Trees (2012) 26:705–714
DOI 10.1007/s00468-011-0637-8

ORIGINAL PAPER

Vessel development and the importance of lateral flow in water transport within developing bundles of current-year shoots of grapevine (*Vitis vinifera* L.)

Youcef Halis · Samah Djehichi · Mohamed Mourad Senoussi

Received: 8 May 2011 / Revised: 11 September 2011 / Accepted: 27 September 2011 / Published online: 13 October 2011

Abstract In the developing xylem bundles of young stems, the presence of immature living vessel elements can strongly restrict or even block axial hydraulic conductance, especially in newly matured vessels. Lateral connections between vessels may provide an alternative pathway for water movement to bypass these closed, living elements. Using the grapevine as a model system, the present study aimed to demonstrate the effects of living vessel elements on water movement patterns, and the importance of lateral flow for effective water conductivity in the developing bundles. Living vessel elements were detected using dye staining and the pattern of vessel development and maturation was then monitored. The importance of lateral flow was confirmed using several approaches: (1) capacity for lateral flow, (2) effect of increasing the distance of water transport, and (3) effect of ion concentrations. Living vessel elements were found along the developing bundles, they occupied a significant proportion of the distal and peripheral parts of the flow path, forming a substantial barrier to apoplastic water flow. Water in the developing xylem bundles could move easily from vessel to vessel and between secondary and primary xylem. Furthermore, data from increasing the transport length and altering the ion concentrations supported the critical contribution of the lateral flow to the total hydraulic conductance within the developing bundles. The hydraulic architecture of the developing xylem bundles is described. The results are discussed in terms of reliability and efficiency of water transport during shoot growth and development.

Keywords Axial flow · Developing bundles · Intervessel contacts · Grapevine · Lateral flow · Lateral pathways · Living vessel elements

Communicated by S. Mayr.

Y. Halis · S. Djehichi · M. M. Senoussi
Laboratory of Biomolecules and Plant Amelioration, Larbi Benmhidi University of Oum El Bouaghi, BP 358, Constantine Road, 04000 Oum El Bouaghi, Algeria
e-mail: samahdjh@gmail.com

M. M. Senoussi
e-mail: senoussi1@yahoo.fr

Y. Halis (✉)
Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biophysical Station, 3240 Nezla, Touggourt, Algeria
e-mail: youcefhal@gmail.com

Introduction

Vessels in most dicotyledonous species do not extend exactly parallel to one another but deviate from their longitudinal course through the stem. Each vessel makes sequential contacts along its course with a number of other vessels (Burggraaf 1972; Fujii et al. 2001; Tyree and Zimmermann 2002; Kitin et al. 2004). Numerous pits are present at these sites of contact, allowing lateral flow of water between vessels in both radial and tangential directions. Different authors have pointed out the importance of lateral flow in plants; Taneda and Tateno (2007) showed that radial flow within the internode contributes significantly to the effective water supply to the leaf. Fujii et al. (2001) and Kitin et al. (2009) demonstrated that lateral transport has potentially important implications in the growth of cambium and differentiating xylem elements. Moreover, the large potential for lateral flow provides a mechanism for rapid and sustainable water movement around blocked vessels, because water can easily bypass vessel occlusions by lateral movement to other functioning

vessels (Tyree and Ewers 1991; Tyree et al. 1994; Tyree and Zimmermann 2002).

Immature vessel elements have been widely recognized to be one of the major factors affecting water movement in developing plant tissues (St Aubin et al. 1986; Frensch and Steudle 1989; Meuser and Frensch 1998). Immature elements are living and turgid cells with a functional plasmalemma within thin unlignified walls (Milburn 1996). They have large vacuoles and dense cytoplasmic contents in which the dictyosomes and elements of rough endoplasmic reticulum are especially numerous (Cronshaw and Bouck 1965; Kitin et al. 2001). These actively growing cells are usually separated from each other and remain as individual cells for a given time before they lose their end walls and cell contents. Living vessel elements with crosswalls and cytoplasm are completely occluded; therefore, they largely impede the apoplastic flow of water. Because of this feature, the presence of living vessel elements can profoundly affect the patterns of water movement within the developing xylem networks. For example, in the developing xylem bundles of young stems, the living vessel elements at the distal end of the newly formed vessels offer a high resistance against the axial movement of water. This would create pressure differential between vessels, which further promotes lateral exchange within the vessel network (Ellmore et al. 2006), and thus, it can be expected that lateral flow would be higher in developing xylem bundles than in mature bundles.

Studies on lateral flow in young stems focus essentially on vascular sectoriality, i.e. water exchanges between different xylem sectors or vascular bundles (Bull et al. 1972; Marshall 1996; Vuorisalo and Hutchings 1996; Orians and Jones 2001; Zwieniecki et al. 2003; Orians et al. 2005; Ellmore et al. 2006). However, lateral flow within a single vascular bundle and its importance for water transport, in particular during xylem development have received relatively little attention. Therefore, this study was conducted to investigate the hydraulic properties of the developing xylem bundles, looking specifically for the role of lateral flow in the effective water transport. We hypothesised that due to the gradual development of the xylem network and the existence of still-living vessel elements, a significant amount of water in the developing xylem bundles is transported in the lateral direction.

In the first part of the present study, we investigated the presence and extent of living vessel elements to better understand the hydraulic architecture of the developing xylem bundles and how this influences the direction of water movement. For this purpose, we used the current-year shoots of grapevine (*Vitis vinifera* L.) and we followed in detail the course of vessel differentiation and maturation along the developing bundles of the stem axis. Grapevine provides an ideal experimental plant for these investigations, because it has relatively wide and also long vessels (Ewers et al. 1990; Schubert et al. 1999; Thorne et al. 2006). Living vessel elements were identified using dye movement method. This technique is based on the mobility of the apoplastic dye: Toluidine blue O (TBO). This dye is a non-penetrating dye in living cells; it cannot cross an intact cell membrane (Chaves et al. 2002). In contrast, TBO is able to pass freely via open vessels and through pit membranes (Shane et al. 2000).

In the second part of the study, we used three experimental approaches to support the hypothesis that lateral flow contributes significantly to water transport in developing bundles. First, we determined the capacity for lateral flow within the developing bundles. An apoplastic dye was directly pulled through the primary vessels and its movement in the secondary vessels was followed. Since any transfer of water from secondary vessels to primary ones must be through lateral transport, the presence of dye in the secondary vessels when the tension occur only in the primary vessels should reflect the existence and the magnitude of lateral flow. Secondly, we compared the effects of increasing the transport distance. Generally, increasing the distance of water transport reduces the hydraulic conductance. But this effect decreases with increased contribution of lateral flow because longer xylem pathway provides more chance for intervessel connections (Taneda and Tateno 2007). Therefore, if lateral flow has a significant contribution to water conductance in the developing bundles, the reduction effect of the distance should be smaller in younger developing stems than in mature stems. Third, we compared the effects of ion concentrations on hydraulic conductance. It was shown that lateral flow is enhanced more than axial flow when xylem fluid is changed from deionized water to ionic solutions (Zwieniecki et al. 2003), i.e. the effect of ions increases with increasing dependence of water conductivity on lateral flow. Thus, the enhancement effect of ions on flow should be greater in developing xylem bundles if there is significant lateral movement in these bundles.

Materials and methods

Plant material

All samples were obtained from mature, field-grown grapevines (*Vitis vinifera* L. 'Cabernet') in the garden of the horticulture at the University of Oum El Bouaghi. Algeria. Current-year shoots were used for the developing xylem experiments; Green shoots, with about 25 internodes and length ranged from 1.3 to 1.6 m, were collected from 3 different plants, placed in plastic bags, and immediately transported to the laboratory. For the mature xylem experiments, 1-year-old stems were used.

Dye movement experiments

The presence and arrangement of living vessel elements along the xylem pathway of the current-year shoots of grapevine were investigated using the following methods: (1) dye-suction method and (2) dye-pressure method. For the suction method, 0.06-m-long segments were cut under water from the centre of the internodes 4–21 counting from the shoot apex. The basal end of the segment was immediately immersed in the xylem lumen-mobile dye (Toluidine blue O, 1% w/v aqueous solution). The upper end was connected to a transparent plastic tubing of the suction pump. The suction force of −120 KPa was applied for a few seconds (5–10 s). Segments were then free-hand sectioned approximately at the middle, and the presence of living vessel elements within the vascular bundles was checked microscopically. In the pressure method, the dye was carried into the dead vessels by using a high pressure force (120 KPa). 0.06-m-long segments were cut under water, and immediately connected to rubber tubing at their lower ends. Toluidine blue dye was pumped into the segments for a few seconds. Xylem tissue was observed in cross sections to determine the mature and immature vessel elements.

All material was examined with a Zeiss KF2 compound light microscope (Carl Zeiss, West Germany). The photomicrographic images were captured using Motic Digital Microscope (DMB1- 2MP, Motic Instruments Inc., Xiamen, China).

Vessel differentiation and maturation

Green stems, consisting of 21 internodes, were cut into segments 0.2 m long. These segments were kept in their original sequence. Each segment was defoliated and the cut proximal end was re-cut underwater and attached to a rubber tube. Toluidine blue O solution was pumped into the segment base at a pressure of 120 kPa. After about 15 min, when the dye was clearly observed on the cut surface of the apical end, the stem segment was cross-sectioned every 5 mm starting from the apical end towards the base. After each 4–6 sections, pressure was applied again for a few seconds. Sections were examined under light microscope and the pattern of xylem differentiation and maturation was monitored from the top to the base. The distance from where a new layer of secondary vessels first appeared at the outermost region of the vascular bundles to the dye loading point was measured. The distance from the point at which the new vessels open (first appearance of dye in the vessel lumens) to the loading point was also recorded. The distances of differentiation and maturation of each new layer of vessels from the stem apex were then calculated. The length of living portion of vessels (the closed portion of the xylem vessels) was measured as the distance from the point where the new vessels first appeared to the point of their openness.

Capacity for lateral flow

In order to detect the lateral movement of water in the developing xylem via vessel-to-vessel pathways, staining was performed by a method somewhat modified from Taneda and Tateno (2007). An apoplastic dye was directly pulled through the primary xylem and its movement in the secondary xylem was followed. Stem segments, consisting of 10 internodes, were collected from positions starting between the 4th and the 13th internode, counted from the shoot apex. The position of the segments ensured that the pathway of axial flow in the secondary vessels was completely blocked, because at the fourth internode only the primary xylem vessels were mature and open, as tested by the dye-pressure method. Each stem segment was defoliated and the cut end of each petiole was sealed using an acrylic based glue (Super Bonder Loctite 409; Loctite Corporation, Rocky Hill, CT, USA). The apical end of the segment was connected to a suction pump through a plastic tubing. The basal end was placed in a 0.1% TBO solution (aqueous, w/v). By using a suction force of −80 KPa, the dye solution was directly pulled through the open primary vessels at the apical end for 15 min. To check for the presence of dye in the secondary vessels, free-hand cross-sections were taken at 0.05, 0.1, 0.15, 0.2 and 0.25 m above the dye injection site. Slides were then viewed under optical microscope, and the number of stained and unstained secondary vessels was counted. Only the functional vessels were considered, the outermost layer of living vessels was not included in the analyses. To confirm that the ascent of dye was not an artifact resulting from a simple passive infusion, movement of TBO under tension from primary vessels was compared with its movement in the absence of tension. Therefore, stem segments were prepared in the same manner as above, but in this case, the dye solution was allowed to flow passively under laboratory conditions for 15 min.

Effect of increasing the transport distance

The method of Taneda and Tateno (2007) was used to demonstrate the significant contribution of lateral flow to water transport in developing xylem bundles. The hydraulic conductance of the stem xylem was measured on progressively shortened stem segments. Segments of 0.35 m length were cut from the current-year shoots at 0.2 m from the shoot apex. Leaves were removed from the stem segment and their petioles were sealed with acrylic based glue. The segment base was cut again and

gently glued to plastic tubing. We avoided using clamps to seal the segments to the tubing to avoid crushing the vessels at the point of stem attachment (Sperry et al. 2005). To remove any air emboli initially present in the vessels, the stem segment was flushed with filtered deionized water at about 150 kPa for at least 15 min. The hydraulic conductance of flushed segment was determined as the mass–flow rate of water divided by the pressure applied (Tyree and Ewers 1991). Deionized water was pushed at 80 KPa pressure through flushed segment until a steady flow was observed. The rate of outflow was measured for 3 min using a balance (model 210, Sartorius). The segment was shortened by successive cuttings off 5 cm from the basal end and the respective values of hydraulic conductance were obtained. Changes of hydraulic conductance in response to changing segment length were compared between developing and mature stem segments. Therefore, the same experiment was repeated with 0.35-m-long segments taken from 1-year-old branches.

Effect of ion concentrations

We followed Zwieniecki et al. (2003) to assess the effect of ion concentrations on the hydraulic conductance. Developing and mature stem segments were used. Unbranched stem segments measuring 0.35 m in length were collected from 1-year-old branches. For the developing stems, segments were taken at 0.2 m from the shoot apex as mentioned above. Leaves were removed and their cut ends sealed with acrylic-based glue. The proximal end was re-cut underwater and attached to a hydraulic system that allowed switching between the solutions supplied to the stem segment. Prior to the first conductivity measurement, deionized distilled water at a positive pressure of 80 KPa was applied until a steady flow was established. Following this, the rate of outflow from the upper end was measured for 3 min using electronic balance. Deionized water was then replaced by 20 mM KCl solution, and the flow rate was remeasured. Finally, the 20 mM KCl solution was replaced by a 50 mM KCl solution and the measurements repeated.

Statistical analysis

All data were obtained at least in triplicate and presented as mean ± standard deviation. Different experimental groups were compared either with the Student's *t* test or with the one-way ANOVA followed by Bonferroni's test for comparisons post hoc. A probability level of $P \leq 0.05$ was considered to be statistically significant. The SPSS software package (SPSS Ver. 15.0, SPSS Inc., Chicago, IL, USA) was used for all tests.

Results

Presence and arrangement of living vessels along the xylem pathway

Young developing stems of grapevine were examined at different heights, and the presence of living vessel elements within the developing xylem could be followed from the base to the top. Dye-pressure and dye-suction methods were used, and results were similar for both techniques. When the apoplastic dye was forced through the xylem network of the stem segments, it distributed throughout the network of open, dead vessels, and this distribution could be restricted only by the closed, living vessel elements. Dead vessels were easily distinguished by colour from living vessels, the dye was observed in both vessel lumens and surrounding fibre cells, whereas for the living vessel elements, dye appeared neither in vessels nor in fibre cells.

In the present investigation, all the tested segments showed the existence of living vessel elements in the xylem pathway. The full maturation of xylem was never observed, even in the oldest segments away from the stem apex. In all the cases, the outermost vessels closer to the cambium remained unstained. The dye pushed through the stem segments could not move through any of these elements, although a high pressure was used, signifying that the outermost vessels were still alive and not open. Figure 1 shows a typical example of cross-sectional images that reflect the presence and arrangement of living vessel elements within the xylem system of young stems of grapevine. However, the same pattern of staining was observed in nearly all the xylem bundles; at least one layer of living vessels occurred in the outermost region of each xylem bundle.

Vessel differentiation and maturation

The progress of differentiation and maturation of the consecutive vessel layers were studied in young grapevine stems. Primary elements appeared at the beginning of xylem differentiation and were fully mature at a few centimetres from the top. Maturation of secondary vessels occurred much later than primary vessels. The first layer of secondary vessels started to appear at 0.08 ± 0.01 m behind the stem apex, but the maturation and openness of this layer started at about 0.16 ± 0.03 m from the apex (Table 1). At a distance of about 0.15 ± 0.03 m from the top, a second layer started to develop at the outermost region of each bundle. Vessels of this layer were larger than that of the first layer. These elements matured to open vessels at a mean distance of 0.35 ± 0.05 m proximal to the top. The differentiation of the third, fourth, and fifth layers was observed to start at about 0.34 ± 0.04,

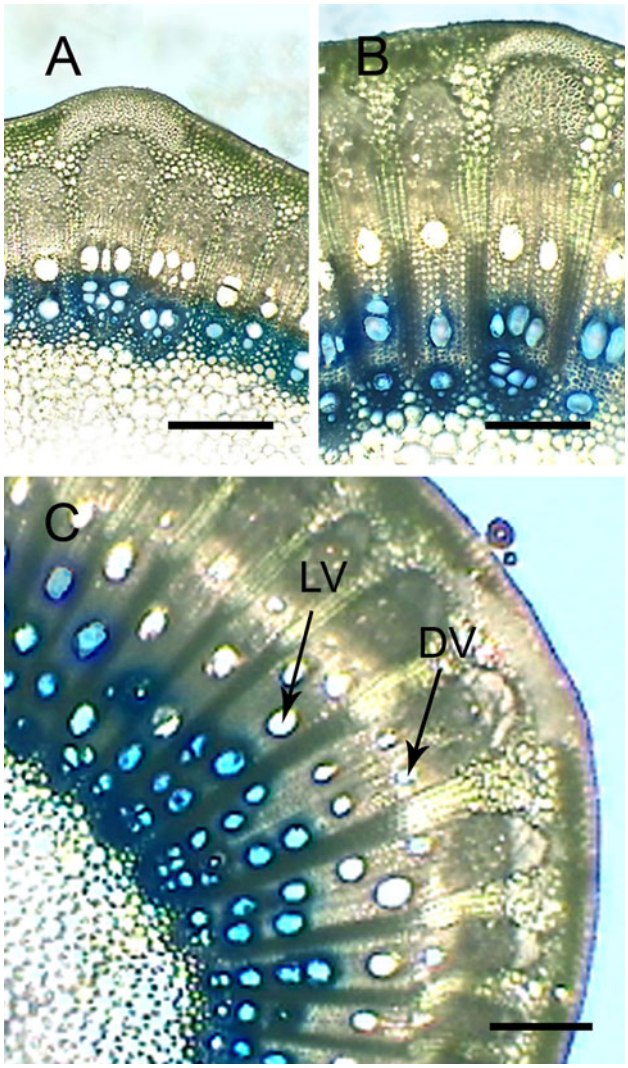

Fig. 1 Microphotographs showing the existence of immature living vessel elements (*unstained vessels*) within the developing bundles of grapevine stems. Since there were no visible differences between the two methods, all pictures are for dye-pressure method. **a** Transverse hand section through the centre of the internode 9, about 0.25 m from top. Only the primary xylem and the first layer of secondary vessels are stained. The second layer at the outermost region adjacent to the cambium is still alive. The section in **b** was obtained 0.2 m below the section in **a**, about 0.45 m from top. At this location, the second layer is fully mature and a third layer with living vessel elements appear at the outermost region. **c** Freehand cross section through the internode 17, about 0.63 m from the top, showing two layers of living vessels at the outermost region of secondary xylem: layer 5 of differentiating xylem vessels (DV) and layer 4 of living xylem vessels (LV). The first, second, and third layers are fully mature. *Scale bars* 400 μm

0.51 ± 0.05, and 0.67 ± 0.06 m from the stem apex, respectively. Maturation of these layers started at about 0.52 ± 0.05, 0.68 ± 0.06, and 0.81 ± 0.09 m, respectively. A schematic diagram showing the pattern of vessel development and maturation is presented in Fig. 2. It is notable that the appearance of each new layer started before the maturation of the previous layer; for example, the third layer appeared at a distance of about 0.34 m whereas the second layer matured at 0.35 m. This may explain why two layers of living vessels could be observed in some cases (Fig. 1c). In addition, the length of living portion of vessels ranged from 0.07 ± 0.02 to 0.19 ± 0.02 m and the longest occurred in the second layer of secondary vessels.

Capacity for lateral flow

The magnitude of lateral flow was evaluated in younger stem segments that had closed secondary network, i.e. the distal ends of all secondary vessels were closed by immature vessel elements while the basal ends were open. When the basal ends of stem segments were immersed in TBO, for simple passive infusion, stained vessels were observed only in the basal portion of segments. Little or no dye was observed beyond a distance of 0.15 m above the loading site (Fig. 3). Results were different when the dye was pulled through the primary xylem at the distal end. The stained vessels were observed in the secondary xylem at the different distances from the injection site. The percentages of stained vessels were more than 60% of the total number of open secondary vessels (Fig. 3). In each vascular bundle, the secondary xylem was stained not only in the inner part adjacent to the primary xylem but also in the middle and outer parts except the outermost vessels (living vessel elements) which did not stain. This suggested that the tension was transmitted from the primary to the secondary xylem and between secondary vessel elements. Although we could not determine how much intervessel contacts occurred along the courses of xylem vessels, the results implied that secondary vessels were hydraulically connected with each other and also with vessels in the primary xylem, and water could be drawn from secondary to primary vessels through lateral pathways.

Effect of increasing the transport distance

To elucidate the importance of lateral flow in water transport during xylem development, the variations in hydraulic conductance with increasing the distance of water transport were investigated on developing and mature stem segments. Overall, mature segments showed significantly higher values of hydraulic conductance than developing segments. For example, the 0.05-m-long segments of mature stems exhibited a mean hydraulic conductance of 17.5×10^{-6} kg s^{-1} MPa^{-1}, about 6.7 times greater than that of the developing stems (2.6×10^{-6} kg s^{-1} MPa^{-1}). The effect of increasing the length of the transport pathway is expressed in Fig. 4 as the percentile decrease in hydraulic conductance relative to that measured in 0.05-m-long segments. Both developing and mature

Table 1 Distances of differentiation and maturation of the successive layers of secondary vessels, from the first layer near the pith to the fifth layer close to the epidermis

Vessel layer	Distance of differentiation (m)	Distance of maturation (m)	Length of living portion (m)
1st layer	0.08 ± 0.01	0.16 ± 0.03	0.07 ± 0.02
2nd layer	0.15 ± 0.03	0.35 ± 0.05	0.19 ± 0.02
3rd layer	0.34 ± 0.04	0.52 ± 0.05	0.17 ± 0.04
4th layer	0.51 ± 0.05	0.68 ± 0.06	0.17 ± 0.02
5th layer	0.67 ± 0.06	0.81 ± 0.09	0.14 ± 0.03

The length of living portion of vessels was determined as the distance from the site of differentiation to the point of maturation. Measurements were made on current-year shoots consisted of 21 internodes. Overall shoot length was 1.01 ± 0.12 m. Values are mean ± SD (n = 6)

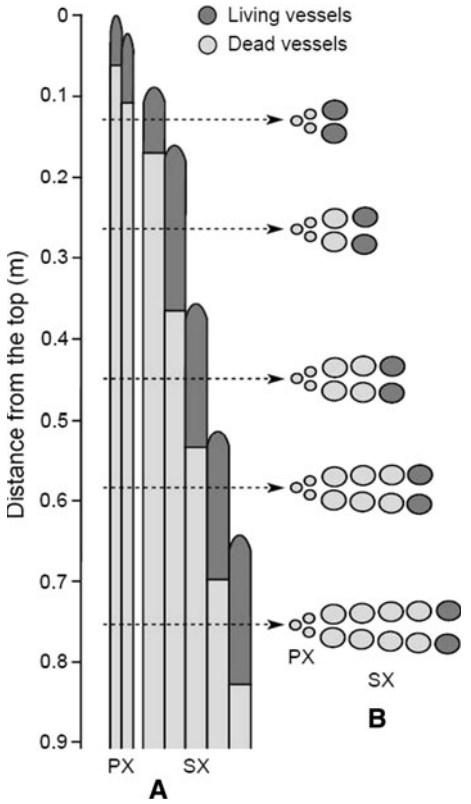

Fig. 2 **a** Diagrammatic longitudinal section showing the normal course of maturation and openness of vessel elements and the arrangement of immature living vessel elements along the developing vascular bundle in younger grapevine stem. Note that the distal end of each vessel is alive. **b** Diagrammatic cross-sections of the vascular bundle. Only the vessel elements are shown for the sake of simplicity. *PX* primary xylem, *SX* secondary xylem. The *horizontal axis* of this figure is not drawn to scale

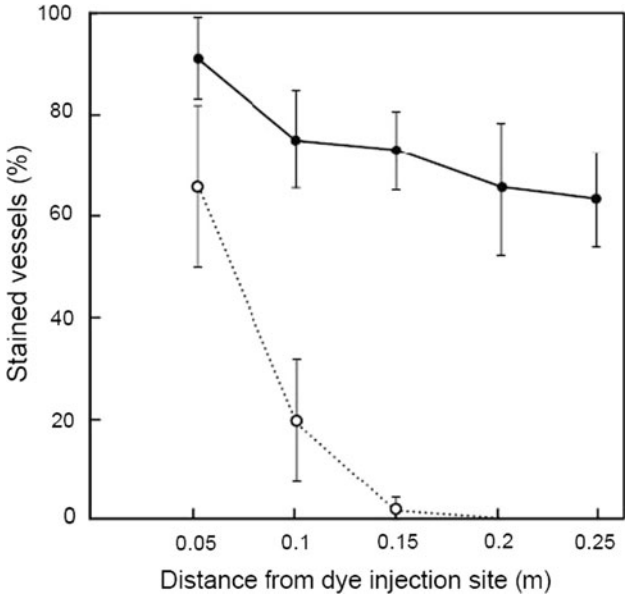

Fig. 3 Results of dye injection experiments for the capacity of lateral movement in the developing bundles. The *vertical axis* represents the mean percentage of stained secondary vessels at the different distances from the injection site. Dye was loaded into the proximal end of the stem segment and allowed to move passively (*open circles*, *dashed line*) or under tension created in the primary xylem (*closed circles*, *continuous line*). *Vertical bars* ±1 SD (n = 10 stem segments)

segments showed a decrease in hydraulic conductance with increasing segment length, but this decrease was significantly lower in the developing segments than in the mature segments (Student t test, paired; $P < 0.05$). When the distance of water transport was increased from 0.05 to 0.3 m, the hydraulic conductance was decreased by 72% in the mature segments while in the developing segments the reduction was only 26% (Fig. 4).

Effect of ion concentrations

The effect of ion concentrations on hydraulic conductance within the developing and mature stem segments are presented in Fig. 5. In the presence of ions in the perfusing solution, the hydraulic conductance increased significantly in the developing segments, while in the mature segments the increment was very weak and did not exceed 3%. When the xylem system was supplied with 20 mM KCl, enhancement of the hydraulic conductance was 10.5 ± 3.1% for developing segments versus 1.4 ± 0.4% for mature segments. In the presence of 50 mM KCl, the hydraulic conductance increased by 12.9 ± 2.6% in the developing segments while in the mature segments only increased by 2.3 ± 0.8%. In addition, it must be noted that

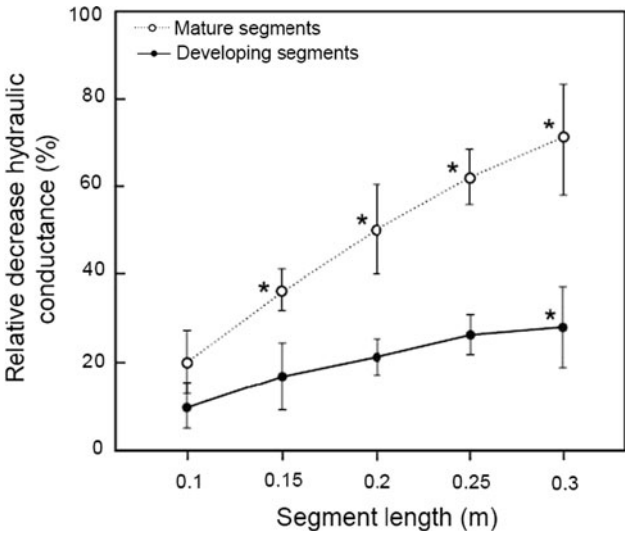

Fig. 4 Changes in hydraulic conductance of developing and mature stem segments in response to the changes in segment length. Data are presented as the percentile decrease in hydraulic conductance relative to that measured in 0.05-m-long segments. *Significant difference from 0.1 m by the Bonferroni multiple-comparisons test at $P < 0.05$. *Vertical bars* ±1 SD ($n = 10$)

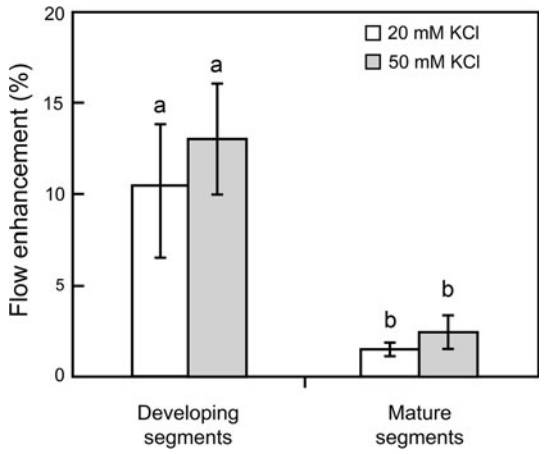

Fig. 5 Enhancement of xylem hydraulic conductance in developing and mature stem segments due to the addition of 20 and 50 mM KCl. Values are mean ± SD; $n = 10$; *different letters* indicate significant differences between treatments; Student t test, $P < 0.05$

for both developing and mature segments, there was not a significant difference in the flow enhancement between 20 mM and 50 mM KCl (Student t test, unpaired; $P < 0.05$).

Discussion

By using the apoplastic dye, Toluidine blue O (TBO), it was possible to distinguish between dead and living vessel elements. TBO stained the cell wall of all dead vessels, because it easily moved within and between dead vessels. Instead, TBO was completely stopped by living elements; when vessel elements were alive and their plasma membranes were intact and functionally active, TBO could not penetrate through these elements. This confirms previous observations on the movement of TBO through xylem networks (Chatelet et al. 2006; Shane et al. 2000; Zanne et al. 2006). However, in this study, dye movement method appeared to be reliable and rapid way not only to identify lateral pathways between vessels, but also to detect living vessel elements in developing xylem networks, at least in grapevine stems.

This study clearly showed the presence and extent of immature, living vessel elements and their influences on the patterns of water flow within the developing xylem of the growing stems. Secondary vessels of grapevine stems were observed to develop with a slow and regular progress. Immature vessel elements were continuously present in the developing vascular bundles, throughout the growing period. The distal portion of each secondary vessel was always alive. This closed, living portion could extend to a significant length (Table 1), signifying that the maturation and openness of vessel elements occurred at considerable distances from the cambium, and consequently, the cambial cells were largely separated from the dead vessels. This isolation from the open secondary xylem might serve to protect the cambial tissues from mobile pathogens and their toxins in the xylem stream (McCulley 1995). However, due to their anatomical arrangement, immature vessel elements could form a significant barrier to apoplastic water movement at the peripheral and terminal ends of the developing secondary xylem (Fig. 6).

The hydraulic architecture of the developing vascular network and the presence of living vessel elements and their locations relative to the water pathway (Fig. 6) suggested that the axial movement of water within the developing bundles was strongly restricted. It was evident that water flow in the newly matured vessels would be completely blocked when encountering immature vessel elements at their distal ends. To bypass these closed elements, water must move laterally to the adjacent inner vessels, where it can spread axially in the vessel lumen before being stopped again by the distal, living portion (Fig. 6). But this requires that mature vessels connect with each other. Indeed, grapevine vessels commonly occur in radial clusters or chains of two to three vessels, where intervessel pits present as bordered pit pairs in a scalariform arrangement and are numerous and much larger than other types of pits in the xylem network (Sun et al. 2006). These clusters provide multiple pathways for water movement around closed vessels (Tyree et al. 1994; Stevenson et al. 2004). This was further supported by the results of the capacity of lateral movement. The results

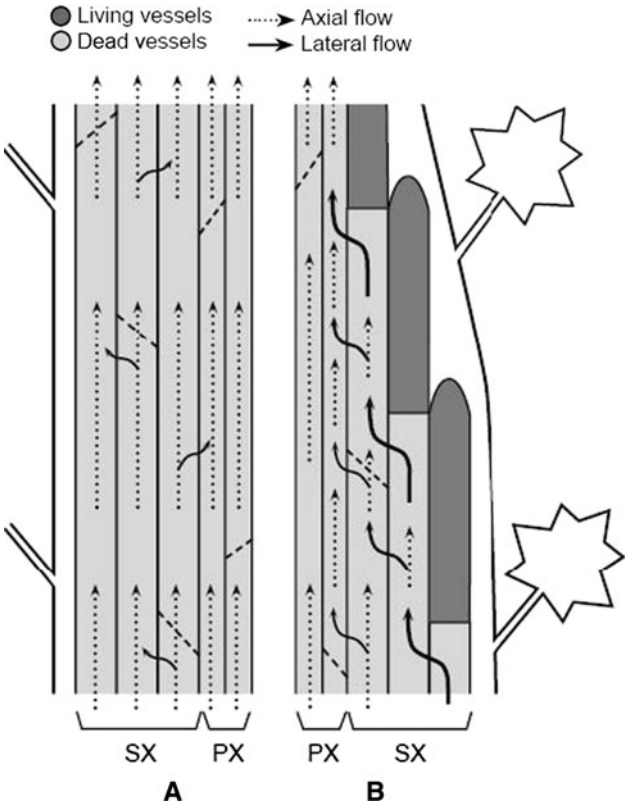

Fig. 6 Schematic presentation of the hydraulic architecture and the patterns of water flow in the mature (**a**) and developing (**b**) vascular bundles. Water flow within the mature bundle is mostly in the axial direction and only very small amounts of water flow laterally between conduits. By contrast, large amounts of water in the developing bundle must be transported in the lateral direction because of the presence of living vessel elements. For further explanation, see text. *PX* primary xylem, *SX* secondary xylem. This figure is not drawn to scale

showed that water in the developing bundles moved easily between vessels, meaning that vessel-to-vessel pathways were distributed between different layers of secondary vessels and between primary and secondary xylem.

The relative decrease in hydraulic conductance in response to increasing distance of water transport was considerably lower in the developing segments compared to mature segments. This could be attributed to the effect of lateral flow. As mentioned above, the majority of secondary vessels in the younger stem segments were closed at their distal ends by living vessel elements. So the pathway of axial flow within these vessels was blocked. It is clear that the contribution of such vessels to the total hydraulic conductance should be only through lateral pathways (Fig. 6). This contribution should be increased with increasing the distance of water transport, since longer pathways provide more intervessel connections (Taneda and Tateno 2007). In addition, the number of secondary vessels in the developing segments increased with increasing segment length. Increasing both the connections between conduits and the number of secondary vessels would enhance conductance and thus compensating for losses in conductive capacity due to the effect of increasing distance. In contrast, almost all secondary vessels in the mature segments were open for the axial movement, and their number was approximately constant along the length of the segment. In this case, increasing length would only increase the resistance to water flow.

The significant contribution of lateral flow for water conduction within the developing vessel networks may explain why the enhancement effect of ions on hydraulic conductance was higher in developing bundles as compared to mature ones. The presence of ions in the xylem fluid facilitate lateral transport between adjacent xylem vessels by changing the hydration status of pectin hydrogels located in bordered pit membranes (Van Ieperen et al. 2000; Zwieniecki et al. 2001a). These authors stated that ions cause shrinkage and swelling of pectin hydrogels, thereby increasing the porosity of the pit membranes and thus decreasing their resistance to water flow. However, since the principal pathway of lateral flow between adjacent vessels is through bordered pit pairs, increasing ion concentrations should substantially increase the conductance of the lateral pathway more than that of the axial pathway and consequently should increase conductance more in the bundles with a higher contribution from lateral pathway to overall flow than those with a lower contribution from lateral pathway.

It is possible that the significant lateral flow seen in the young stems may be due to the dominance of shorter vessels within their developing xylem bundles. Shorter vessels exhibit high resistance to axial flow because water would have to pass through more end walls (Comstock and Sperry 2000; Zwieniecki et al. 2001b). Increased axial resistance favours lateral flow between adjacent vessels (Ellmore et al. 2006). However, we exclude this possibility because vessels of grapevine are among the longest and widest vessels found in plants (Zimmermann and Jeje 1981; Salleo et al. 1985; Ewers et al. 1990). Although it has been reported that end wall resistivity averaged more than 50% of the stem axial resistance in grapevine (Sperry et al. 2005; Wheeler et al. 2005; Hacke et al. 2006), this does not mean that end wall resistivity is sufficiently high to promote significant amounts of lateral flow, as observed in our developing stems. It seems very logical that the total axial resistance due to end wall and lumen resistivities is negligibly small compared to axial resistance due to closed living elements. Thus, it may be suggested that the degree of xylem development and the presence of living vessel elements might be the dominant factors affecting patterns of lateral flow in developing stems. It is already known that lateral flow is affected by several factors including size and density of intervessel pits, vessel width and length, and

pressure gradients (Orians et al. 2004). In addition to these factors, the developmental state of xylem is another important factor that deserves further attention.

In conclusion, it is apparent from this and other studies that living vessel elements have a significant influence on water transport and distribution. We suggest that living vessel elements are a very important factor that cannot be neglected, and the water transport across developing bundles, in respect to these elements, appears to be more complex than that of mature bundles. To model water and nutrients exchanges in the developing xylem, it is not sufficient to consider dead conduits only; immature living conduits are a significant part of the developing xylem pathway and must be included in the study of water transport within developing plant organs. In addition, the insights provided by this study further emphasize the importance of lateral flow in plants. In addition to its roles in supplying water, maintaining the functional water transport system, and controlling water and particle movement, lateral flow has important roles in water transport efficiency and reliability during shoot growth and development.

Acknowledgments We thank Abdelaziz Labidi and Belgacem Hadid for their support and assistance. Special thanks are extended to Drs. Hocine Bensaha, Mohamed Amine Benhadia, Mohamed Hocine Benaissa, Nouredine Slimani and the anonymous referees for helpful comments on earlier versions of this manuscript. We are also grateful to Sofiane Amira, Ali Lahcini, Abdessalem Bougafla and Saci Djouwahi for their technical support.

References

Bull TA, Gayler KR, Glasziou KT (1972) Lateral movement of water and sugar across xylem in sugarcane stalks. Plant Physiol 49:1007–1011

Burggraaf PD (1972) Some observations on the course of the vessels in the wood of *Fraxinus excelsior* L. Acta Bot Neer 21:32–47

Chatelet DS, Matthews MA, Rost TL (2006) Xylem structure and connectivity in grapevine (*Vitis vinifera*) shoots provides a passive mechanism for the spread of bacteria in grape plants. Ann Bot 98:483–494

Chaves T, Regalado AP, Chen M, Ricardo CP, Showalter AM (2002) Programmed cell death induced by (β-D-galactosyl)3 Yariv reagent in *Nicotiana tabacum* BY-2 suspension-cultured cells. Physiol Plant 116:548–553

Comstock JP, Sperry JS (2000) Theoretical considerations for optimal conduit length for water transport in vascular plants. New Phytol 148:195–218

Cronshaw J, Bouck GB (1965) The fine structure of differentiating xylem elements. J Cell Biol 24:415–431

Ellmore GS, Zanne AE, Orians CM (2006) Comparative sectoriality in temperate hardwoods: hydraulics and xylem anatomy. Bot J Linn Soc 150:61–71

Ewers FW, Fisher JB, Chiu ST (1990) A survey of vessel dimensions in stems of tropical lianas and other growth forms. Oecologia 84:544–552

Frensch J, Steudle E (1989) Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). Plant Physiol 91:719–726

Fujii T, Lee SJ, Kuroda N, Suzuki Y (2001) Conductive function of intervessel pits through a growth ring boundary of *Machilus thunbergii*. IAWA J 22:1–14

Hacke UG, Sperry JS, Wheeler JK, Castro L (2006) Scaling of angiosperm xylem structure with safety and efficiency. Tree Physiol 26:619–701

Kitin P, Sano Y, Funada R (2001) Analysis of cambium and differentiating vessel elements in *kalopanax pictus* using resin cast replicas. IAWA J 22:15–28

Kitin PB, Fujii T, Abe H, Funada R (2004) Anatomy of the vessel network within and between tree rings of *Fraxinus lanuginosa* (Oleaceae). Am J Bot 91:779–788

Kitin P, Fujii T, Abe H, Takata K (2009) Anatomical features that facilitate radial flow across growth rings and from xylem to cambium in *Cryptomeria japonica*. Ann Bot 103:1145–1157

Marshall C (1996) Sectoriality and physiological organization in herbaceous plants: an overview. Vegetation 127:9–16

McCulley M (1995) How do real roots work? Plant Physiol 109:1–6

Meuser J, Frensch J (1998) Hydraulic properties of living late metaxylem and interactions between transpiration and xylem pressure in maize. J Exp Bot 49:69–77

Milburn JA (1996) Sap ascent in vascular plants: challengers to the Cohesion theory ignore the significance of immature xylem and the recycling of Munch water. Ann Bot 78:399–407

Orians CM, Jones CG (2001) Plants as resource mosaics: a functional model for predicting patterns of within-plant resource heterogeneity to consumers based on vascular architecture and local environmental variability. Oikos 94:493–504

Orians CM, van Vuuren MM, Harris NL, Babst BA, Ellmore GS (2004) Differential sectoriality in long distance transport in temperate tree species, evidence from dye flow, 15 N transport, and vessel element pitting. Trees 18:501–509

Orians CM, Smith SDP, Sack L (2005) How are leaves plumbed inside a branch? Differences in leaf-to-leaf hydraulic sectoriality among six temperate tree species. J Exp Bot 56:2267–2273

Salleo S, LoGullo MA, Oliveri F (1985) Hydraulic parameters measured in 1-year-old twigs of some Mediterranean species with diffuse-porous wood. J Exp Bot 36:1–11

Schubert A, Lovisolo C, Peterlunger E (1999) Shoot orientation effects vessel size, shoot hydraulic conductivity and shoot growth rate in *Vitis vinifera* L. Plant Cell Environ 22:197–204

Shane MW, McCully ME, Canny MJ (2000) Architecture of branch root junctions in maize: structure of the connecting xylem and the porosity of pit membranes. Ann Bot 85:613–624

Sperry JS, Hacke UG, Wheeler JK (2005) Comparative analysis of end wall resistivity in xylem conduits. Plant Cell Environ 28:456–465

St Aubin G, Canny MJ, McCully ME (1986) Living vessel elements in the late metaxylem of sheathed maize roots. Ann Bot 58:577–588

Stevenson JF, Matthews MA, Greve LC, Labavitch JM, Rost TL (2004) Grapevine susceptibility to Pierce's disease. I. Relevance of hydraulic architecture. Am J Enol Vitic 55:228–237

Sun Q, Rost TL, Matthews MA (2006) Pruning-induced tylose development stem of current-year shoots of *Vitis vinifera* (Vitaceae). Am J Bot 93:1567–1576

Taneda H, Tateno M (2007) Effects of transverse movement of water in xylem on patterns of water transport within current-year shoots of kudzu vine, *Pueraria lobata*. Funct Ecol 21:226–234

Thorne ET, Young BM, Young GM, Stevenson JF, Labavitch JM, Matthews MA (2006) The structure of xylem vessels in grapevine and a possible passive mechanism for the systemic spread of bacterial disease. Am J Bot 93:497–504

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol 119:345–360

Tyree MT, Davis SD, Cochard H (1994) Biophysical perspective of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to disfunction? IAWA J 15:335–360

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap, 2nd edn. Springer, Berlin

Van Ieperen W, Van Meeteren U, Van Gelder H (2000) Fluid ionic composition influences hydraulic conductance of xylem conduits. J Exp Bot 51:769–776

Vuorisalo T, Hutchings MJ (1996) On plant sectoriality, or how to combine the benefits of autonomy and integration. Vegetation 127:3–8

Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Intervessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. Plant Cell Environ 28:800–812

Zanne AE, Sweeney K, Sharma M, Orians CM (2006) Patterns and consequences of differential vascular sectoriality in 18 temperate trees and shrub species. Funct Ecol 20:200–206

Zimmermann MH, Jeje AA (1981) Vessel-length distribution in stems of some American woody plants. Can J Bot 59:1882–1892

Zwieniecki MA, Melcher PJ, Holbrook NM (2001a) Hydrogel control of xylem hydraulic resistance in plants. Science 29:1059–1062

Zwieniecki MA, Melcher PJ, Holbrook NM (2001b) Hydraulic properties of individual xylem vessels of *Fraxinus americana*. J Exp Bot 52:1–8

Zwieniecki MA, Orians CM, Melcher PJ, Holbrook NM (2003) Ionic control of the lateral exchange of water between vascular bundles in tomato. Ann Bot 54:1399–1405