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Contributed Paper

Culm internodal Anatomy of the Tribe Oryzeae (Poaceae) in Thailand

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ABSTRACT

Culm internodal anatomy of Oryzeae found in Thailand, namely *Hygroryza aristata, Leersia hexandra, Oryza meyeriana* var. *meyeriana* and var. *granulata, O. minuta, O. ridleyi, O. rufipogon, O. sativa,* and *Zizania latifolia,* was investigated by transverse sectioning using the paraffin method. The general characters found in most taxa were the intercellular cavities, the hollow center of the culm, and the vascular bundles arranged in rings. The sclerenchyma ring was present only in some taxa. A description of culm internodal anatomy is provided for each taxa. In addition, an identification key is presented based on the presence and the arrangement of intercellular cavities, the presence of collapsed cells, the number of parenchyma cell layers in the cortex between the innermost vascular bundle ring and the hollow center, the number of vascular bundle rings, the presence of a sclerenchyma ring, and the number of fiber cell layers in the sclerenchyma ring. However, culm internodal anatomy alone could not be used to distinguish two varieties of *O. meyeriana* and three species in the *O. sativa* group (*O. minuta, O. rufipogon,* and *O. sativa*).

Keywords: culm transverse section, grasses, *Hygroryza*, internode, *Leersia*, micromorphology, *Oryza*, stem, *Zizania*

1. INTRODUCTION

Oryzeae Dumort. is a tribe in the grass family (Poaceae) which contains 12 genera and around 70 species worldwide [1]. In Thailand, 10 taxa representing nine species within four genera (*Hygroryza* Nees, *Leersia* Sw., *Oryza* L., and *Zizania* L.) are recorded [2]. Various studies have examined the morphology [3], micromorphology [4, 5], leaf anatomy [6], and molecular phylogeny [7, 8] of the tribe, however culm anatomy is not favoured for investigations; only two species in the tribe Oryzeae (*L. oryzoides* (L.) Sw. and *Z. aquatica* L.) were studied by Brown [9], while de Wet selected two species from *Oryza* and *Potamophila* R.Br. [10] as representatives of this tribe. Watson and Dallwitz briefly reported

that the culm anatomy of the genus *Leersia* and *Oryza* had one or two vascular bundle rings [11]. In spite of those studies, there is no intensive study of the culm anatomy for this tribe.

Previous investigations have reported that some characters of culm anatomy are less useful for determining relationships at the family, tribe, or genera levels [12], however, a study of the culm anatomy in *Bouteloua* Lag. and its relatives revealed that the culm contained valuable characters and suggested close relationships between some *Bouteloua* species [13]. In addition, culm anatomical characters including epidermis and transverse sections were used in creating a key to Andean woody bamboo species [14]. Moreover, an investigation of the vegetative anatomy in the subtribe Ischaeminae regarded culm outline and the central cavity in ground tissue as particularly distinct characters [15]. Therefore, the current study aims to investigate the anatomy of the culm internode in an attempt to describe, compare, and construct an identification key for wild Thai Oryzeae in order to support species distinction, especially when the plants lack reproductive parts.

2. MATERIALS AND METHODS

Plant materials of nine taxa in four genera within the tribe Oryzeae found in Thailand were collected from the field throughout Thailand. The voucher specimens are listed in Table 1.

For the investigation of culm anatomy, culm internodes (5 cm above the ground) of plants that were either flowering or fruiting were selected, and the modified paraffin method [16] was used to prepare permanent slides. Afterwards, the anatomical features of culm transverse sections were photographed under light microscopes (Olympus BX43 and DP21), and described using grass terminology following Ellis [17] and Metcalfe [12].

Table 1. List of voucher specimens used in this study. Herbarium acronyms are given in parentheses.

Taxon	Voucher specimen
Hygroryza aristata (Retz.) Nees ex Wright & Arn.	Thailand: Phatthalung, P. Sumanon & P. Traiperm 2 (BKF) Thailand: Bangkok, P. Sumanon & P. Traiperm 19 (BKF)
Leersia hexandra Sw.	Thailand: Kamphaeng Phet, P. Sumanon & P. Traiperm 5 (BKF) Thailand: Chiang Mai, Traiperm *et al.* 564 (BKF)
Oryza meyeriana (Zoll. & Moritzi) Baill. var. *meyeriana*	Thailand: Phatthalung. P. Sumanon & P. Traiperm 1 (BKF)
O. meyeriana var. *granulata* (Nees & Arn. ex G. Watt) Duist.	Thailand: Phetchabun, P. Sumanon & P. Traiperm 3 (BKF) Thailand: Phrae, P. Sumanon & P. Traiperm 16 (BKF) Thailand: Chiang Mai, P. Sumanon & P. Traiperm 18 (BKF)
O. minuta J. Presl	Thailand: Kanchanaburi, P. Sumanon & P. Traiperm 12 (BKF) Thailand: Nonthaburi, P. Sumanon & P. Traiperm 14 (BKF) Thailand: Kanchanaburi, P. Sumanon & P. Traiperm 15 (BKF)
O. ridleyi Hook. f.	Thailand: Saraburi, P. Sumanon & P. Traiperm 11 (BKF) Thailand: Chumphon, P. Sumanon & P. Traiperm 13 (BKF)
O. rufipogon Griff.	Thailand: Kamphaeng Phet, P. Sumanon & P. Traiperm 8 (BKF) Thailand: Chiang Mai, Traiperm *et al.* 565 (BKF) Thailand: Roi Et, Traiperm *et al.* s.n. (BKF)
O. sativa L.	Thailand: Kamphaeng Phet, P. Sumanon & P. Traiperm 10 (BKF) Thailand: Pathumthani, P. Sumanon & P. Traiperm 28 (BKF)
Zizania latifolia (Griseb.) Turcz. ex Stapf	Thailand: Krabi, P. Sumanon & P. Traiperm 30 (BKF)

3. RESULTS AND DISCUSSION

3.1 General Characters

Culm internodal outlines of Oryzeae taxa were either circular, elliptic, or oval, with a hollow area in the center. A thin membrane composed of stellate parenchyma was also present in the hollow of the culm (Figure 5A). Culm epidermis was composed of small, lignified cells with a thick cuticle in most taxa, except in *H. aristata.* Prickles were normally present on the epidermal surface for all taxa, while papillae were ornamented on epidermal surfaces of *O. minuta, O. rufipogon,* and *O. sativa* (Figure 1). Ground tissue consisted of many layers of large, thin-walled parenchyma cells extending towards the hollow center of the culm, with intercellular cavities in some taxa. Vascular bundles were round to elliptic, and arranged in one to three rings around the culm. Two circular metaxylem and one lysigenous cavity were always present in the first order vascular bundles (Figure 2) while a sclerenchyma ring appears in some taxa. Tables 2 and 3 show the main differences in culm anatomy between the genera and *Oryza* species, respectively.

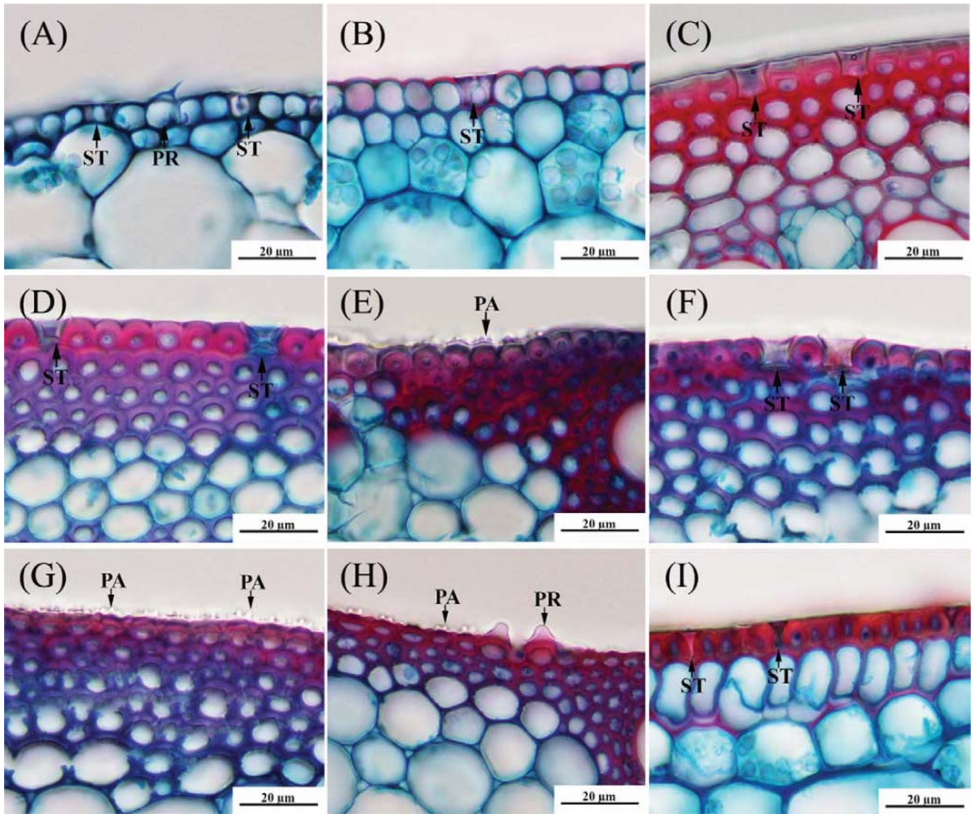

Figure 1. Epidermis of culm in transverse sections of Thai Oryzeae taxa: (A) and (B) *H. aristata;* (C) *L. hexandra;* (D) *O. meyeriana* var. *meyeriana;* (E) *O. minuta;* (F) *O. ridleyi;* (G) and (H) *O. rufipogon;* (I) *Z. latifolia.* Abbreviations: PA-papillae, PR-prickle, ST-stomata.

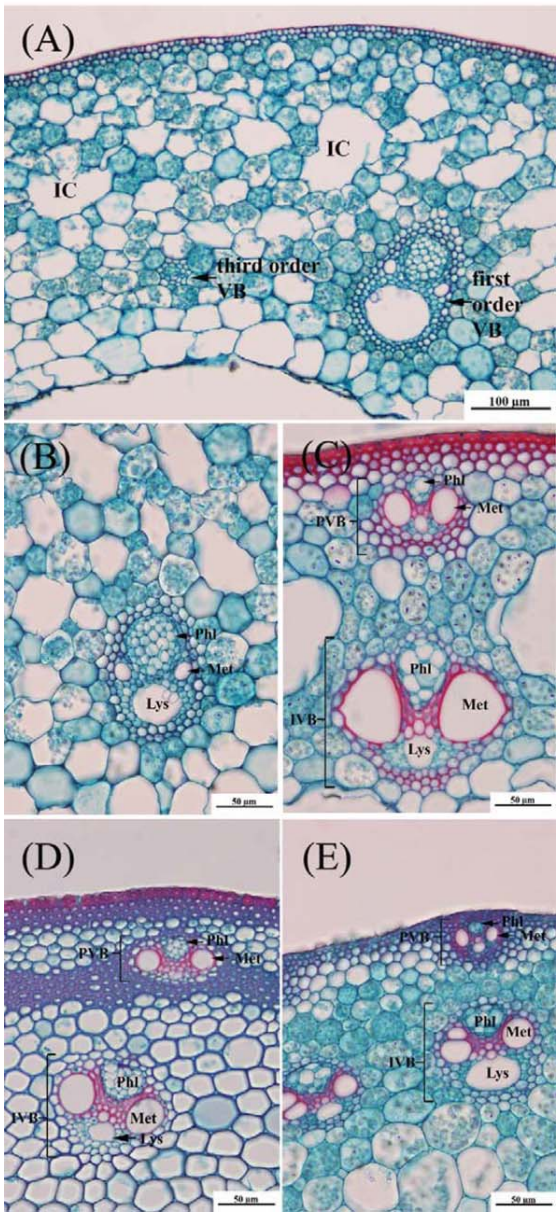

Figure 2. Culm transverse sections showing vascular bundles in some Thai Oryzeae taxa: (A) *H. aristata* showed both first order vascular bundles and third order vascular bundles, which are situated between first order vascular bundles; (B) *H. aristata* with a first order vascular bundle shown in detail; (C) *L. hexandra;* (D) *O. meyeriana* var. *meyeriana;* (E) *O. minuta.* Abbreviations: IC-intercellular cavity, VB-vascular bundle, IVB-innermost vascular bundle, PVB-peripheral vascular bundle, Lys-lysigenous cavity, Met-metaxylem vessel, Phl-phloem.

Table 2. The main anatomical differences of culm transverse sections separating the generic taxa in the tribe Oryzeae.

Characters	*Hygroryza*	*Leersia*	*Oryza*	*Zizania*
Epidermis	Non-lignified cells	Lignified cells	Lignified cells	Lignified cells
Vascular bundle ring	1	2	2-3	3
Average diameter of metaxylem vessels of first vascular bundles (μm)	15.7 ± 3.3	53.4 ± 9.7	35.9 ± 9.4	46.7 ± 7.9
Sclerenchyma ring	Absent	Absent	Absent or present	Present
Intercellular cavities	Numerous, scattered in ground tissue	Arranged in rings alternating with vascular bundles	Absent or present, arranged in rings alternating with vascular bundles	Numerous, from collapsed cells between epidermis and collenchyma ring

Table 3. The main anatomical differences of culm transverse sections separating taxa in the genus *Oryza*.

Characters	*O. ridleyi*	*O. meyeriana* var. *meyeriana*	*O. meyeriana* var. *granulata*	*O. minuta*	*O. rufipogon*	*O. sativa*
Outermost vascular bundle ring	Embedded in the inner sclerenchyma ring separating columns of parenchyma cells from one another	Embedded in the inner sclerenchyma ring separating columns of parenchyma cells from one another	Embedded in the inner sclerenchyma ring separating columns of parenchyma cells from one another	Embedded in sclerenchyma layers near epidermis	Embedded in, or situated near, sclerenchyma layers near epidermis	Embedded in, or situated near, sclerenchyma layers near epidermis
Sclerenchyma ring	Present; 2-3 layers of cells	Present; 5-6 layers of cells	Present; 4-6 layers of cells	Absent	Absent	Absent
Intercellular cavities	Absent	Absent	Absent	Absent	Present or absent	Present or absent

The anatomical characters of culms were used to construct an identification key for Oryzeae found in Thailand, as shown below. Although culm anatomy could separate most investigated species in the four genera, these characters were insufficient to clearly distinguish the species of the *O. sativa* group, which contained *O. minuta, O. rufipogon* and *O. sativa,* as well as the two varieties of *O. meyeriana.*

A key to the species based on culm transverse section

1. Intercellular cavities scattered irregularly in ground tissue.....*H. aristata*

1. Intercellular cavities arranged in rings or sometimes absent
2. Innermost vascular bundles adjoin thick-arched sclerenchyma layers of lignified cells at the side near the lysigenous cavity.....*Z. latifolia*
2. Innermost vascular bundles adjoin thin-walled parenchyma cells of ground tissue
3. One to two layers of cells between the innermost vascular bundles and the hollow center.....*L. hexandra*
3. Three or more layers of cells between the innermost vascular bundles and the hollow center
4. Sclerenchyma ring absent.....*O. minuta, O. rufipogon, O. sativa*
4. Sclerenchyma ring present
5. Sclerenchyma ring undulated, consists of two to three layers of fiber cells; prickles and papillae absent.....*O. ridleyi*
5. Sclerenchyma ring nearly straight, consists of four to six layers of fiber cells; prickles and papillae present.....*O. meyeriana* var. *meyeriana,* var. *granulata*

3.2 Culm Internodal Anatomical Description for Nine Taxa of Thai Oryzeae

Genus *Hygroryza* Nees

***Hygroryza aristata* (Retz.) Nees ex Wright & Arn. (Figure 1A-B, 2A-B and 3A).**

The culm outline of this species was circular, and its epidermis composed of small non-lignified cells with thin cuticles, further subtended by one layer of small cells with thickened, non-lignified walls. Epidermal cells were 5.4-8.9 µm thick and 5.0-7.4 µm wide. Prickles and stomata were also present on the epidermal surface. Ground tissue consisted of many layers of large, thin-walled parenchyma cells extending towards the hollow center of the culm. Collapsed cells were absent. Intercellular cavities were scattered irregularly throughout ground tissue. Vascular bundles were elliptic, arranged in one ring near the hollow center. Two metaxylem vessels and large lysigenous cavity were easily distinguished in the first order vascular bundles. The diameters of first order vascular bundles were 92.9-163.6 µm long and 73.7-114.3 µm wide. Metaxylem vessels were circular, 16.7-20.0 µm long and 9.8-17.8 µm wide in diameter. Third order vascular bundles were sometimes present between the first order bundles, between 36.4-59.3 µm long and 36.1-51.7 µm wide in diameter. No distinct lysigenous cavity or metaxylem vessels were noticed in third order bundles. There were no sclerenchyma rings accompanying any vascular bundles.

Genus *Leersia* Sw.

***Leersia hexandra* Sw. (Figure 1C, 2C and 3B).**

Culm outline was elliptic or ovate, and its epidermis composed of small, lignified cells with thick cuticles, further subtended by one to two layers of small cells with thickened, lignified walls. The lignification of the epidermal cells occurred in all directions, but the tangential wall on the outer side was the thickest. Epidermal cells were 7.3-8.4 µm thick and 4.4-7.2 µm wide. Prickles and stomata were also present on the epidermal surface. Ground tissue consisted of many layers of thin-walled, rounded parenchyma cells extending towards the hollow center of the culm, with one to two layers of cells between the innermost vascular bundles and the hollow center. Collapsed cells were absent. Intercellular cavities were present five to six cells layers below the epidermis, around the periphery of the culm and alternating with the innermost vascular bundles. Vascular bundles were round to elliptic, arranged in two rings. The innermost vascular bundles

were 117.7-163.1 µm long and 100.3-160.1 µm wide in diameter. Metaxylem vessels of these bundles were circular, around 54.2-71.1 µm long and 34.6-57.2 µm wide in diameter. The outermost vascular bundles or peripheral vascular bundles were smaller than those of the innermost circle; these peripheral bundles were 57.2-82.0 µm long and 66.5-101.1 µm wide in diameter. All vascular bundles were accompanied by sclerenchyma fibers.

Genus *Oryza* L.

Plants in this genus had circular, elliptic, or ovate culm outlines with a hollow center. Intercellular cavities were absent or present. Vascular bundles were round to elliptic, arranged in two rings. Sclerenchyma ring was absent or present, bounding three to four layers of cells on the inner side, alternating with the outermost vascular bundles.

***Oryza meyeriana* (Zoll. & Moritzi) Baill.**
a. *Oryza meyeriana* var. *meyeriana*
(Figure 1D, 2D and 3C).

Culm outline was elliptic. Epidermal cells were 7.4-9.4 µm thick and 5.8-8.4 µm wide. The lignification of the epidermal cells occurred in all directions, but the tangential wall on the outer side was the thickest. The epidermal layers were composed of small, lignified cells with thick cuticles, and were subtended by three to four layers of sclerenchyma cells. A sclerenchyma ring was nearly straight, consisted of five to six layers of sclerenchyma fibers and three to four layers of cells bounded on the inner side, alternating with the outermost vascular bundles. Prickles and stomata were also present on the epidermal surface. Collapsed cells were absent. Inner ground tissue consisted of many layers of thick-walled parenchyma cells at the outer part near the sclerenchyma ring, and large thin-walled parenchyma cells at the inner part, extending towards the hollow center of the culm. Intercellular cavities were absent. Vascular bundles were round to elliptic, arranged in two to three rings. The innermost vascular bundles were 80.4-128.1 µm long and 101.1-127.6 µm wide in diameter, embedded in parenchyma layers of inner ground tissue. Metaxylem vessels of these bundles were circular with a diameter 31.2-39.2 µm long and 30.1-37.1 µm wide. The outermost vascular bundles or peripheral vascular bundles were smaller than the innermost (35.5-48.3 µm long and 45.6-81.5 µm wide in diameter), embedded in the inner sclerenchyma ring separating the columns of parenchyma cells from one another. All vascular bundles were accompanied by sclerenchyma fibers.

b. *Oryza meyeriana* var. *granulata* (Watt) (Nees & Arn. ex G. Watt) Duist.
(Figure 3D).

Culm outline was circular or elliptic. Epidermal cells were 6.3-9.1 µm thick and 5.6-7.8 µm wide. The lignification of the epidermal cells occurred in all directions, but the tangential wall on the outer side was the thickest. The epidermal layers were composed of small, lignified cells with thick cuticles, and were subtended by two to three layers of sclerenchyma cells. A sclerenchyma ring was nearly straight, consisting of four to six layers of sclerenchyma fibers, and two to three layers of cells were bounded on the inner side, alternating with the outermost vascular bundles. Prickles and stomata were also present on the epidermal surface. Collapsed cells were absent. Inner ground tissue consisted of many layers of thick-walled parenchyma cells at the outer part near the sclerenchyma ring, and large thin-walled parenchyma cells at the inner part, extending towards the hollow center of the culm. Intercellular cavities

were absent. Vascular bundles were round to elliptic, arranged in two to three rings. The innermost vascular bundles were 65.5-101.1 µm long and 76.2-118.5 µm wide in diameter, embedded in parenchyma layers of inner ground tissue. Metaxylem vessels of these bundles were circular with diameters 23.9-32.4 µm long and 18.7-32.2 µm wide. The outermost vascular bundles or peripheral vascular bundles were smaller than the innermost bundles (38.7-56.7 µm long and 50.9-90.2 µm wide in diameter), embedded in the inner sclerenchyma ring separating the columns of parenchyma cells from one another. All vascular bundles were accompanied by sclerenchyma fibers.

***Oryza minuta* J. Presl (Figure 1E, 2E and 3E).**

Culm outline was elliptic or ovate. Epidermal cells were 4.5-7.3 µm thick and 6.2-8.8 µm wide. The lignification of the epidermal cells occurred in all directions. The epidermal layers were composed of small, lignified cells with thick cuticles, and were subtended by two to four layers of sclerenchyma cells. Sclerenchyma rings and collapsed cells were absent. Prickles, papillae and stomata were also present on the epidermal surface. Ground tissue consisted of many layers of thin-walled parenchyma cells extending towards the hollow center of the culm. Intercellular cavities were absent. Vascular bundles were round to elliptic, arranged in two rings. The innermost vascular bundles were 117.7-160.8 µm long and 91.3-139.5 µm wide in diameter, embedded in parenchyma layers of inner ground tissue. Metaxylem vessels of these bundles were circular with diameters 33.9-44 µm long and 29.4-42.4 µm wide. The outermost vascular bundles or peripheral vascular bundles were smaller than the innermost bundles (33.3-59.7 µm long and 38.8-81.4 µm wide in diameter), embedded in sclerenchyma layers. All vascular bundles were accompanied by sclerenchyma fibers.

***Oryza ridleyi* Hook. f. (Figure 1F and 3F).**

Culm outline was elliptic. Epidermal cells were 6.1-10.2 µm thick and 4.9-8.4 µm wide. The lignification of the epidermal cells occurred in all directions, but the tangential wall on the outer side was the thickest. The epidermal layers were composed of small lignified cells with thick cuticles, and were subtended by two to three layers of sclerenchyma cells. A sclerenchyma ring was undulated, consisting of two to three layers of sclerenchyma fibers, and three to four layers of cells bounded on the inner side, alternating with the outermost vascular bundles. Stomata were also present on the epidermal surface. No prickles or papillae were present on the epidermis. Collapsed cells were generally absent, but sometimes present. Inner ground tissue consisted of many layers of large thin-walled parenchyma cells, extending towards the hollow center of the culm. Intercellular cavities were absent. Vascular bundles were round to elliptic, arranged in two rings. The innermost vascular bundles were 75.6-140.2 µm long and 85.3-148.3 µm wide in diameter, embedded in parenchyma layers of inner ground tissue. Metaxylem vessels of these bundles were circular with diameters 28.2-48.9 µm long and 25.7-47.8 µm wide. The outermost vascular bundles or peripheral vascular bundles were smaller than the innermost bundles (26.3-73.9 µm long and 32.6-95.7 µm wide in diameter), embedded in the inner sclerenchyma ring separating the columns of parenchyma cells from one another. All vascular bundles were accompanied by sclerenchyma fibers.

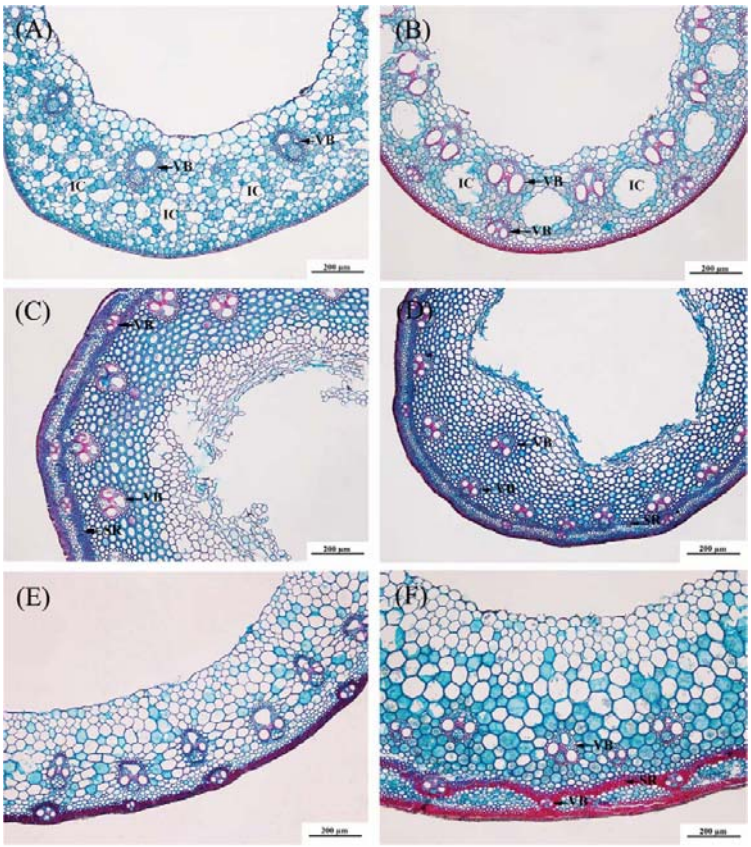

Figure 3. Culm transverse sections of six Thai Oryzeae taxa: (A) *H. aristata;* (B) *L. hexandra;* (C) *O. meyeriana* var. *meyeriana;* (D) *O. meyeriana* var. *granulata;* (E) *O. minuta;* (F) *O. ridleyi.* Abbreviations: IC-intercellular cavity, SR-sclerenchyma ring, VB-vascular bundle.

***Oryza rufipogon* Griff. (Figure 1G-H and 4A-B).**

Culm outline was circular to elliptic. Epidermal cells were 3.6-8.4 µm thick and 5.1-7.8 µm wide. The lignification of the epidermal cells occurred in all directions. The epidermal layers were composed of small lignified cells with thick cuticles, and were subtended by two to five layers of sclerenchyma cells. Sclerenchyma rings and collapsed cells were absent. Prickles, papillae and stomata were also present on the epidermal surface. Ground tissue consisted of many layers of thin-walled parenchyma cells extending towards the hollow center of the culm. Intercellular cavities were absent or present seven to eight cells layers below the epidermis, situated between the outermost and innermost vascular bundle rings, alternating with vascular bundles. Vascular bundles were round to elliptic, arranged in two rings. The innermost vascular bundles were 77.7-173.9 µm long and 67-168.9 µm wide in diameter, embedded in parenchyma layers of inner ground tissue. Metaxylem vessels of these bundles were circular with diameters 21.8-50.9 µm long and 20.2-55.2 µm wide. The outermost vascular bundles or peripheral vascular bundles were smaller than the innermost bundles (41.4-68.9 µm long and 64.4-88.8 µm wide in diameter), embedded in sclerenchyma layers or situated near the sclerenchyma layers. All vascular bundles were accompanied by sclerenchyma fibers.

***Oryza sativa* L. (Figure 4C-D).**

Culm outline was elliptic. Epidermal cells were 4.2-6.9 µm thick and 5.3-8.1 µm wide. The lignification of the epidermal cells occurred in all directions. The epidermal layers were composed of small, lignified cells with thick cuticles, and were subtended by two to three layers of sclerenchyma cells. Sclerenchyma rings and collapsed cells were absent. Prickles, papillae and stomata were also present on the epidermal surface. Ground tissue consisted of many layers of thin-walled parenchyma cells extending towards the hollow center of the culm. Intercellular cavities were absent or present five to six cells layers below the epidermis around the periphery of the culm, situated between the outermost and innermost vascular bundle rings and alternating with vascular bundles. Vascular bundles were round to elliptic, arranged in two rings. The innermost vascular bundles were 144.4-198.7 µm long and 106.7-150.6 µm wide in diameter, embedded in parenchyma layers of inner ground tissue. Metaxylem vessels of these bundles were circular with diameters 43.6-71.3 µm long and 32.7-53.3 µm wide. The outermost vascular bundles or peripheral vascular bundles were smaller than the inner bundles (55.8-84.0 µm long and 77.6-114.4 µm wide in diameter), embedded in sclerenchyma layers or situated near the sclerenchyma layers. All vascular bundles were accompanied by sclerenchyma fibers.

Genus *Zizania* L.

***Zizania latifolia* (Griseb.) Turcz. ex Stapf (Figure 1I, 4E-F and 5).**

Culm outline was elliptic. Epidermal cells were 7.2-10 µm thick and 3.6-5.3 µm wide. The lignification of the epidermal cells occurred in all directions. The epidermal layers were composed of small lignified cells with thick cuticles, and were subtended by three to four layers of thin-walled cells and loose parenchyma, mostly consisting of collapsed cells between the epidermis and a sclerenchyma ring. Intercellular cavities were present between collapsed cells. This zone was bounded on its inner side by a sclerenchyma ring, consisting of three to five layers of cells. Inner ground tissue consisted of many layers of thin-walled parenchyma cells extending towards the hollow center of the culm. A thin membrane composed of stellate parenchyma cells was sometimes present in the hollow center. Vascular bundles were round to elliptic, arranged in three rings. The innermost vascular bundles were 215.5-296.6 µm long and 137.3-191.6 µm wide in diameter, embedded in parenchyma layers of inner ground tissue. Metaxylem vessels of these bundles were circular with diameters 45.3-62.2 µm long and 36.7-45 µm wide; the side of bundles near lysigenous cavities were surrounded by thick-arched sclerenchyma layers of lignified cells. The middle vascular bundles were smaller than the innermost with diameters 113.3-150.7 µm long and 83.8-99.1 µm wide. Those of the outermost circle or peripheral vascular bundles were embedded in a sclerenchyma ring, and were smaller than the other circles, with diameters 63.2-89.5 µm long and 52.5-91.4 µm wide. The vascular bundles in the outermost circle were composed of xylem and phloem, which were easily distinguishable. All bundles were accompanied by sclerenchyma fibers.

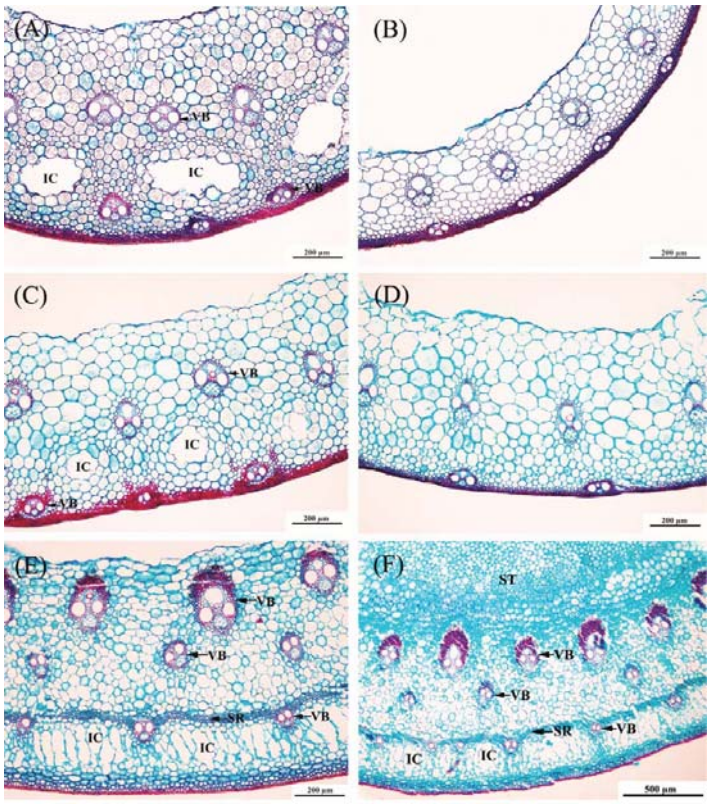

Figure 4. Culm transverse sections of three Thai Oryzeae taxa: (A) *O. rufipogon* with intercellular cavities; (B) *O. rufipogon* without intercellular cavities; (C) *O. sativa* with intercellular cavities; (D) *O. sativa* without intercellular cavities; (E) *Z. latifolia;* (F) *Z. latifolia* with stellate parenchyma. Abbreviations: IC-intercellular cavity, SR-sclerenchyma ring, ST-stellate parenchyma, VB-vascular bundle.

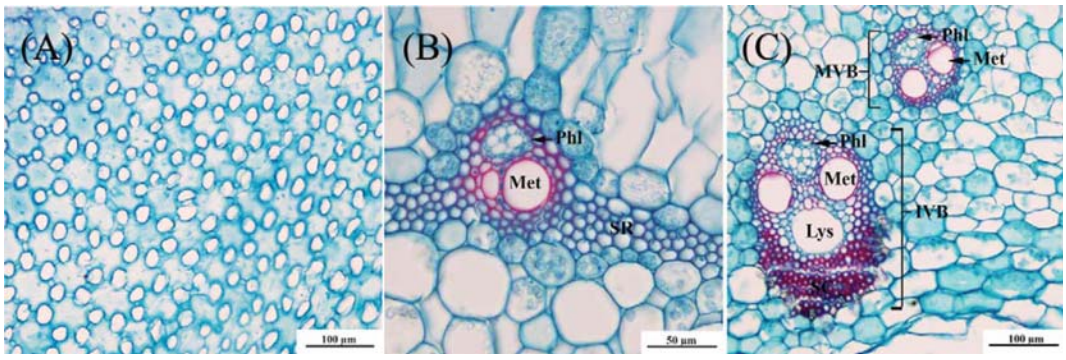

Figure 5. Details of culm transverse sections of *Z. latifolia*: (A) stellate parenchyma; (B) peripheral vascular bundle embedded in a sclerenchyma ring; (C) innermost vascular bundle and middle vascular bundle. Abbreviations: IVB-innermost vascular bundle, MVB-middle vascular bundle, Lys-lysigenous cavity, Met-metaxylem vessel, Phl-phloem, SC-sclerenchyma cells, SR-sclerenchyma ring.

3.3 The Taxonomic Value of Culm Internodal Anatomical Characters

The culms of grasses contain only a few characters, making them generally unfavorable for identification and classification. Metcalfe stated that culm anatomy lacks informative characters for taxonomic studies [12]. However, some characters can be used to indicate environmental factors of where the plants grow [8]. All culms of taxa observed in this study had hollow centers, but a previous study of grass internodes reported an Oryzeae species with a solid culm [9]. However, only two species of Oryzeae, *L. oryzoides* and *Z. aquatica,* were observed in that study. The former had a hollow culm and the latter had a solid culm. Unfortunately, their study did not show pictures of the culm. Thus, we hypothesize that the solid culm found in *Z. aquatica* might be the same as the culms observed in this study, which were hollow, but sometimes contained a thin membrane in the hollow making it look like a solid culm. Thin membranes were composed of stellate parenchyma. Each cell was polygonal in shape with small pores aligned around the cell between the edges of each cell (Figure 5A). It is assumed that this membrane is associated with air storage, as is normally found (as diaphragms) in plants with hollow stem.

Intercellular cavities were present in all species that grow in aquatic environments, such as in paddy fields (*O. rufipogon, O. sativa* and *L. hexandra*), at the edges of ponds or swamps (*Z. latifolia*), or floating on water (*H. aristata*). These cavities provide air storage, and the arrangement is different in each species. Numerous intercellular cavities of *H. aristata* were scattered in the ground tissue of the culm (Figure 3A), while those of *Z. latifolia* were arranged around the periphery between the epidermis and sclerenchyma ring (Figure 4E-F). The three species found in paddy fields had similar intercellular cavity arrangements, but in two *Oryza* species, the appearance of this feature was not constant (intercellular cavities were sometimes absent). For example, culms from one *O. sativa* accession (P. Sumanon & P. Traiperm 10) contained intercellular cavities (Figure 4C), while those of another accession (P. Sumanon & P. Traiperm 28) lacked these cavities (Figure 4D). It is assumed that this difference is related to environmental conditions or the genetics of the plants. It was suggested that the presence of air spaces in roots and stems are related to oxygen transportation for plants under lodging stress [17, 18]. Therefore, this feature might indicate the ability of plants to survive under such stress, and be a useful trait for plant breeders. Further studies are needed to confirm the relationship of intercellular spaces to the physiological abilities of the plants.

There was an obvious size difference in the diameter of metaxylem vessels of *H. aristata* compared to other Oryzeae taxa (Figure 2). Transverse sections showed that the metaxylem diameters of *H. aristata* first order vascular bundles were smaller (<20 μm long) than those of other taxa (>20 μm long). Xylem vessels play an important role in water transportation, however, *H. aristata,* which is an aquatic plant, had the smallest vessels.

Some culm anatomical characters were found to be useful for generic classification and specific identification, including the number of vascular bundle rings, the presence of sclerenchyma rings, the number of sclerenchyma cell layers, and the arrangement of intercellular cavities. Nonetheless, a key to species based on culm anatomy could not identify the two varieties of *O. meyeriana* and the three species of the *O. sativa* group, which contained *O. sativa, O. rufipogon,* and *O. minuta*. Our previous study on lemma micromorphology [5] showed that the two

varieties of *O. meyeriana* could be distinguished, but the three species of *O. sativa* could not be separated using the key obtained from this study.

Rice (*O. sativa*) is one of the most economically important crops in Thailand, however, there are challenges in rice cultivation and breeding to obtain new cultivars with high yields and environmental stress tolerances. Because of the importance of rice, studies of the members in the genus *Oryza* and also in the tribe Oryzeae (which are wild relatives and can provide genetic resources for cultivated rice), are valuable for rice improvement. Many genes that are associated with biotic resistance, abiotic tolerance, and high yield have been found in wild rice species [20, 21]. The anatomy of plants provides a foundation for understanding plant function, and can indicate the physiological abilities of plants. For instance, lodging is one of the major problems in rice cultivation, leading to low yields. Prior studies have shown that air spaces in roots and stems correspond with oxygen transportation for plants under lodging stress [18, 19]. Culms of rice and its close relative *(O. rufipogon)* were found both with and without intercellular spaces in this study. If this character is related to genetic traits and plays a role against stress, it could be used as an indicator for rice improvement. Moreover, many layers of thick-walled parenchyma cells were found in the inner ground tissue of two varieties of *O. meyeriana*, which we assume strengthens plant toughness. In addition, a sclerenchyma ring found in two varieties of *O. meyeriana* and *O. ridleyi*, which is also related to plant structural support. Therefore, intensive studies of the function of these anatomical characters are required.

4. CONCLUSION

The culm anatomy of Oryzeae was described and some characters were recognized as valuable for key construction. Here, an identification key for Thai Oryzeae based on culm transverse sectional characters is provided, however, this key could not clearly distinguish some taxa from their close relatives (*i.e.*, two varieties of *O. meyeriana* and the three species of the *O. sativa* group, namely *O. sativa, O. rufipogon,* and *O. minuta*). Moreover, the anatomical characters which are considered as important traits for some physiological abilities are mentioned. These features need further intensive studies to confirm their roles, which could be related to stress tolerance and might be useful for rice breeding programs.

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