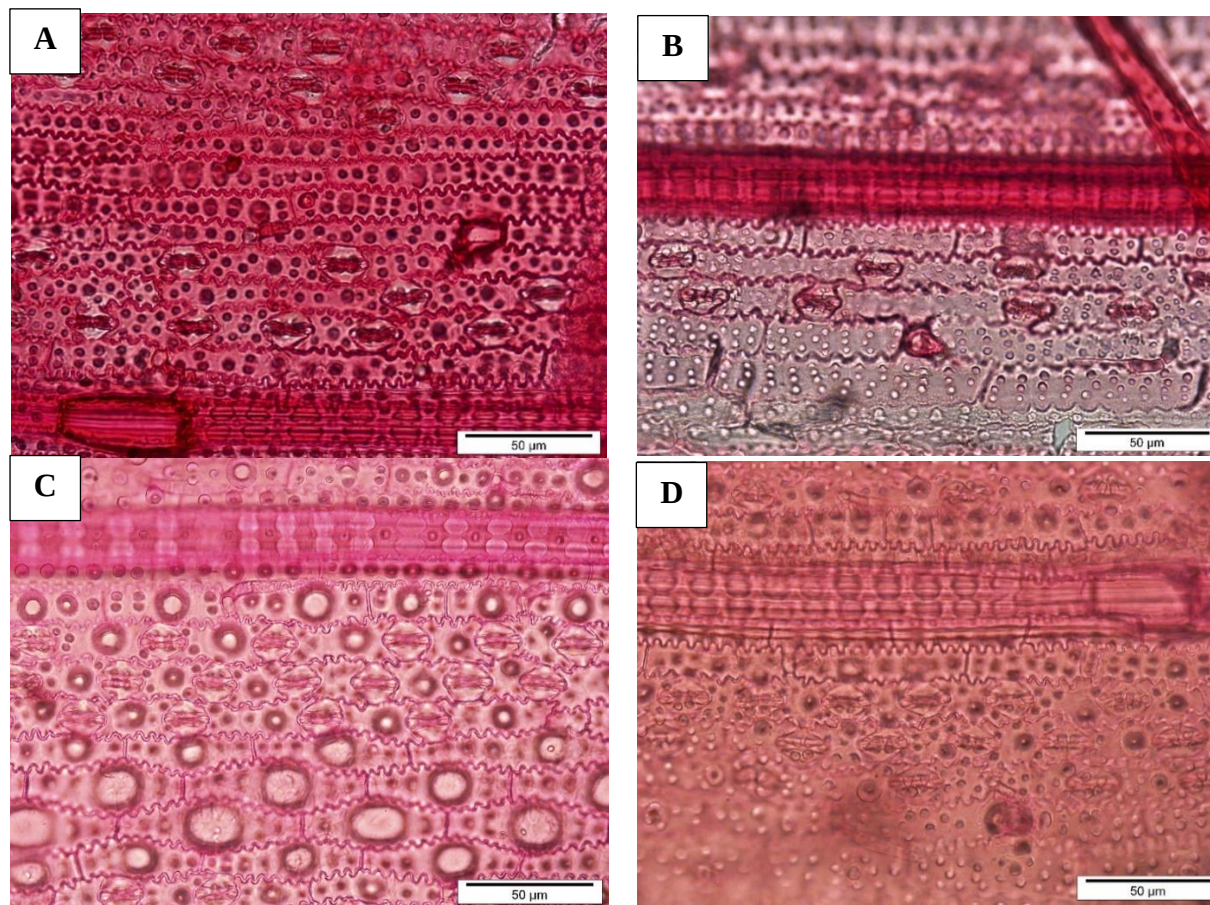


Figure 5: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-31 abaxial and adaxial surfaces. **C-D:** UNIMAS-32 abaxial and adaxial surfaces.

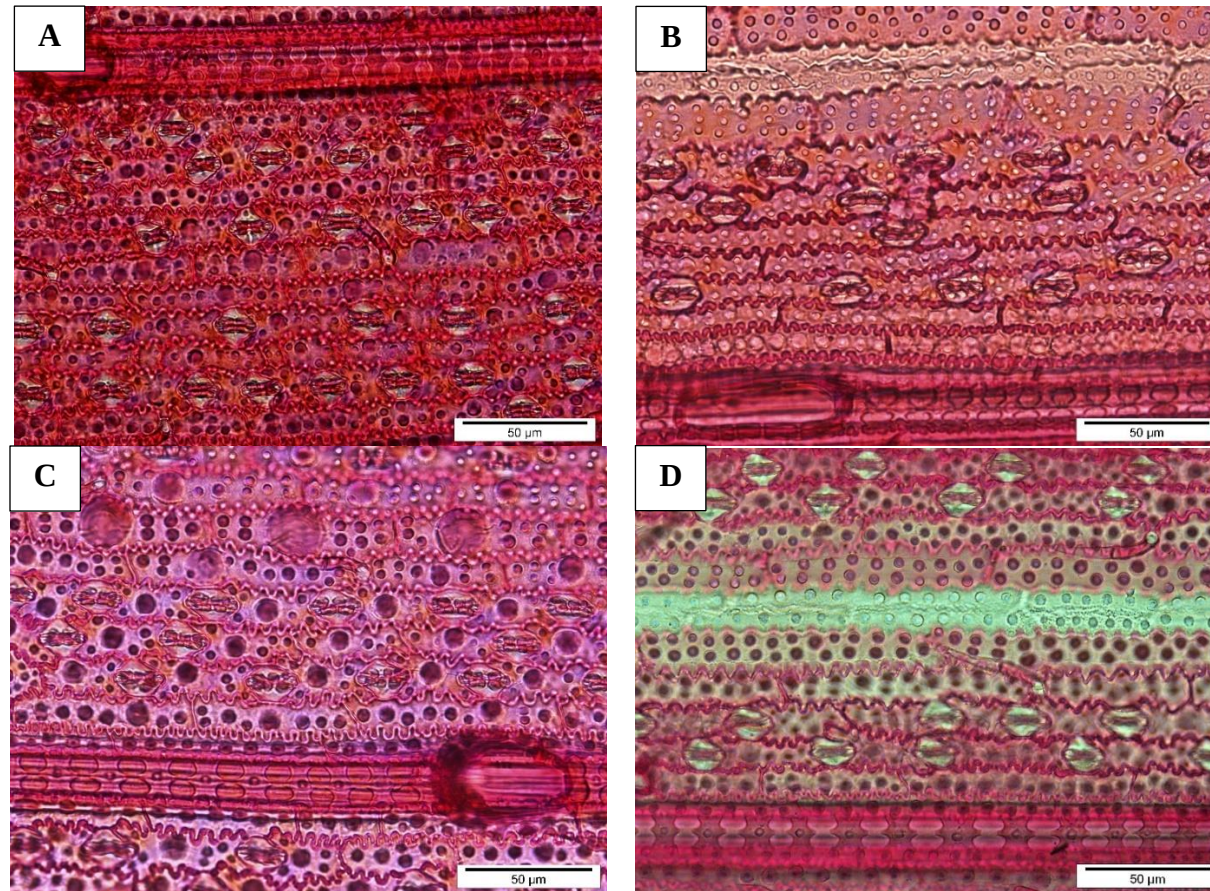


Figure 6: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-33 abaxial and adaxial surfaces. **C-D:** UNIMAS-34 abaxial and adaxial surfaces.

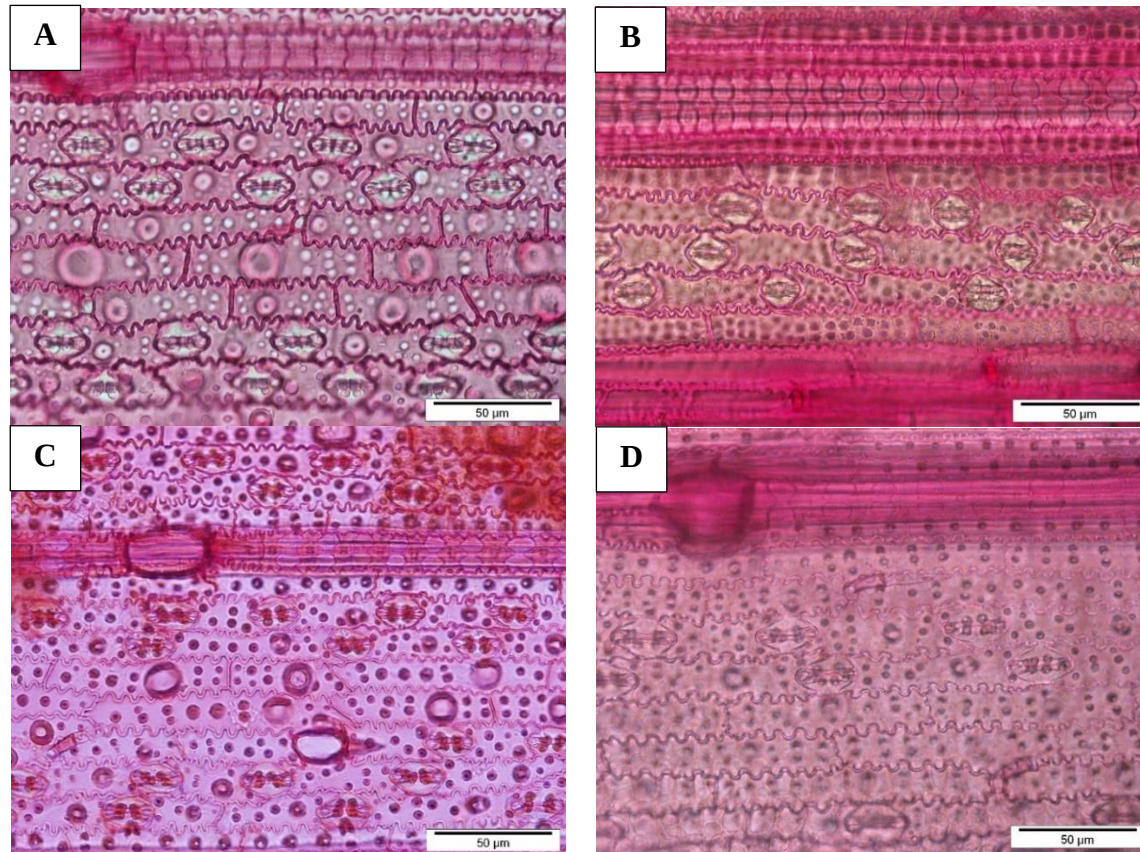


Figure 7: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-35 abaxial and adaxial surfaces. **C-D:** UNIMAS-36 abaxial and adaxial surfaces.

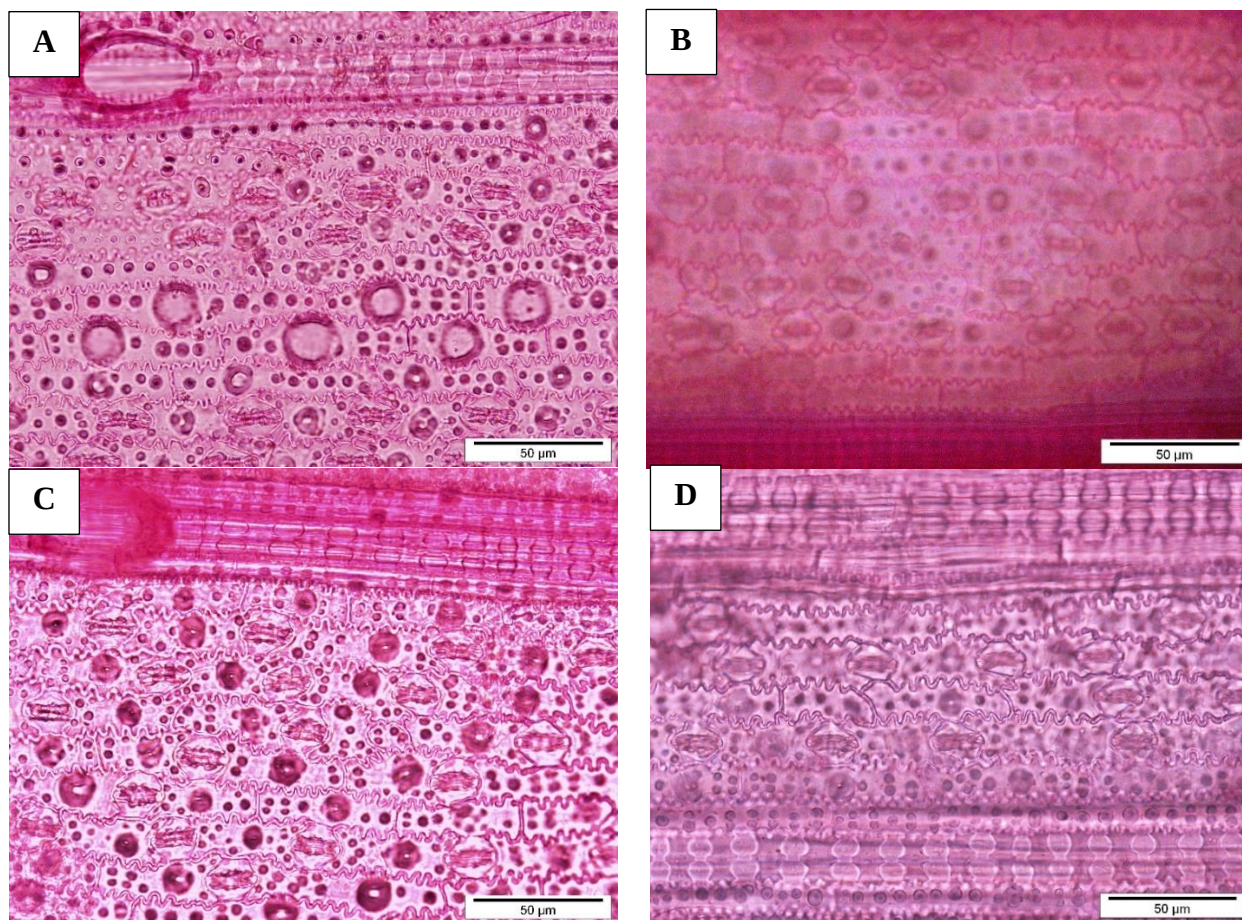


Figure 8: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-37 abaxial and adaxial surfaces. **C-D:** UNIMAS-38 abaxial and adaxial surfaces.

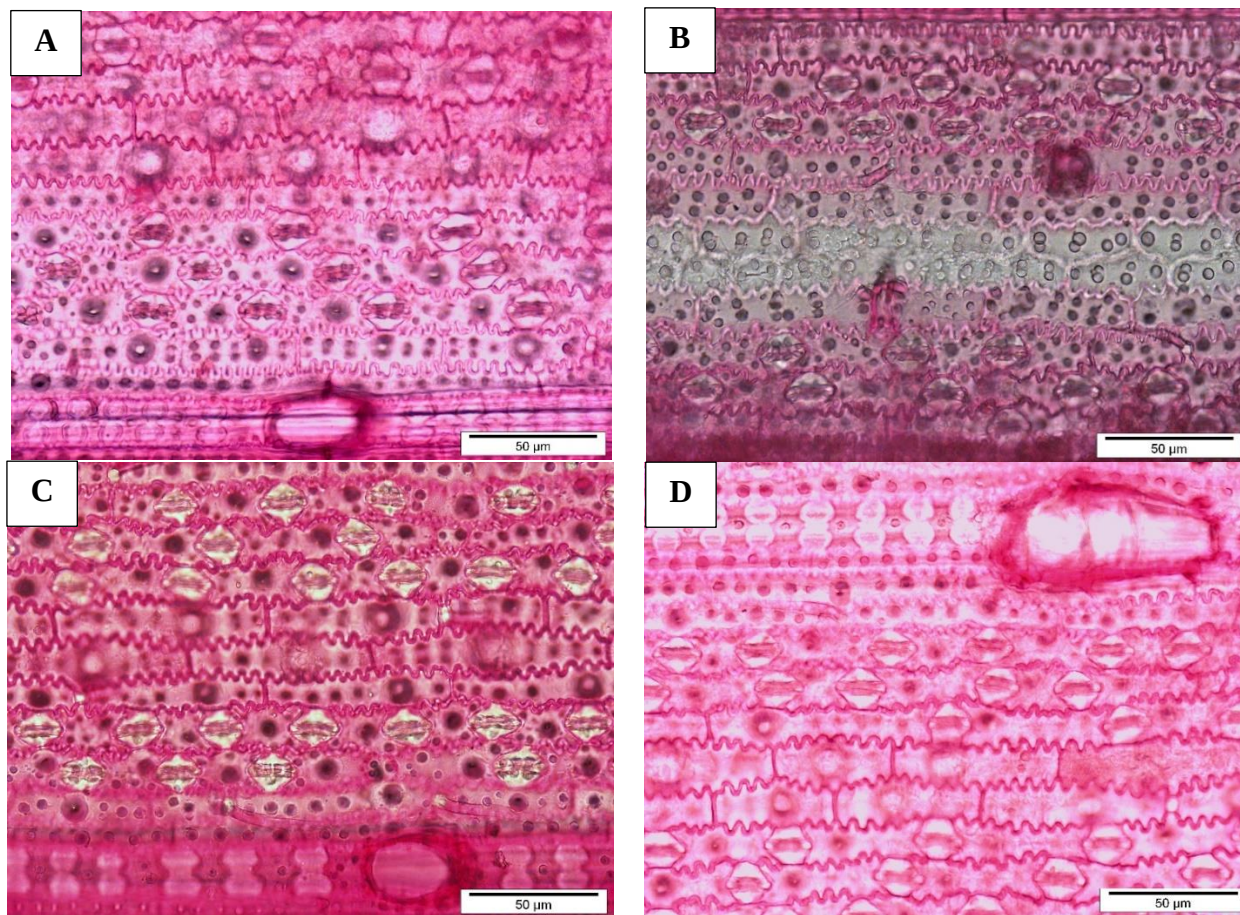


Figure 9: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation.
 Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-39 abaxial and adaxial surfaces. **C-D:** UNIMAS-40 abaxial and adaxial surfaces.

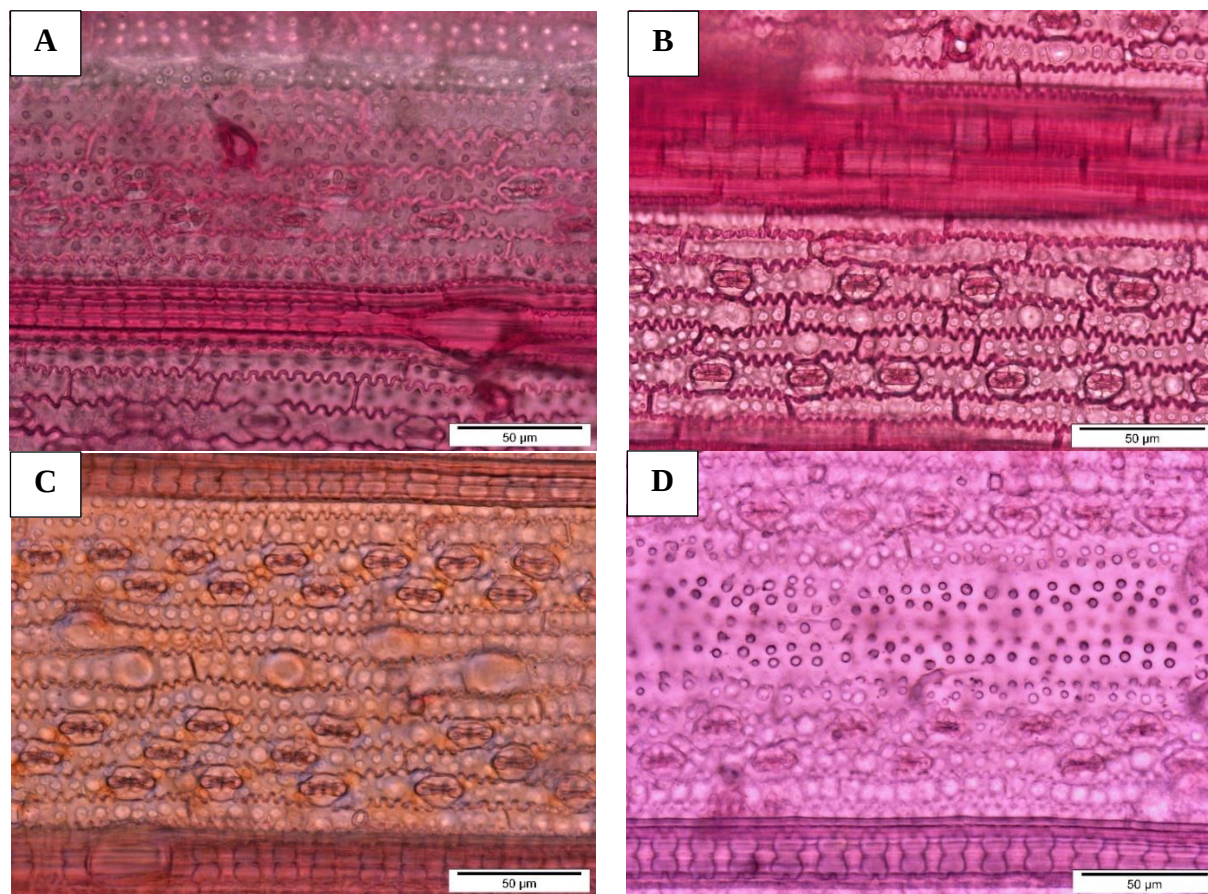


Figure 10: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-41 abaxial and adaxial surfaces. **C-D:** UNIMAS-42 abaxial and adaxial surfaces.

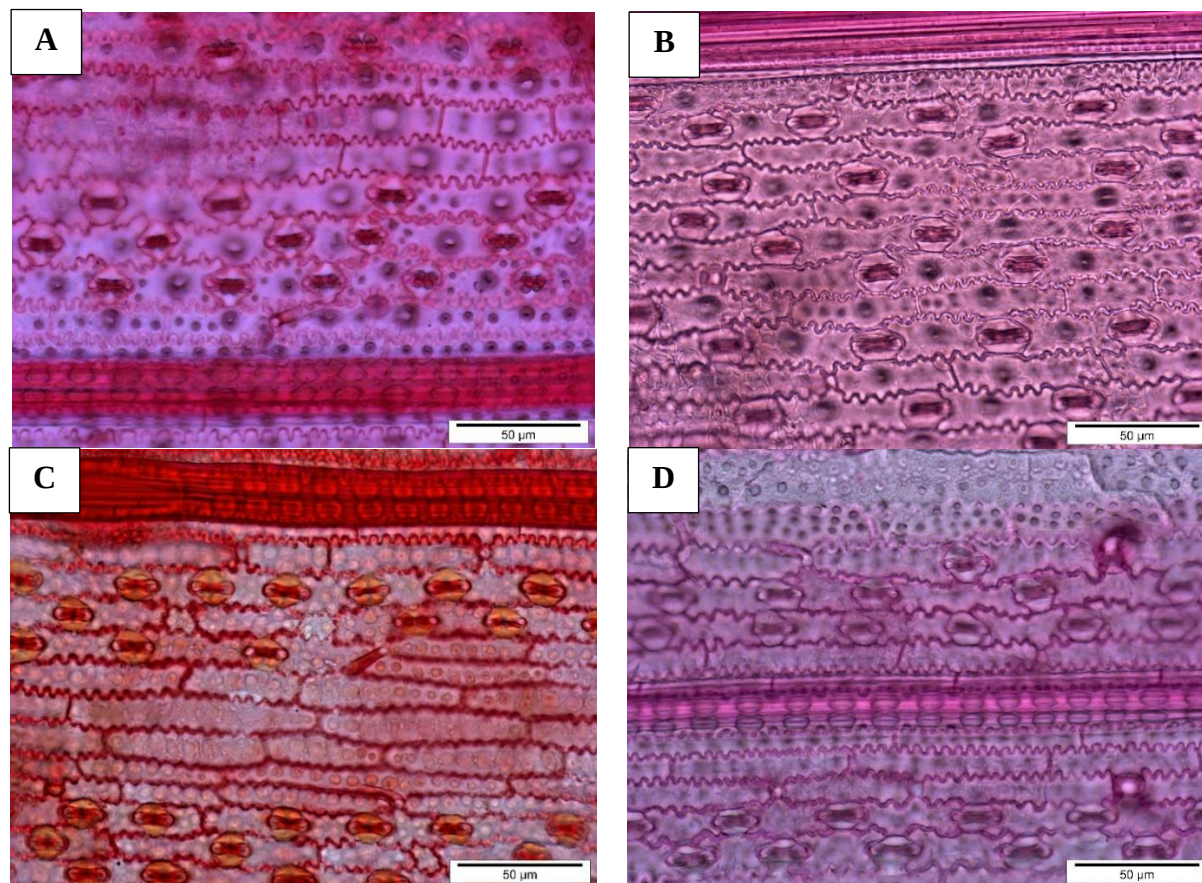


Figure 11: The Abaxial and Adaxial Leaves Surfaces of 22 Rice Accessions in First Generation. Remarks: Magnification= 60x. Scale= 50 µm. **A-B:** UNIMAS-43 abaxial and adaxial surfaces. **C-D:** UNIMAS-44 abaxial and adaxial surfaces.

APPENDIX 8: THE MIDRIB ANATOMY

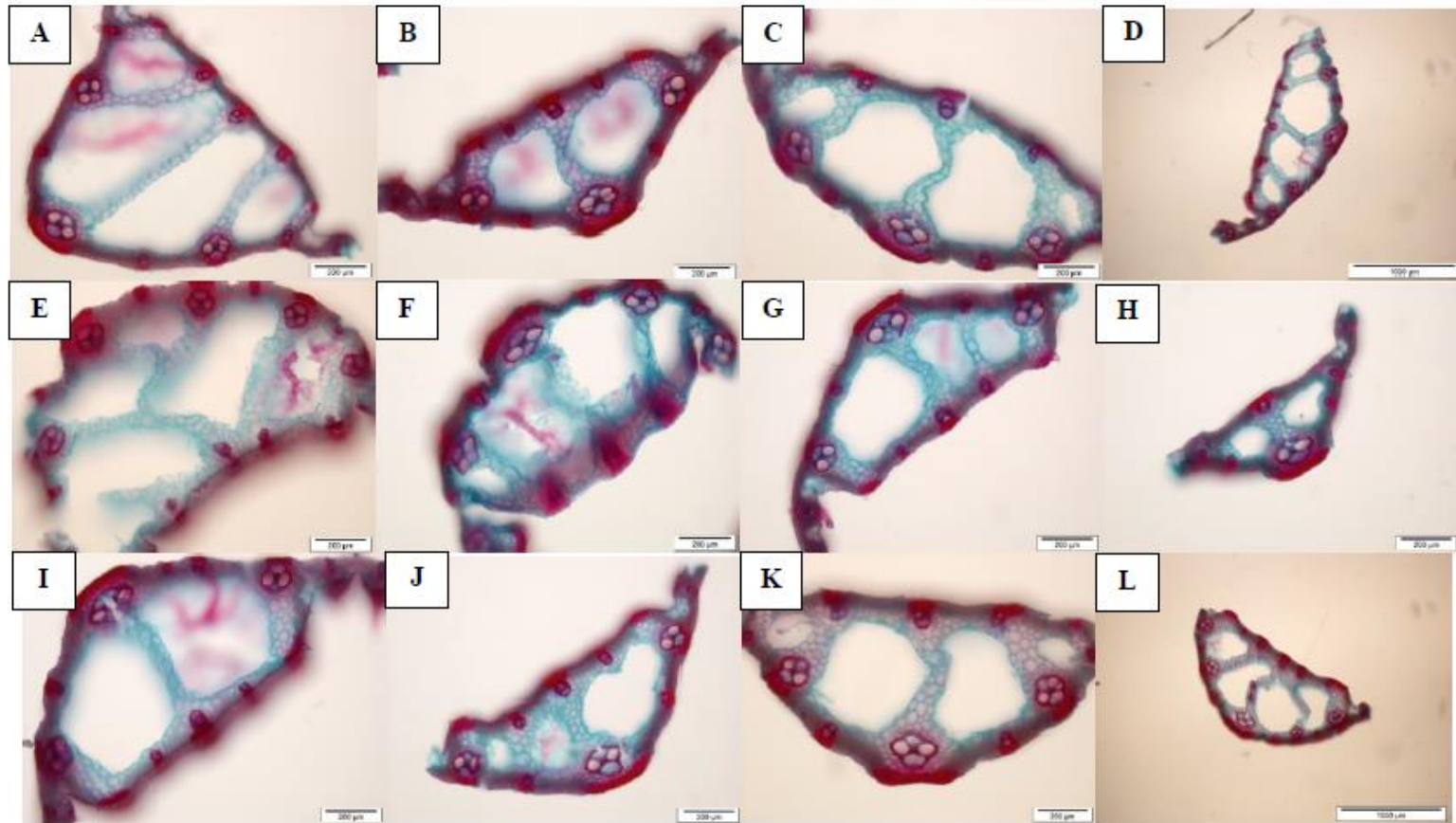


Figure 1: The Midrib Anatomy of Lowland Rice Accessions in First Generation. Scale bar indicates the measurement in micrometres. **A-L** represent midrib of accession UNIMAS-23 – UNIMAS-34.

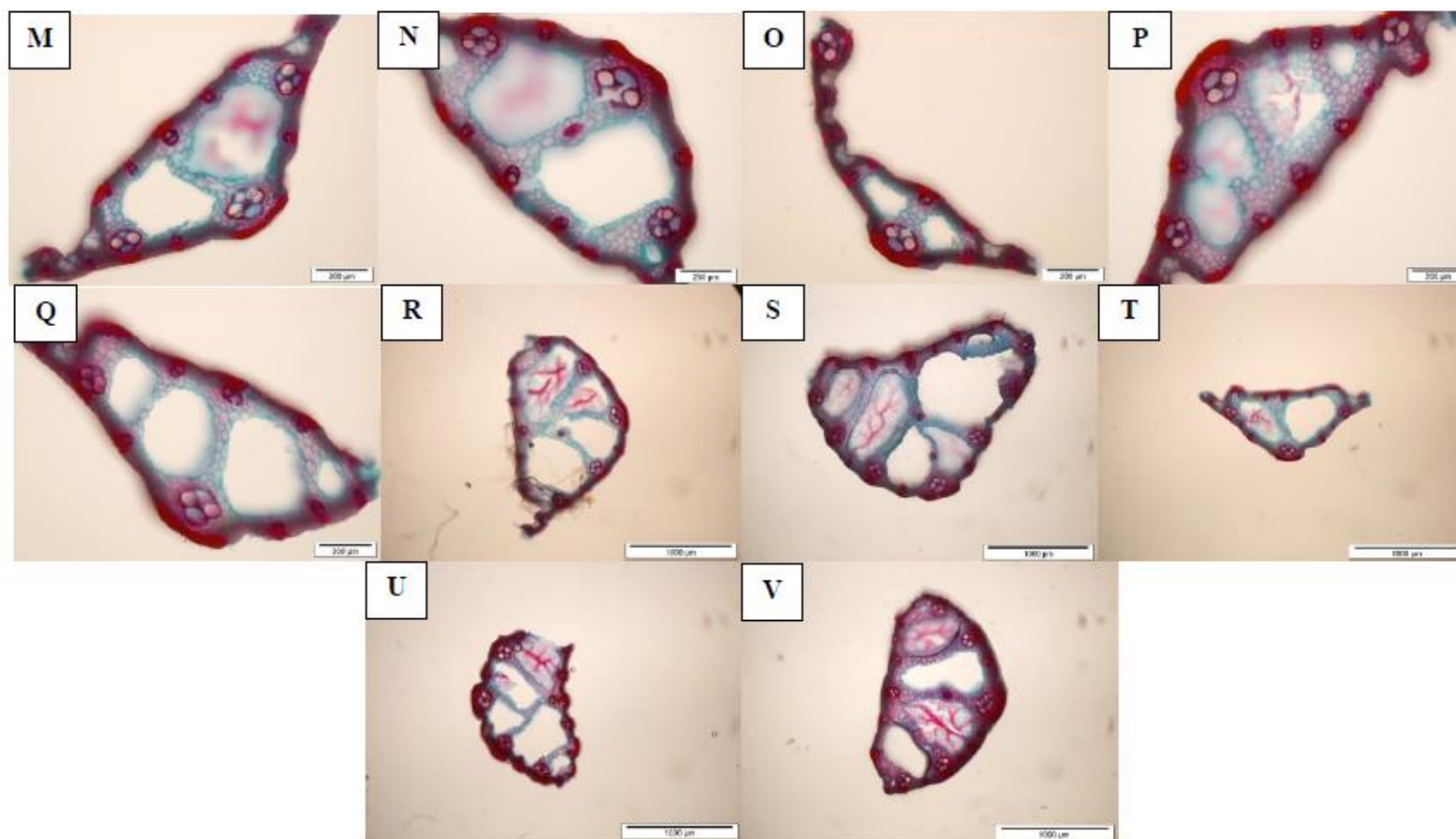


Figure 2: The Midrib Anatomy of Lowland Rice Accessions in First Generation. Scale bar indicates the measurement in micrometres. **M-V** represent midrib of accession UNIMAS-35 – UNIMAS-44.

APPENDIX 9: THE ROOT ANATOMY

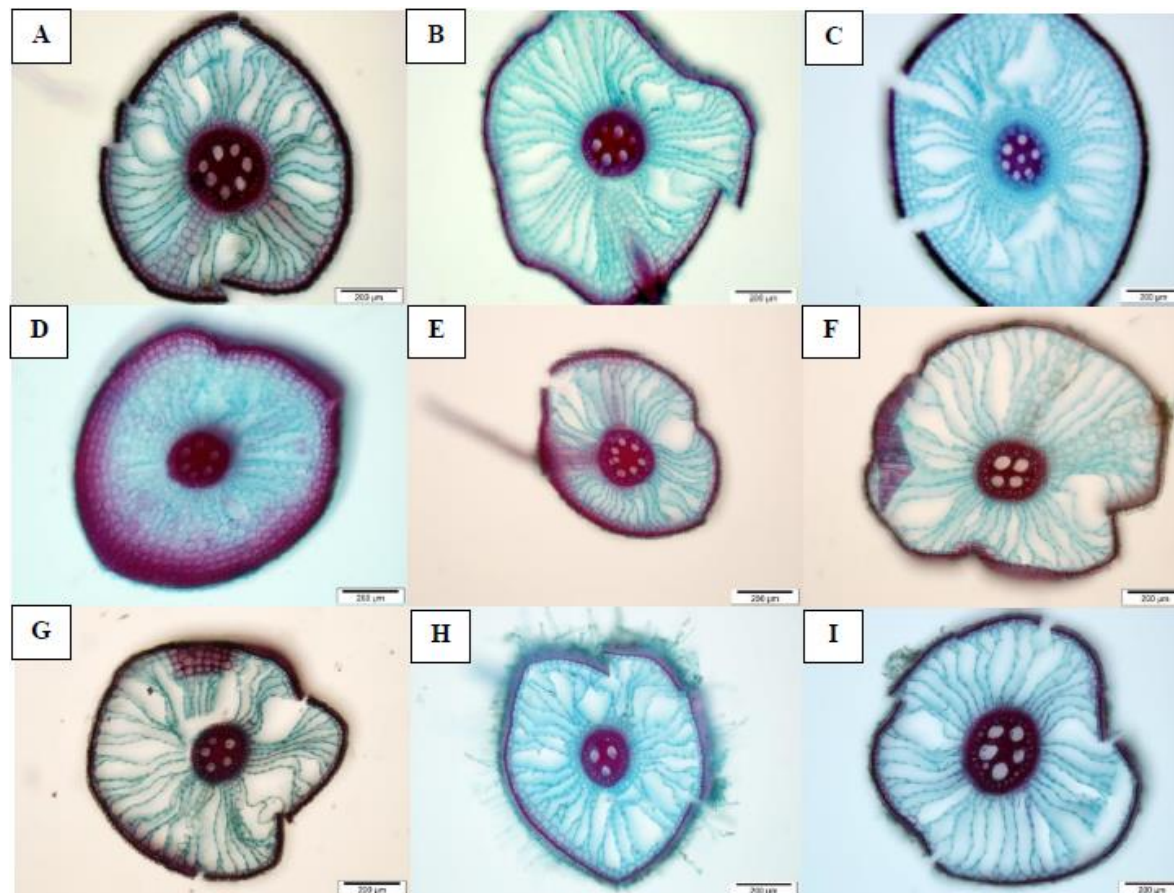


Figure 1: The Root Anatomy of Lowland Rice Accessions in First Generation. Scale bar indicates the measurement in micrometres. **A-I** represent midrib of accession UNIMAS-23 – UNIMAS-31.

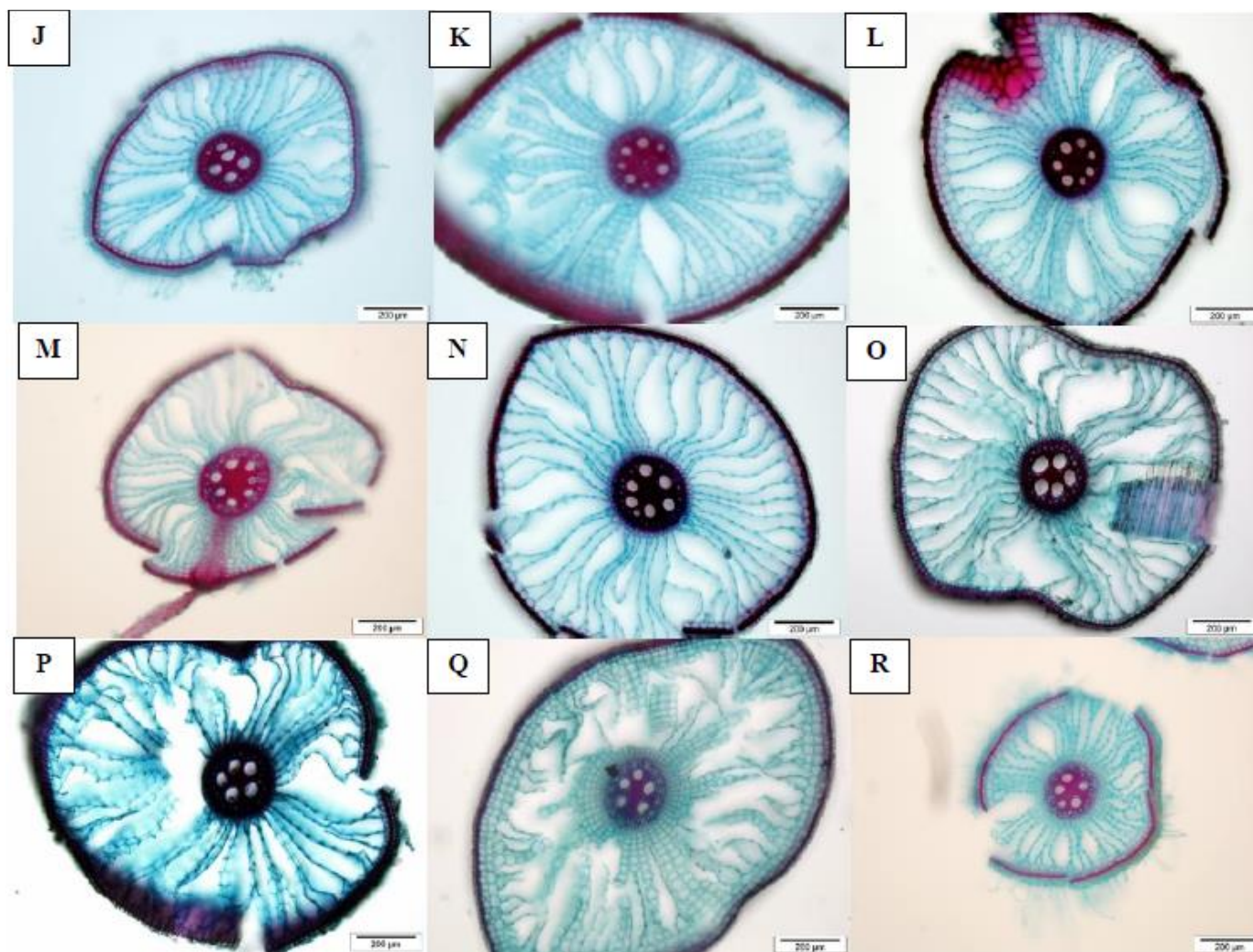


Figure 2: The Root Anatomy of Lowland Rice Accessions in First Generation. Scale bar indicates the measurement in micrometres. **J-R** represent midrib of accession UNIMAS-32 – UNIMAS-40.

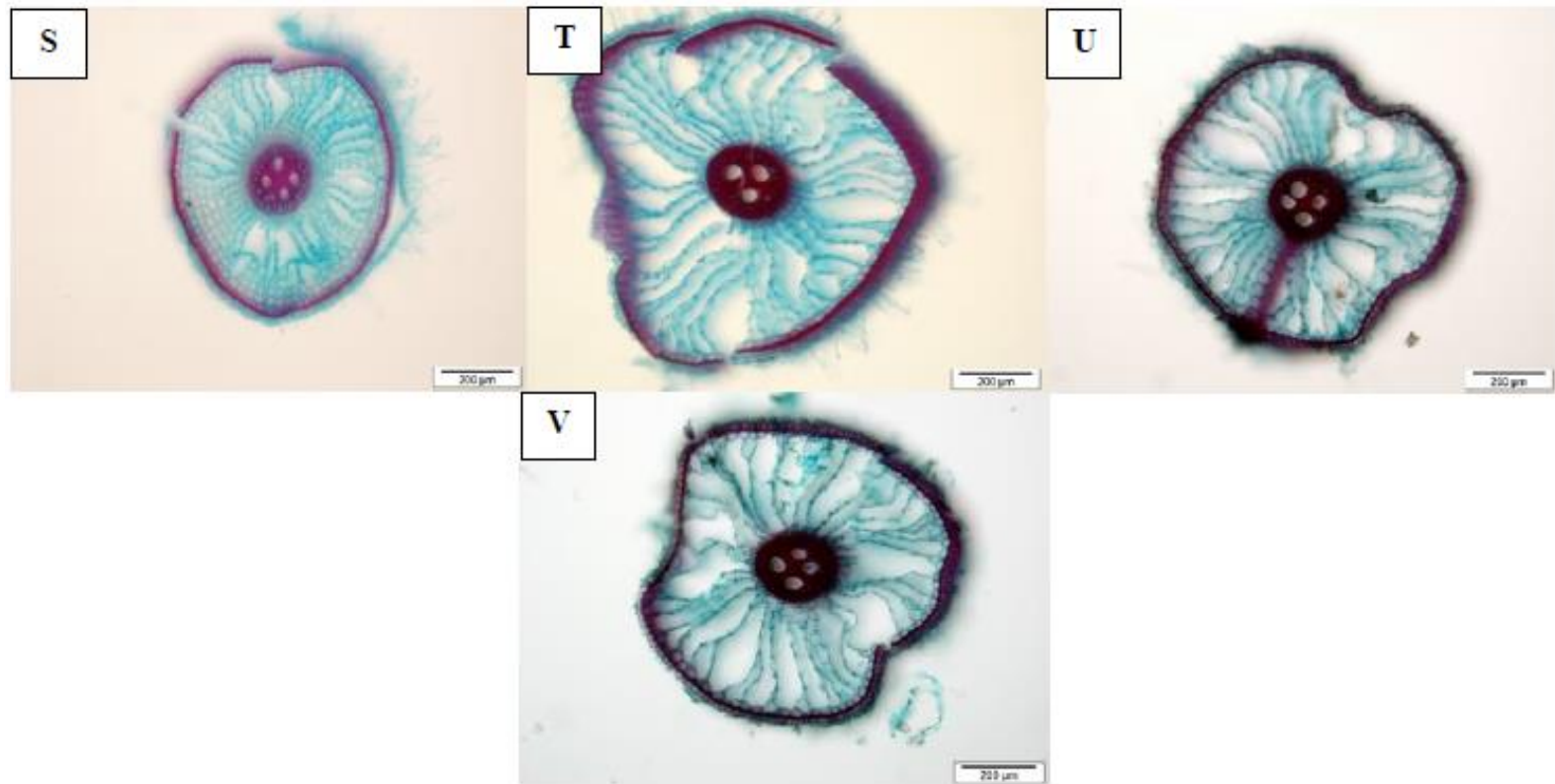


Figure 3: The Root Anatomy of Lowland Rice Accessions in First Generation. Scale bar indicates the measurement in micrometres. S-V represent midrib of accession UNIMAS-41 – UNIMAS-44.

APPENDIX 10: THE BANDING PATTERN OF THE SEVEN POLYMORPHIC SSR MARKERS

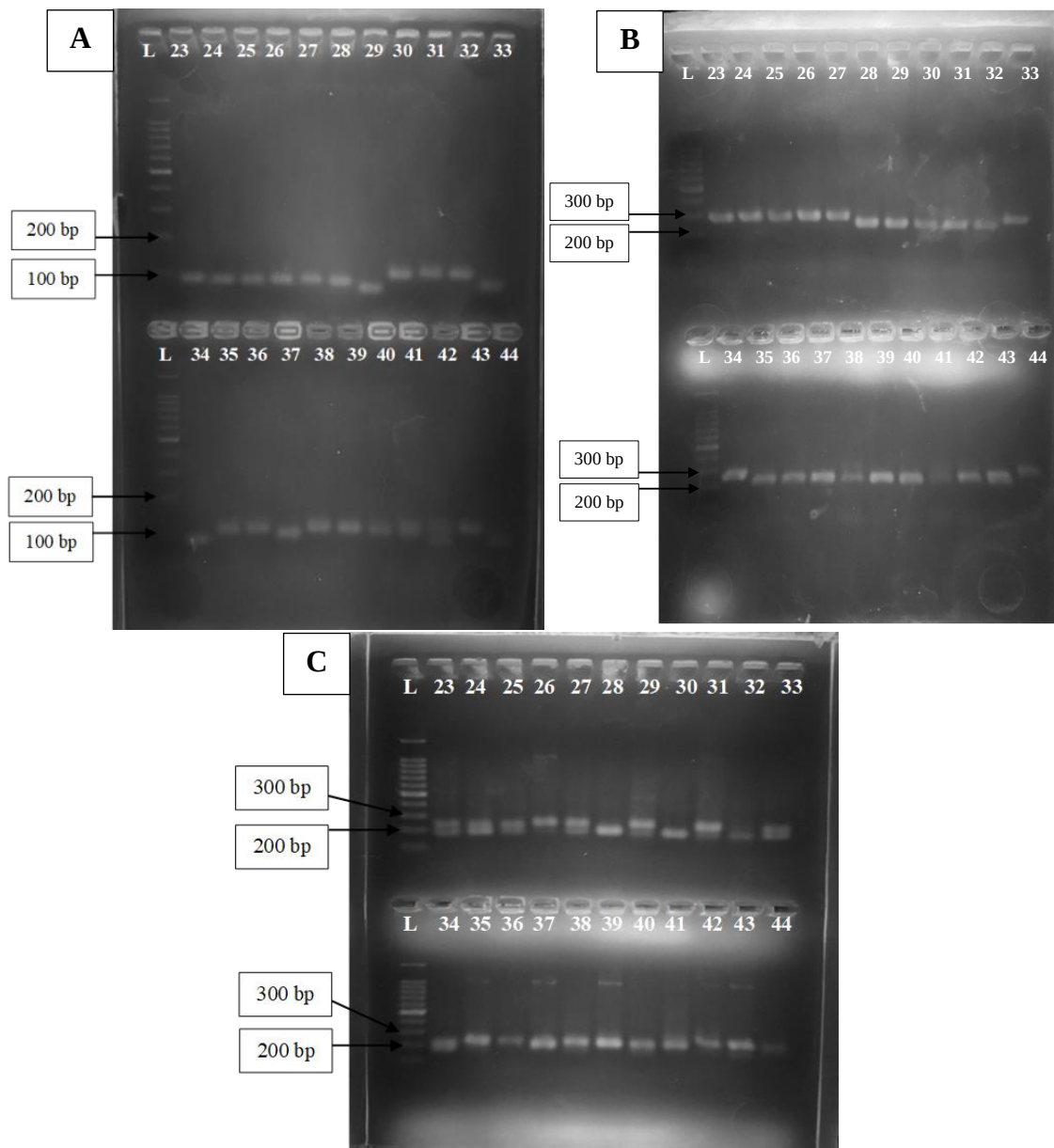


Figure 1: The Banding Pattern of Polymorphic SSR Markers. **A:** RM1, **B:** RM489, **C:** RM552. **L**= 100 bp ladder. The number represents the 22 UNIMAS collections from UNIMAS-23 to UNIMAS-44.

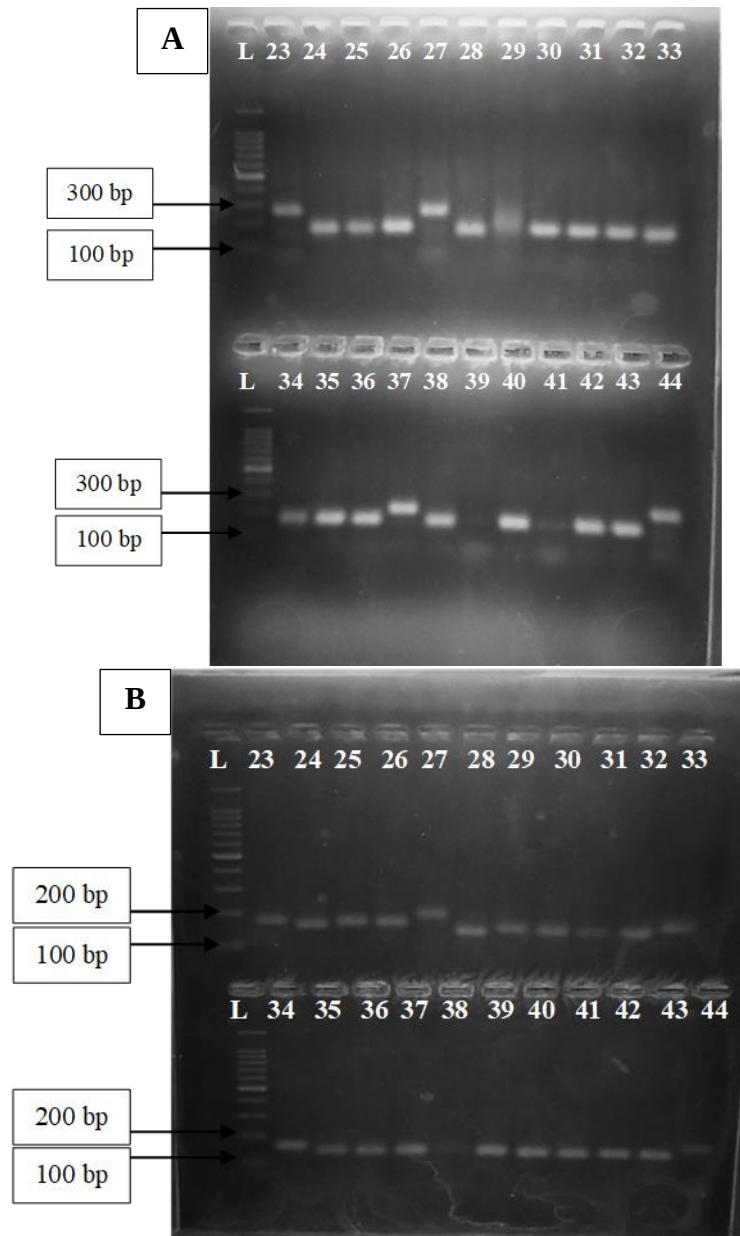


Figure 2: The Banding Pattern of Polymorphic SSR Markers. **A:** RM444, **B:** RM257. **L=** 100 bp ladder. The number represents the 22 UNIMAS collections from UNIMAS-23 to UNIMAS-33.

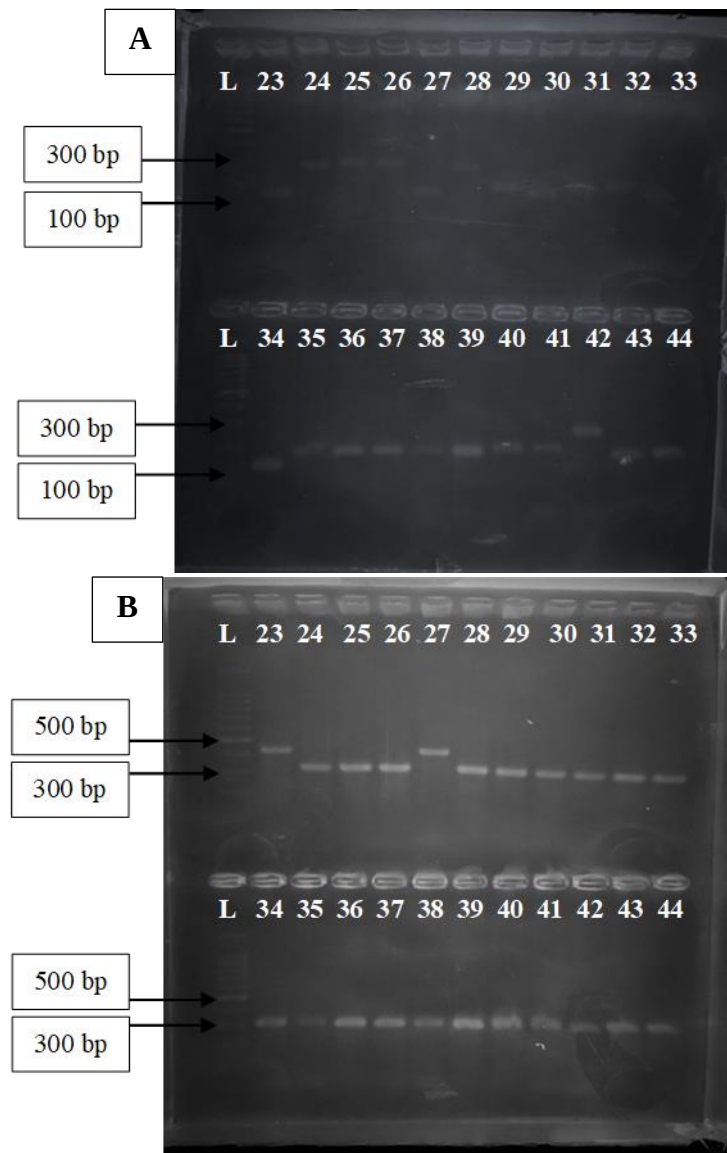


Figure 3: The Banding Pattern of Polymorphic SSR Markers. **A:** RM481, **B:** RM166. **L=** 100 bp ladder. The number represents the 22 UNIMAS collections from UNIMAS-23 to UNIMAS-33.

APPENDIX 11: THE GENETIC DISTANCE OF SSR MARKERS

Table 5: The Genetic Distance of 22 Lowland Rice Accessions Using Seven SSR Markers

23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	
0																						23
8	0																					24
6	2	0																				25
6	2	0	0																			26
2	8	8	8	0																		27
11	5	5	5	11	0																	28
10	6	8	8	10	7	0																29
13	7	9	9	13	6	5	0															30
10	8	8	8	10	5	4	5	0														31
11	9	9	9	11	4	5	4	1	0													32
8	6	4	4	10	9	6	7	8	9	0												33
9	7	5	5	11	8	7	6	9	8	1	0											34
9	5	7	7	9	6	3	6	5	6	9	10	0										35
9	5	7	7	9	6	3	6	5	6	9	10	0	0									36
9	9	11	11	9	10	3	8	7	8	9	10	4	4	0								37
9	7	7	7	9	8	5	6	3	4	7	8	4	4	6	0							38
10	8	10	10	10	9	4	5	4	5	10	11	3	3	3	3	0						39
11	7	9	9	11	8	3	4	3	4	9	10	2	2	4	2	1	0					40
10	8	10	10	10	9	4	5	4	5	10	11	3	3	3	3	0	1	0				41
12	4	6	6	12	5	6	5	6	7	10	11	3	3	7	5	4	3	4	0			42
11	7	9	9	11	8	3	4	3	4	9	10	2	2	4	2	1	0	1	3	0		43
3	7	5	5	5	8	9	10	9	8	7	6	8	8	8	8	9	10	9	11	10	0	44

* The number 23-24 represents the accessions number UNIMAS-23 to UNIMAS-44.