ANALYSES OF CANE TISSUE PROFILES IN *VITIS* SPP.

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Abstract. The work was carried out with the aim of analysing the size of cane tissues of the grapevine cultivar 5BB (*Vitis berlandieri x V. riparia*) and (CS, *Vitis vinifera* L.) at the microscopic level. Cane tissues of both cultivars were assessed. Cuttings were taken at the same cane diameter of CS and 5BB during the winter period 40 days after leaf fall. The cuttings were fixed in a FAA solution for microscopy. In this study, the cultivars showed significant differences in the parameters of the vascular tissues. A strong relationship was detected between the profile parameters of different tissues (xylem vessel diameter, xylem, phloem, primary phloem fibre, xylem vessel density). The CS cultivar showed wider xylem vessel diameters than the 5BB cultivar. The relationship between xylem vessel size in CS and 5BB cultivars and tissue profiles are discussed. We suggest that the anatomical profiles of cane tissues can provide useful information for further investigations of grapevines.
Keywords: *cross-sections, cuttings, vascular tissues, grapevine*

Introduction

Histological analysis allows researchers to study the cellular structure and organisation of grapevine canes. This can reveal information about the arrangement of vascular tissues, pith, cortex, and epidermis, providing insights into plant growth and development. It can also assess the presence and distribution of carbohydrates, such as starch and sugars, within different cane tissues. This information aids in understanding carbohydrate partitioning, storage capacity, and mobilisation patterns (Zapata et al., 2004; Castro et al., 2021). Tissue profiling, along with molecular techniques, can help to identify structural or biochemical attributes associated with resistance mechanisms. For example, the presence of specific chemical compounds or structural barriers in cane tissues may provide defense against fungal infections or insect infestations (Liu et al., 2015; Bortolami et al., 2021).

Potential biomass accumulation and balance between the source and sink is regulated by the structure and function of the shoot system (Torregosa et al., 2021). Because of its commercial and agricultural interest, the genus *Vitis* has been thoroughly studied in terms of its unique hydraulic structure in its xylem and phloem structures partly associated with its liana habit (Holbrook et al., 2001; Brodersen et al., 2010, 2013). Studies on the structure and anatomy of xylem tissues have indicated a link in *Vitis* spp. to their origins (Pouzoulet et al., 2020), disease tolerance (Ramsig et al., 2021; Battiston et al., 2022), rootstock effects (Santarosa et al., 2016), grafting type (Battiston et al., 2022) and water stress responses (Lovisolo and Schubert, 1998; de Souza et al., 2022).

The goal of this study was to investigate the cane tissues by quantifying characteristics such as number, width, diameter, and density of the tissues of xylem, phloem and pith in two different *Vitis* origins.

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Materials and Method

Canes of Cabernet Sauvignon (CS, *Vitis vinifera*) and Kober 5BB (*Vitis berlandieri* x *V. riparia*) were taken at approximately the same height and diameter during the winter period (40 days after completion of leaf fall). Segments of 0.5 cm were fixed in a formaldehyde/glacial acetic acid/ethyl alcohol (FAA, 5/10/50; Sigma-104, St Louis,) solution in 50 ml brown bottles with low light transmission for analysis. Cane width was around 10 mm.

Characterization of the stem anatomy

Cane tissues were processed according to the protocol of Hacke (2015). Before preparation for cross-sections, the epidermis on the samples was cleaned under a stereo microscope (Olympus SZ61, Japan). To prevent tissues disruption and intertwining during thin the cross sectioning, the surface of the samples was covered with a tape made on epoxy embedded material. Cross-sections were taken using a classical sliding semi-automatic microtome (Reichert Jung, Germany). Samples were cut into no more than 100 µm. Observations of cane tissues were taken under a microscope (Olympus SZX7, Olympus CX41, Olympus Corp., Japan) equipped with a digital camera (Olympus LC20, Japan). LC microsoftware (Tokyo, Japan) was used for cane morphometric measurements.

Sections were stained using the revised methods of different researchers (Bond et al., 2008; Hacke, 2015). Two different staining methods were used. The first method is double stain safranin and bromophenol blue. The cross-sections washed three times with distilled water were kept in 96% ethanol at a ratio of 1/1 for 10 minutes. Then kept in dipped in 1g/L safranin solution for 20 minutes, rinsed three times in deionized water to remove excess stain, dipped in 0.75% bromophenol blue solution (with 10% glycerol and 10% acetic acid) for 25 minutes and again rinsed three times in deionized water. The second method was double stain bromophenol blue and fast green. The cross-sections were washed two times with distilled water kept in 50% ethanol for 5 minutes and then kept in dipped in 0.75% bromophenol blue solution (with 10% glycerol and 10% acetic acid) for 10 minutes and rinsed three times in deionized water for remove excess stain. Then dipped in fast green solution (0.5 g fast green in 50 ml with 100% alcohol) for 10 minutes.

Assessment in microscopy

Cross-sectional measurements of the widths of xylem, phloem and phloem fibres, vessel diameter and density, ray number and distance between the two rays, and pith width were carried out on the cane cross-sections, as described by Scholz et al. (2013). How these measurements were taken is shown in *Figures 1 and 2*. For each cultivar, at least 10 cross-sections from each stem part, then 120 from different stem parts, making approximately 600 cross-sections were prepared.

Calculations of the widths for xylem, phloem and phloem fibres along with pith were performed on at least 40 different cross-sections from each stem, then 90 from each cultivar (*Fig. 1 and 2*). Tangential vessel diamters were calculated by a minimum of 25 vessels from each section, resulting in a total of 250 vessels from each cultivar. Vessel density was calculated by 4 area of interest (AOI, *Fig. 1a, c*) per cross-section and 40 per cultivar bu counting the average number of vessels per 1 mm^2. The number of rays and distance between the two rays were measured from at least 100 times in a cultivar.

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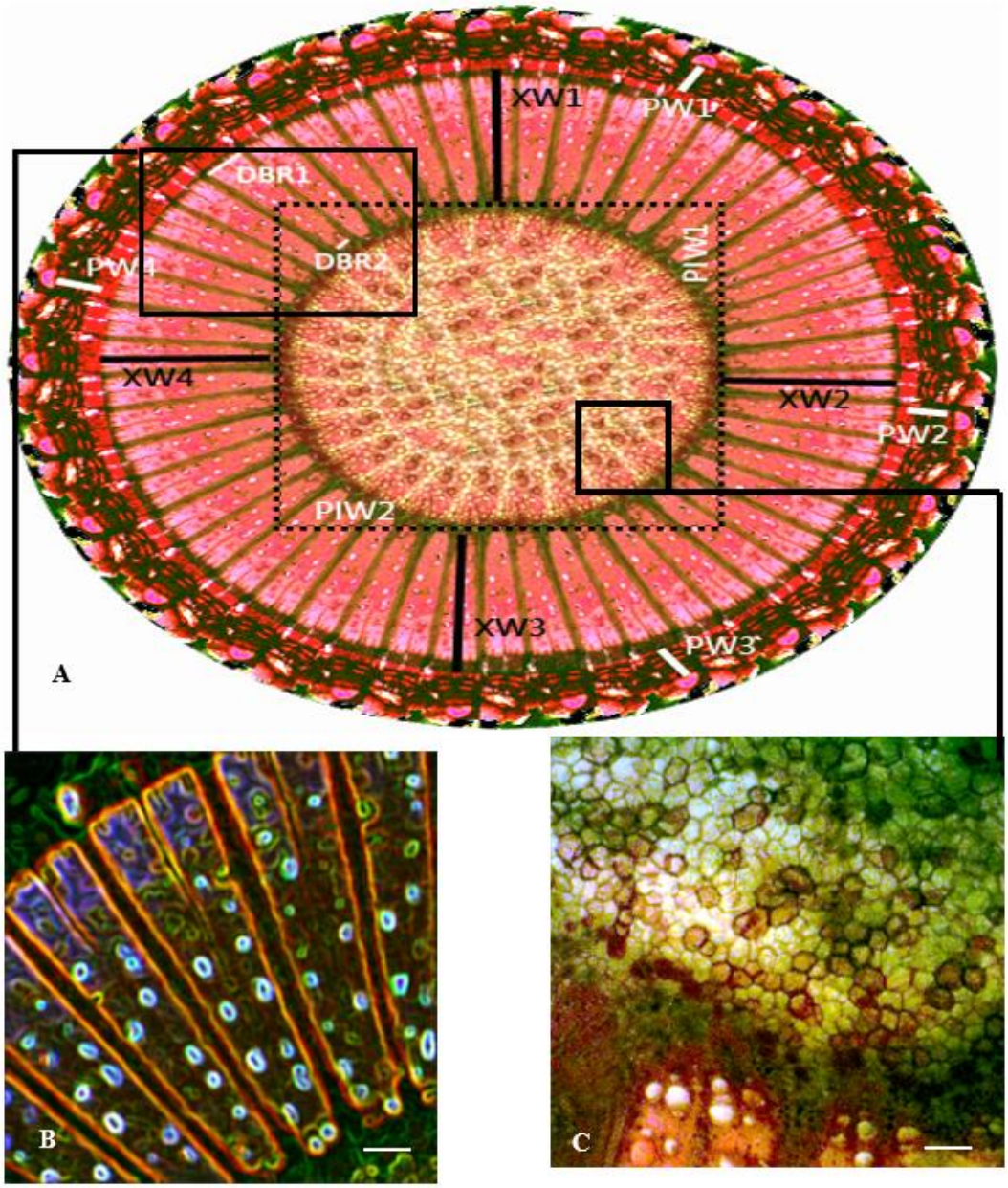

Figure 1. *Illustration of representative cane tissues of a grapevine stem. A) A nonmetric image showing how the measurements were taken from the cane. B) Enlarged view of the xylem with rays between them, C) Pith profile from cane of Vitis vinifera cv. Cabernet Sauvignon after double staining with bromophenol blue and fast green. Abbreviations; DBR, distance between xylem rays; XW, xylem width; PW, phloem width; PIW, pith width, AOI. Area of interest. Scale bar, 200 µm*

Statistical analysis

The data were evaluated by ANOVA (analysis of variance) and by multiple comparison of means, using least significant difference (LSD) test at $p < 0.05$ with the aid of Minitab.

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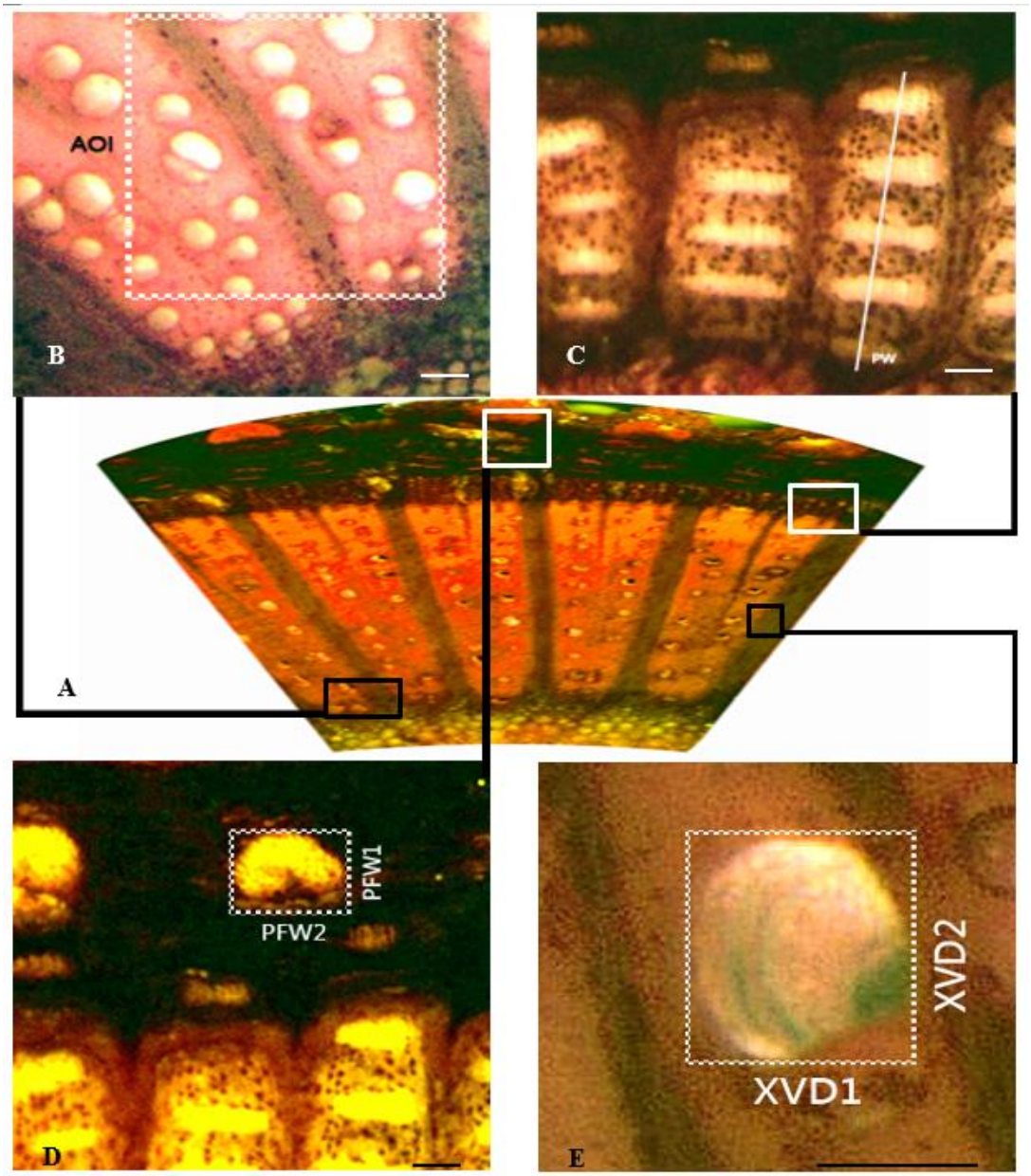

Figure 2. *Cross-sections for the measurement of cane tissues from Cabernet Sauvignon (Vitis vinifera L.). A) Nonmetric image resulting from staining treatment using the LC micro software showing anatomical characteristics from cane. B) The vessel density in the area of interest (AOI) as the total numbers of vessels per 1 mm^2. All vessels outside the AOI were excluded. C) Phloem width (PW) was calculated by measuring the arithmetic mean of four different points (PW1, PW2, PW3 and PW4) of the phloem profile. D) Phloem fiber width (PFW) was calculated by measuring the average of the minimum (PFW1) and maximum (PFW2) diameters for phloem fibers. E) Xylem vessel diameter (XVD) was calculated as the mean value of the minimum diameter (XVD1) and the maximum diameter (XVD2). Scale bar, 100 µm*

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Results

Our study allowed to detect a cultivar-spesific modifications in the anatomy of 1-year-old cane tissues. Statistical analysis showed that there were significant differences in most of the measurements taken for both '5BB' and 'CS' cultivars, except for the ray number and distance between the rays (*Table 1*). The results indicate that CS had a wider xylem with larger vessels, but lower vessel density compared to 5BB. Phloem was also larger in CS than in 5BB. On the other hand, pith in 5BB cross-sections were wider than those in CS.

Table 1. *Anatomical parameters obtained from the 1-year old cane tissues of Cabernet Sauvignon and 5BB rootstock*

Measurements of tissues profiles	**Cultivars**		
	Codes of listed anatomy profiles	**5BB (*V. berlandieri x V. riparia*)**	**Cabernet Sauvignon (*Vitis vinifera*)**
Xylem width (mm)	XW	2,20 b*	3,07 a
Xylem vessel diameter (µm)	XVD	74,53 b	94,54 a
Xylem vessel density (count/mm^2)	XVDN	39,67 a	31,01 b
Distance between rays (µm)	DBR	399 a	421 a
Ray number (count/section)	RN	34,2 a	35,8 a
Phloem width (µm)	PW	647 b	803 a
Phloem fiber width (µm)	PFW	278,3 b	342,4 a
Pith width (mm)	PIW	3,66 a	2,99 b

*Means in each column followed by the same letters are not significantly different at p< 0.0

Although a similar pattern can be observed in cane tissue development between grapevine and other woody plants, xylem, and phloem structure in *Vitis* is considered unique (Jacobsen et al., 2015). They are initiated early in the growing season and new ones are formed during most of the season (Halis et al., 2012). Because they are repeatedly formed as the season progresses, they are observed to expand and differentiate or to be alive and form secondary walls or to become hydraulically functional (Jacobsen et al., 2015). An increase in the size of xylem is naturally expected as the stem becomes thicker (Zimmermann and Jeje, 1981; Lovisolo and Schubert, 1998). In the CS canes, xylem contained wide vessels compared to 5BB canes, even though the samples were around 10 mm. It should be emphasized that several studies carried out in some species have shown that the variation in xylem vessel size within genotypes changed across different environments (Fisher et al., 2007; Palliotti et al., 2014). A high variability in both vessel diameter and vessel density between the genotypes emphasized that the vessel dimensions can be affected by cultivar characteristics. The differences observed among the grapevine cultivars agreed with data reported in previous studies (Pouzoulet et al., 2014, 2017; Gerzon et al., 2015). We observed similar vessel densities for 'Cabernet Sauvignon' by Pouzoulet et al. (2017). Quintana-Pulido et al. (2018) reported that Cabernet Sauvignon had wide vessels and 34.52 vessels per mm^2. Similar results were also observed in the work of Jelmini et al. (2021) between Chardonnay and Merlot not only in the diameter of xylem vessels buat also in the widths of phloem and xylem.

Similar to the observations of Jacobsen et al. (2015), the vessel network within grapevines, 5BB and CS, indicated some uncommon features. Large diameter vessels,

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especially in CS showed no lateral connection to other large vessels. Observation on the xylem sections suggest a tendency of an abrupt termination in the big vessels and a connection of one wide vessel to the next at the end. Brodersen et al. (2013) stated that these connections between these large vessels are made by relays of vessels that are shorter and smaller vessels, a possible indication of hydraulic function in grapevines.

Conclusion

Study of the structure and function of vasculature network in grapevines using cross-sectional profiles presents its own challenges, but also presents a potential to increase our understanding of plant physiology. Our data represent a step that can be useful for understanding the anatomical characteristics of grapevine canes. However, further interpretation and expansion of the results is still limited because environmental conditions and particularly disease contamination are known to cause considerable differences in the dimensions and functions. Continuing in research using microscopy would help scientists in making more advancement in the field of histology of grapevines.

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