Leaf Anatomy of Vetiver Grass Supporting the Potentially C Sequestration

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Abstract

Investigation of internal leaf structures can give a primary explanation of the potentially C sequestration of the plant. This research aimed to descript internal leaf structures of 11 vetiver provenances and observe some relative structures. The results found that all 11 provenances had a similar pattern of vascular bundle arrangement with the ratio of 1:3:1:3:1 for large:small:medium:small:large and Kranz structure which is similar to C4 plants. Like aquatic plants, large lysigenous intercellular spaces in vetiver leaves strongly related aeration system. Evident of aerenchyma at cortex layer could confirm gas circulation from leaves to roots and encouraged deeply root penetration of vetiver by avoiding hypoxia condition. Moreover, "Humidity-induced convection" and "Venturi-induced convection" were assumed as a strategy to gain more gas circulation of vetiver. Especially, fiber (sclerenchyma) in bundle caps of Loei provenance extended from abaxial to adaxial surface believed playing a dual function of mechanic and hydraulic (a short cut of water pathways) which could retain stomata open and longer gas exchange. Angle of leaf wings reflected an adaptive high radiation and was useful for provenance classification.

In conclusion, the internal leaf structures attributed all 11 vetiver provenances to sequester more C and especially Loei provenance.

Keywords: Kranz structure, lysigenous intercellular space, arenchyma, vascular bundle.

Introduction

Investigating internal leaf structures can predict efficiency of C assimilation, photosynthetic mechanisms, environment during the plant grown and subsequently potentially C sequestration. Leaves present palisade and spongy mesophyll normally indicate to C3 plants, which the photosynthetic capacity is lower than C4 plants (Larcher, 2001). C4 plants, a few groups of both dicots and monocots, display a modified leaf anatomy that is termed Kranz structure. It is characterized by elongated mesophyll cells that radiate from a single layer of large parenchymatous bundle-sheath cells containing starch and enlarged chloroplasts (Gunning and Steer, 1996). This normally forms a second bundle sheath layer, though in some grasses the primary vascular bundle sheath is itself recruited for this purpose. Taxonomic character in Poaceae, as double sheaths often occur in festucoid grasses and single sheaths in panicoid grasses, though there are exceptions (Lu and Liu, 2003; Twiss et al., 1969). In particular, internal leaf structures can change toward environmental variation. For example, sun leaves usually are smaller and thicker with more and better defined palisade cells, and more chloroplasts. They frequently have more hairs as well. Sun leaves rarely have chloroplasts in their epidermal cells, but chloroplasts are common in the epidermises of shade leaves (Rand, 2001). Leaves with thick cuticle, multiple epidermis or depressed stomata often found in xerophytes, whereas large intercellular spaces normally present hydrophytes or long term flooding-tolerant plants

(Schulze et al., 2002). High laticifers, crystals or tannins serve in high grazing plants (Tomlinson and Fisher, 2005).

Therefore, this research aimed to investigate and descript internal leaf structures of 11 vetiver provenances, which the results tentatively induced to the prediction on the potentially C sequestration.

Objectives

1 To descript internal leaf structures of 11 vetiver provenances.

2 To investigate some relative structures promoting vetiver in C sequestration.

Materials and methods

Plant taxa

This study conducted on eleven provenances of two vetiver species, *Chrysopogon nemoralis* and *C. zizanioides*. *C. nemoralis* consists of 6 provenances: Kamphaeng Phet 1 (KP1), Loei (LI), Nakhon Sawan (NS), Prachuabkhirikhan (PK), Ratchaburi (RB) and Roi Et (RE); whereas *C. zizanioides* has 5 provenances: Kamphaeng Phet 2 (KP2), Phraratchathan (PT), Songkhla 3 (SK), Sri Lanka (SL) and Surat Thani (ST). All plants were grown on loamy sand on November 2004 at the experimental plots of the Regional Office 3, Land Development Department, Muang District, Nakhon Ratchasima, Thailand (15°05′N, 102°13′E, 167 m a.s.l.) Each plot was of 2 x 10 m. Plants were given manures in an early stage and allowed naturally grown continually for 3 years. During November 2004 to 2007, the mean annual temperature ranged between 23.1 and 33.3°C with the average annual precipitation about 94.38 mm per month and the average annual evaporation about 4.84 mm per day (Nakhon Ratchasima Meteorology Station; Appendix A).

Leaf cross section

Fresh leaves were free-handed section and strained with safranin solution to provide transverse section slides. The leaves were observed internal structures through a light microscope.

Results

1. Kamphaeng Phet 1 provenance: (Plate 1: Fig.1-2).

Angle of leaf wings: Angle of leaf wings was steeply about 45° with curve wings(at the ends).**Epidermis:**Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles. **Mesophyll:** Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves. **Vascular bundle arrangement:** Three sizes of vascular bundles could be classified, large (L), medium (M) and small (S), and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L.:**Xylem** composes of 4 vessels paralleled with lamina by1 on the left, 1 on the right, and 2 on the top (nearby a small intercellular space).**Phloem** Sieve tubes and companion cells located lower xylem. **Bundle sheath**:Two layers of parenchyma surrounded phloem and xylem: (i) the inner was large cells of fibers containing chloroplasts; (ii) and the outer was chlorenchyma cells radiating from the inner (Kranz structure). **Bundle cap**: All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the

bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 9 - 10, 8, and 2 fiber cell layers which were for the large, medium and small vascular bundle.

2. Loei provenance: (Plate 2: Fig. 3-4).

Angle of leaf wings was steeply about 45° with curve wings (from middle to end).**Epidermis**:Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles.**Mesophyll:**Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves. **Vascular bundle arrangement**: Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L.**Xylem**: Xylem composes of 4 - 5 vessels paralleled with lamina by 1 - 2 on the left, 1 - 2 on the right, and 1 - 2 on the top (nearby a small intercellular space). **Phloem** Sieve tubes and companion cells located lower xylem.**Bundle sheath**: Two layers of parenchyma surrounded phloem and xylem: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure).**Bundle cap**: All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended from the large and medium vascular bundles towards both epidermises. The thickness of bundle caps from phloem to lower epidermis was about 6 - 7, 6 - 7 and 2 - 3 fiber cell layers which were for the large, medium and small vascular bundle; and the thickness of bundle caps from xylem to upper epidermis was about 3 - 4 fiber cell layers.

3. Nakhon Sawan provenance: (Plate 3:Fig. 5-7).

Angle of leaf wings: Leaf wings attached together like a U - shape upside down (∩). **Epidermis:** Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles.**Mesophyll:**Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves. **Vascular bundle arrangement:**Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L. **Xylem** composes of 3 vessels paralleled with lamina by 1 on the left, 1 on the right, and 1 on the top (nearby a small intercellular space).**Phloem:** Sieve tubes and companion cells located lower xylem. **Bundle sheath**: Two layers of parenchyma surrounded phloem and xylem: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure).**Bundle cap:** All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 5 - 6, 4 - 5, and 1 - 2 fiber layers which were for the large, medium and small vascular bundle.

4. Prachuabkhirikhan provenance: (Plate 4:Fig.8-10).

Angle of leaf wings was steeply about 45° with curve wings (from middle to end). **Epidermis:** Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles. **Mesophyll:**Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves. **Vascular bundle arrangement:**Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L.**Xylem** composes of 4 vessels paralleled with lamina by 2 (or 1) on the left, 1 on the right, and 1 (or 2) on the top (nearby a small intercellular space). **Phloem:**Sieve tubes and companion cells located lower xylems.**Bundle sheath:** Two layers of parenchyma surrounded phloems and xylems: (i) the inner was large cells of fibers containing chloroplasts; and (i) the outer was chlorenchyma cells radiating from the inner (Kranz structure).**Bundle cap:** All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 10 - 11, 9 - 10 and 6 - 7 fiber cell layers which were for the large, medium and small vascular bundle.

5. Ratchaburi provenance: (Plate 5: Fig.11 -13).

Angle of leaf wings was steeply about 45°. **Epidermis:** Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles. **Mesophyll:**Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves.**Vascular bundle arrangement:**Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L. **Xylem** composes of 5 vessels paralleled with lamina by 1 on the left, 2 on the right, and 2 on the top (nearby a small intercellular space). **Phloem:**Sieve tubes and companion cells located lower xylems. **Bundle sheath:** Two layers of parenchyma surrounded phloems and xylems: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure). **Bundle cap:** All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 6 - 7, 5 - 6 and 1 - 2 fiber cell layers which were for the large, medium and small vascular bundle.

6. Roi Et provenance: (Plate 6: Fig.14-17).

Angle of leaf wings was about 60° with curve wings (from middle to end). **Epidermis:** Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles.**Mesophyll:**Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves. **Vascular bundle arrangement:** Three sizes of vascular bundles could be classified, large,

medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L.**Xylem:**Xylem composes of 4 vessels paralleled with lamina by 1 on the left, 1 on the right, and 2 on the top (nearby a small intercellular space).**Phloem**:Sieve tubes and companion cells located lower xylems.**Bundle sheath**:Two layers of parenchyma surrounded phloems and xylems: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure).**Bundle cap:** All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 6 - 7, 6 - 7 and 2 - 3 fiber cell layers which were for the large, medium and small vascular bundle.

7. Kamphaeng Phet 2 provenance: Plate 7: Fig. 18 - 20).

Angle of leaf wings was about 60° without curve wings. **Epidermis:**Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles. **Mesophyll**: Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves.**Vascular bundle arrangement**: Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attachning with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L. **Xylem** composes of 4 vessels paralleled with lamina by 1 on the left, 1 on the right, and 2 on the top (nearby a small intercellular space).**Phloem:** Sieve tubes and companion cells located lower xylems. **Bundle sheath:** Two layers of parenchyma surrounded phloems and xylems: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure). **Bundle cap:** All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 8 - 9, 5 - 6 and 2 - 3 fiber cell layers which were for the large, medium and small vascular bundle.

8. Phraratchathan provenance: (Plate 8: Fig. 21 - 23)

Angle of leaf wings was steeply about 45° without curve wings. **Epidermis:**Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles.**Mesophyll**:Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves.**Vascular bundle arrangement**: Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L. **Xylem** composes of 4 vessels paralleled with lamina by 1 on the left, 1 on the right, and 2 on the top (nearby a small intercellular space).**Phloem**: Sieve tubes and companion cells located lower xylems. **Bundle sheath**: Two layers of parenchyma surrounded phloems and xylems: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure). **Bundle cap**: All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle

caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 7 - 8, 7 - 8 and 1 - 2 fiber cell layers which were for the large, medium and small vascular bundle.

9. Songkhla 3 provenance: (Plate 9 :Fig. 24 -26).

Angle of leaf wings was very wide (over than 90°).**Epidermis**:Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles.**Mesophyll**: Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves.**Vascular bundle arrangement**: Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L.**Xylem** composes of 4 vessels paralleled with lamina by 1 on the left, 1 on the right, and 2 on the top (nearby a small intercellular space).**Phloem**:Sieve tubes and companion cells located lower xylems. **Bundle sheath:** Two layers of parenchyma surrounded phloems and xylems: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure). **Bundle cap:** All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 6 - 7, 6 - 7 and 1 - 2 fiber cell layers which were for the large, medium and small vascular bundle.

10. Sri Lanka provenance: (Plate 10: Fig.27 - 29).

Angle of leaf wings was steeply about 45° with curve wings (at the ends) **Epidermis:** Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles. **Mesophyll**: Large lysigenous intercellular spaces were found in the mesophyll of mature leaves by positioning upper the small vascular bundles,but the spaces were not prominently in developing leaves. **Vascular bundle arrangement**: Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with aaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L. **Xylem** composes of 4 vessels paralleled with lamina by 1 on the left, 2 on the right, and 1 on the top (nearby a small intercellular space). **Phloem**: Sieve tubes and companion cells located lower xylems.**Bundle sheath**:Two layers of parenchyma surrounded phloem and xylem: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure). **Bundle cap** :All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 4 - 5, 7 - 8 and 1 - 2 fiber cell layers which were for the large, medium and small vascular bundle.

11. Surat Thani provenance: (Plate 11: Fig 4.30-32).

Angle of leaf wings was very steep (less than 45°). **Epidermis**: Both upper and lower epidermis were single layer with thick cuticle, by the outer walls were thicker than the inner. Stomata were greater in the lower epidermis by setting bellow the spaces between the small vascular bundles. **Mesophyll:** Large lysigenous intercellular spaces

were found in the mesophyll of mature leaves by positioning upper the small vascular bundles, but the spaces were not prominently in developing leaves.**Vascular bundle arrangement**:Three sizes of vascular bundles could be classified, large, medium and small, and arranged as a single row across laminas by attaching with abaxial surface. The vascular bundles arranged by alternating by sizes with the ratio of 1:3:1:3:1 for L:S:M:S:L. **Xylem** composes of 4 - 5 vessels paralleled with lamina by 1 - 2 on the left, 1 on the right, and 1 - 2 on the top (nearby a small intercellular space).**Phloem:** Sieve tubes and companion cells located lower xylems. **Bundle sheath:** Two layers of parenchyma surrounded phloems and xylems: (i) the inner was large cells of fibers containing chloroplasts; and (ii) the outer was chlorenchyma cells radiating from the inner (Kranz structure). **Bundle cap:** All vascular bundles, except the middle of the small size, had well developed bundle caps, which composed of small and thick phloem fibers. By the bundle caps extended to lower epidermis. The thickness of bundle caps from phloem to lower epidermis was about 5 - 7, 7 - 8 and 1 - 2 fiber cell layers which were for the large, medium and small vascular bundle.

Plate 1: Internal Leaf Structure of Kamphaeng Phet 1 **provenance**

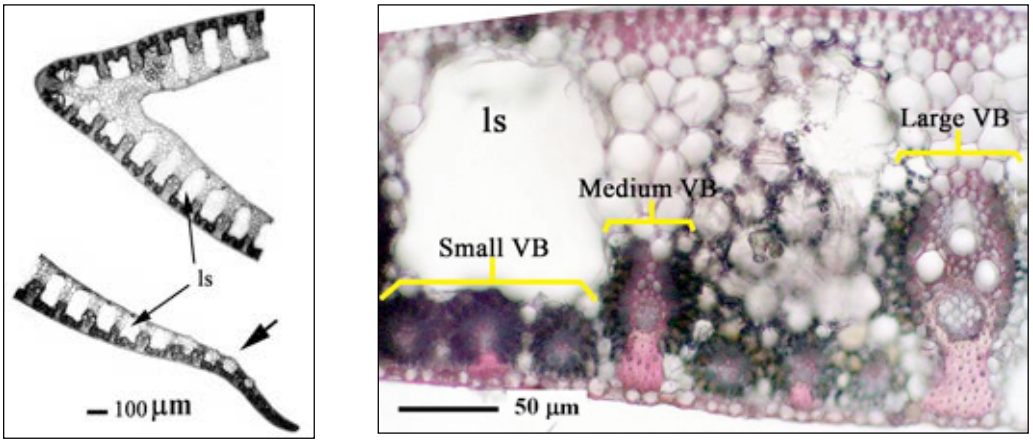

Figure 1 TS of Kamphaeng Phet 1 leaves showing a steep angle of leaf wings (~45°) with curve wings at the ends (large arrow), and large intercellular spaces (ls).
Figure 2 TS of a developing leaf showing lysigenous intercellular spaces and a lysis of parenchyma cells.

Plate 2 Internal Leaf Structure of Loei provenance

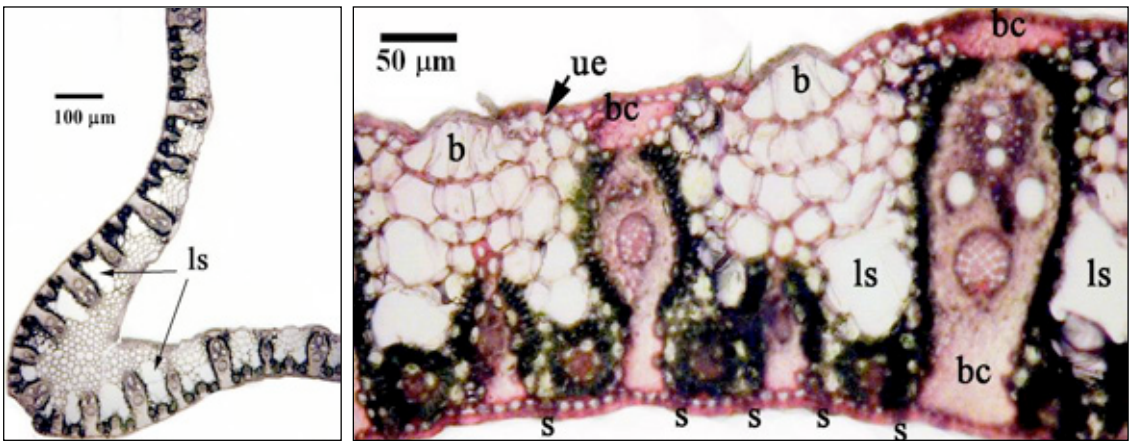

Figure 3 TS of Loei leaf showing a steep angle of leaf wings (~45°) with curve (from middle to end), and lysigenous intercellular spaces (ls).
Figure 4 TS of a developing leaf showing an early stage of parenchyma lysis.

Plate 3 Internal Leaf Structure of Nakhon Sawan provenance

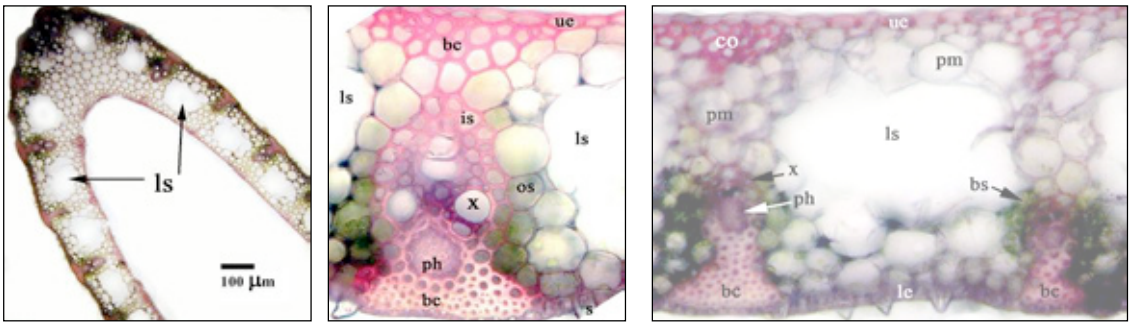

Figure 5 TS of Nakhon Sawan leaf showing two leaf wings like a U - shape upside down (∩) and lysigenous intercellular spaces (ls).
Figure 6 TS of a large vascular bundle in a developing leaf.
Figure 7 TS of a developing leaf showing parenchyma lysis.

Plate 4 Internal Leaf Structure of Prachuabkhirikhan provenance

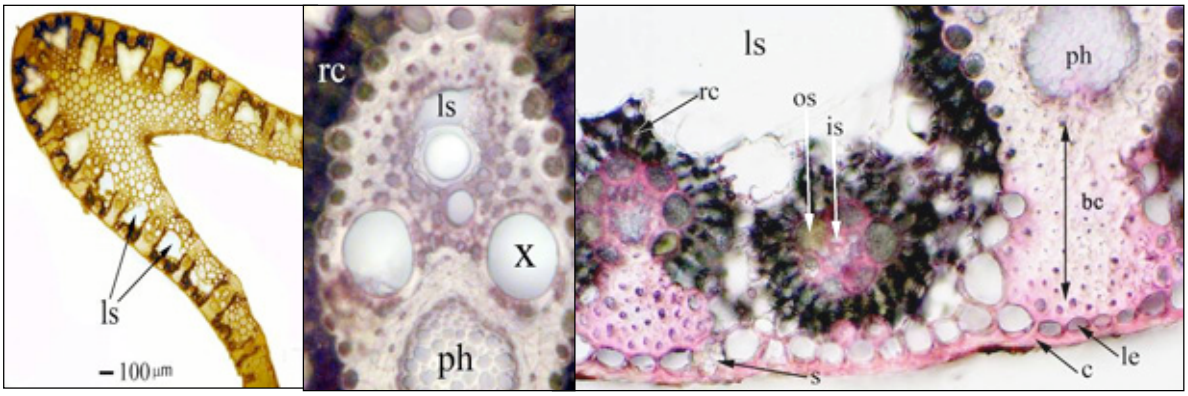

Figure 8 TS of Prachuabkhirikhan leaf showing a steep angle of leaf wings (~45°) with curve (from middle to end), and lysigenous intercellular spaces (ls).
Figure 9 Focus on a large vascular bundle.
Figure 10 Two sizes of vascular bundles, large and small.

Plate 5 Internal Leaf Structure of Ratchaburi provenance

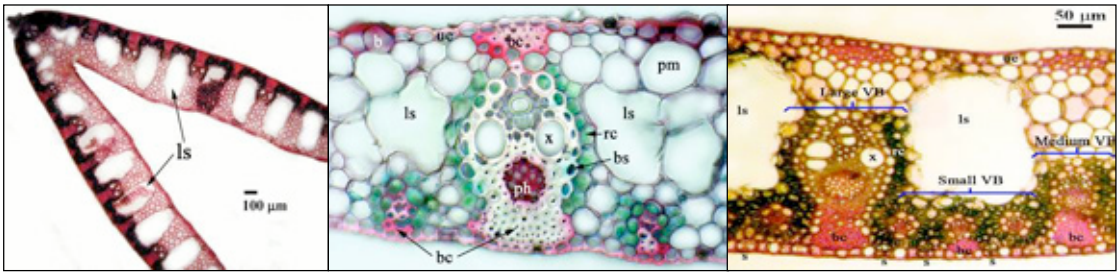

Figure 11 TS of Ratchaburi leaf showing a steep angle of leaf wings (~45°) without curve, and lysigenous intercellular spaces (ls).
Figure 12 TS of a developing leaf showing an early stage of parenchyma lysis.
Figure 13 TS of a mature leaf comparing three sizes of vascular bundles, large, medium and small.

Plate 6 Internal Leaf Structure of Roi Et provenance

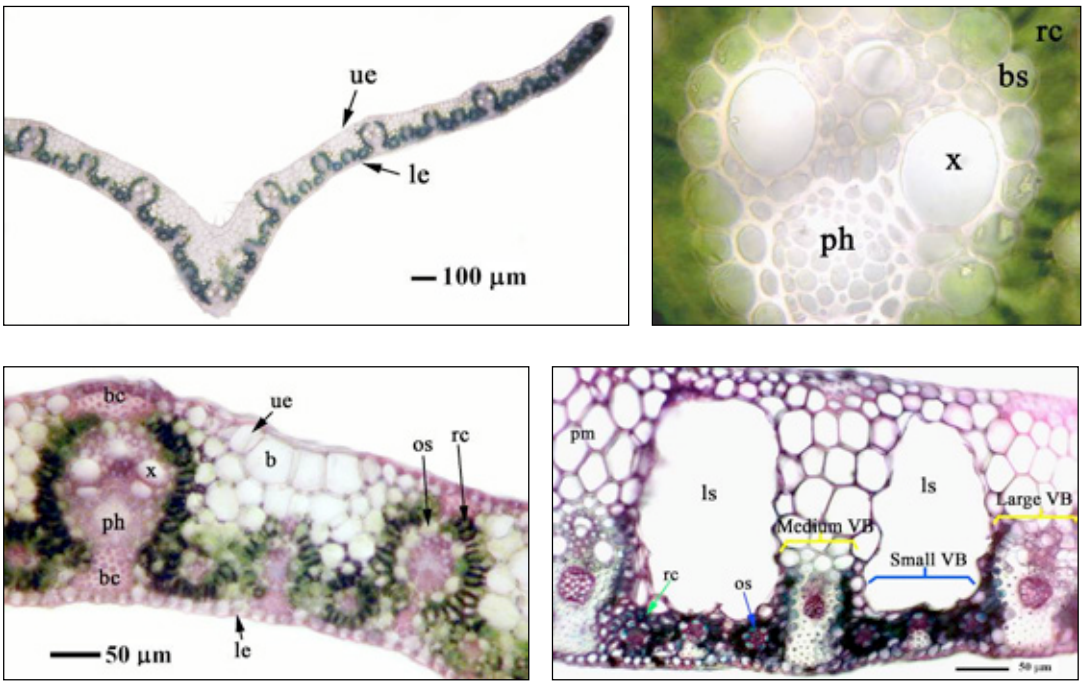

Figure 14 TS of Roi Et leaf showing a wide angle of leaf wings (~ 60°) with curve wings (from middle to end).
Figure 15 Focus on a large vascular bundle.
Figure 16 TS of a developing leaf.
Figure 17 TS of a mature leaf showing the three sizes of vascular bundles.

Plate 7 Internal Leaf Structure of Kamphaeng Phet 2 provenance

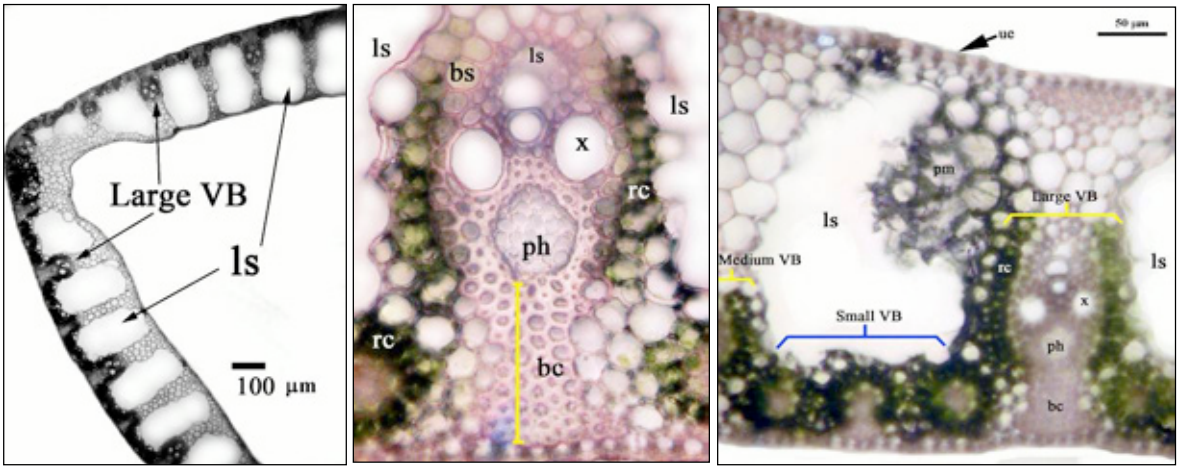

Figure 18 TS of Kamphaeng Phet 2 showing a wide angle of leaf wings (~ 60°) without curve wings, and lysigenous intercellular spaces (ls).

Figure 19 Focus on a large vascular bundle.
Figure 20 TS of a mature leaf showing parenchyma lysis.

Plate 8 Internal Leaf Structure of Phraratchathan provenance

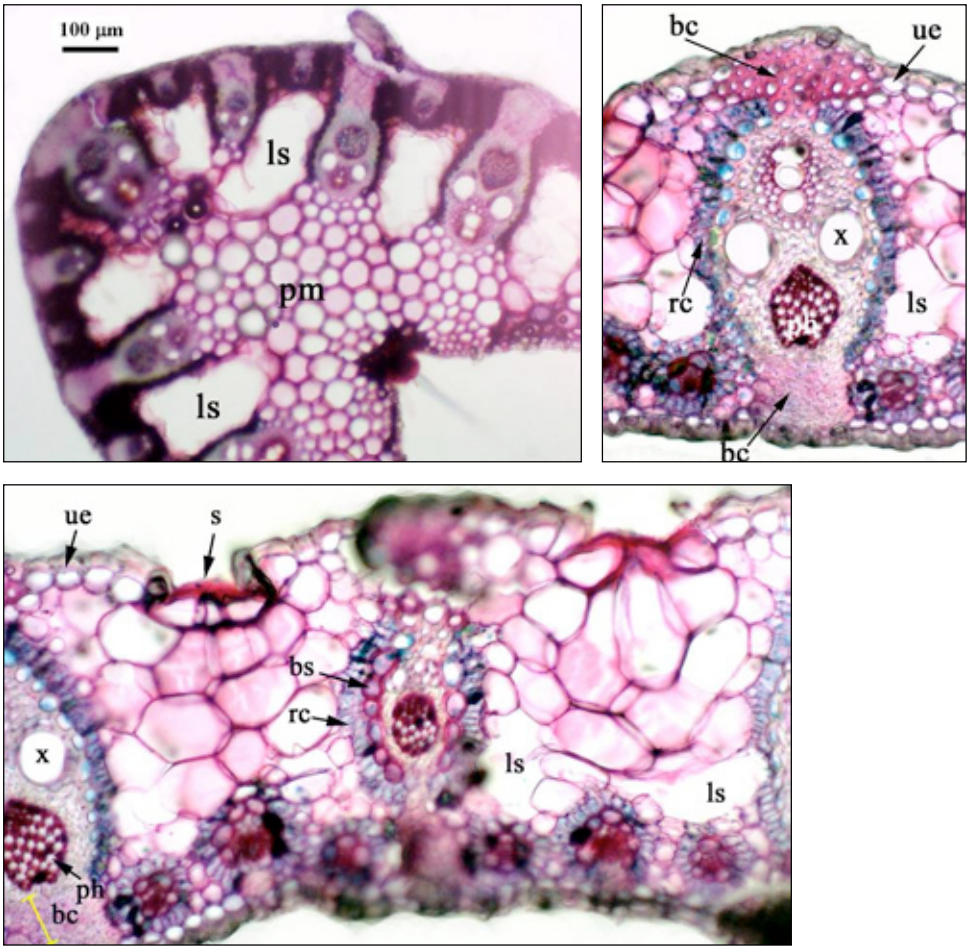

Figure 21 TS of Phraratchathan leaf showing a steep angle of leaf wings (~45°) without curve wings, and lysigenous intercellular spaces (ls).
Figure 22 Focus on a large vascular bundle in a developing leaf.
Figure 23 TS of a developing leaf showing an early stage of parenchyma lysis.

Plate 9 Internal Leaf Structure of Songkhla 3 provenance

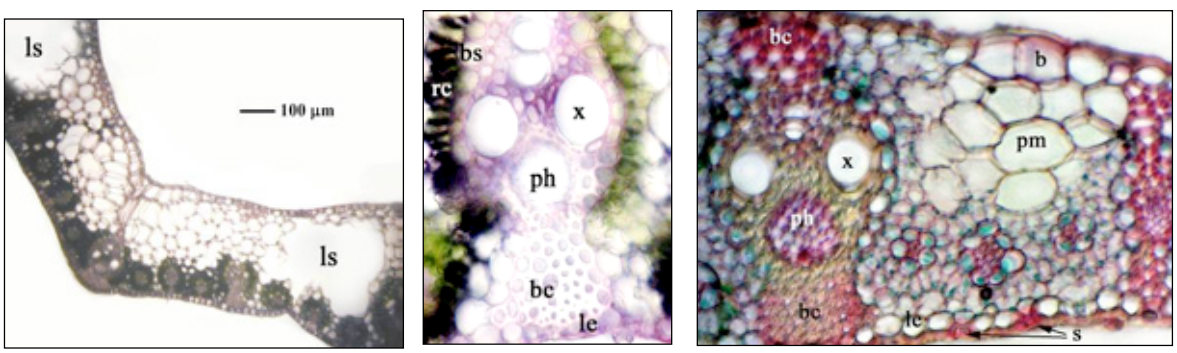

Figure 24 TS of Songkhla 3 leaf showing a wide angle of leaf wings (> 90°), and lysigenous intercellular spaces (ls).

Figure 25 Focus on a large vascular bundle.
Figure 26 TS of a developing leaf showing an early stage of parenchyma lysis.

Plate 10 Internal Leaf Structure of Sri Lanka provenance

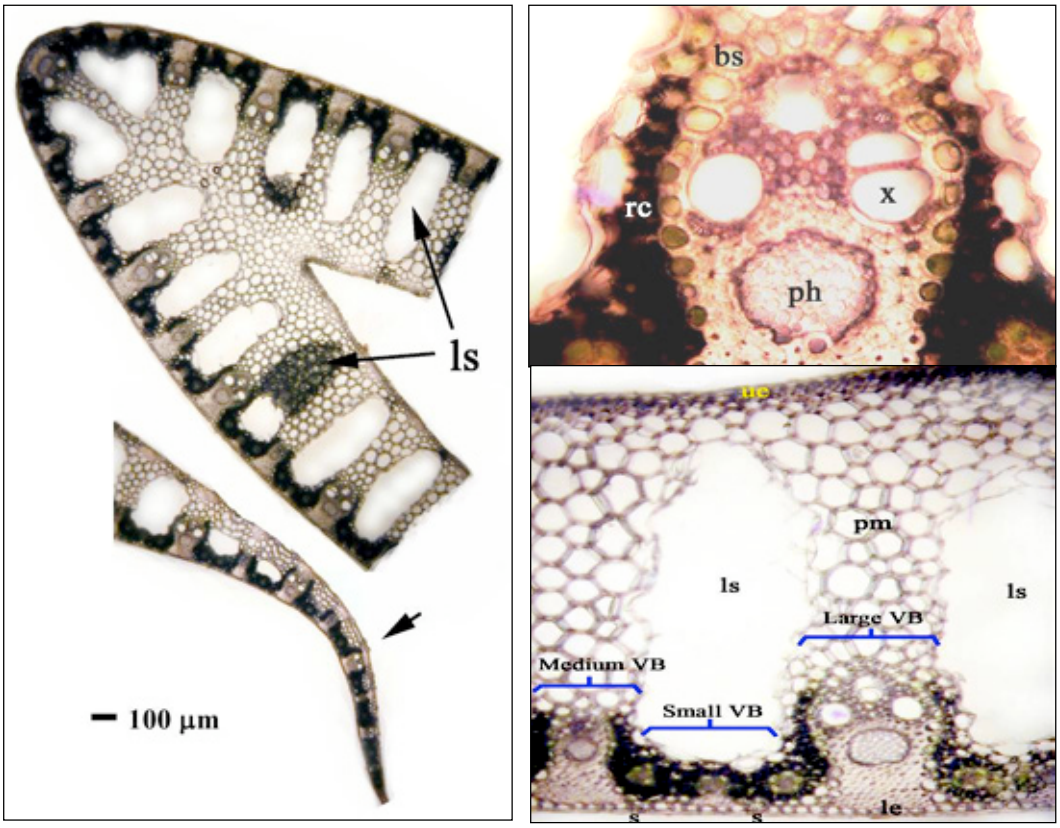

Figure 27 TS of Sri Lanka leaves showing a steep angle of leaf wings (~45°) with curve wings (at the ends), and lysigenous intercellular spaces (ls)
Figure 28 Focus on a large vascular bundle.
Figure 29 TS of a mature leaf showing large lysigenous intercellular spaces and three sizes of vascular bundles, large, medium and small.

Plate 11 Internal Leaf Structure of Surat Thani provenance

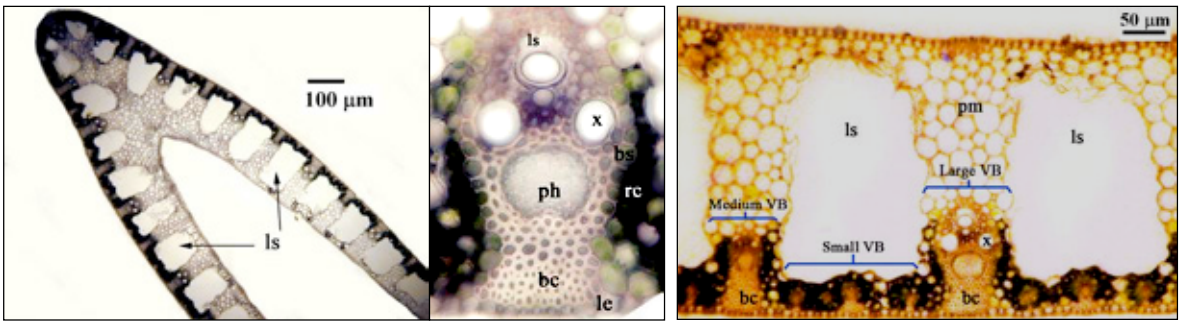

Figure 30 TS of Surat Thani leaf showing a very steep angle of leaf wings (< 45°), and lysigenous intercellular spaces (ls).
Figure 31 Focus on a large vascular bundle.
Figure 32 TS of a mature leaf showing large lysigenous intercellular spaces and three sizes of vascular bundles, large, medium and small.

Discussion

1. Comparing angle of leaf wings among 11 provenances

Angle of leaf wings was different among 11 provenances, but it could be benefit in provenance classification as shown in Table 1. Steep angle of leaf wings, except SK provenance, indicated an adaptation of vetiver leaves to high radiation such as very steep angle of ST provenance (< 45°). However, SK provenance seemed to have high radiant tolerance with wide angle of leaf wings (> 90°).

Table .1 Classification of vetiver provenance based on angle of leaf wings.

Angle of leaf wings	Vetiver provenance
< 45°	ST
~45° without curve wings	RB and PT
~45° with curve wings (from middle to end)	LI and PK
~45° with curve wings (at the ends)	KP1 and SL
~60° without curve wings	KP 2
~60° with curve wings (from middle to end)	RE
> 90°	SK
U - shape upside down (∩)	NS

2. Leaf anatomy comparison of vetiver to other grasses

More study, we investigated seven grass species which had leaf characters similar vetiver, by the ratio of vascular bundles and leaf transverse sections were shown in Table 2 and Figure 33. Comparative arrangement of vascular bundles between vetiver and the selective grasses, it could find that only paragrass and sugarcane which the arrangement was similar vetiver (the ratio of 1:3:1:3:1 for L:S:M:S:L), but differ in particular. By leaves of sugarcane had more abundant chloroplasts in Kranz structure and lignified cells in bundle caps, and very large bulliform cells; while leaves of paragrass had very large bulliform cells and very less parenchyma cells. Moreover, chloroplast contents, lignified cells and parenchyma cells of vetiver did not prominent, if comparing to selective grasses.

Table 2 Vascular bundle arrangement of some C4 grasses.

Common name	Vascular bundle	
	Arrangement	ratio
Paragrass (*Brachiaria mutica* (Forsk.) Stapf)	L:S:M:S:L*	1:3:1:3:1
Lemongrass (*Cymbopogon citratus* (DC.) Staph)	L:S:L	1:3:1
Maize (*Zea mays* L.)	L:S:L	1:6:1
Cogongrass (*Imperata cylindrical* (L.) P. Beauv.)	L:S:M:S:M:S:M:S:M:S:L	1:1:1:1:1:1:1:1:1:1:1
Sugarcane (*Saccharum officinarum* L.)	L:S:M:S:L	1:3:1:3:1
Goose grass (*Eleusine indica* (L.) Gaertn)	L:S:L	1:4:1
Guinea grass (*Panicum maximum* Jacq.)	L:S:L	1:10:1

*L = large vascular bundle, M = medium vascular bundle, S = small vascular bundle.

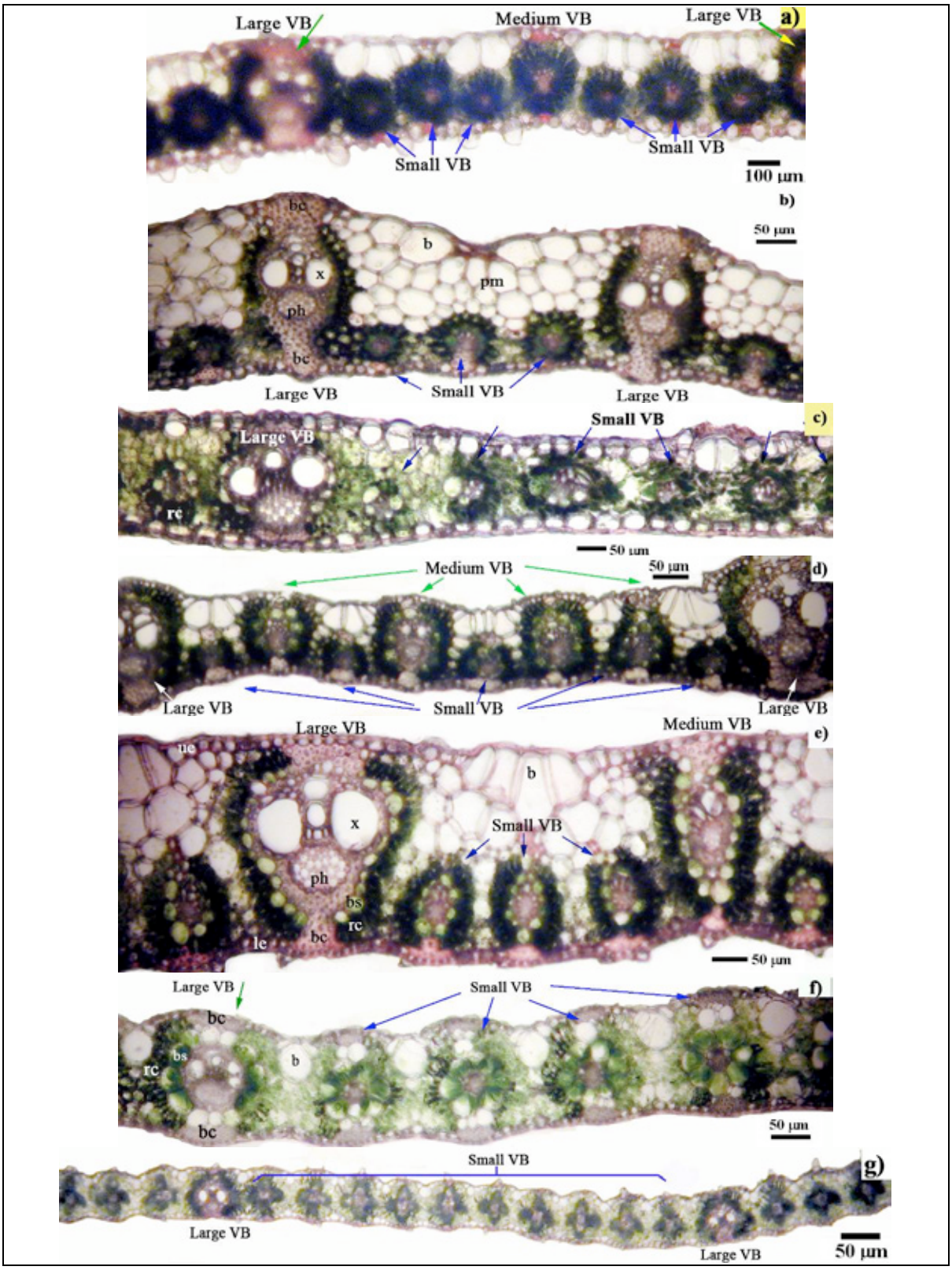

Figure 33 TS of Leaves of (a) paragrass, (b) lemongrass, (c) maize, (d) cogongrass, (e) sugarcane, (f) goose grass and (g) guinea grass.

3. Fibers and hydraulics within leaves

Moreover, I found only LI provenance has bundle caps (sclerenchyma) extend from the large and the medium vascular bundles towards both epidermises. These bundle sheath extensions are termed girders which afford mechanical support to the leaf and are a xeromorphic feature. Moreover, I believe that sclerenchyma acts as a short cut of water pathways resulting sustainable higher photosynthetic rates (Figure 34). *Gnetum gnemon***,** a forest understory species, also has large lysigenous intercellular spaces and the fibers were proved by fluorescent dyes in an additional function of hydraulics (Tomlinson and Fisher, 2005; Figure 35). This apoplastic transport system is responsible for maintaining a high internal humidity and consequently stomata tend to remain open, by promoting more extensive gas exchange. Two species of conifer and cycad, *Podocarpus dispermis* and *Sciadopitys verticillata* also had sclereids act as a hydraulic short cut through the mesophyll tissues, which exhibited much higher the hydraulic conductance (K_{leaf}) as well as greater net CO_2 assimilation rate (A_{max}) (Brodribb et al., 2007; Figure 36). General plants with no facilitating water-conducting sclereids, the length of hydraulic pathway (D_m) (from veins across the mesophyll to where it evaporates from the leaf) exerted a controlling influence over K_{leaf} and secondarily A_{max}, by the D_m traversed by the transpiration stream (Brodribb et al., 2007). However, water moves in a continuous path from soil through the roots, stems, and leaves to the atmosphere at rates also determined by gradients in water potential and the resistances to flow. The water potential ψ^J at any site J in this pathway is governed by: (1) ψ^{soil}; (2) ψ of other possible sources of water, such as tissues storage; (3) the resistances to water flow from parts of the plant downstream and to parts upstream; and (4) the rate of water movement (Nobel and Jordan, 1983). Water moves in response not only to evaporation from the leaves but also to the exchange of water between the tissues of a plant and the xylem adjacent to these tissues. The amount and kinetics of this exchange is determined by the capacitance (water potential to water content) of the storage tissue C^J and the resistance T_U^L to flow between such tissues and the xylem (Nobel and Jordan, 1983).

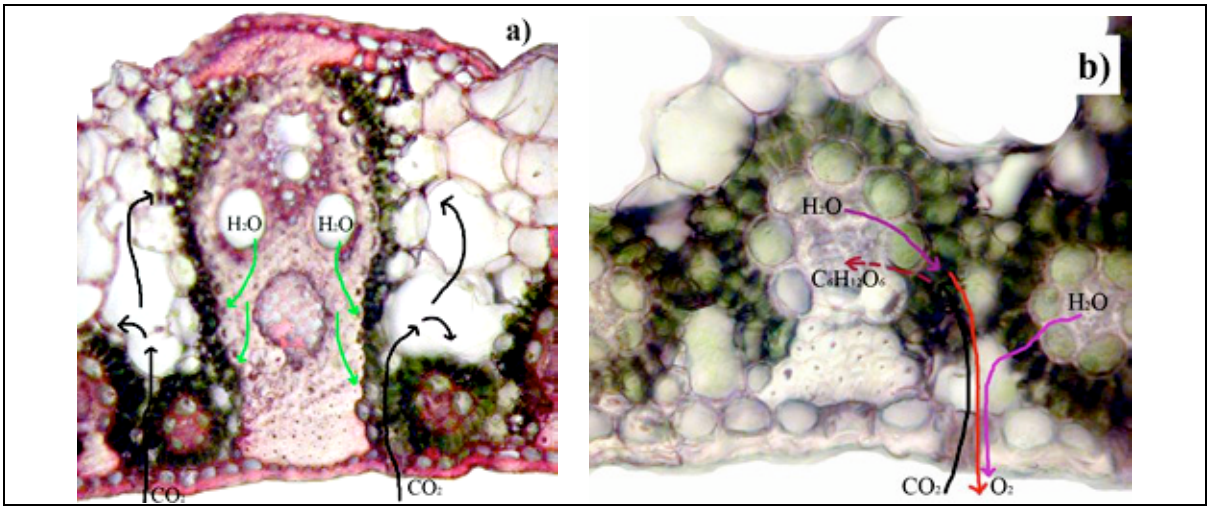

Figure 34 Schemes of CO_2 and H_2O distribution to photosynthetic sites of LI leaf: (a) at Kranz of the large vascular bundle; and (b) at Kranz of the small vascular bundles.

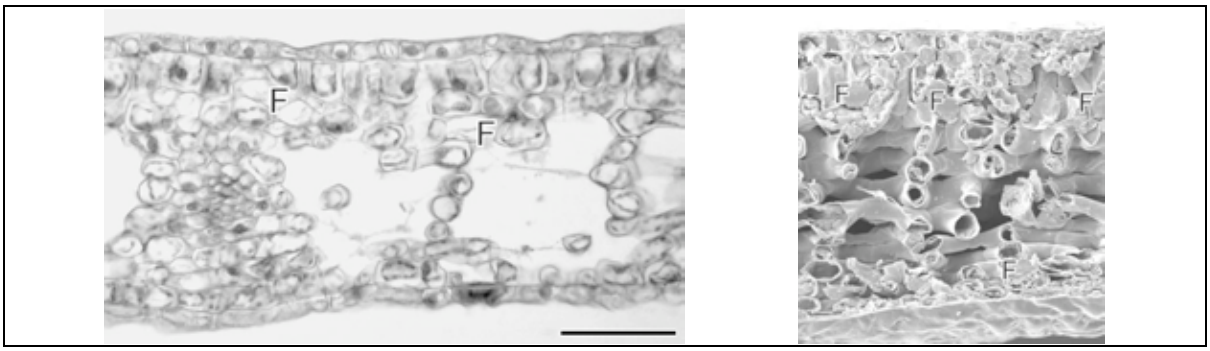

Figure 35 Mature fully expanded lamina of *Gnetum gnemon*. (Left) TS of Lamina immature but intercellular space system of mesophyll fully established, fibers (F) thin-walled and highly vacuolated and therefore obscure. (Right) An SEM transverse view of leaf tissue with mature fibers (F). Scale bar = 100 µm. (Tomlinson and Fisher, 2005).

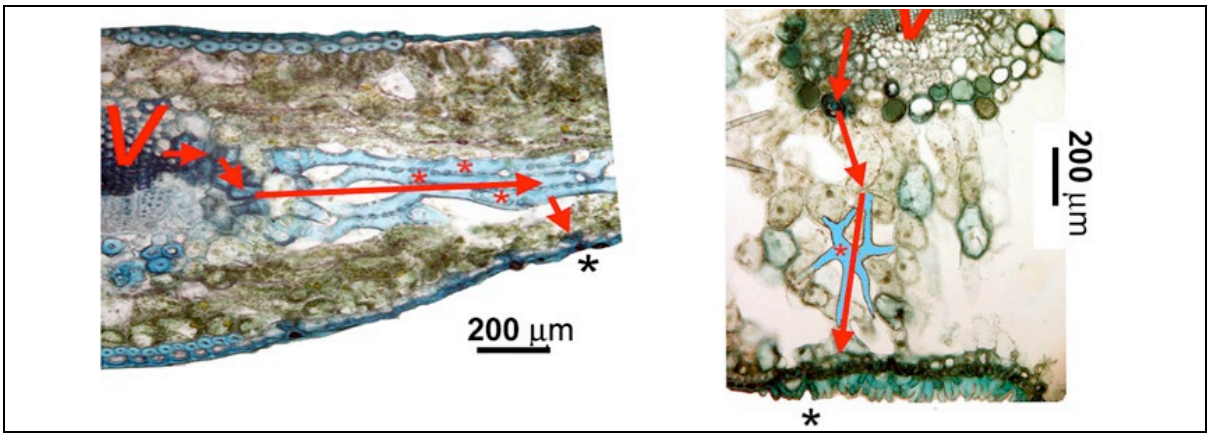

Figure 36 Leaves of two species of conifer and cycad, *Podocarpus dispermis* and *Sciadopitys verticillata* show hydraulic flow path (from the midrib (V) to the stomata (black asterisks) facilitate by water-conducting sclereids (Brodribb et al., 2007).

4. Lysigenous intercellular spaces and aeration system

Leaves of all 11 provenances found large lysigenous intercellular spaces being upper the small vascular bundles, which were similarly water plants, typha, banana or even bamboo leaves (Figure 37). The lysigenous intercellular spaces appeared even developing leaves but with a small size and the size developed larger in mature leaves, which was opposite to bulliform cells. I guessed that vetiver used bulliform cells functioning for water and/or gas accumulation before the lysigenous intercellur space development. In principal, large lysigenous intercellular spaces within leaves of water plants provide to eliminate hypoxia/anoxia effects at roots by promoting oxygen from leaves to roots. Aerenchyma at cortex layer of roots is another evidence for sustaining oxygen to roots, which was found in vetiver roots, also. I extended study on vetiver roots and found the parenchyma cells at cortex layer were lysed to aerenchyma and the parenchyma cells at the inner vascular cylinder were lysed to intercellular spaces, as shown an example of LI provenance in Figure 34. Large lysigenous intercellular spaces at leaves and aerenchyma at roots could be evident to confirm a great aeration system of vetiver as well as a potential of deeply root penetration. Similar prolonged flooding, oxygen concentrations at deep soils are very low but vice versa for ethylene concentrations, which does not support for general plant grown,

except some plants such as vetiver. Under hypoxia conditions, ethylene formation increases, and it accumulates in and around roots and submerged shoots because of its low solubility in water. Ethylene concentrations of 0.1 - 0.5 ppm are sufficient to induce formation of intercellular space-rich tissues by programmed cell death (PCD) or apoptosis. PCD does not take place in differentiated older cells, rather an aerenchyma is initiated already at the end of the elongation zone of the organ. Formation of aerenchyma is lytic, e.g. in maize, *Luronium*, and *Nymphoides*, but usually schizogenous in petioles, e.g. of *Caltha*, *Rumex*, or *Filipendula*. Formation of aerenchyma is not restricted to helophytes and submerged plants; even terrestrial plants, such as maize and sunflower, may develop aerenchyma in roots and the basal part of the shoot. In many helophytes (such as rice and arrowhead), the formation of aerenchyma is genetically fixed (constitutive), and the induction of PCD is not dependent on oxygen deficiency or ethylene accumulation. Aerenchyma formation caused by hypoxia commonly results in irregular air spaces, whereas those constitutively developed show regular patterns of air channels. Aerenchyma allows air circulation in tissues, additionally supported by pressure ventilation.

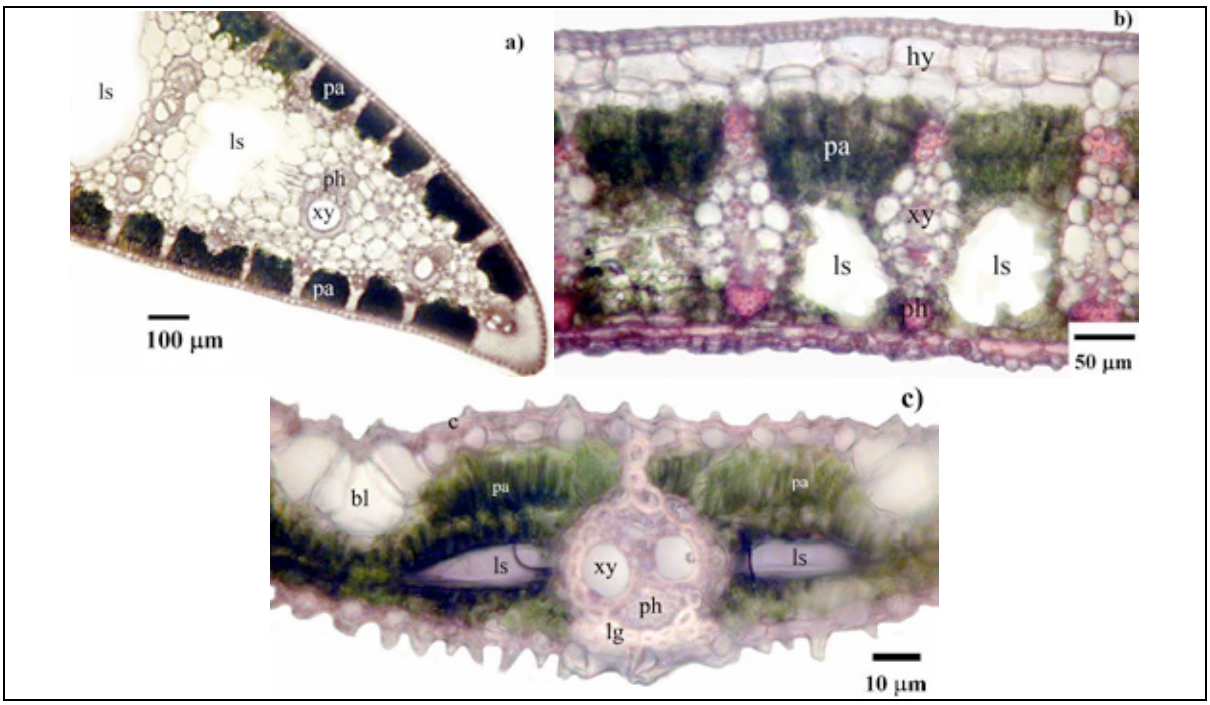

Figure 37 Leaf TS of (a) typha (*Typha angustifolia*), (b) banana (*Musa sapientum* Linn.) and (c) Phai ruak (*Thyrsostachys siamensis* Gamble.).

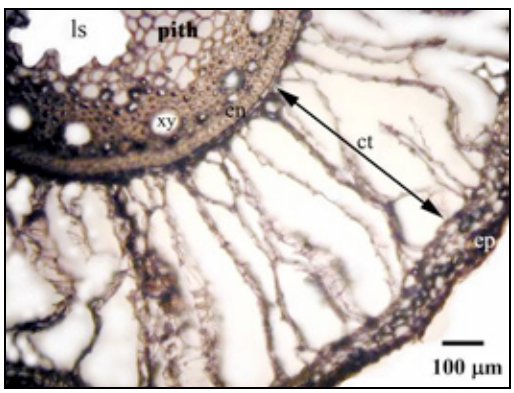

Figure 38 TS of vetiver root from LI provenance.

Primary roots of terrestrial plants usually cannot tolerate hypoxia and die. Hypoxia-resistant plants (e.g. maize, ash, willow, *Forsythia*, *Rumex palustris*) are able within a few days to produce adventitious roots with a well-developed aerenchyma from basal shoot parts or the lower nodes. These roots do not penetrate as deeply into the soil as the primary root system into a well-aerated substrate.

The individual cell layers of young adventitious roots are differently supplied with oxygen. The exodermis is the only cell layer which is usually oxygen-free; cell walls of this layer are often suberinised, thus preventing diffusion of oxygen from the interior of the root to the external medium. Formation of aerenchyma not only guarantees the aerenchyma of tissues, but also reduces the number of oxygen-consuming cells in that tissue.

Lytic aerenchyma formation (i.e. from disintegration of cells; Drew et al., 2000) occurs selectively in the cortex of adventitious roots, starting in those parts of the tissues that are least supplied with oxygen. Lysis of cells often requires not more than 24 h. Ethylene induces this process more or less independently of normoxia or hypoxia, but hypoxia stimulates ethylene synthesis. Inhibition of ethylene synthesis, on the other hand, suppresses formation of aerenchyma. The genus *Rumex* comprised hypoxia-sensitive (*R. acesota, acetosella*) as well as hypoxia-tolerant species (*R. palustris*). In the flooding-tolerant *R. palustris* ethylene production is relatively low and almost the same under aerobic and anaerobic conditions. The internal ethylene concentration slightly decreases even long-term submergence. In flooding-intolerant sorrel, however, internal ethylene concentration increases with the duration of submergence. Low concentration of ethylene increases elongation growth, while a long-term increasing ethylene concentration, as in *R. acetosella*, causes premature senescence of the whole organ.

It has been shown that ethylene as a signal induces the protein kinase signal transduction pathway via a G-protein, Ca^{2+} and inositol-P, which leads to synthesis of lytic enzymes, e.g. cellulase and hemicellulase (Saab and Sachs, 1996). Calcium activates endonucleases, so that the cell death is caused by a controlled breakdown of nucleic acid (by an endonuclease, activated by caspases) and not through breakdown of cell membranes, as after cell damage. Formation of aerenchyma is promoted by further effectors. For example, mechanical resistance of a heavy soil stimulates ethylene synthesis by the growing root and scarcity of minerals increases the sensitivity of the plant tissue to ethylene. Figure 39 shows a model of the biochemical processes that take place in the formation of aerenchyma.

To promote gas circulation, vetiver was suggested maintaining oxygen to roots via aeration systems called “Humidity-induced convection (HIC)” and “Venturi-induced convection (VIC)”, by the evidence was a pith cavity at culms. Such *Phragmitis australis*, an aquatic macrophyte, can maintain aeration system through both HIC and VIC mechanism (Amstrong et al., 1996; Figure 40 and Figure 41). According to different humidity, for HIC, gas can ventilate from atmosphere into plant parts via stomata of living culms and exit the plant via broken/die culms, so the gas volumes depend on drier air and more light. VIC implicates wind speeds and different pressures between inside and outside the plant, by both influx and efflux of gas stream are via broken/die culms. Aeration system of vetiver was assumed to strongly influencing with HIC and VIC mechanism because of anatomy supporting. Even large intercellular spaces can encourage aeration system within vetiver and rhizosphere, however, it must trade-off with parenchyma loss.

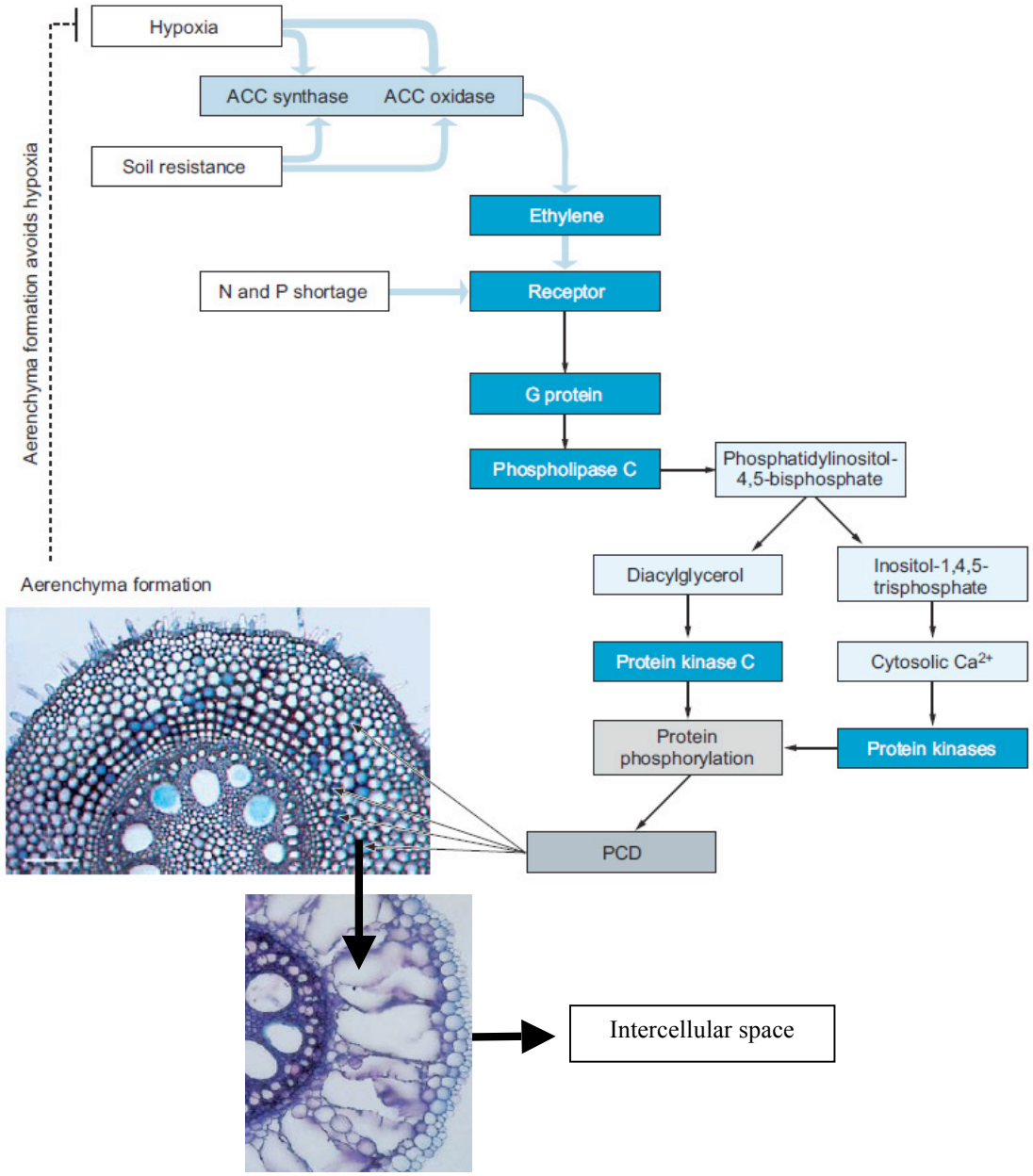

Figure 39 Signal chain that leads to programmed cell death (PCD) upon aerenchyma formation, including environmental factors that control it (Drew et al., 2000).

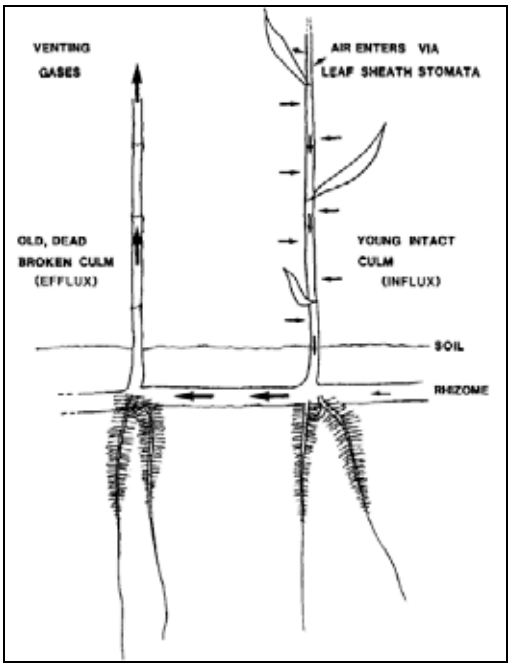

Figure 40 *Phragmites:* humidity-induced convection. The difference in humidity between the sub-stomatal gas-space of the plant and the drier atmosphere favours the inward diffusion of O_2 and N_2 through leaf sheath and nodal stomata. A pressurised flow of gases is induced through the rhizome because of stomatal resistance to baekflow. The throughflow results in increased diffusion of O_2 into root and rhizosphere (Armstrong et al., 1996).

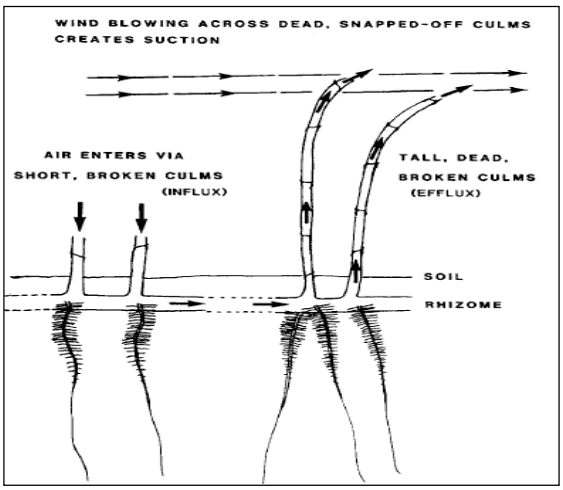

Figure 41 *Phragmites:* Venturi-induced convection. A suction pressure, ΔP (*Pa*) is created by the wind such that $\Delta R = \left(\frac{3}{4}\right)\rho X^4$, where ρ is the density of air (approx. 1.2- 1.25 kg m^{-3}) and V, the wind velocity (ms^{-1}). The convective flow through the rhizome results in an increased diffusion of oxygen to root and rhizosphere (Armstrong et al., 1996).

Conclusions

1. Internal leaf structures of 11 vetiver provenances were different in particularly such as angles of leaf wings, bundle cap present/thickness, and vessel numbers, which could be benefit in provenance classification.

2. This study found all 11 provenances had uniformly and regularly vascular bundle arrangement with the ratio of 1:3:1:3:1 for Large:Small:Medium:Small:Large.

3. Like C4 plants, all 11 provenances had Kranz structure surrounding vascular bundles which confirmed high efficient photosynthetic rates.

4. LI provenance has great bundle caps extend to abaxial and adaxial surfaces, which believed that sclerenchyma acts as a short cut of water pathways and strength.

5. Large lysigenous intercellular spaces were found in mature leaves, but very small sizes in developing leaves, which suggested relative gas circulation in roots and encouraged deeply root penetration. Consistently, I found that aerenchyma at root cortex and air cavity at pith were strong evidences of aeration system from leaves to roots. To avoid hypoxia/anoxia, large lysigenous intercellular spaces at laminas were a character of aquatic plants or long term flooding-tolerant plants by transporting O_2 from leaves to roots. Moreover, I guessed atmospheric O_2 could pass into vetiver via a pith cavity at culms by the theories calling “Humidity-induced convection” and “Venturi-induced convection”.

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