The application of various anatomical techniques for estimating hydraulic conductivity in tomato fruit pedicels

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Deficit irrigation (DI) and partial root zone drying (PRD) are techniques recently used for saving water. Davies *et al*. [1] suggested that the smaller effect of PRD on fruit growth, compared with DI, might be a reduction of the xylem area in fruit pedicels, which restrict the movement of chemical signals from shoot to fruit. We tried to compare different methods in analyzing the anatomical basis of the hydraulic conductivity of tomato fruit pedicels.
Tomato plants (*Lycopersicon esculentum*, cv. Alisia Craig) were grown in a growth chamber. Both PRD and DI treatments received 50% of the water given to fully irrigated (FI) plants. In PRD treated plants water was applied only to one half of the root system, and the wet and dry sides were periodically alternated. Tomato pedicels were collected at the stage of final fruit growth, and examined as follows:

a. Pedicels were cut into 2-3mm long segments, air-dried and coated with platinum using an Emitech K 550 sputter coater. Observations were carried out with a SEM Hitachi S-4700.
b. Pedicels were hand sectioned along their longitudinal and transverse axis and the total xylem area of sections was examined using fluorescence microscopy (Excitation Filter BP 340-380 nm/Suppression Filter LP 425).
c. Pedicels were cut into 2-mm^3 pieces, fixed in Karnovsky's fixative, post-fixed in osmium tetroxide, dehydrated, embedded in LR white resin and sectioned on an ultramicrotome using a diamond knife. The sections were stained with uranyl acetate and lead citrate. Observations were carried out using a JEOL JEM- 1210 TEM.
d. Resin embedded material was cut at 0.5µm with a glass knife, stained with 0.5% toluidine blue O, and mounted in DPX.
e. Pedicel samples were macerated in Franklin's solutions for 48h at 60ºC, washed in tap water, stained in 1% safranine and examined with LM in water.
f. X-ray tomography: fruit pedicels were scanned with the microCT scanner at the UCGT-Laboratory of Ghent University (Belgium) and the tomographic reconstruction process was performed using the Octopus and VGStudio Max programmes.

As xylem hydraulic resistance strongly depends on the number and diameter of xylem vessels along the transport path, it is possible to calculate the hydraulic conductance based on the number and diameter of xylem vessels as measured on transverse sections prepared for LM or SEM (Fig 1a). Longitudinal sections of tomato fruit pedicels (fig 1b and 1d) show a strong reduction of xylem in the abscission zone, and a reduction of the hydraulic conductance is therefore likely. These sections also revealed a very specific shape of the abscission zone, which makes it very difficult to calculate the exact number and diameter of vessels passing this zone based on transverse serial sections. In zones before and after the

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abscission zone, there is a large portion of secondary xylem and it is possible to measure the diameter of all cells on transverse sections, but it is hard to distinguish between vessels and tracheids, and between conductive and non-conductive cells types [2]. TEM (Fig 1c) images allows us to distinguish vessels from other cell types, but the sample size for TEM is limited to 2- 4mm$_2$, which makes it impossible to obtain a complete picture of much larger cross sectional areas of the abscission zone in fruit pedicels (about 20mm$_2$). The maceration technique (Fig 1e) is a very good method for measuring the length and width of vessel elements, but not for estimating the total number of vessel elements in each pedicel zone. The microCT images (Fig 1f) provide useful 3D information. Unfortunately, the microCT resolution of 2,3µm that we obtained was too low to distinguish the various cell types, which makes it impossible to determine the hydraulic conductance.

Because of the very complex structure of the abscission zone, none of the methods that we applied was suitable for estimating the hydraulic conductance in tomato pedicels. NanoCT techniques that allow a higher resolution (<1µm) would seem most useful, in combination with 3D confocal imaging and experiments for staining conductive cells in xylem.

1. W. J. Davies et al J. Exp. Bot. **51** (2000) p1617–1626.
2. D. Rančić et al. J. Microscopy **232** (2008) p618–622
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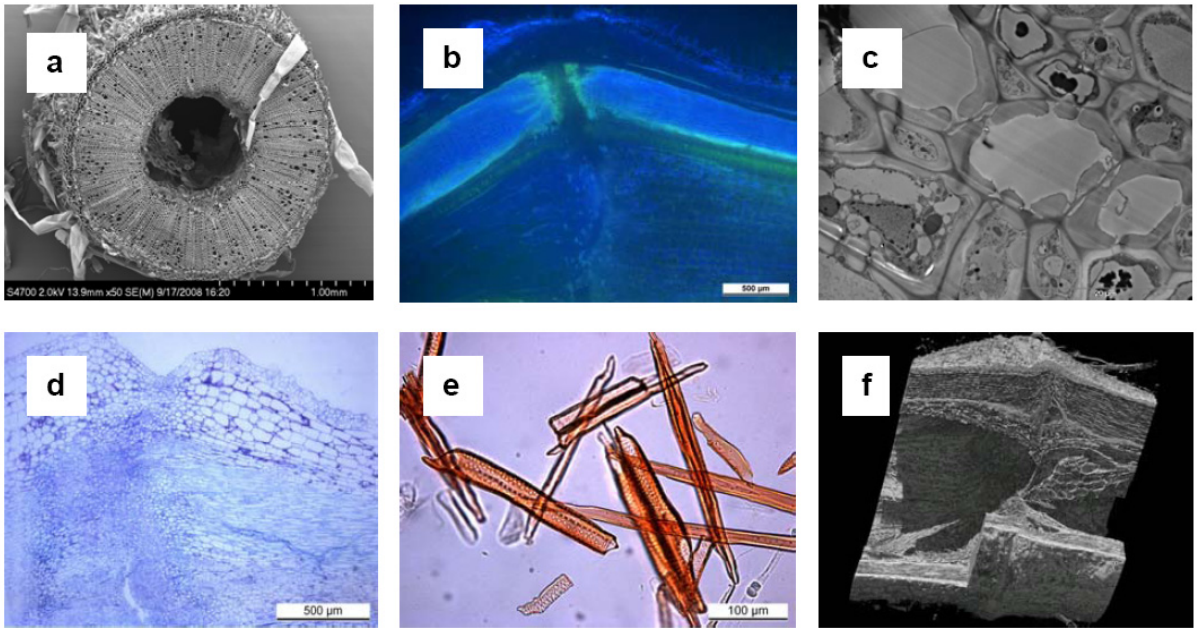

Figure 1. a-Scanning electron microscopy, b-Longiutdinal hand section, fluorescence microscopy, c-Transmission electron microscopy, d- Longiutdinal semi-thin sections, light microscopy, e-Maceration, f-X-rays tomography

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