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Research paper

How do newly matured vessels start conducting water? The significance of lateral pathways for connecting newly matured vessels to the transpiration stream

Youcef Halis [1,4], Mohammed L. Benhaddya[1], Hocine Bensaha[2] and Mohamed M. Senoussi[3]

[1]Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biophysical Station, 3240 Nezla, Touggourt, Algeria; [2]Unité de Recherche Appliquée en Énergies Renouvelables, URAER, Centre de Développement des Énergies Renouvelables, CDER, 47133, Ghardaïa, Algeria; [3]Laboratory of Biomolecules and Plant Amelioration, Larbi Benmhidi University of Oum El Bouaghi, Constantine Road, 04000, Algeria; [4]Corresponding author (youcefhal@gmail.com) orcid.org/0000-0002-5521-2145

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Despite the long history of research on xylem structure and function, there are no reports in the literature explaining how xylem vessel elements began conducting water just after their maturation. This study was conducted to demonstrate the anatomical arrangement of newly matured vessels, looking specifically for the first pathways connecting newly matured vessels to the transpiration stream. Using the developing stems of *Paraserianthes lophantha* (Willd.) I.C.Nielsen as the experimental system, the course of vessel differentiation and maturation along the developing bundles was followed by using the dye-pressure method. Water pathways from newly matured vessels to other functioning vessels were directly visualized by the technique of single-vessel dye injection. Some isolated newly matured vessels from the transpiration stream were detected using two apoplastic tracers. The results of this study converge to support the hypothesis that the movement of water in the newly matured vessels depends completely on lateral contacts with other functioning vessels via vessel-to-vessel paths or vessel relays. In cases where the lateral pathways were absent, the flow within the newly matured vessels was substantially blocked resulting in a significant hydraulic isolation of the newly matured vessels. These results might contribute to a better understanding of the pattern of water movement within the developing xylem systems, and underscore that xylem vessels start conducting water through lateral transport, although their primary function is the axial transport.

Keywords: immature living vessels, intervessel contacts, lateral flow, newly matured vessels, vessel relays, xylem development.

Introduction

The main and primary function of xylem vessels in angiosperms is the rapid long-distance transport of water and essential materials (Bailey 1953, Carlquist 1975, 1988, Tyree and Ewers 1991, Tyree and Zimmermann 2002, Sperry 2003). Water moves in the xylem vessels predominantly in the axial direction, following the pathway of least resistance. There is general agreement that the axial conductance for water flow is much greater and faster compared with the lateral conductance (Comstock and Sperry 2000, van Ieperen et al. 2000, Nijsse et al. 2001, Tyree and Zimmermann 2002).

However, newly matured vessel elements may represent an exception to this dominant pattern, as water in these elements might be transported almost entirely in the lateral direction. This is because the pathway of axial flow in the newly matured vessels is completely blocked by the existence of immature living vessel elements at their distal ends. Living vessel elements with cross-walls and cytoplasm are completely occluded, and consequently, they substantially block the apoplastic flow of water. In a related paper, Halis et al. (2012) demonstrated the presence and extent of immature living vessel elements and their influences on the patterns of water flow within the developing xylem bundles of the current-year shoots of grapevine. By using staining and hydraulic techniques, they suggested that lateral pathways contribute largely to water conduction within the developing vessel network. Although Halis et al. (2012) have

proposed a simplified model of the hydraulic architecture of the developing stem bundles, these authors did not provide direct evidences for the pattern of water movement within and from the newly matured vessels.

In the present study, the findings of Halis et al. (2012) were extended by investigating the hydraulic properties of newly matured vessel elements, focusing especially on how these elements start conducting water. Our working hypothesis was that the movement of water in the newly matured vessel elements depends completely on lateral contacts with other functioning vessels. To confirm this, we used developing stems of *Paraserianthes lophantha* to (i) examine the course of vessel differentiation and maturation along the developing bundles; and (ii) determine how newly matured vessels contribute to the total hydraulic conductance, by following the water flow pathways from the newly matured vessels to the rest of open xylem network. Furthermore, it is possible that some newly matured vessels remain unconnected, for a certain time, to the transpiration stream if they have no functional connections with open xylem network. To test this, we examined the functional connections of the newly matured vessels to the transpiration stream based on the movement of two apoplastic tracers.

Materials and methods

Plant material

Stem samples were collected from the Fabaceae species (*Paraserianthes lophantha* (Willd.) I.C.Nielsen) during the growing season (May 2017) from 3-year-old trees that were growing in the experimental station, CRSTRA-Touggourt, Algeria. Current-year shoots, with about 20 internodes and length ranging from 86 to 113 cm, were cut in the morning, placed in black plastic bags and brought to the laboratory where they were analyzed the same day. The fast-growing stems of *P. lophantha* were selected for this study because they have relatively wide vessels that develop within distinct layers along the developing xylem bundles. Samples tested in this study were obtained from five different plants.

Pattern of vessel differentiation and maturation

The dye-pressure method (Halis et al. 2012) was used to follow the course of vessel differentiation and maturation along the developing bundles of the stem axis. An apoplastic dye was forced through the stem segments to trace the dead xylem network in order to distinguish between dead and living vessel elements. This was because apoplastic dyes cannot enter living cells, as they cannot cross the intact cell membranes (Chaves et al. 2002), whereas apoplastic dyes are able to pass freely via open vessels and through pit membranes (Shane et al. 2000). Developing stems of 20 internodes were defoliated and cut underwater into segments of 5 cm length. These segments were kept underwater in their original sequence. The cut basal end of each segment was re-cut underwater and immediately attached to a plastic tube that connected to a reservoir filled with a filtered (0.2 μm) solution of toluidine blue O (0.1% w/v in deionized water). The reservoir was connected to a tube coming from the air compressor with a manometer to regulate the pressure (Sperry 1993, Vogt 2001). The dye solution was pumped into the segment base for 30 s at a pressure of 120 kPa. Preliminary experiments indicated that 30 s was a sufficient time for dye to stain all the functional pathways. High pressure was used to remove any air emboli initially present in vessels, thus ensuring that the stain would be distributed over the whole network of open dead vessels (Sperry et al. 1988). After that, each segment was cross-sectioned every 1 cm starting from the apical towards the basal end. Sections were examined and digitally imaged with Motic Digital Microscope (DMB1–2 MP, Motic Instruments Inc., Xiamen, China). Clear xylem bundles were selected and followed in the successive sections, and the number of stained vessels within the selected bundles was counted. It should be noted that the number of samples tested in this experiment was 10 developing stems (two stems from each sampled tree).

Tracing water flow paths from newly matured vessels to other functioning vessels

The patterns of water movement from the newly matured vessels to the rest of open xylem network were investigated using the microcapillary technique (also known as single-vessel dye injections) (Zwieniecki et al. 2001, Brodersen et al. 2013, Pratt et al. 2015, Venturas et al. 2016). Stem segments of 15 cm were cut at different distances from the shoot apex. At the basal end of each segment and using a stereomicroscope and a glass microcapillary, the dye solution was injected into the open lumen of a newly matured vessel located at the second layer of vessels from the cambium (Figure 1). As observed by Halis et al. (2012) on grapevine and confirmed here on stems of *P. lophantha* during the application of dye-pressure method, the vessels of the first layer adjacent to the cambium were still alive and closed for the free movement, while the vessels of the second layer were mostly newly matured vessels with distal ends closed by immature living elements. Thus, any particles that injected into the vessels of the second layer will flow freely within these newly matured vessels until reaching the distal closed portion (Figure 1).

The glass microcapillary was prepared by pulling capillary tubes to a fine point using a pipet puller (Model 700 C, David Kopf Instruments, Tujunga, CA, USA). Because the vessel diameters of the tested species ranged between 40 and 60 μm (measured by the image-analysis software ImageJ, NIH, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/), the glass tip was subsequently broken to achieve a tip diameter of less than 50 μm. The microcapillary tip was inserted into the open lumen of a newly matured vessel as explained above. Fast-setting, water-insoluble epoxy glue (Kafuter Epoxy AB Glue Adhesive, Guangdong Hhngda New Materials Technology Co., Ltd, Guangdong, China) was used to fix and stabilize the capillary tip,

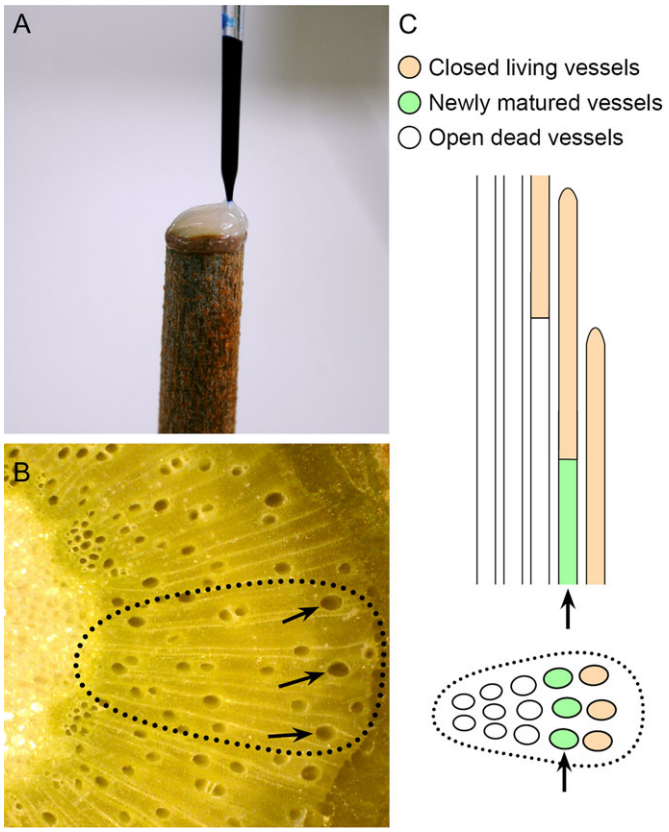

Figure 1. Utilization of the microcapillary technique to follow the water pathway from the newly matured vessels to the rest of open xylem network. (A) The glass microcapillary was inserted into the open lumen of a newly matured vessel and fixed by epoxy glue. (B) The newly matured vessels (black arrows) at the cut surface of the proximal end of the stem segment. (C) Diagrammatic cross and longitudinal sections of a vascular bundle, only the secondary vessels are drawn for the sake of simplicity. The diagram is not drawn to scale. Black arrows indicate the newly matured vessels where the microcapillary was inserted.

and also to seal the entire proximal end (Figure 1). The microcapillary tube was attached to a plastic tubing (0.5 mm inner diameter, Clay Adams, Parsippany, NJ, USA) that connected to a reservoir of toluidine blue solution. By using a compressed air-tank with pressure gauge and regulator, the dye solution was pushed through the selected vessel, at a pressure of <30 kPa, until the dye exited the distal end of segment. When the dye did not exit from the distal end, the pressure was stopped after 2 min. The stem segment was then cross-sectioned every 5 mm starting from the dye injection site (proximal end). Slides were viewed under optical microscope, and the pathway of dye was then followed. Motic Digital Microscope was used to examine slides and to capture photomicrographic images. In cases where the dye failed to move within the selected newly matured vessel, the tested samples were excluded. In the present investigation, the number of successful samples where the dye clearly moved within the selected vessels was 15 stem segments.

Functional connections between newly matured vessels and the transpiration stream

The principle of this test was to apply a tension force only to the open xylem network and see whether such tension could be transferred to the newly matured vessels through intervessel pit pathways. The test was conducted on stem segments containing one layer of newly matured vessels in their developing vascular bundles. These segments were prepared as follows: defoliated stem segments consisting of 10 internodes were collected from positions starting between the 5th and the 16th internode, counted from the shoot apex. Each segment was shortened by successive cuttings of 1 cm from both distal and proximal ends. With each incision, newly cut ends were cross-sectioned and examined under light microscope. At the distal part, incisions were made until the first appearance of the fourth layer of secondary vessels in the outermost region of the vascular bundles. While at the proximal part, incisions were made until a point where the xylem bundles contained five distinct layers of secondary vessels (Figure 2). At the proximal end of these prepared segments, the outer layer adjacent to the cambium (fifth layer from the pith) contained the closed living vessels. The second layer from the cambium (fourth layer from the pith) contained the newly matured vessels, and of course, the distal ends of these newly matured vessels were closed by the immature living conduits (the fourth layer of vessels closer to cambium at the distal end). The remaining vessels of the first, second and third layers from the pith were mature and open along the stem segment. Thus, when a negative pressure was applied at the distal end, the tension force will extend only along the inner layers of open-dead vessels (layers 1–3), while the newly matured vessels of the fourth layer will receive the tension force only through the lateral connections (see Figure 2).

To test for the existence of functional connections between newly matured vessels and other open vessels, the distal end of the prepared segment was connected to the suction tube of a vacuum pump with vacuum gauge and regulator (KNF Neuberger, Freiburg, Germany). The basal end was immersed in safranin-O dye (1% w/v aqueous solution) and the suction force of −80 kPa was applied for 30 s (Figure 2). If the newly matured vessels were laterally connected to the open dead vessels, they would be stained red with safranin. If any newly matured vessels were not connected to open xylem, they would remain unstained. However, unstained vessels could be just closed living conduits. So, to avoid this confusion, a second dye (toluidine blue O) was used to distinguish between the closed living vessels and the open newly matured vessels (Figure 2).

A small segment of 3 cm was cut from the basal part of the tested segment (Figure 2). Within this small segment, the distal closed part (living vessels) of the fourth layer of vessels was assumed to be removed. So the newly matured vessels were open along this small segment. The distal end of the small

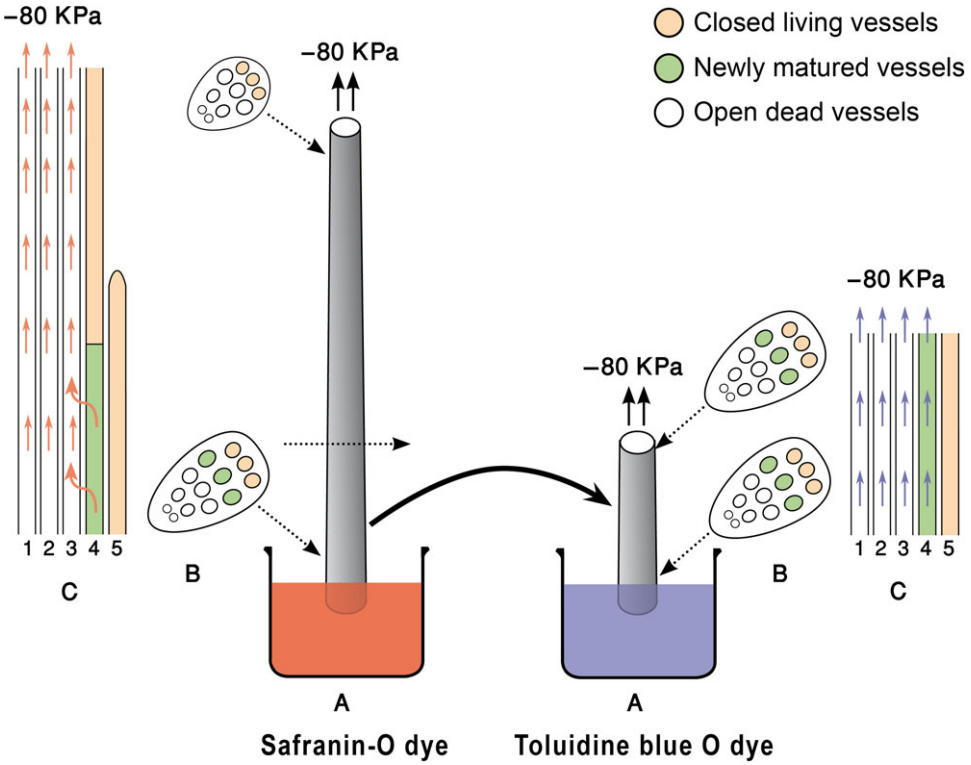

Figure 2. Schematic diagram showing how to evaluate the functional connections between newly matured vessels and other open vessels. (A) The proximal end of the stem segment was immersed in the dye solution while the tension force was applied to the distal end. (B) Diagrammatic cross-sections of a vascular bundle at both ends of the tested segment. (C) Diagrammatic longitudinal section of a vascular bundle (numbers denote the consecutive vessel layers from the pith). Diagrams are not drawn to scale.

segment was connected to the suction tube while the basal end was immersed in toluidine blue O dye. The suction force was applied for only a few seconds at a pressure of −80 kPa (Figure 2). A free-hand cross-section was taken at the middle of the small segment to check for the presence of dyes. All the newly matured vessels that were laterally connected to the open dead vessels would be stained by the two dyes and would appear red-brown in cross-sections. The newly matured vessels that were still unconnected to open xylem would be stained only by the toluidine blue O and would appear blue in cross-sections. The living vessels closer to the cambium would remain unstained. The number of samples investigated in this experiment was 15 stem segments.

Results

Pattern of vessel differentiation and maturation

The growing stems of *P. lophantha* were examined at different heights and the progress of maturation of secondary vessels in the developing bundles was followed from the top to the base. When a high pressure was applied on the small segments, the apoplastic dye was spread throughout the network of open, dead vessels, and only immature living vessels remained unstained in the cross-sections. At a distance close to the shoot apical meristem, only a few open-dead vessels (stained vessels) were observed in the inner part of the xylem bundles (Figure 3), whereas the outermost part closer to the cambium contained one layer of unstained living vessels. The number of stained open vessels increased gradually with increasing distances from the shoot apex. However, the unstained living vessels continued to appear as one outermost layer along the stem axis (Figure 3). New layers of secondary vessels were sequentially formed along the developing bundles. One explanation for this staining pattern was that, along the developing xylem bundles, the distal portion of each secondary vessel is alive and closed for the free movement of water. This could be summarized in a schematic diagram as presented in Figure 3. Another explanation could be that the whole secondary vessel closest to the cambium was still alive and not connected. When several vessels were connected in a file, the vessels closest to the basal area were mature but the vessels closest to the distal part of the branch were still alive.

Tracing water flow paths from newly matured vessels to other functioning vessels

Using the microcapillary technique, the apoplastic dye was injected in the lumen of a newly matured vessel and followed by microscopy to determine the patterns of water movement within and from the newly matured vessels. Results demonstrated that, in all cases, the dye moved axially within the newly matured vessels for a limited distance then flowed laterally to adjacent

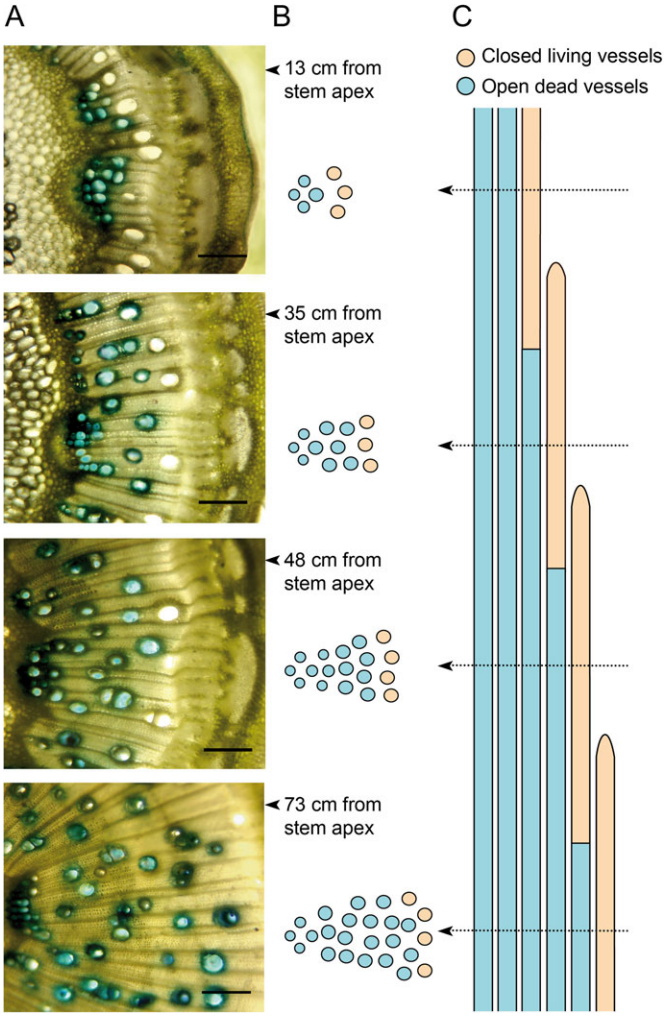

Figure 3. (A) Microphotographs showing the arrangement of mature dead vessels (stained vessels) and immature living vessels (unstained vessels) within the developing xylem bundles at different locations along the shoot axis. Scale bars = 400 μm. (B) Diagrammatic cross-sections of developing xylem bundle as observed in A. (C) Diagrammatic longitudinal section showing the pattern of differentiation and maturation of secondary vessels along the developing xylem bundle. Diagrams are not drawn to scale.

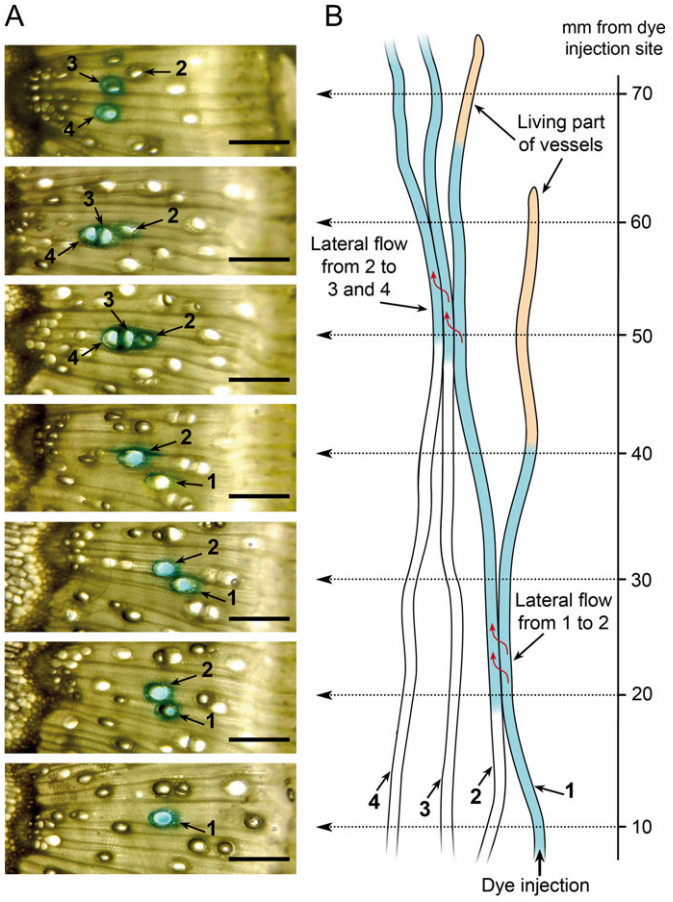

Figure 4. Showing the simple vessel-to-vessel pathway from the newly matured vessel to the adjacent vessels. This pathway was traced by the technique of single-vessel dye injection. (A) Successive cross-sections from the point of injection to 70 mm above. The dye appears firstly in the newly matured vessel then it moves laterally to the adjacent vessels via simple vessel-to-vessel contacts. Scale bars = 400 μm. (B) Reconstruction of the successive cross-sections to show the arrangement of xylem vessels and the pathway of the injected dye (light blue). Vessels are numbered from the beginning to the end of dye pathway (from the site of dye injection to the last staining vessel). The horizontal axis of this diagram is not drawn to scale.

vessels through intervessel pit pathways. The direct movement within a single vessel was not observed, indicating that all vessels tested by this technique were closed at their distal ends by immature living vessels. The simple vessel-to-vessel contact was the dominant pathway connecting newly matured vessels to the open xylem network, because this simple pathway was observed in 73.3% of the 15 stem samples tested in this experiment (11 cases of the 15 samples). Figure 4 shows a typical pattern of water flow from the newly matured vessels to other open vessels through the simple vessel-to-vessel contacts. In this case, the dye moved through the newly matured vessel (Figure 4, vessel No. 1) until it reach a zone where the newly matured vessel was in direct contact with a neighboring vessel (20 mm from dye injection site). At this location, dye moved laterally to the adjacent vessel (Figure 4, vessel No. 2) where it could spread axially within the vessel lumen. After a short distance (40 mm from injection site), no dye was observed in the newly matured vessel (Figure 4, vessel No. 1), indicating that the dye had reached the living part of vessel No. 1. Further away from this location, vessel No. 2 was in contact with two other vessels, where the dye was transmitted laterally towards these vessels (Figure 4, vessels No. 3 and 4). Similarly, the dye had disappeared from vessel No. 2 at a few distances, which indicated that vessel No. 2 was still alive at this location. However, a possibility that cannot be ruled out is that a vessel end wall could occur at the distal living part of vessels. For example, it is possible that at about 40 mm from the injection site in Figure 4, vessel No. 1 had an end wall and it was connected to another vessel that had not matured yet. However, this could not be confirmed as cross-sections were performed every 5 mm.

In a few samples, lateral movement from the newly matured vessels to other open ones was observed to occur through the so-called vessel relays (Figure 5). This type of lateral pathway was observed only in 26.6% of the tested samples (4 cases of the 15 samples). Lateral pathways through vessel relays differed somewhat from those via a simple vessel-to-vessel contacts. In contrast to the direct movement through vessel-to-vessel contacts, the dye solution, by way of vessel relays, could travel between the separated large-diameter vessels through a radial chain of small-diameter vessel elements. At a short distance from the dye injection site, the blue coloration was observed only in the newly matured vessel where the dye was firstly injected (Figure 5, vessel No. 1). At a location above, the stain was also observed in a series of interconnected small vessels that form a continuum from the newly matured vessel to the neighboring vessel (Figure 5, vessel No. 2). At few distances above, the dye continued to move axially within the vessel No. 2, while it gradually disappeared from vessel No. 1, meaning that the dye had reached the living portion of this vessel.

Functional connections between newly matured vessels and the transpiration stream

Two apoplastic tracers were used in this experiment. When the first tracer (safranin-O dye) was sucked into the segment at high negative pressure via the open vessels at the distal end, the red color was observed in most of the newly matured vessels at the basal end (red-brown vessels in Figure 6). This indicated that the tension force that occurred in the open xylem network was transmitted to the newly matured vessels via lateral pathways. However, some newly matured vessels were observed to be isolated from the open xylem network. These vessels were stained only by the second dye (toluidine blue) when the distal portion of the segments were removed, i.e., when the closed living part of these vessels was removed (Figure 6). The absence of the first stain (red color) in these elements indicated that the tension force did not reach the isolated vessels due to the absence of lateral contacts with the rest of the open vessel network.

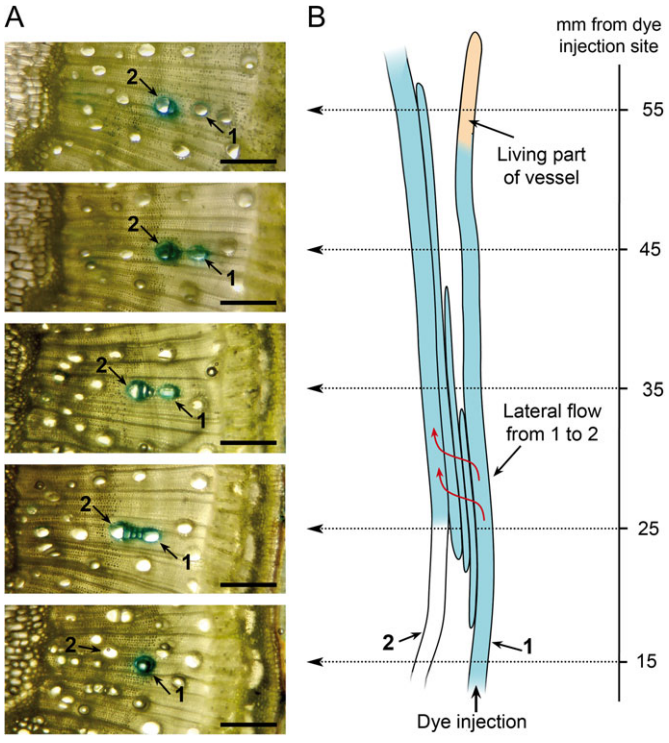

Figure 5. Showing the movement of dye from the newly matured vessel to an adjacent vessel through the pathway of xylem vessel relays. This pathway was traced by the technique of single-vessel dye injection. (A) Successive cross-sections from the point of injection to 55 mm above. The dye appears firstly in the newly matured vessel then it moves laterally to adjacent vessel through vessel relays. Scale bars = 400 μm. (B) Reconstruction of the successive cross-sections to show the arrangement of xylem vessels and the pathway of the injected dye (light blue). Vessels are numbered from the beginning to the end of dye pathway. The horizontal axis of this diagram is not drawn to scale.

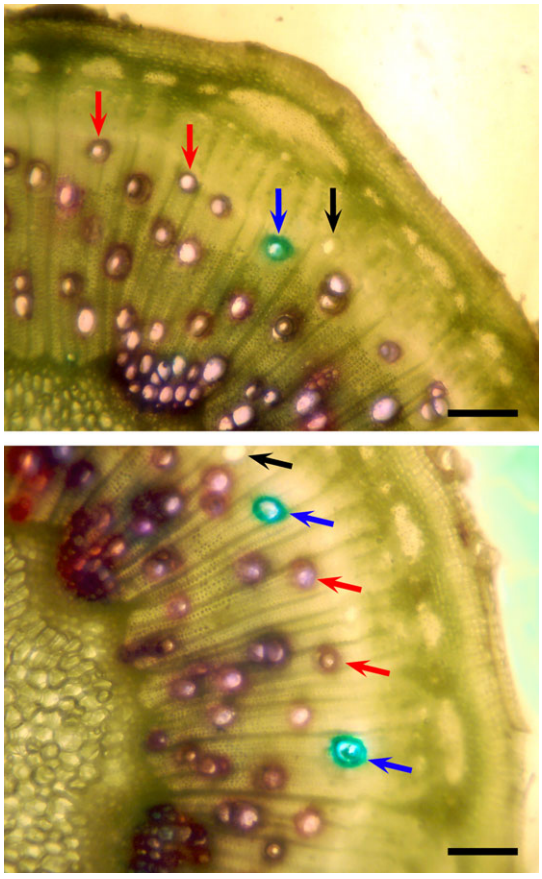

Figure 6. Functional connections between newly matured vessels and the transpiration stream based on the staining pattern by using two apoplastic tracers. The microphotographs show that most of newly matured vessels were laterally connected to other open vessels (red arrows). Some newly matured vessels were isolated from the transpiration stream (blue arrows). The black arrows denote the immature living vessels. Scale bars = 400 μm.

Discussion

Consistent with the observations on grapevine stems (Halis et al. 2012, Jacobsen et al. 2018), secondary vessels of the young growing stems of *P. lophantha* followed a regular pattern of differentiation and maturation. Secondary vessels developed gradually within successive layers along the developing xylem bundles. Each secondary vessel element did not immediately mature after its differentiation, but rather, it remained alive with an intact and functional cytoplasm and plasma membrane for a certain time period. Consequently, the closed, living vessel elements would be present continuously in the developing vascular bundles as long as the vascular cambium is active. The gradual pattern of xylem development might be an important issue of plant hydraulic function. Jacobsen et al. (2018) demonstrated that the gradual development and maturation of vessel elements and the differences in the timing of vessel transitions to functionality and post-functionality might represent a possible mechanism for xylem to respond to changing conditions and hydraulic requirements.

The gradual development of the xylem network and the presence of living vessel elements and their position in relation to the water pathway (Figure 3) suggested that the axial pathway of water within the newly matured vessels was completely blocked. The contribution of newly matured vessels to the total hydraulic conductance therefore required that water moved laterally from these elements to the open xylem network. Remarkably, this was clearly observed in the single-vessel dye injection experiments. The apoplastic dye that was injected into newly matured vessels followed almost the same pattern of movement. The dye moved laterally from newly matured vessels to neighboring open vessels to bypass the closed, living vessels. In this study, different types of lateral pathways were observed. The dominant type was the simple vessel-to-vessel contacts. The direct contacts between vessels result from the tangential and radial deviations in the course of xylem vessels (Kitin et al. 2004, Halis et al. 2014). It is known that vessels might arrange in a zigzagging manner, helical pathway or even might twist around one another (Burggraaf 1972, Chaney and Kozlowski 1977, Fujii 1993, Fujii et al. 2001, Tyree and Zimmermann 2002). At the site of intervessel contacts, water has to cross pit membranes to pass from one vessel element to another, and thus, intervessel pit membranes might play a major determining role not only for the transport and distribution of materials, but also as safety valves for the control and regulation of movement of water, gas and particles (Crombie et al. 1985, Tyree and Sperry 1989, Tyree and Ewers 1991, Kitin et al. 2004, 2009, Choat et al. 2006, Taneda and Tateno 2007, Lens et al. 2013).

In addition to the direct intervessel connections, the vessel relays represent another type of lateral pathway. Vessel relays were observed in many cases in the present investigation, which confirmed the importance of these elements in providing functional connectors between spatially discrete vessels. The term xylem vessel relays was firstly mentioned by Brodersen et al. (2013) in their study on grapevine stems. These authors defined the vessel relays as radial chains of short, narrow diameter vessel elements connecting the larger diameter vessels. They demonstrated that the presence of vessel relays increases xylem network connectivity and limits the isolation of large-diameter vessels. They also suggested that because of the differences in vessel relays between species, these anatomical features could contribute to disease and embolism resistance in some species (Brodersen et al. 2013). In fact, xylem relays have been previously reported by Carlquist (1984) as grouping of vessels in radial multiples. He showed that these multiples could be regarded as a way of providing alternate conduits whereby water can be carried in case one or several vessels in a group are incapacitated.

It is important to note that in some cases of the single-vessel dye injections, the dye solution failed to advance through the selected newly matured vessels. The dye was observed only near the dye injection site (data not shown), signifying that the apoplastic pathway was blocked. The most likely explanation is that the tested vessels were still alive and nonconducting. We have considered these samples as failed tests because the microcapillary was wrongly inserted into an immature living vessel. In fact, it was a challenging issue to confirm whether the selected vessel was a newly matured vessel or not. This could be confirmed only after checking the pattern of dye movement at the end of the test.

The use of two apoplastic tracers allowed us to determine the functional connectivity between newly matured vessels and the rest of the open vessel network. Although most newly matured vessels were shown to be connected to the transpiration stream, some newly matured vessels could remain isolated. Our results showed that the apoplastic dye moved within some newly matured vessels only when removing the closed living part at the distal end. This could be explained by the fact that some vessels had matured and lost their end walls and cell contents and formed hollow tubes, but they did not contribute to overall water transport because they were still isolated from the transpiration pull. Of course, this isolation could be attributed to the absence of lateral contacts at this site of the xylem network. In light of these observations, it might be suggested that any water and particles found in the newly matured vessels did not move upward unless there were lateral pathways connecting the newly matured vessels to the open xylem network. In cases where the lateral pathways were absent or have not yet developed, the flow within the newly matured vessels was substantially blocked. Thus, the solution within the isolated newly matured vessels might remain unmixed with the general sap. However, it must be mentioned that the absence of lateral contacts does not mean that newly matured vessels were permanently isolated. Isolation

might occur only for a certain amount of time until the distal living vessels become functional. In other words, newly matured vessels that were identified as nonfunctional and still isolated to lateral flow were only temporarily isolated until vessels to which they were connected in a file become functional. Additionally, lateral contacts could exist below the newly matured vessels meaning that these elements would receive the tension from the bottom, and thus solutions in this special case might move towards the bottom. If this is happening, it is therefore surprising that at a certain place at a certain moment within the developing xylem network, sap can move from top to bottom, in the opposite direction to the conventional sap flow.

Our results raised two important questions. (i) Does the sap solution in the newly matured vessels differ from that in the open functional vessels? (ii) Do substances coming from the newly matured vessels affect the chemical properties of sap in the open xylem network? One can expect that the remnants of protoplasm and vacuole contents including hydrolytic enzymes would be the main components occurring in the newly matured vessels. It is well known that at the final stages of maturation of xylem conduits, the large central vacuole collapse causes hydrolytic enzymes to invade the cytoplasm and attack various organelles, resulting in the degradation of cell contents and parts of the cell walls (Fukuda 2000, Déjardin et al. 2010, Bollhöner et al. 2012). In the developing xylem of grapevine roots, Mapfumo et al. (1993) have stated that some newly matured vessels contained remnants of degenerated protoplasm, which would impede water flow. Schenk et al. (2017) have speculated that after cell death during conduit development, some insoluble lipid surfactants, including phospholipids and proteins, could remain in xylem sap and in pores of intervessel pit membranes and deposit on vessel wall surfaces. Based on this, it is possible to suppose that within the developing xylem network, sap composition could differ between newly matured vessels and the rest of open xylem network.

On the other hand, the first materials leaving newly matured vessels toward the open network could be the reason why the xylem sap contains certain kind of macro-molecules such as enzymes, proteins and carbohydrates (Wilson 1923, Biles and Abeles 1991, Satoh et al. 1992). The effect of these macromolecules remains an interesting question and needs further investigation. Some authors believed that the presence of macromolecules could have physical inhibitory effects on xylem water transport especially when these molecules accumulate at pit membranes (Neumann et al. 2010). In contrast, other authors argued that the presence of macro-molecules in the xylem sap does not necessarily have a negative impact on the xylem function. Schenk et al. (2017) demonstrated that the existence of lipid-based surfactants in xylem sap and in pit membranes may contribute to stabilize nanobubbles under negative pressure, hence avoiding embolism formation. Several studies demonstrated the presence of a specific set of proteins in the xylem sap and their important role for maintaining xylem function and for plant responses to pathogens and other stresses (Buhtz et al. 2004, Dafoe and Constabel 2009, Krishnan et al. 2011, Ligat et al. 2011).

Based on the current results, it is quite clear that the contribution of newly matured vessels in water transport depends on the degree of lateral connections between the newly matured vessels and the open vessel network. Within the same developing xylem bundle, the newly matured vessels differed in their ability to transport water as a result of the differences in the presence and extent of intervessel pitting. Some newly matured vessels were fully connected to the open xylem network, while others were still isolated and presumably remained filled with breakdown products. Gradually with the maturation of the distal parts, lateral contacts would be formed allowing the breakdown products to move from the newly matured vessels to the rest of open xylem network. Thus, the beginning of conducting water by the newly matured vessels could affect the chemical and hydraulic of the sap in the rest of open xylem vessels.

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Conflict of interest

None declared.

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