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RESEARCH ARTICLES

Low variations in anatomical characters of lowland and upland rice from Sarawak, Malaysian Borneo

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Abstract
Oryza sativa L. or commonly known as rice belongs to the family of Poaceae. In Malaysia, rice is normally cultivated either as lowland or upland rice. The present study was undertaken with the objective to characterise and document the variations in anatomical traits of leaf, midrib and the root of 22 lowland and 22 upland rice accessions. The leaf, midrib and root anatomy of the lowland and upland rice accessions have the same fundamental anatomical structure. Stomata were found abundant on the abaxial surface as compared to the adaxial surface in general. It is interesting to note that the upland rice accessions, in general, had higher stomatal density on the adaxial surface. In addition, the upland rice accessions, in general, had larger root and stele diameters as compared to lowland rice accessions. The variation in root diameter is associated to the changes in the number and size or width of cortical cells and stele diameter. Wider stele may allow higher plant water status under water deficit, due to higher hydraulic conductivity. In addition, greater xylem diameter (indicated by larger stele diameters) is linked to better axial conductance, which improved rice's ability to absorb and hold more water during water-limiting conditions. These traits are possibly advantageous for upland rice for efficient water capture, especially under water-deficit stress.

Keywords Anatomy · Lowland rice · *Oryza sativa* · Sarawak · Upland rice

Introduction

In Malaysia, cultivated rice has been classified into lowland and upland rice. Lowland rice is commonly planted in a field with well irrigation conditions. On the contrary, upland or dryland rice is planted in a field with naturally rain-fed, well-drained soils with no surface water accumulation and having undulated topography (Department of Agriculture 2005). Rice is genetically diverse and shows wide variation in plant, leaf, as well as root architecture. Anatomical characteristics may also contribute to the diversity.

Under partial submergence or waterlogging conditions, plants develop anatomical responses such as generation of aerenchyma in tissues, which facilitates the transport of oxygen from shoots to roots (Colmer 2002; Colmer and Voesenek 2009). Under water deficit condition, stomatal conductance can be influenced by leaf anatomical traits such as stomatal density and size (Ouyang et al. 2017). Plant closes stomata to prevent major water loss, consequently, reduces photosynthesis (Ouyang et al. 2017). Improving the leaf structure may improve photosynthetic efficiency. However, very little effort has been made to characterise the diversity of leaf anatomical features within *Oryza*. Moreover, the functional significance of leaf anatomical structure, and its regulation is still not very clear for lowland and upland rice, especially Sarawak rice.

Plant roots are crucial for absorption and translocation of water and nutrients. Varietal differences in root morphological characteristics have been reported in cultivated rice. Studies on the anatomy of roots have shown that the associated traits such as root xylem diameter are related to yield and crop adaptation to different water regimes (Phule et al.

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Table 1 List of rice landraces with its locality and global positioning system reading

Lowland Landraces				Upland Landraces			
Name	Location	GPS	Division	Name	Location	GPS	Division
UNISR2018-02 Padi Pandan	Kg Paon Gahat	0° 57' 0" North, 110° 39' 0" East	Serian	UNISR2018-06 Padi Belawi Pandan	Kg Paon Gahat	0° 57' 0" North, 110° 39' 0" East	Serian
UNISMT 2018-21 Padi Hitam	Kg Pueh	1° 51' 0" North, 109° 41' 0" East	Kuching	UNISR2018-10 Padi Belawi	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	UNIPDW 2018-42 Padi Merjat	Kg Tabuan Rabak	1° 18' 39" North, 110° 20' 59" East	Kuching
UNIPDW2018- 48 Padi Kihuai	Kg Mambong	1° 22' 0" North, 110° 21' 0" East	Kuching	UNIPDW 2018-16 Padi Merjat	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	UNIPDW 2018-17 Padi Pandan Wangi	Kg Pesak	1° 45' 0" North, 110° 46' 0" East	Kuching
UNISAMA 2018- 45 Padi Sabak Hitam	Kg Baru	1° 33' 28" North, 110° 22' 22" East	Kota Samarahan	UNISRA 2018-36 Padi Badawi	Kg Sg Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman
UNISAMA 2018- 47 Padi Sabak Angin	Kg Baru	1° 33' 28" North, 110° 22' 22" East	Kota Samarahan	UNISRA 2018-39 Padi Sempang	Kg Melugu Tengah	1° 06'14" North, 111°23'57" East	Sri Aman
UNISRA 2018-38 Padi Arang	Kg Sg Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman	UNISRA 2018-41 Padi Mawang	Kg Melugu Tengah	1° 06' 14" North, 111° 23' 57" East	Sri Aman
UNISRA 2018-40 Padi Selasih	Kg Melugu Skim	1°07'10" North, 111°25'33" East	Sri Aman	UNISRA 2018-29 Padi Limbang	Kg Sg Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman
UNISRA 2018-26 Padi Kanowit	Kg Melugu Skim	1° 07' 10" North, 111° 25' 33" East	Sri Aman	UNISRA 2018-30 Padi Chelum	Kg Sg Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman
UNISRA 2018-24 Padi Bajong	Kg Panggil	1°05'48" North, 111°23'23" East	Sri Aman	UNISRA 2018-31 Padi Bario	Kg Sg Tenggang	1° 04' 48" North, 111° 03' 28" East	Sri Aman

Table 2 List of four simple sequence repeat markers used for pre-selection of rice accessions

SSR	Primer sequence	Chromosome	Annealing temperature (°C)
RM1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	1	55
RM279	F: GCGGGAGAGGGATCTCCT R: GGCTAGGAGTTAACCTCGCG	2	55
RM489	F: ACTTGAGACGATCGGACACC R: TCACCCATGGATGTTGTCAG	3	55
RM335	F: GTACACACCCACATCGAGAAG R: GCTCTATGCGAGTATCCATGG	4	55

2019). In comparison to lowland rice, upland rice typically has thicker roots and penetrates more deeply into the soil (Phule et al. 2019). Sarawak has diverse rice varieties (Yeo et al. 2018). The anatomical features of root for Sarawak rice are unknown, thus far.

This study was conducted to identify the differences of the anatomical traits between the lowland and upland rice landraces in Sarawak.

Materials and methods

Field sample collection

A total of 22 rice landraces (11 lowland and 11 upland rice landraces) were collected from 10 different localities across different divisions in Sarawak (Table 1). Ten seeds of each rice landrace were germinated and transplanted into germination tray containing planting medium consisting of topsoil, compost and sand (3:2:1). Assuming the seeds for each landrace were heterogenous, each seedling was considered as one accession. In total, there were 220 accessions (110 lowland and 110 upland rice accessions) available for selection to proceed to anatomical characterisation.

Seedling selection using simple sequence repeat markers

Plant DNA was extracted from leaf samples of 220 rice accessions following Doyle and Doyle (1987). Four SSR markers (Table 2) randomly chosen from RiceGenes database (www.gramene.org), were used to genotype the 220 rice accessions to select plants (to reduce the number of accessions) for anatomical characterization without replication. Polymerase Chain Reaction (PCR) was carried out following Zhu et al. (2012). The amplified PCR products were electrophoresed on 2% agarose gel, at 90 V in 1 X TAE buffer. Following electrophoresis, ethidium bromide was used to stain the agarose gel and gel was visualized under ultraviolet transilluminator.

From the 10 accessions of each landrace, individuals having polymorphic genotypes based on the four SSR markers were selected for anatomical characterisation. If only one genotype was observed based on the markers, one individual was chosen randomly. The selected accessions were transplanted into pots (13 inches, 5 gallons) containing planting medium consisting of topsoil, compost and sand (3:2:1). For lowland rice accessions, flooding was maintained at 8 cm from soil surface. For upland rice accessions, drainage holes were drilled at the bottom of the pot to drain excess water. Following Sarawak's main rice planting season, the experiment was carried out from October 2018 to June 2019.

Anatomical observation

Anatomical samples of plant materials such as leaves, midribs and roots were prepared based on Cutler et al. (1978) with modifications. Three slides were observed for leaf samples, and two slides each for midrib and root samples. For the preparation of the leaf sample, the 5th leaf was selected and hand-sectioned by using a scalpel. Only the middle part of the leaf was used. To remove the chlorophyll, the leaf section was soaked in 70% Clorox (bleach) solution until the leaf turned white. The leaf section was rinsed with distilled water and stained using Safranin O. Then, the stained leaf section was dehydrated by soaking in 50% ethanol for 3–5 min and rinsed with distilled water. The dehydration process was repeated with 60%, 70%, 80%, 90% and 95% ethanol. The midrib on the middle part of the 5th leaf was hand-sectioned using a microtome blade. The section was processed using the same protocol as described above except, Alcian Blue was used for staining and a drop of hydrochloric acid was added into 70% ethanol used for dehydration. For the root, sample was collected after harvesting plant. Mid-section of the root was selected randomly and hand-sectioned using a microtome blade and processed as described for leaf midrib. Microscopic slides were prepared using the wet mount technique (Karuppaiyan and Nandini 2006). Clear cross-sectioned of leaf, midrib and root tissues were photographed using Olympus DP72 camera, attached to Olympus BX151 light microscope.

Epidermal characteristics of leaf such as the length and width of long cell, the shape, arrangement, cell outline and surface ornamentation were studied under compound microscope. The measurement and characteristics of the stomatal complex which comprises stomata and the shape of subsidiary cells were recorded. The stomatal density (SD) was calculated based on Paul et al. (2017) using the equation:

$$SD = \frac{1}{S \times \pi r^2}$$

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

The length and width of a leaf midrib were measured and the shape was described. The diameter of the root and stele were recorded. A Java-based image processing program (ImageJ) was used to process all anatomical measurements under 60x magnifications (Schneider et al. 2012).

To compare the anatomical difference between the two rice ecotypes, quantitative measurements (length of epidermal cell, width of epidermal cell, length of stomatal complex, width of stomatal complex, stomatal density, midrib width, root diameter and stele diameter) were analysed using Student's t-test. Wilcoxon Signed Rank Test was used to compare the quantitative measurements of anatomical characters between abaxial and adaxial leaf surfaces, within the two rice ecotypes. IBM SPSS Statistics Version 20 was used for statistical analysis.

Results

Based on the SSR marker genotype, 44 rice accessions were selected from the 220 accessions. The 44 accessions consisted of 22 lowland rice accessions designated as UNIMAS-23–UNIMAS–44 and 22 upland rice accessions designated as UNIMAS-01–UNIMAS–22.

Variations in epidermal traits

Clear differences in epidermis tissue were not observed between rice accessions in the present study (Supplementary Table 1). The long cells of abaxial and adaxial leaf surfaces were rectangular. Cells and appendages.

including long and short cells (comprised of silica cells and phellem cells) and stomata with guard cells were observed. The arrangement of cells was linear, elongated with alternating long and short cells. The lateral walls showed that the cell outline was undulated, and trichomes were found present (Fig. 1I).

Supplementary Tables 2 and Supplementary Table 3 tabulated the length of the long cells (LEPC) on the abaxial leaf surface for lowland and upland rice accessions, respectively. The abaxial LEPC for lowland rice accessions ranged from 53.20 µm (UNIMAS-32) to 79.66 µm (UNIMAS-29) (Supplementary Table 2) and 58.73 µm (UNIMAS-05) to 138.88 µm (UNIMAS-13) for upland rice accessions (Supplementary Table 3). On the adaxial leaf surface, the LEPC for lowland rice accessions ranged from 47.94 µm (UNIMAS-37) to 104.94 µm (UNIMAS-28) (Supplementary

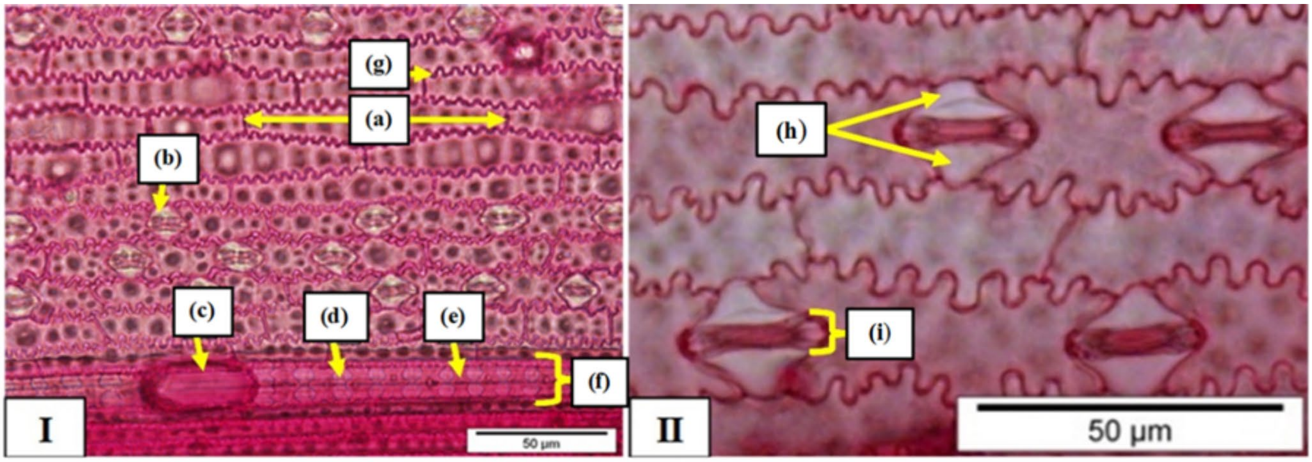

Fig. 1 The leaf anatomy of *Oryza sativa*. (I) A representative of epidermal structure in leaf sample of different rice accessions in this study; (a) Rectangular long cell; (b) Stomatal complex; (c) Trichome; (d) Silica cell; (e) Phellem cell; (f) Short cell row comprised of silica cell and phellem cell; (g) Undulated long cell outline. Magnification: 20x. Scale bar: 50 µm. (II) A representative of the stomatal complex observed in different accessions in this study; (h) A pair of triangular-shaped subsidiary cells; (i) A pair of guard cells. Magnification: 20x. Scale bar: 50 µm

Table 3 Comparison of measurements for epidermal and stomatal complex traits between leaf samples of lowland and upland rice accessions

Surface	Traits	Lowland	Upland
Abaxial	*LEPC (µm)	67.10 ± 1.38	80.43 ± 3.44
	*WEPC (µm)	14.71 ± 0.362	16.52 ± 0.682
	LSC (µm)	19.06 ± 0.38	19.54 ± 0.44
	WSC (µm)	14.82 ± 0.32	14.41 ± 0.39
	SD (unit/mm^2)	362.45 ± 17.70	363.27 ± 8.54
Adaxial	LEPC (µm)	69.60 ± 3.42	73.30 ± 2.92
	WEPC (µm)	13.45 ± 0.32	14.80 ± 0.64
	LSC (µm)	19.66 ± 0.47	18.83 ± 0.52
	WSC (µm)	14.66 ± 0.31	14.66 ± 0.50
	*SD (unit/mm^2)	265.77 ± 14.62	311.55 ± 8.93

* indicates significant different at $p < 0.05$ (Student's t-test). The values indicate the means ± standard error.

LEPC: Length of epidermal cell; WEPC: Width of epidermal cell; LSC: Length of stomatal complex; WSC: Width of stomatal complex; SD: Stomatal density

Table 2). Meanwhile for upland rice accessions recorded a range from 47.65 µm (UNIMAS-07) to 97.83 µm (UNIMAS-08) (Supplementary Table 3).

The LEPC of the abaxial leaf epidermis for the lowland rice accessions analysed using Student's t-test, was significantly shorter (average = 67.10 µm) than those of upland rice accessions (average = 80.43 µm) (Table 3). At the adaxial leaf epidermis, however, the LEPC was similar between the leaf samples of lowland (average = 69.90 µm) and upland (average = 73.30 µm) rice accessions (Table 3). Comparing the LEPC of the abaxial and adaxial leaf epidermis, they were similar for lowland and upland rice accessions (Table 4).

The width of the long cell (WEPC) on abaxial and adaxial leaf surfaces of the lowland rice accessions were ranging from 12.05 µm (UNIMAS-27) to 17.95 µm (UNIMAS-32) and 10.46 µm (UNIMAS-44) to 16.15 µm (UNIMAS-29), respectively (Supplementary Table 2). For upland rice accessions, the WEPC of abaxial and adaxial leaf surfaces were ranged from 11.23 µm (UNIMAS-21) to 23.90 µm (UNIMAS-16) and 8.98 µm (UNIMAS-13) to 21.03 µm (UNIMAS-03) (Supplementary Table 3), respectively.

Statistical analysis using Student's t-test showed a significant difference between the WEPC on the abaxial leaf surface of lowland and upland rice accessions, but not on the adaxial leaf surface (Table 3). Abaxial leaf surface of upland accessions were found to have wider WEPC compared to lowland accessions (Table 3). Comparing the WEPC between the abaxial and adaxial leaf surfaces within lowland and upland rice accessions using Wilcoxon Signed Rank Test, significant difference was observed (Table 4), where WEPC on the abaxial surface was wider in both lowland and upland rice accessions (Table 3).

Table 4 Comparison of measurement for epidermal and stomatal complex traits between abaxial and adaxial surfaces of leaf samples within lowland and upland rice accessions

Traits	Lowland	Upland
LEPC (µm)	-2.50 ± 17.51	7.12 ± 20.09
WEPC (µm)	1.26 ± 2.10*	1.72 ± 3.89*
LSC (µm)	-0.60 ± 2.13	0.71 ± 2.18
WSC (µm)	0.16 ± 1.36	-0.25 ± 2.51
SD (unit/mm^2)	96.69 ± 98.52*	51.73 ± 58.22*

* indicates significant different at $p < 0.05$ (2-tailed Wilcoxon Signed Rank Test). The values indicate the means ± standard error.

LEPC: Length of epidermal cell; WEPC: Width of epidermal cell; LSC: Length of stomatal complex;

WSC: Width of stomatal complex; SD: Stomatal density

Diversity of stomatal complex

The stomatal complex is composed of two guard cells (GuC), flanked by two subsidiary cells (SuC) (Fig. 1II). All lowland and upland rice accessions were having dumbbell-shaped GuC and triangular-shaped SuC (Supplementary Table 1).

The length of stomata (LSC) on abaxial leaf surface of lowland rice accessions ranged from 15.49 µm (UNIMAS-33) to 23.13 µm (UNIMAS-35) and 16.78 µm (UNIMAS-34) to 26.20 µm (UNIMAS-36) on adaxial leaf surface (Supplementary Table 2). Upland rice accessions recorded LSC on abaxial and adaxial leaf surfaces from.

13.86 µm (UNIMAS-02) to 23.77 µm (UNIMAS-09) and 14.20 µm (UNIMAS-02) to 24.09 µm (UNIMAS-19), respectively (Supplementary Table 3). The LSC of lowland and upland rice accessions analysed using Student's t-test were statistically similar on both abaxial and adaxial leaf epidermis (Table 3). Comparing LSC between abaxial and adaxial leaf epidermis also shown similar for both lowland and upland rice accessions (Table 4).

For lowland rice accessions, the stomata width (WSC) on abaxial and adaxial leaf surfaces ranged from 12.42 µm (UNIMAS-25) to 19.03 µm (UNIMAS-28) and 12.93 µm (UNIMAS-33) to 17.68 µm (UNIMAS-37), respectively (Supplementary Table 2). Meanwhile, the WSC of upland rice accessions on abaxial and adaxial leaf surfaces recorded from 10.86 µm (UNIMAS-02) to 18.51 µm (UNIMAS-19) and 10.55 µm (UNIMAS-02) to 19.33 µm (UNIMAS-19),

respectively (Supplementary Table 3). The WSC on the abaxial and adaxial leaf epidermis analysed using Student's t-test were statistically similar between lowland and upland rice accessions (Table 3). Comparison of WSC between abaxial and adaxial leaf surfaces was also similar for lowland and upland rice accessions (Table 4).

Stomatal density (SD) on the leaf samples of the lowland rice accessions ranged from 192 unit/mm2 (UNIMAS-41) to 546 unit/mm2 (UNIMAS-33) for abaxial leaf surface (Supplementary Table 2). On the adaxial leaf surface of lowland rice accessions, SD ranged from 162.00 unit/mm2 (UNIMAS-31 and UNIMAS-36) to 385.00 unit/mm2 (UNIMAS-40) (Supplementary Table 2). For leaf samples from upland rice accessions, the SD on the abaxial surface ranged from 269 unit/mm2 (UNIMAS-03) to 423 unit/mm2 (UNIMAS-02) (Supplementary Table 3). SD on the adaxial leaf surface, on the other hand, ranged from 223 unit/mm2 (UNIMAS-17) to 392 unit/mm2 (UNIMAS-12) (Supplementary Table 3). The SD on the abaxial epidermis was not significantly different between the leaf sample from lowland and upland rice accessions (Table 3). The SD on the adaxial leaf surface, however, denoted significant difference when analysed using Student's t-test, where lowland rice accessions recorded lower SD as compared to upland rice accessions (Table 3). Comparing the SD between the abaxial and adaxial leaf surfaces within lowland and upland rice accessions using Wilcoxon Signed Rank Test, significant difference was observed (Table 4), where SD was found higher on the abaxial leaf surface in both lowland and upland rice accessions (Table 3).

Variations in midrib cell structures

The shape of the midrib across the 44 rice accessions revealed no variation, where all accessions showed a concave-shaped midrib outline for the abaxial side and flat-shaped midrib outlines for the adaxial (Supplementary Fig. 1 and Supplementary Fig. 2). The midrib was comprising of vascular bundle, sclerenchyma strand and parenchyma. The general structure of midrib observed for leaf samples from the lowland and upland rice accessions is illustrated in Fig. 2.

The midrib width for the lowland and upland rice accessions ranged from 321.00 µm (UNIMAS-37) to 1422.22 µm (UNIMAS-41) and 777.50 µm (UNIMAS-15) to 2188.99 µm (UNIMAS-19), respectively (Supplementary Table 4). It was observed that the midrib width of leaf sample from the lowland rice accessions was significantly narrower when analysed using Student's t-test, as compared to the midrib sample from the upland rice accessions (Table 5).

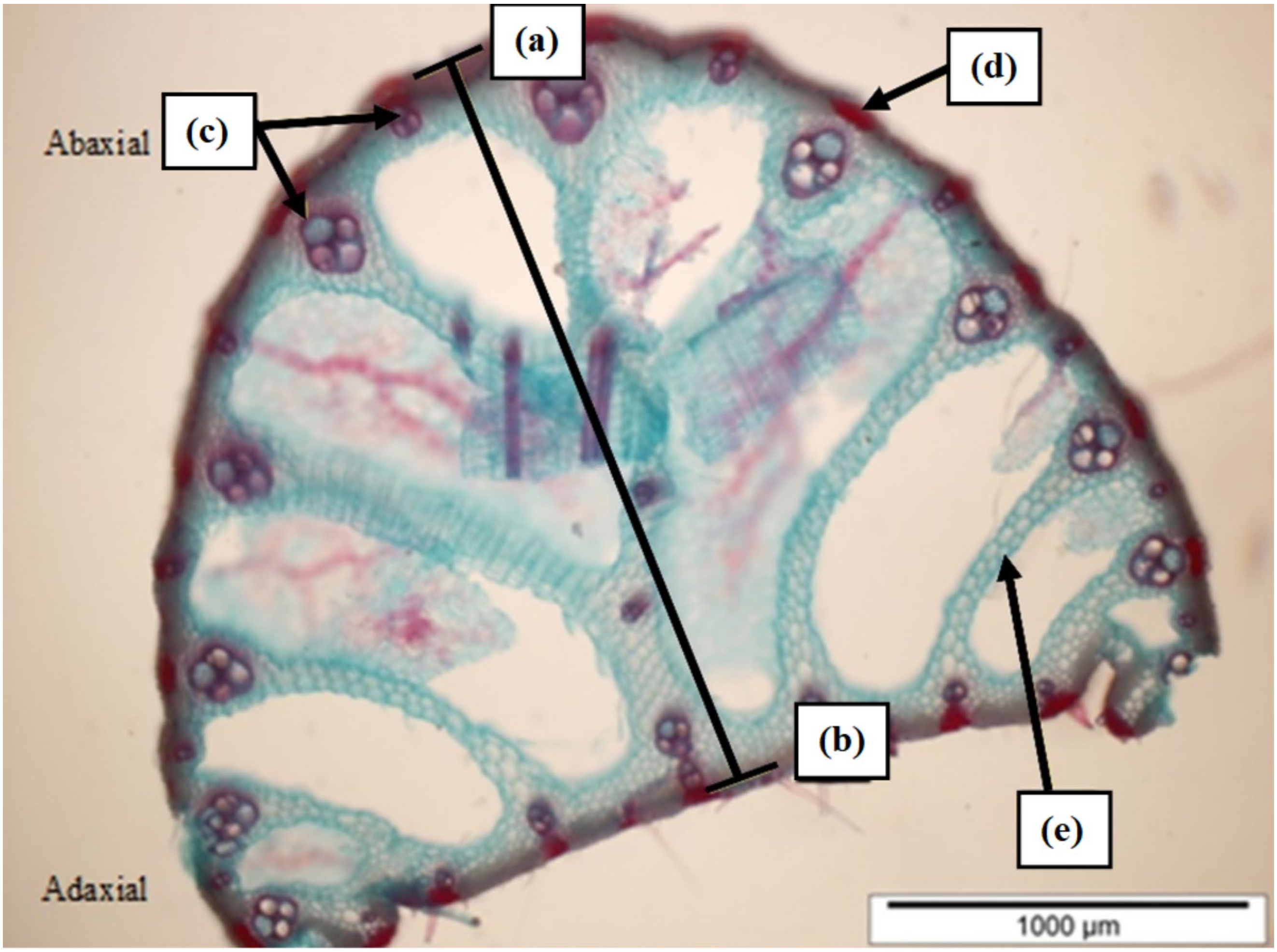

Fig. 2 A representative midrib anatomical structure observed in leaf samples of different rice accessions in this study. (a) to (b): Midrib width; (c) Vascular bundle; (d) Sclerenchyma strand; (e) Parenchyma. Magnification: 10x. Scale bar: 1000 µm

Table 5 Comparison of midrib width, root diameter and stele diameter between root samples from lowland and upland rice accessions

Part	Traits	Lowland	Upland
Midrib	*Width (µm)	750.94 ± 55.27	1093.34 ± 62.77
Root	*Diameter (µm)	866.08 ± 34.86	1072.01 ± 41.33
	*Stele diameter (µm)	224.18 ± 6.72	298.91 ± 9.79

* indicates significant difference at $p < 0.05$ (Student's t-test). The values indicate the means ± standard error.

Variations in Root

The root anatomical structure of the lowland and upland rice accessions showed no variation between the 44 rice accessions in tissue types (Supplementary Fig. 3 and Supplementary Fig. 4). The anatomical features of the transverse section of roots revealed the epidermis, exodermis, sclerenchyma layer, mesodermis and well-developed aerenchyma (Fig. 3I).

The root diameter of the lowland rice accessions ranged from 580.70 µm (UNIMAS-27) to 1123.56 µm (UNIMAS-37) (Supplementary Table 4). For upland rice accessions, the root diameter ranged from 778.00 µm (UNIMAS-02) to 1427.47 µm (UNIMAS-17) (Supplementary Table 4).

The stele of root had vascular cylinder comprises endodermis, fibre, pericycle, metaxylem, protoxylem and phloem (Fig. 3II). The range of stele diameter was from 176.37 µm (UNIMAS-30) to 293.54 µm (UNIMAS-23) and 213.55 µm (UNIMAS-22) to 399.91 µm (UNIMAS-13) (Supplementary Table 4) for root sample from lowland and upland rice accessions, respectively.

This study observed that the root and stele diameter from lowland rice accessions were smaller as compared to those from upland rice accessions when analysed using Student's t-test (Table 5).

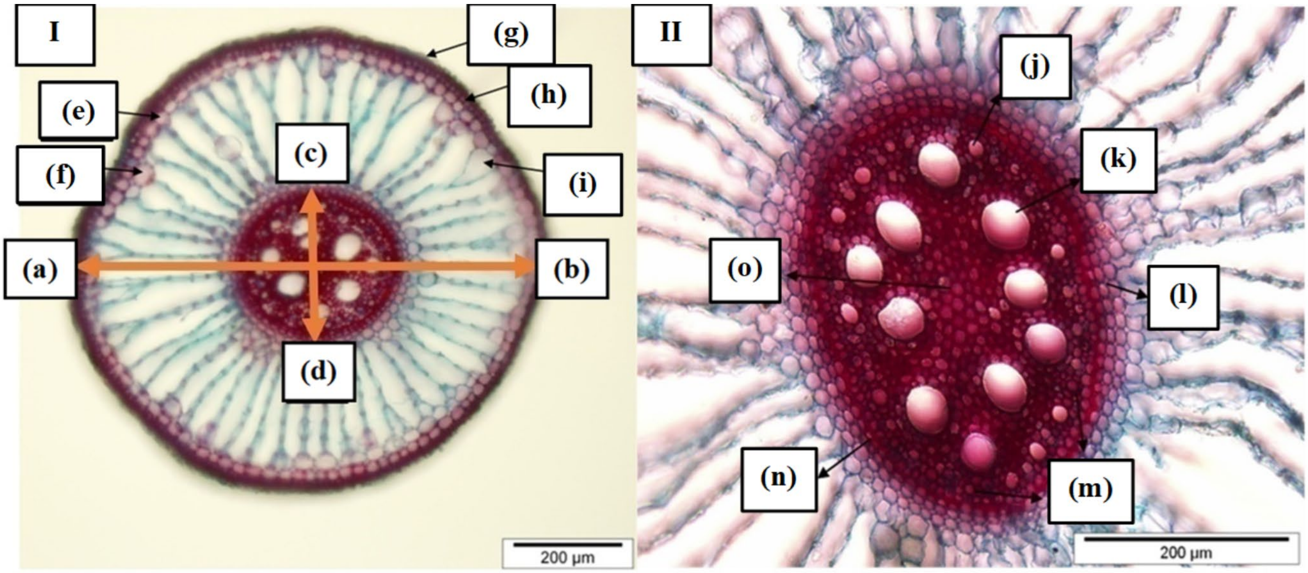

Fig. 3 A representative of root and root stele as observed in the root sample of different rice accessions in this study. I: Root anatomical structure; (a) to (b): Root diameter; (c) to (d): Stele diameter; (e) Sclerenchyma layer; (f) Mesodermis; (g) Epidermis; (h) Exodermis; (i) Aerenchyma. II: Enlargement of root stele; (j) Protoxylem; (k) Metaxylem; (l) Endodermis; (m) Phloem, (n) Pericycle; (o) Fibre. Magnification: I = 10x, II = 40x. Scale bar: I = 200 µm, II = 200 µm

Discussion

Distinctive anatomical variations were not observed among lowland and upland rice accessions. Like in other Poaceae, the studied accessions have the expected anatomical traits, particularly in epidermal cell structures, midrib and root cell structures (Islam et al. 2009; Kadam et al. 2015; Luo et al. 2012). The leaf surface of the 44 rice accessions has been observed to have stomata with guard cells, long cells, short cells comprised of silica cells and phellem cells. The long cell was observed dominating the various cells.

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

One of the important research areas in current rice science is stomatal study. Variations or any changes in stomatal traits can influence water and carbon flux, which may contribute significantly to creating rice that is more tolerant to climate change (Chatterjee et al. 2020). Stomata are the major organs which function to control water and gaseous exchange, hence making it the potential target for modification to increase and sustain rice yield with minimal water input (Dittrich et al. 2019; Franks et al. 2015; Flexas 2016). In the present study, stomata were found abundant on the abaxial surface as compared to the adaxial surface in almost all accessions, in line with the findings of Chatterjee et al. (2020).

It is interesting to note that the upland rice accessions, in general, has higher SD on adaxial surface as compared to lowland rice accessions (Table 3). This is contradicting to previous studies where upland rice is frequently been correlated with a lower stomatal density when compared to lowland rice due to their capacity to conserve water and drought-resistant qualities (Ouyang et al. 2017; Sandar et al. 2022). However, many plant species are able to adjust their stomatal development, with stomatal size and density adapted to the local conditions in response to continual changes in environmental stimuli (Bertolino et al. 2019). Given the primary function of stomata is mediating gas exchange by regulating carbon uptake and transpiration of plants, changes in stomatal development and opening can reveal adaptive links to the environmental conditions around the plant (Driesen et al. 2020; Richardson et al. 2017). The 22 upland rice accessions may be interesting for breeding lowland rice accessions with higher SD to adapt to climate change. High temperature and carbon dioxide enrichment will occur as a result of global climate change, and increasing in stomatal density at warmer areas may boost the rate for transpiration as a way of evaporative cooling (Hill et al. 2015).

The upland rice accessions, in general, wb ere having larger root and stele diameters in comparison to lowland rice accessions (Table 5). The variation in root diameter is due to changes in the number and size or width of cortical cells and stele diameter (Xu et al. 2022). A stele is the central part of the root system that contains vascular tissue (i.e. xylem and phloem). Wider stele may allow an efficient water capture, especially in a water-deficit condition (Phoura et al. 2020). In addition, greater xylem diameter (which indicates larger stele diameters) is linked to better axial conductance, which improved rice's ability to absorb and hold more water during water-limiting conditions due to thicker suberized endodermis (Henry et al. 2012). These traits are possibly advantageous for upland rice for plant adaptation to dry environments.

In summary, comparison between anatomical traits of lowland and upland rice accessions has revealed that lowland accessions have recorded a lower SD on adaxial surface with narrower midrib width. A lower SD may help the accessions for water conservation during high temperatures. Upland rice accessions typically have a larger root diameter. A large root diameter is a crucial trait to aid in better water absorption, especially for upland accessions which are grown in a well-drained soil.

Conclusion

In this study, the anatomical features of the 22 lowland and 22 upland rice landraces collected from the North-Western region of Sarawak were successfully determined and analysed. The studied accessions were found to have the expected anatomical traits, particularly in epidermal cell structures, midrib cell structures and root cell structures like in other Poacea. Stomatal density was interestingly higher on adaxial surface of upland accession in response to climate change. Midrib width was found to be narrower in lowland accession. The difference in root and stele diameter is as expected; upland rice accessions were found to be larger in diameter than lowland accessions, which is an excellent trait for water absorption during water stress condition. This study provided a potential reference for studying the utilization of Sarawak rice landraces.

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Declarations

The authors have no relevant financial nor nonfinancial interests to disclose.

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