Functional anatomy of vascular tissue as a tool to understand transport of water and assimilates into developing fruit

Dragana Rančić*, Ilinka Pećinar, Radenko Radošević, Maja Terzić, Sofija Pekić Quarrie

1 Faculty of Agriculture-University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

ABSTRACT: The yield of crop plants and fruit growth depends, to a large extent, on the efficiency of the vascular tissue, in optimal conditions, as well as in stress conditions. Most of the material on which fruit growth depends is transported from the stem into the fruit through the fruit pedicel, by the xylem and phloem, so the anatomy of fruit pedicel is an important factor in understanding water transport from stem to fruit. This paper provides an overview of micro-morphological research of the tomato fruit pedicel using various methods in the Laboratory for Functional Anatomy of Crop Plants at the Faculty of Agriculture, Belgrade University. Such an approach is important for understanding transport mechanisms as important physiological processes occurring during fruit growth.

Key words: tomato, microscopy, hydraulic conductance, xylem, phloem, fruits

INTRODUCTION

Understanding the transport of water is important for understanding growth and development of the parts of the crop important for yield. As the growth of organs such as fruits and tubers, depends on accumulation of water, it also requires coordination between the long-distance transport of water and dissolved substances by the phloem and xylem, and short-distance transport of water at the level of individual cells (Matthews & Shackel 2005). Beside the transport of water and assimilates, the complex of the vascular tissue is also involved in the transport of chemical signals, especially in drought conditions, as the concentration of phytohormones in certain organs and tissues during growth and development is usually correlated with the transport of assimilates from the site of synthesis (source) to the sites of consumption (sink). Hydraulic architecture at the whole plant level is therefore important for understanding the mechanism whereby assimilates and water are transported to the fruit, contributing to determine the nutritive value of fruit in optimal or in drought conditions.

FUNCTIONAL ANATOMY OF VASCULAR TISSUE IN FRUIT PEDICEL

Knowledge of the mechanism for transport of water and dissolved substances is necessary in understanding the processes that occur in organs and tissues during the growth and maturation of the fruit. Fruit growth of many crop plants, including tomato, depends on the accumulation of water, assimilates and ions transported through the vascular tissue of the pedicel towards the fruit (Lee 1989). Pedicels are important for tomato yield, as growth of fruits depends on the uptake of water, nutrients and assimilates, which are all transported from other parts of the plant to the fruit via the xylem and phloem tissue of the pedicel (Van Ieperen et al. 2003; Matthews & Shackel 2005). As the growth of fleshy fruits is to a great extent the result of water accumulation, for understanding of fruit developmental processes it...
is important to understand water transport as well as coordination between long distance transport of water and solutes and short distance transport, such as water absorption of the individual fruit cells (Matthews & Shackel 2005). Understanding of the vascular anatomy could, therefore, be the key factor in understanding the transport of water, assimilates and signaling molecules within the plant and consequently their effects on yield. In this paper we provide an overview of the application of various anatomical techniques and methods useful for studying the hydraulic network in plants together with data on tomato fruit pedicels that we used in our research in the Laboratory for Functional Anatomy of Crop Plants at the Faculty of Agriculture, Belgrade University.

**Use of electron microscopy in understanding vascular anatomy of tomato fruit pedicels.** Transmission electron microscopy (TEM), although time-consuming, following the procedure of fixation, post-fixation, dehydration, embedding in resins, sectioning on an ultramicrotome and contrasting with uranyl acetate and lead citrate, allows details to be observed at very high magnification on TEM (Fig. 1). TEM images allow vessels to be distinguished from other cell types, though the sample size for TEM is limited to a few mm², which makes it impossible to obtain a complete picture of much larger cross-sectional areas.

Scanning electron microscopy (SEM) allows the analysis of dry samples without any complicated sample preparation or need to prepare thin sections, especially if xylem tissue is observed. Alternatively, critical point drying or freeze drying could be used to avoid the collapse of thin-walled cells such as parenchyma or phloem cells. This method is useful for observing relatively large samples (Fig. 2-1), as well as the fine details of cell structure (Fig. 2-2).

**Studying xylem anatomy of tomato fruit pedicels using microcasting method.** The silicone microcasting technique developed by André et al. (1999) enables the investigation of relatively long segments of intact vessels and in combination with SEM enables the fine details of secondary cell walls to be investigated (e.g. Kittin et al. 2004). The microcasting technique involves the preparation of a silicon cast that infiltrates into xylem cells and polymerizes. After chemical dissolution of the actual cell walls of the xylem tissue, the silicon casts can be observed with light or SEM (Fig. 3). This method enables visualization of the inner vessel walls, vessel connections and can be used to determine vessel length distributions, though it should be emphasized that tracheids cannot be visualised using the microcasting technique (André et al. 1999; Van Ieperen et al. 2003). Therefore, this method does not allow us to accurately estimate the overall xylem hydraulic conductivity in plant parts.

**Studying xylem anatomy of tomato fruit pedicels using a maceration method.** Individual tracheids and vessel elements can also be studied by a maceration technique using light microscopy and SEM (Fig. 4). An easy and efficient method for maceration is immersing the tissue in a mixture of equal volumes of glacial acetic acid and hydrogen peroxide and moderate heating. After washing in tap water and staining i.e. in safranin, samples could be examined with a light microscope (Fig. 4-1), or samples cold be dried and, after coating, observed in SEM (Fig 4-2). The maceration technique is a very good method for measuring the length and width of vessel elements, but information about the three dimensional vessel networks becomes lost after maceration.

**Use of fluorescence microscopy in the analysis of xylem transport.** Xylem tissue plays a crucial role in long-distance water transport from roots to leaves. Water transport through plants could be investigated using eosin Y, a water-soluble xylem mobile dye which easily flows through vessels and can be used as a xylem tracer (Baum et al. 2000). After a short time of immersing the base of a freshly-sectioned stem with transpiring leaves in an aqueous solution of eosin, the stem can be cut transversally or longitudinally and sections investigated using an epifluorescent microscope (340-380nm). Lignin in the xylem in UV light shows autofluorescence and

Figure 1. Transmission electron microscopy of xylem in tomato pedicel (a-c) and section through abscission zone. a) and b) cross section, d) and e) longitudinal section, c) vessel pit, f) abscission zone, asterisk indicates separated cells, V-vessel lumen.
emits blue light, but after eosin staining the fluorescence and emitted light is yellow. As eosin flows only through functional xylem elements, comparing blue areas with yellow areas is possible to get information about number of functional and non-functional xylem elements.

We used this method to visualize the hydraulic network of tomato fruit pedicels and especially to understand better the xylem structure across the abscission zone of tomato plants (Rančić et al. 2010). Studies of eosin uptake with fluorescence microscopy in the abscission zone of tomato fruit suggested that all visible xylem on the cross sections was functional, but the large majority of xylem vessels before and after the abscission zone were not functional for water transport. According to our analysis in tomato fruit pedicels only, 15-25% xylem was functional for water transport (Fig. 5) in the two genotypes.
investigated: wild type tomato and its ABA-deficient genotype flacca. The average diameter of functional xylem elements in both genotypes was about 8-10 µm. This indicates that the hydraulic conductivity of the fruit pedicel in both genotypes is similar and the larger area of non-functional xylem in the wild type fruit pedicels compared with the ABA-deficient genotype probably has an important mechanical role to support much larger and heavier fruits. Dye uptake studies suggested also that a reduced-irrigation treatment significantly altered the number and width of functional xylem elements in the fruit pedicel, especially in the abscission zone, which indicates that drought can modify xylem architecture and thus an environmentally-produced change in the hydraulic property of the pedicel may affect development of the fruit (Rančić et al. 2010).

**Xylem anatomy and calculating hydraulic conductance.** Xylem hydraulic resistance strongly depends on the number and radii of xylem vessels along the transport path (Nobel 1983). Based on anatomical measurements, the theoretical axial xylem conductance (K) could be calculated via the Hagen-Poiseuille law, according to which the hydraulic conductivity is proportional to the fourth degree of capillary radius: \( Kh = \frac{\pi r^4}{8\eta l} \text{ m}^4 \text{MPa}^{-1} \text{s}^{-1} \), where \( Kh \) is hydraulic conductivity, \( r \) - the radius of the capillary, \( l \) - length of the capillary, \( \eta \) - the viscosity of water. If we ignore the length of tracheas, considering their lower significance, and take into account that the viscosity of water at 20°C is 1.002x10^{-3} Pa, the equation takes the form: \( Kh = \frac{\pi r^4}{8} \), enabling hydraulic conductance to be calculated only on the basis of vessel radius. According to this equation the narrower vessels are much less effective in water transport than wide ones, indicating the significant effect of several large tracheas in determining the total hydraulic conductivity of a plant organ. For example, vessels with a diameter of 40 µm have the same conductivity as 16 tracheas of diameter 20 µm, or as 256 vessels of diameter 10 µm (Tyree & Zimmermann 2002). From this equation, it is also evident that small changes in radius lead to major changes in the hydraulic conductivity, i.e. if the capillary radius decreases by a factor of two, volume flow rate will decrease by a factor of 16.

We estimated the hydraulic conductance based on the number and diameter of the water transporting xylem elements (i.e. tracheary elements, including vessels and tracheids) before and after the abscission zone in tomato fruit pedicels (Rančić 2011). According to this analysis, calculations of the hydraulic conductance based upon measurements of all xylem elements viewed in cross sections led to an overestimation of hydraulic conductivity because only a small percentage of the xylem area in tomato fruit pedicels is functional from a hydraulic point of view.

**Evaluation of phloem properties by fluorescence imaging.** Although the xylem is generally regarded as the major water supplier for growth and transpiration of organs, it is estimated that the fruit are supplied mainly by the phloem. Water and assimilate transport via the

![Figure 4](image_url)

**Figure 4.** Macerated xylem tissue of tomato fruit pedicel

4.1. Light microscopy of macerated xylem tissue. Perforated plates are arrowed

4.2. SEM microscopy of macerated xylem tissue: Pitted vessel element with single perforated plate (a) and pitted and helical vessel element (b) in secondary xylem
phloem could be visualized using an aqueous solution of the fluorescent dye 6,5-carboxyfluorescein (Xiao et al. 2009; Gillaspé et al. 1993). Carboxyfluorescein is a simplest-mobile tracer capable of long-distance phloem transport as it does not diffuse via the cell membrane into the surrounding tissue and allows identification only of phloem cells active in transport. We used carboxyfluorescein to evaluate the hydraulic properties of phloem in tomato pedicels (Rančić 2011). Dye applied to the terminal tomato leaflet penetrated through the stomata in leaf parenchyma and was transported by the phloem to other parts of the plant. A week later, fruit pedicels were cut off the plant, and tracer distribution on transverse sections of tomato fruit pedicel was determined using epifluorescence microscopy. The phloem efficiency was calculated based on data for phloem area on transverse sections of tomato fruit pedicels in the zone near the stem and data for dry fruit weight in the final stage of fruit development, as the ratio of these two values (modified according to Bertin et al. 2002). It is believed that ABA stimulates assimilate intake into the fruit and accelerates fruit maturation (Kojima et al. 1995; Buta & Spaulding 1994), possibly by increasing sugar intake by the phloem or releasing glucose from stored carbohydrates (Kojima et al. 1995). Our study showed that phloem efficiency in the final stage of fruit growth in the flacca mutant was about 30% less than in the wild type, and this could explain a fruit dry mass almost twice as large in the wild type, indicating the important role of ABA in the fruit sink activity (Rančić et al. 2010).

Water and solute transport into developing fruit - a new hypothesis. In some species (e.g. Platypuntia), the phloem is the most dominant tissue, while in other fruits such as the tomato, apple, kiwi, and grapes the amount of water uptake via the xylem decreases with fruit ageing, and the phloem will gradually become the most important transporting tissue (Bondada et al. 2005). It has been suggested that xylem reduction in the abscission zone could be the major structural cause of a switch from xylem to phloem transport in some fruits during ripening (Lee 1989; André et al. 1999). A restricted xylem connection between shoot and fruit and the consequential hydraulic isolation of the fruit from the rest of the plant could be both advantageous and disadvantageous for developing tomato fruits (Van Ieperen et al. 2003). Davies et al. (2000) suggested that xylem reduction in the abscission zone of the fruit pedicels might restrict the free movement of chemical signals from shoot to fruit. In berry fruits, xylem and phloem participate in the supply of fruit with water, but with a different amount depending on the stage of fruit development. In young fruits of some species such as some cacti (Nobel & De La Barrera 2000), phloem is the dominant source, while in other fruits such as tomato (Ho et al. 1987), apple (Lang 1990), kiwi (Dichio et al. 2003) and grape (Greenspan et al. 1994; Matthews & Shackel 2005; Keller et al. 2006), during fruit growth a transition from xylem to predominantly phloem transport occurs. In grapes, for example, xylem water is the main source of water for green berries with the phloem contributing less than 10% (Greenspan et al. 1994), while after the beginning of ripening, phloem input represents more than 80% of the total amount of water (Creasy et al. 1993; Keller et al. 2006). Transfer from xylem to phloem water transport for the ripening fruit has also been observed during tomato fruit development (Ruan & Patrick 1995). It is estimated that the tomato fruit is supplied

**Figure 5.** Use of fluorescent microscopy in the analysis of xylem transport
5-1 Autofluorescence of xylem in unstained cross section of tomato pedicel
5-2 Fluorescence of xylem after eosin staining: non-functional xylem is blue and functional xylem is yellow
mainly by the phloem from 85-95% of the total water input during development of the fruit, while water input via the xylem almost completely stopped approximately 25 days after flowering (Ehret & Ho 1986a, b; Ho et al. 1987; Van Ieperen et al. 2003).

Experiments with visualization of xylem transport in fruit pedicels of tomato and grape demonstrated that vascular elements of the xylem in the pedicel abscission zone remain intact and apparently functional during development (Bondada et al. 2005, Rancic et al. 2010). As the tomato fruit has a low transpiration (Guichard et al. 2005) due to a thick cuticle (Andrews et al. 2002) and lack of stomata (Jonson et al. 1992), only calyx transpiration has a significant impact on the xylem inflow into tomato fruit (Ehret and Ho 1986a, b; Araki et al. 1997). It is known that starch accumulates in the pericarp of tomato fruit 13-14 d after flowering, and rapid accumulation of hexose begins 23-25 d after flowering (Ruan & Patrick 1995), while at the same time fruit water intake by the xylem almost completely stops (Ehret & Ho, 1986a, b; Ho et al. 1987; Van Ieperen et al. 2003). As the hydraulic conductivity of the pedicel did not change during the period from 11 to 35 d after anthesis (Van Ieperen et al. 2003), stopping of xylem transport is probably not a consequence of reducing xylem functionality, as was previously considered. According to Rancic et al. (2010), the cessation of water xylem input into the fruit can be explained by the fact that flow through the xylem exists, but in the reverse direction. Considering that the fruit is a plant organ with a large sink activity, it is possible that the excess of water which enters the fruit by the phloem is forwarded by the xylem into the stem preventing xylem transport in the direction toward the fruit (Figure 6). In line with this hypothesis are the data of Ehret and Ho (1986a,b) and Ho et al. (1987), who reported that significant xylem transport in tomato fruit occurs only at night when the phloem transport is at a minimum (Jonson et al. 1992). As tomato fruit growth is also limited by the elasticity of cell walls of the exocarp (Matthews et al. 1987), increased phloem water intake may increase the pressure inside the fruit (Matthews et al. 1987). Restoring water by the xylem makes fruit less susceptible to plant water status (Van Ieperen et al. 2003) and can reduce their vulnerability to cracking as it serves as a overflow mechanism (Choat et al. 2009). This is especially important in cases of re-watering after prolonged exposure to drought. According to this finding, the relative importance of flow by the xylem and phloem during development of the fruit can be reversed and is probably more dependent on the sink/source relationships, and not on the physical loss of continuity or xylem conductivity. A similar conclusion was provided for berry fruit of other cultivated plants, such as vine grape (Keller et al. 2006, Choat et al. 2009) and plum (Matthews & Shackel 2005).

**CONCLUSION**

The functional anatomy of vascular tissue could be a useful tool in understanding physiological processes and the transport of water and assimilates at the whole plant level. The best approach in these investigations is using a combination of several different techniques, as each of the described methods has its advantages and disadvantages. For the fine micro-morphological details of xylem and phloem tissue, i.e. pits, perforated plates etc., SEM and TEM observations are recommended. Experiments with visualization of xylem and phloem transport are useful for investigation of the vascular tissue in whole organ sections. Estimating the theoretical hydraulic conductivity should be based on functional analysis of the xylem on larger samples, if possibly whole-organ cross sections, to minimize overestimation due to various portions of non-functional xylem elements. The area of functional phloem by itself could not be used for estimating phloem transport, as phloem functionality depends on the activity of live cells, so in estimating phloem efficiency the dry weight of fruits should also be considered. For details of the inner surface of vessels and their connections, as well as for vessel length measurements, the microcasting technique could be used, but this technique is not suitable for tracheid investigations. For investigating outer surface details, the diameter and length of single vessel elements as well as of tracheids, maceration in combination with light or SEM microscopy is recommended.

In the case of tomato fruit pedicels, cessation of xylem transport during fruit development is probably not a consequence of physical loss of continuity or reducing xylem conductivity in the tomato fruit abscission zone, as was as previously considered, but could be explained
by reversing the direction of xylem water flow, as a way for forwarding the excess of water which enters the fruit by the phloem back into the stem, to prevent fruit cracking.

Acknowledgements — This study was supported by the EU Commission (FP7 REGPOT project AREA, No 316004) and the Serbian Ministry of Education, Science and Technological Development (project TR 31005).

REFERENCES


Rančić D, Pećinar I, Pekić Quarrie S, Terzić M, Duletić Laušević S. 2011. Evaluation of phloem properties of tomato fruit pedicel by fluorescence


Prinos gajenih biljaka i rast plodova u velikoj meri zavisi od efikasnosti provodnog sistema, kao u optimalnim uslovima, tako i u uslovima vodnog deficita. Većina materija od kojih zavisi rast plodova, transportuje se u plod kroz peteljku ploda ksilemom i floemom, pa je tako anatomija peteljke važan faktor za razumevanje transporta vode i asimilata. U ovom radu dat je pregled mikromorfoloških istraživanja peteljki ploda paradajza primenom različitih metoda. Ovakav pristup je važan za razumevanje transportnih mehanizama kao važnih činilaca fizioloških procesa značajnih za rast i sazревanje ploda.

**Ključne reči:** paradajz, mikroskopija, hidraulična provodljivost, ksilem, floem, plodovi

---

**REZIME**

**Značaj funkcionalne anatomije u istraživanjima transporta vode i asimilata u plodove u razvoju**

Dragana RANČIĆ, Ilinka PEČINAR, Radenko RADOŠEVIĆ, Maja TERZIĆ, Sofija PEKIĆ QUARRIE

Prinos gajenih biljaka i rast plodova u velikoj meri zavisi od efikasnosti provodnog sistema, kao u optimalnim uslovima, tako i u uslovima vodnog deficita. Većina materija od kojih zavisi rast plodova, transportuje se u plod kroz peteljku ploda ksilemom i floemom, pa je tako anatomija peteljke važan faktor za razumevanje transporta vode i asimilata. U ovom radu dat je pregled mikromorfoloških istraživanja peteljki ploda paradajza primenom različitih metoda. Ovakav pristup je važan za razumevanje transportnih mehanizama kao važnih činilaca fizioloških procesa značajnih za rast i sazревanje ploda.

**Ključne reči:** paradajz, mikroskopija, hidraulična provodljivost, ksilem, floem, plodovi