Comparison of light and fluorescence microscopy for xylem analysis in tomato pedicels during fruit development

D. RANČIĆ*, S. PEKIĆ QUARRIE*, M. TERZIĆ*, S. SAVIĆ† & R. STIKIĆ*

*Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Serbia  
†Faculty of Biofarming, Megatrend University, Maršala Tita 39, 24300 Bačka Topola, Serbia

Key words. Fluorescence microscopy, pedicel, tomato, xylem.

Summary

The xylem hydraulic connection between shoot and fruits has previously been investigated, but contradictory conclusions were drawn about the presence of a flow resistance barrier in the pedicel. In this paper we were studying effect of the drought on the functional xylem vessels in the pedicels of tomato fruit. Commercial tomato genotype was grown in cabinet conditions under two watering regimes (full and deficit irrigation). An aqueous solution of eosin Y were used to visualize the path of water movement through tomato fruit pedicel and fluorescence microscopy observations were done on transversal and longitudinal sections. Dye uptake studies suggested that in well watered plants and in plants exposed to drought, a large majority of xylem vessels are not functional in water transport. Reduced-irrigation treatment significantly altered number and width of functional xylem elements in the fruit pedicel, especially in the abscission zone. This indicates that drought modifies xylem architecture and, thus, environmentally produced change in the hydraulic property of pedicel may affect fruit development.

Introduction

The anatomy of tomato fruit pedicels becomes an important factor for understanding physiological events during fruit development, especially under drought conditions. The xylem hydraulic connection between shoot and fruits has previously been investigated, but contradictory conclusions were drawn about the presence of a flow resistance barrier in the pedicel. In most varieties of tomato, a knuckle is found midway along the pedicels that contains a future abscission zone (Lee, 1989; André et al., 1999; Van Ieperen et al., 2003), whereas Malone & Andrews (2001) recently reported the opposite. Water stress during growth may influence xylem hydraulic resistance by inducing the development of xylem vessels with a smaller diameter (Lovisolo & Schubert, 1998). Xylem hydraulic resistance strongly depends on the number and radii of xylem vessels along the transport path (Nobel, 1983). According to Davies et al. (2000), there may be hydraulic and chemical isolation of fruits from the rest of the plant.

However, as the physiological significance of these effects is unclear, we are studying functional xylem vessels with an appropriate dye tracer. The major objective of this paper was to develop the method for detailed anatomical characterization of fruit pedicels of tomato in response to drought stress during fruit development, by using both light and fluorescence microscopy.

Methods

Commercial tomato genotype SUNPACK was grown in cabinet under well-watered (FI), deficit irrigation (DI) and partial root drying (PRD) conditions. During fruit development, samples of flower and fruit pedicels were collected in the flower stage, early (fruit diameter 3–5 mm), mid (fruit diameter 1–2 cm) and late (yellowing fruit of diameter 4–6 cm) fruit phases. A 1% aqueous solution of eosin Y was used to visualize the path of water movement through pedicels (Eichhorn et al., 1986). The base of the freshly cut pedicels of tomato fruit was placed in 1 mL of eosin and left to perfuse for 1 h. The pedicels were then removed from the dye solution and cross-sectioned at three points: at the abscission zone and 5 mm before and after it. Some longitudinal sections of the abscission zone were also made on some pedicels. Total xylem area was examined using bright-field microscopy and functional xylem area using fluorescence microscopy (LEICA DMSL with UV filter A, excitation Filter BP 340–380 nm/Suppression Filter LP 425). Xylem vessels autofluoresce blue in UV light.
but if stained with eosin they fluoresce yellow. Bundles that appeared yellow by fluorescence microscopy were assumed to be functional for water transport. Sections were photographed with a digital camera (LEICA DC 300; Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) and measurements were taken using an image analysis system (LEICA IM 1000 software; Leica Microsystems Wetzlar GmbH). Data were subjected to statistical analysis using the $t$ test (SigmaPlot; Jandel Scientific GmbH, Erkrath, Germany).

**Results and discussion**

Cross sections of flower pedicels showed the structure typical of the **Solanacea** stem anatomy, with bi-collateral phloem elements. With aging, xylem in fruit pedicels, before and after abscission zone, formed a continual ring with numerous xylem vessels and external and internal phloem elements on both sides of the ring (Figs 1 and 2). At early fruit stages, xylem area was around $3.5 \times 10^5 \, \mu m^2$ in the zone near the stem, about half this ($1.9 \times 10^5 \, \mu m^2$) in the zone near the fruit and only $0.4 \times 10^5 \, \mu m^2$ at the abscission zone (Fig. 3(a)). By the final sampling stage, xylem area had increased over 15 times in the zone near the stem and about 75 times in the zone near the fruit. However, the xylem area during fruit development increased only about sixfold at the abscission zone. In the abscission zone, compared with other zones, the xylem area was less developed in all developmental stages of fruit and never formed a ring (Figs 4 and 5). In the stage of final, mature fruit, the xylem area near the stem was about $24.4 \times 10^5 \, \mu m^2$, near the fruit it was about $19.9 \times 10^5 \, \mu m^2$ and in the abscission zone only $2.4 \times 10^5 \, \mu m^2$ (Fig. 3(c)). The most evident effects of drought on tomato fruit pedicels was a reduction of xylem area in almost all phases of fruit development, whereas the PRD treatment in early fruit pedicels reduced xylem development in all zones, and in later developmental phases increased xylem areas. However, no
changes in xylem area were significant between treatments in all zones during fruit development (Fig. 3).

Studies of dye uptake with fluorescence microscopy suggested that in well-watered plants and those under drought conditions, the large majority of xylem vessels are not functional for water transport. Functional analysis of xylem vessels on tomato fruit pedicels indicated that drought increased the proportion of functional xylem before and after the abscission zone: plants under full irrigation had 21–29%, plants under drought 28–44% and PRD-treated plants 31–66% functional as a percentage of total xylem area. These results are partially in agreement with those of Van Ieperen et al. (2003), who suggested that most of the xylem vessels proximal and distal to the abscission zone are involved in xylem water transport, and with André et al. (1999), who suggested that secondary xylem production and lignification in the pedicel at fruit maturity are mostly mechanical responses, ensuring the support of heavy organs like fruits. The only significant difference between PRD and control plants was in the functional xylem area in the zone near the fruit (Fig. 3). In the early stages of fruit development, PRD reduced the functional xylem area in this zone (about 80–90%), but in the later phases functional xylem was increased (about 50%).

Studies of dye uptake in the abscission zone suggested that all visible xylem seen on cross sections seemed to be functional. The same effect on xylem area, above and below the abscission zone of PRD-treated plants, was noticeable, but not significant: compared with control plants, PRD reduced xylem areas in early–mid stages during fruit development to 30%, and whereas in the final stage, the xylem area was increased by 30%. The result was an increase of xylem area in the abscission zone of tomato pedicels during fruit development by up to 5–6 times in control and drought plants and up to 10 times in PRD-treated plants. According to Van Ieperen et al. (2003), in the abscission zone only primary vessels are formed during the early phases of pedicel development. The primary character of
the vessels in the abscission zone also suggests that hydraulic resistance of the pedicel abscission zone is determined at an early stage of fruit development, and the small number of vessels through the abscission zone makes the xylem system in the pedicel more vulnerable to water stress. According to Nobel (1983), hydraulic resistance strongly depends on the number and radii of xylem vessels along the transport path, and water stress during growth may influence xylem hydraulic resistance by inducing the development of xylem vessels with smaller diameters (Abdel-Rahim et al., 1998; Lovisolo & Schubert, 1998). A restricted xylem connection between shoot and fruit and the consequential hydraulic isolation of the fruit from the rest of the plant may have clear advantages as well as disadvantages for tomato fruit development.
reduction may be a limiting factor for transport of apoplastic water to the fruit and restricted xylem connections may reduce the transport of apoplastically mobile ions, which may also lead to negative effects on fruit development (Ho et al., 1987; Adams & Ho, 1993), but by contrast, xylem reduction may protect fruits from water stress chemical signals that could be harmful for fruit and seed development.

Conclusion

Reduced-irrigation treatments significantly affected the area of functional xylem in the tomato fruit pedicel, especially in the zone near the fruit. Anatomical investigations showed that hydraulic isolation of tomato reproductive organs might occur in earlier stages (flowering and early fruit phase) of fruit development. These results appear to confirm the theory of hydraulic isolation of tomato fruits and show that a PRD reduced-irrigation treatment significantly alters xylem anatomy in the fruit pedicel. Reducing the functional xylem area in early fruit growth phases significantly under drought indicates that drought modifies xylem architecture, and this environmentally induced change in the hydraulic property of the pedicel may affect fruit development.

It is also evident from our data that for functional anatomy studies, light microscopy should be combined with fluorescence microscopy. As the abscission zone is very complex, and not so easy to study on transverse and longitudinal sections, it will be better to use confocal microscopy for histological investigations in this zone.

Acknowledgement

We thank EU FP6 projects, WATERWEB and CROPWAT, for the financial support and Dr. Steve Quarrie for useful suggestions.

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