

## **HISTOLOGICAL CHARACTERIZATION OF XYLEM VESSEL ON STEMS AND CAVITATION VULNERABILITY IN AVOCADO ROOTSTOCKS (*Persea americana* Mill) AND HASS VARIETY IN NURSERY PLANTS.**

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Recent anatomic studies on avocado trees (Reyes-Santa Maria *et al*, 2002) show the existence of differences in anatomy of xylem vessels among the different avocado varieties. In order to determine if these differences occur in nursery plants of Hass grafted on rootstocks of different races, the following aspects were determined through histological sections on stems: frequency of xylem vessels (vessels mm<sup>-2</sup> xylem area), average diameter of vessels (µm), relative hydraulic conductivity and vulnerability rate to cavitation that different plant materials would show. Mexicola, Nabal, Zutano and Nachlat 3 rootstocks as well as the Hass variety presented significant differences regarding frequency and average diameter of xylem vessels. Nevertheless, no differences were found among rootstocks studied in terms of relative hydraulic conductivity, which would suggest an adjustment between the diameter of vessels and their frequency. Although the results obtained are opposite to those obtained by Reyes- Santa María *et al* (2002), this could occur due to the physiological and chronologic age of the material evaluated.

## **CARACTERIZACIÓN HISTOLÓGICA DE VASOS XILEMÁTICOS A NIVEL DE TALLO Y VULNERABILIDAD A LA CAVITACIÓN EN PORTAINJERTOS DE PALTO (*Persea americana* Mill) Y LA VARIEDAD HASS EN PLANTAS DE VIVERO.**

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Recientes estudios anatómicos realizados en palto (Reyes- Santa Maria *et al*, 2002) describen la existencia de diferencias en la anatomía de vasos xilemáticos entre las distintas razas de palto. A objeto de definir si estas diferencias se presentan a nivel de plantas de vivero de Hass injertada sobre portainjertos de distintas razas, se procedió a determinar en cortes histológicos a nivel de tallo: la frecuencia de los vasos del xilema (vasos mm<sup>-2</sup> de área de xilema), el diámetro promedio de los vasos (µm), la conductividad hidráulica relativa y el índice de vulnerabilidad a la cavitación que presentarían los distintos materiales vegetales. Tanto los portainjertos Mexícola, Nabal, Zutano, Nachlat 3, como la variedad Hass presentaron diferencias significativas en cuanto a la frecuencia y diámetro promedio de vasos xilemáticos. Sin embargo, entre los portainjertos estudiados no se encontró diferencias en términos de la conductividad hidráulica relativa, lo que sugeriría un ajuste entre el diámetro de los vasos y su frecuencia. En relación al índice de vulnerabilidad a la cavitación, el máximo valor lo obtuvo el portainjerto Mexícola y el menor Nachlat 3. Si bien, los resultados obtenidos en

esta investigación son inversos a los obtenidos por Reyes- Santa Maria *et al* (2002), esto podría deberse a la edad fisiológica y cronológica del material evaluado.

Palabras clave: *Persea americana* Mill, vasos xilemáticos, portainjertos, histología, cavitación, conductividad hidráulica

## INTRODUCTION

Angiosperm plants have xylem vessels, which are responsible for long-distance transport of water and nutrients. During vascular development, single cells fuse into linear strings. After fusion and formation of a secondary cell wall, those tracheary elements lose their nucleus and cell content, forming the xylem vessel (Nijssse, 2004).

The transport of water in plants, through xylem vessels, occurs with the aid of the gradient of water potential between soil and atmosphere. According to the tension-cohesion theory, evaporation in leaves generates tensions in the nearest zones to the places of evaporation (Martinez-Vilalta and Piñol, 2003; Tyree, 1997). The high cohesion between water molecules permits that these tensions are transmitted through the entire plant and, therefore, makes transport possible (Steudle, 1995).

When the water column of the xylem experiences extremely-negative tensions, especially under water stress or frost, the cavitation phenomenon appears, a rupturing of the water column under extremely low tensions (Zimmerman, 1983; Reyes-Santamaria *et al.*, 2002).

Consequently, air bubbles introduce in the tracheid and vessel ducts, causing embolism (Tyree and Sperry, 1989; Fanvi Shen *et al.*, 2002). The introduction of air in the water column increases the resistance to its flow and triggers responses in the plant such as stomatal closing, foliage loss and sometimes may cause the death of the plant (Kavanagh and Zaerr, 1997).

The present study has the objective of determining through histological sections in stems, the frequency of xylem vessels (vessels  $\text{mm}^{-2}$  of xylem area), the average diameter of vessels ( $\mu\text{m}$ ), relative hydraulic conductivity and the vulnerability rate to cavitation which would present rootstocks of different strains and Hass variety.

## MATERIALS AND METHODS

The rootstocks used were Mexicola, Nabal, Nachalt 3 and Zutano. For the evaluation of the Hass variety, a total of 8 scions were collected from 2 adult trees of the Hass variety of the Experimental Station La Palma, Faculty of

Agricultural Sciences of Pontificia Universidad Catolica de Valparaiso during September.

The experiment was conducted through a Completely Randomized Design (CRD) with 4 replications for each rootstock.

The protocol used to obtain the samples was the following: though a transversal section in stem axis, samples of 3 cm long from the rootstock stem zone at 20 cm high and middle third of Hass scions were sectioned. Then, they were fixed in FAA solution (10 formalin: 5 glacial acetic acid: 50 ethanol), allowing a reaction for at least 48 hours to achieve a correct fixation.

The protocol for preparation of samples to be observed under microscope was carried out at the Histology laboratory of the Pontificia Universidad Catolica de Valparaiso, and consisted in making progressive alcohol dehydration (50<sup>o</sup>, 70<sup>o</sup>, 95<sup>o</sup> and 100<sup>o</sup> for 30 minutes each). Then, a paraffin embedding was applied and through a microtome, the blocks were engraved in sheets of 14 µm, making transversal sections in the axis to observe the vessels.

The dyeing of the samples was made with safranin and then they were tagged with *Fast Green*.

The samples were observed using an optical microscope (Olympus™ model BX40) equipped with a camera (Sony™ CCD-Iris model DXC-107A) and a camera adapter (Sony™ CMA-D2). The signal of the adapter was entered into a personal computer through a video capture card and WinTv™ software (Hauppauge Computer Works).

All photographs were taken with a 10x target at a resolution of 640 x 480 pixels and the image analysis was made with Scion Image™ Beta 4.02 software (Scion Corporation).

To get the measures, first the photograph scale was obtained. This associates the real dimensions of vessels in µm with photograph pixels. An object of known dimensions was chosen to be photographed to subsequently determine its length in pixels. A 250 x 250 µm cell of a Neubauer camera was used. Through the software, 100 measures of one of the cell sides were made, determining that with such image resolution and the objective of the microscope, an average of 258.1 pixels in the photograph represent 250 µm. The scale obtained was 1.032 pixels per each µm.

The frequency of the xylem vessels (mm<sup>-2</sup> vessels of xylem area) was determined through a linear function as from 16 times the mean of 80 samples of 250 x 250 µm per sample. The diameter of vessels (µm) was obtained from the mean of 150 individual vessels per sample.

The variation in the efficiency during the water conveyance was evaluated through Relative Hydraulic Conductivity (RHC) of vessels, estimated as from the Hagen-Poiseuille modified equation (Fahn *et al.*, 1986; Reyes Santamaría *et al.*, 2002):  $RHC = r^4 FV (\mu\text{m}^4 \times 10^6)$ , where “r” is the radius of the vessel measured in  $\mu\text{m}$  and “FV” is the frequency of vessels. This formula considers the pressure values inside the xylem ducts as insignificant.

The susceptibility to damages during the conveyance of water was evaluated through the Vulnerability Rate to Cavitation (IV) proposed by Reyes Santamaría *et al.* (2002), this is:  $IV = DV / FV$ , where “DV” is the diameter of vessels and “FV” their frequency.

The relative hydraulic conductivity and the vulnerability rate to cavitation were determined by an average of individual values of the 4 replications per each rootstock.

Variance analyses were conducted to determine differences between means, and if differences are detected, the Duncan Multiple Comparison Test was used.

## RESULTS AND DISCUSSION

Table 1 shows the results obtained for the different diameters evaluated in the rootstocks as to dimensions, hydraulic distribution and characteristics of xylem vessels.

Significant differences were observed in the diameter of vessels among all the rootstocks. The West Indian rootstocks “Nachlat 3” presented the vessels of smaller diameter, the Guatemalan rootstocks Nabal was medium and those Mexican showed the widest vessels, although there are significant differences between these two (Figure 1).

Significant differences were detected in the frequency of vessels of the studied rootstocks. “Nachlat 3” has the highest frequency of vessels. No significant differences were found in the frequency of vessels between “Zutano” and “Nabal” rootstocks, which have medium frequencies. Mexicola has the lowest frequency of vessels.

Table 1: Anatomical and hydraulic characteristics of xylem vessels of rootstocks of avocado trees studied

| Rootstock | Strain | Diameter ( $\mu\text{m}$ ) | Frequency (Vessels $\text{mm}^{-2}$ of xylem area) | Relative Hydraulic Conductivity ( $\mu\text{m}^4 \times 10^6$ ) | Vulnerability Rate |
|-----------|--------|----------------------------|--|---|--------------------|
| Mexicola  | M      | 48.4 d*                    | 52.8 a   | 18.3 a  | 0.93 c             |
| Nachlat 3 | A      | 38.9 a                     | 93.3 c   | 13.2 a  | 0.43 a             |
| Zutano    | M x G  | 42.9 c                     | 66.6 b   | 14.5 a  | 0.65 b             |
| Nabal     | G      | 40.4 b                     | 67.3 b   | 11.0 a  | 0.63 b             |

\*Equal letters in the same column indicate there are no statistical differences of significance of 0.05. M: Mexican, A: West Indian, G: Guatemalan, M x G: Mexican and Guatemalan hybrid.

Due to the previous analysis, there would be an inverse relation between diameter and density of vessels from the different rootstocks according to the strain they belong, as reported by Reyes Santamaría *et al* (2002). The array of strains obtained in this trial is different to that study. The relation obtained in this research is that the Mexican strain has the widest vessels and lowest frequencies, oppositely to the West Indian strain, which has the narrowest vessels and highest frequencies. The rootstocks with Guatemalan characteristics ("Nabal" and "Zutano" hybrid) would have medium anatomical characteristics.

No significant differences were detected among the rootstocks regarding relative hydraulic conductivity. This suggests an adjustment between the diameter and frequency of vessels, which would allow keeping a similar relative conductivity among rootstocks, as reported by Reyes Santamaría *et al* (2002).

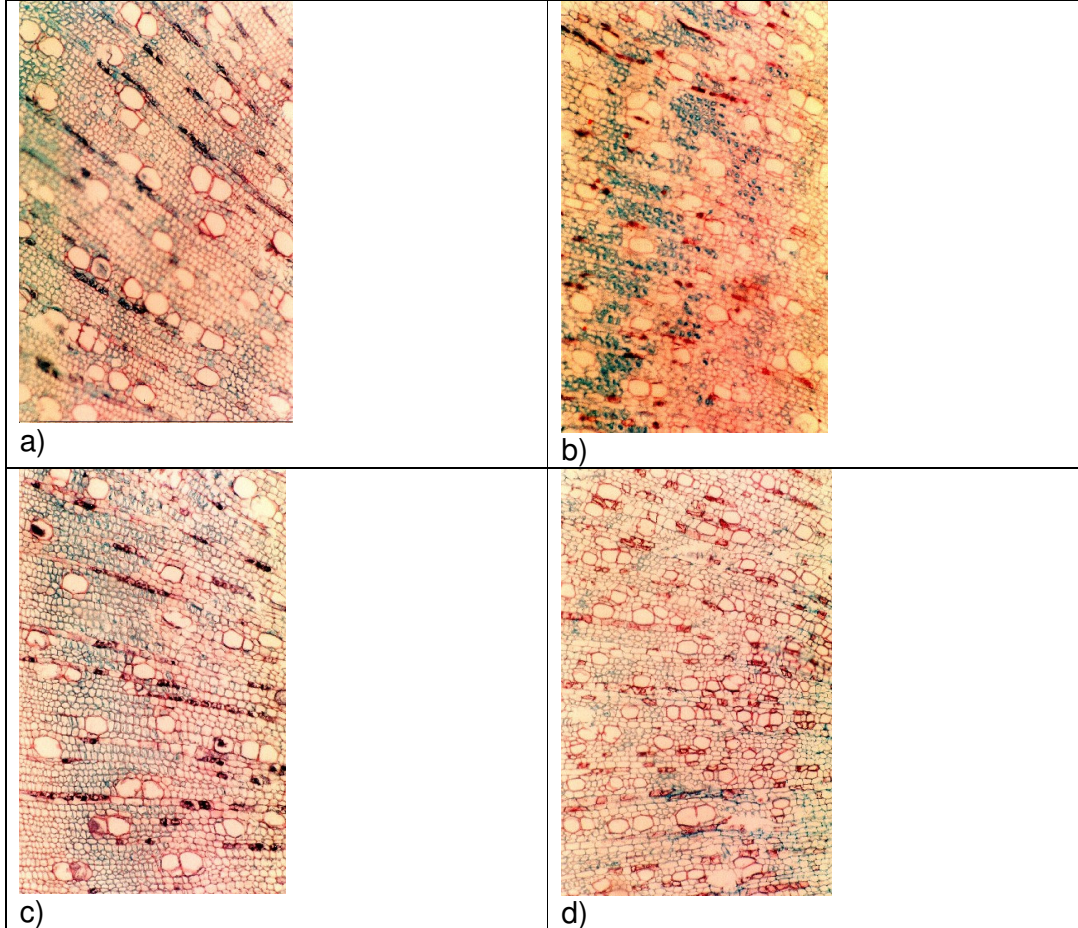


Figure 1: Microphotographs at 10 x which details the xylem vessels of plant stems of avocado rootstocks: a) Mexicola, b) Zutano, c) Nabal and d) Nachlat 3.

Significant differences were detected in the vulnerability rate to cavitation among the different rootstocks. The West Indian rootstock “Nachlat 3” stands out because of having the lowest vulnerability rate to cavitation, the rootstocks “Zutano” and “Nabal” were medium; whereas “Mexicola” obtained the highest vulnerability rate.

In addition, an analysis was conducted to compare the dimensions and distribution of xylem vessels among the different Hass rootstocks and scions. Table 2 shows the results obtained for the mean separation.

Table 2: Comparison of diameter and frequency of vessels among the different avocado rootstocks studied and Hass scions

| Plant material | Diameter ( $\mu\text{m}$ ) | Frequency (Vessels $\text{mm}^{-2}$ ) |
|----------------|----------------------------|---------------------------------------|
| Mexicola (T)   | 48.4 e*                    | 52.8 a                                |
| Nachlat 3 (T)  | 38.9 b                     | 93.3 c                                |
| Zutano (T)     | 42.9 d                     | 66.6 b                                |
| Nabal (T)      | 40.4 c                     | 67.3 b                                |
| Hass (P)       | 28.3 a                     | 163.2 d                               |

\*Equal letters in the same column indicate there are no statistical differences of significance of 0.05. (T): Stems of rootstocks of 0.5 cm in diameter, measured on 20 cm high. (P): Scions proper for grafting

Table 2 shows significant differences among all the stems of rootstocks and Hass scions regarding diameter and frequency of xylem vessels. Hass scions have narrower vessels and with greater frequency than any studied rootstock. This may occur since stem tissues of rootstocks have already hardened at 6 months of age; in contrast, the scions are still herbaceous, non-hardened tissues in which xylem is still not completely developed. The anatomical differences observed among all the rootstocks and Hass variety could have implications in the total hydraulic conductivity of the plant once grafted.

## CONCLUSIONS

Significant differences were detected among the diameters of vessels of all the studied rootstocks.

The West-Indian rootstock "Nachlat 3" showed the vessels with lowest diameter. The Guatemalan rootstocks were medium and the Mexican had the widest vessels.

Nachlat 3 has the highest frequency of vessels. Zutano and Nabal have medium frequencies and the Mexicola rootstock the lowest.

An inverse relation was detected between the diameters and densities of vessels, as proposed by Reyes Santamaría *et al.* (2002), although the array of strains is different.

No significant differences were detected among the rootstocks in relation to relative hydraulic conductivity, which suggests an adjustment between the diameter of vessels and their frequency, which would allow keeping a similar relative conductivity among rootstocks studied.

Significant differences were found in the vulnerability rate of the rootstocks. The "Nachlat 3" rootstock has the lowest vulnerability rate to cavitation. "Zutano" and "Nabal" had medium rates and "Mexicola" had the highest rate.

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