Unusual Metaxylem Tracheids in Petioles of Amorphophallus (Araceae) Giant Leaves

ZYGMUNT HEJNOWICZ*
Department of Biophysics and Cell Biology, Silesian University, Jagiellonska 28, Katowice 40-032, Poland

INTRODUCTION

Studies of the morphological and mechanical peculiarities of the petioles of the giant solitary leaves of Amorphophallus titanum and A. gigas (Araceae) (Hejnowicz and Barthlott, 2005) revealed an unusual feature of the petiole xylem, which deserved a separate presentation.

The Amorphophallus petiole is in the form of a cylindrical shell of compact tissue with an aerenchymatous core (Hejnowicz and Barthlott, 2005). In both parts, numerous vascular bundles occur. They are orientated longitudinally but neighbouring bundles are connected by anastomoses. In the core the bundles are located in the parenchymatous strands along edges of big intercellular cavities of honeycomb aerenchyma. No apparent differences in the vascular bundles were noted between the two investigated species.

Vascular bundles are collateral. On a xylem side of the bundle there are several lignified narrow tracheary elements and a characteristic, relatively wide canal surrounded by unlignified, parenchymatous cells. These canals and the narrow tracheary elements are the objects of this study.

MATERIALS AND METHODS

The two petioles of Amorphophallus titanum Becc. and one of A. gigas Teijsm & Binn used in this study were taken from plants growing in a glasshouse in the Botanical Garden of Bonn University (Hejnowicz and Barthlott, 2005). The leaves were accessible for research only when they collapsed due to senescence. Thus no developmental studies were possible. Scanning electron microscopy (SEM) and optical microscopy was the same as in Hejnowicz and Barthlott (2005). To detect lignin, a hand section of fresh tissue was mounted in a large drop of saturated ethanol solution of phloroglucinol to which 20% HCl was added. Additionally, to show lignified secondary walls in semi-thin sections, the sections were stained by PAS and toluidine blue (O’Brien and McCully, 1981) and were inspected by polarizing microscopy. The lignified secondary walls could be recognized by a bluish glaze (unlignified birefrigent walls, e.g. collenchyma, showed a reddish glare). Maceration was performed using a 1 : 1 mixture of acetic acid and hydrogen peroxide (30%) at 100°C for 2–10 h (depending on the degree of required maceration). To clear the hand-made longitudinal sections or macerated material, lactic acid (60%) was used. For autofluorescence of lignin, short blue light was used for excitation in an epi-fluorescence microscope. Means are accompanied by standard errors.

RESULTS

Vascular bundles

The vascular bundles in the shell and the core are qualitatively similar (Fig. 1A and B). In a cross-section of each bundle, there is a characteristic unlignified canal located...
opposite the phloem (Figs 1, 2A and B). Its diameter ranges from 75 to 200 μm (mean = 144 ± 30 μm, n = 39 for A. gigas). The narrowest canals occur in the most peripheral bundles. For the present purpose it is called a ‘xylemic canal’. Between the phloem and the xylemic canal there are one to four typical narrow tracheary elements (NTEs), 8–40 μm in diameter (mean = 27 ± 10 μm, n = 43 for A. gigas), with lignified secondary walls (Fig. 1). The secondary wall in an NTE is mainly in the form of bars (Fig. 2C and D) so that the wall varies from annular to scalariform. The bars are close to each another, which indicates that the elements do not belong to the protoxylem. No doubt, the thickenings of the NTEs were formed after the elongation of the petiole had ceased and therefore the NTEs should be classified as metaxylem elements.

Xylemic canals (wide tracheary elements)

In cross-sections, the canals resemble small cavities that occur at the periphery of the aerenchyma (Hejnowicz and Barthlott, 2005, fig. 30). However, in a living petiole, the xylemic canals are filled with water instead of air. The evidence for water being present in the canals is given by a simple experiment in which one end of a freshly cut segment of petiole is brought into contact with an aqueous solution of methylene blue immediately after it is cut; the stain diffused into the canals. The xylemic canal is surrounded by parenchyma cells (Fig. 2B and C), much narrower than those forming longitudinal diaphragms separating the aerenchyma cavities (Fig. 2A). The length of these parenchymatous cells is variable. It may be >100 μm (Fig. 2C) or as low as 20 μm.

Some xylemic canals seen in cross-sections are divided into two parts, often of different size (Figs 1C, D and 2F), by a seemingly longitudinal cell wall. This wall, in contrast to the canal lateral wall, has birefringent secondary thickenings that contain lignin. Further examination (cross-section series, longitudinal hand-made sections, partially macerated material manipulated under dissecting microscope) showed that the seemingly longitudinal wall in the xylemic canal is in fact a steeply oriented partition between overlapping, tapering ends of two cells. This means that a xylemic canal, when considered three-dimensionally, represents a series of wide tracheary elements (WTEs) with un lignified lateral walls but with lignified joint end walls. Such an interpretation is supported by the observations that, on maceration, the parenchymatous cells surrounding a WTE can be separated from the WTE wall, and that bar-type thickenings may occur locally on the lumen side of the lateral surface of a WTE (Fig. 2G, L and M).

The partitions were examined on WTE files dissected from a partially macerated core material transferred to lactic acid for clearing. The length of partitions is variable; where it could be measured it varied from 5 to 15 mm (mean = 11 ± 3 mm, n = 8). Each partition contains bar-like thickenings that cross the partition (Fig. 2H) called bars. Some partitions also have the thickenings along their border (Fig. 2H). These are called margin thickenings. The partition is a double wall between two adjacent (successive) WTEs. Thus a single thickening is a feature of an individual WTE (Fig. 2H). The bars are oriented obliquely (sometimes transversely) with respect to the WTE axis. When the orientation is oblique, the bars on opposite faces of a partition cross one another (like fissured canals in a pair of bordered pits), which means that the bars have the same chiral configuration in the two contacting cells (Fig. 2H and J). The bars and margin thickenings are a few micrometres wide. The distance between bars is variable, from one bar width to a few times more than
the bar width. The partitions are very variable in terms of the margin thickenings, from complete lack of such thickenings to different combinations of partial margin thickenings in the two contacting WTEs. An individual WTE may have a margin thickening along the partition border on one (e.g. left) side, often only locally, but not on the opposite side (Fig. 2J). Bars often join margin thickenings, but not always. The bars not joining the margin thickenings may extend on the lateral wall beyond the partition. Figure 2I shows a fragment of WTEs with a partition, in which bars of one WTE (these inclined with respect to cell axis, similar to the middle portion of the letter Z) are
connected by margin thickenings on both sides, while those belonging to the next WTE and inclined in an opposite direction (S) extend beyond the partition on the right side. It is interesting that all bars showed the autofluorescence of lignin within the partition but these parts of bars, which extended beyond the partition, did not show the autofluorescence (compare J1 and J2 in Fig. 2).

Examination of whether the partition represents a perforation plate of a vessel led to a negative answer; the primary wall between the bars is always maintained (Fig. 2H), though it is reticulated. This means that the WTE should be classified as tracheids. Since the partitions are very steep and the lignified margin thickenings make them non-stretchable, it is inferred that the WTEs mature after the elongation of the petiole is completed (at a particular level). It is thus concluded that WTEs are metaxylem tracheids.

It was not possible to measure directly the length (L) of WTEs (the distance between two partitions) on fixed material, though probably the task would be possible if the bundles were dissected from turgid material. Unsuccessful attempts to find two successive partitions in WTE files 30 mm long showed that \( L \geq 30 \) mm. However, it was possible to estimate \( L \) indirectly. The partitions were found in 12% of cross-sectioned WTEs (n=433, cross-sections separated by >30 cm along petiole axis). Assuming that the mean length of partitions (\( L_p \)) is 11 mm, \( L \) would be 91 mm (\( L = L_p / 0.12 \)). The WTEs taken as metaxylem tracheids are thus unusual not only because their lateral walls are unligified, but also because of their size, both diameter and length. No doubt they are the largest tracheids now known.

**Narrow tracheary elements**

The WTEs are always accompanied by NTEs (Figs 1 and 2B, arrow and C). The latter are often in direct contact with the WTE (Figs 1B, C and 2C), otherwise an NTE and a WTE are separated by one or two parenchyma cells (Figs 1D and 2B). The direct contact occurs in 85% of bundle cross-sections. This means that each WTE, which is a very long element, is in direct contact with NTEs along most of its length.

At the NTE/WTE contact site the two primary walls of each cells are maintained (Fig. 2C and D). This contact wall is often covered by more deposits than the remaining portions of the NTE wall. It is interesting that at the contact with WTE (at least in those instances in which the contact places could be seen in the SEM) the bars of NTEs were triangular in cross-section, and the tips of triangles were directed towards the partition, as if to minimize the area of the contact, while beyond the contact they were hemispherical and with the base directed towards the wall (Fig. 2D).

In bundles dissected to investigate their longitudinal aspects and inspected after clearing in lactic acid, it could be seen that the NTEs form longitudinal strands, which are continuous along the bundle. The number of NTEs in a strand (at a particular level) varies along the bundle, mostly from one to three. Lateral extensions of the NTE strands occur within some transverse ‘diaphragms’ of the aerenchyma with bundle anastomoses (Hejnowicz and Barthlott, 2005). These extensions connect two longitudinal bundles. However, the transverse branch of NTE is not accompanied by a branch of WTE.

The NTEs are fusiform and their mutual lateral contacts are very long. In fact, the NTEs are too long to be measured in the dissected, longitudinal strands. It was also not possible to obtain complete (undamaged) isolated NTE by maceration. Nevertheless, judging from the frequency of the occurrence of fusiform tips along the bundles, the mean length of NTEs could be estimated as 10–30 mm. The NTEs within transverse ‘diaphragms’, or in the proximity of such lateral extensions, may be relatively short but are also fusiform. No perforation plates were observed in the NTEs, even in the short ones from lateral extensions. Presumably then NTEs are tracheids rather than vessel members, similar to WTEs.

**Protoxylem**

The protoxylem elements were obliterated and could not be recognized in cross-sections. However, in longitudinal optical sections of dissected bundles immersed in lactic acid, they could be recognized as flattened and stretched helical wall thickenings (Fig. 2K). Only in a few cases, could the position of these obliterated protoxylem elements be specified. They occurred in direct contact with the WTE at a site opposite to a group of NTEs. Interestingly, at least in one case of the WTE which was in contact with an obliterated protoxylem element, there were local bar-type thickenings oriented transversely on the inner surface of the WTE (Fig. 2L).

In the case of one WTE, inspected under SEM in a longitudinal section, there was a neighbouring narrow tracheary element that could be recognized when looking from inside the WTE (Fig. 2M, right side). The wall between the WTE and the narrow element contained some material that increased electron reflection so the wall appeared more whitish in SEM. This wall was split longitudinally (at the left edge of the narrow element); the bars of the narrow element were broken at the split and extended slightly beyond the split edge (Fig. 2M, arrowhead). They were less densely arranged than in a typical NTE. There were also local bars on the inner surface of the WTE (two bars are marked by short arrows in Fig. 2M). It was not possible to ascertain the position of this narrow element. Most probably it was on the phloem side of the WTE, i.e. the normal position of NTE. Presumably the considered narrow element matured in the final phase of petiole elongation so that the bars were only slightly displaced one from the other due to the elongation occurring after they were formed. This element could be a transient between the proto- and metaxylem narrow elements.

**DISCUSSION**

**Xylemic canals as elements of super apoplasm**

The lumina of trachery elements are part of the apoplasm, and to single out this part the term ‘super apoplasm’ was coined (Romberger et al., 1993). Super apoplasm denotes...
relatively big apoplastic capillaries surrounded by cell walls in which a fast flow of water is possible, in contrast to microcapillaries within cell walls, where the flow is strongly limited. Two kinds of super apoplasm can be distinguished: lumina of tracheary elements, and water-filled intercellular spaces. The best-known examples of the latter are the carinal canals of *Equisetum* stems (Bierhorst, 1958), intrastelar canals of *Isoëtes* (Romeo et al., 2000) and protoxylem lacunas in monocotyledons (Buchholz, 1921; Canny, 2001). In fact, carinal and intrastelar canals are in contact with proxylem and thus also represent protoxylem lacunas. The flow of water along these lacunas was confirmed by Buchholz (1921) for several monocotyledons, for *Equisetum* by Bierhost (1958), and by Dong et al. (1997) for sugarcane. The structural difference between the two types of super apoplasmic capillaries (tracheary elements versus intercellular spaces) lies in the way in which the capillary is secured against collapse. This is done either by lignin in the secondary wall (tracheary super apoplasm) or by turgor pressure in surrounding parenchyma (intercellular super apoplasm). WTEs are the first example of turgor-supported capillaries, which develop from cell lumina.

**Peculiarities of metaxylem in Amorphophallus**

The extensive comparative study of primary xylem, by Bierhorst and Zamora (1965), does not include *Amorphophallus*. In the anatomical descriptions of the *Amorphophallus titanum* inflorescence, the xylem elements in the inflorescence axis (Boecker, 1998), in flowers (Boecker et al., 1998) and in spathes (Napp-Zinn and Scheferhoff, 1998) are classified as vessel members. The only peculiarity of the wide putative vessel members in the inflorescence axis mentioned is: ‘mit dünnen, spanangenartigen leiterformig angeordneten Wandverdickungen ... oft unvollständig ausgebildet’ (Boecker, 1998, p. 50). Araceae are considered as having vessels in roots and tracheids in shoots (Watson and Dallwitz, 1992; Keating, 2003). It should be, however, be mentioned that in *Arisaema*, all the xylem, both in petioles (which are also long) and in roots, is composed of tracheids (Ko et al., 1990).

The peculiarities of the WTEs in *Amorphophallus* petiole are: (1) in their huge dimensions; (2) in their un lignified lateral walls; (3) they are surrounding by turgid parenchyma cells providing the ‘pipe wall’; (4) they have lignified end walls; (5) they have contact with a strand of narrow metaxylem tracheids.

(1) The great length of the WTE is surely achieved by symplastic (Erickson, 1986) elongation of the metaxylem cells before they mature. As it is known, metaxylem cells start to differentiate and become morphologically distinguishable very early. However, the maturation process is slow. In *Libocedrus* root, metaxylem cells become detectable about 100 μm from root initials but remain immature structurally and functionally until they are displaced as far as 15 mm from the root tip (Wilcox, 1962). In the root, the elongation zone is relatively short; therefore, despite the delayed maturation, the metaxylem elements do not attain considerable length. However, if they occur in organs with long-lasting intercalary elongation, such as internodes or petioles of some plants, their length would increase proportionally to the final length of the organ, and might attain a value so high that it would become impossible to measure. This is probably the reason why records of the length of metaxylem elements are not listed (as far as the author is aware). Maybe, the WTEs in *Amorphophallus* petioles are the champions.

The WTEs are not only very long but are also very wide. Similarity of the xylemic canals to the aerenchyma cavities indicates that the growth patterns leading to the large transverse dimensions of WTE on one hand, and to the air cavities on the other hand, may be similar. Presumably, the growing WTE fills the space between parenchymatous cells that otherwise would represent an intercellular space. Probably, when a large cell is surrounded by much smaller cells, the larger size of this cell is not so much the matter of its excessive growth, as of such a pattern of growth of the whole tissue that the larger cell can fill the free space available between the small cells. Such a pattern of growth occurs in ovules in which the relatively big embryo sac develops at a site of ‘pure’ tension in the centre of the nucellar tissue in the ovule (Lintilhac and Jensen, 1974). Similarly, during the development of wide vessels of the ring porous wood, there is a tensile tissue stress, both in the radial and tangential directions in the cambium zone (Hejnowicz, 1980). If transverse growth of wide vessels of ring porous wood is similar to that of the WTEs in the *Amorphophallus* petiole, the concept that vessel members in wood grow intrusively along longitudinal edges between surrounding cells (Romberger et al., 1993) should be reexamined.

(2) Typical metaxylem tracheary elements have a lignified secondary wall (in the form of either bars or a layer with pits). As far as the author is aware, the WTEs in *Amorphophallus* petioles are the first example of mature tracheary elements, which function in water transport without lignified lateral walls. Why then does strengthening by secondary wall and lignification occurs in other tracheary elements? It seems to be primarily an adaptation to resist the effects of the negative pressure in water filling the elements during fast transpiration. Such the pressure may result in a collapse of the wall and/or the embolism—the separation of water from the wall (Kramer and Boyer, 1995). Lignification provides stiffness without decreasing the adhesion of water to the wall (in contrast to suberization). Though lignin is often considered to be a hydrophobic substance, it is partly hydrophilic; when reabsorbed on cellulose fibres it retains the wetability of the fibre surface (Lee and Luner, 1972).

(3) The lack of lignified secondary lateral wall in WTE shifts the function of ‘pipe walls’ to the turgid parenchyma, in which the WTE is embedded. This indicates that parenchyma can assure sufficient stability to the ‘pipe’ functioning in the petiole in its normal environment.

(4) The occurrence of the lignified partitions of WTE in which the primary wall is maintained surely acts against a flow. One may thus ask why are they not perforated? Probably, the single long WTE in an *Amorphophallus* petiole may be considered to be an analogue of a single xylem vessel composed of many vessel members. Then the
partitions would correspond to the non-perforated end walls of the terminal vessel members. The end walls of a vessel limit the extension of gas if the latter appears in the vessel due to negative pressure (in xylem sap). Presumably, the end walls of the WTE are developed to limit gas embolism in the WTE file. To fulfill this function they must be stiff by themselves in contrast to the lateral walls which are stiffened by neighbouring turgid parenchyma cells. The Amorphophallus petioles may be interesting from the view point of the cohesion-tension mechanism (Wei et al., 1999; Steudle, 2001).

(5) The analogy between the WTE and intercellular super apoplasm of the type of protoxylem lacunae, is strengthened by the occurrence of narrow lignified tracheary elements in contact with the wide apoplasmic canals. This is protoxylem in the case of lacunae, and NTEs in case of WTEs. Probably the NTEs, which are stiff by themselves, assure refilling of a bigger capillary if a gas cavity is formed.

ACKNOWLEDGEMENTS
I thank Professor A. Sievers (Bonn) for friendly support, Professor W. Barthlott (Bonn) for the help in collecting materials, Mr H.-J. Ensikat (Bonn), Mrs Ewa Kolano and Mr Jerzy Karczewski (Katowice) for technical assistance, and Dr Dorota Kwiatkowska (Wroclaw) for her comments on the manuscript. I thank anonymous reviewers for their helpful criticisms.

LITERATURE CITED