THE VASCULAR CONNECTIONS IN GRAFTED PLANTS UNDER EXAMINATION

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Abstract

Aims: Decreasing longevity of vineyards due to the increase in the infection of different grapevine trunk diseases is a growing concern, and could be related to the quality of grafting. The main aim of this study was to evaluate the use of xylem hydraulic conductivity measurements as a potential indicator for the quality of vascular connections in the graft junction of grapevine plants. For that purpose, two specific trials were carried out: (1) the quality of different batches of plants whose subjective quality was previously known from the nursery's experience was evaluated, and (2) the vascular connections between the rootstock and the scion were evaluated on a different set of plants grafted using different grafting techniques.

Methods and Results: (1) The XYL´EM conductivity meter was used to measure the specific hydraulic conductivity (K_s) of plants from six different batches whose quality was subjectively defined from the nurseryman experience. Hydraulic conductivity was measured in one-year-old dormant plants, which were kept in a cold chamber since leaving the nursery, first on the whole plant, then on the rootstock and finally at the graft junction. Results showed that two of the six batches evaluated had significantly lower values than the others. (2) After reflecting on the previous experience, we decided to perform the measurements in growing plants, for which we used different batches of plants grafted using different techniques but keeping the same scion (Airén) and the same rootstock (110 R) in all of them. The grafting techniques used were 'omega', 'full cleft', 'manual whip and tongue', 'mechanical whip and tongue', and 'V'. This time, K_s measurements were only carried out on the whole plant by determining the water flow from the scion to the rootstock. Results revealed a tendency that the *maWT, meWT and V* grafts had higher K_s values than *FC* or *OM* grafts, which corresponds to higher growth rates according to results previously collected on a field trial performed on the same type of grafted plants.

Conclusions: Hydraulic conductivity measurements may be a useful trait for the evaluation of vascular connections between the rootstock and the scion in grapevine.

Significance and Impact of the Study: To the best of our knowledge, there are no similar studies in grapevine combining hydraulics measurements with grafting techniques. Results suggest that the hydraulic functioning at the graft junction could be an interesting tool to measure the quality/quantity of vascular connections on grafted plants, and be used to characterize batches in experimentation or, even, to evaluate batches from the nurseries as a quality control.

Keywords: Grapevine, *Vitis vinifera* L., omega graft, whip and tongue, alternative graft

Introduction

The grafting of *Vitis vinifera* L. cultivars onto American rootstocks tolerant to phylloxera is a powerful and advantageous adaptation tool (Ollat *et al.,* 2016), being widely used in most of the wine-growing regions since the entry of this pest into Europe from the 1860s onwards. Since then, grafted plants have taken over new vineyards, even in free phylloxera areas around the world. Initially, grafting was carried out directly in the field, on already rooted rootstocks. However, due to the great effort required for this labour, bench machine-aided grafting became more widespread at the beginning of the 80's and it is still the most common method of propagating vine plants globally (about 99% in Europe) (Birebent, 2017). W*hip and tongue* and *full cleft* grafts were popular bench techniques at first, despite the difficulty of mechanizing them and their requirements in terms of experience and time to be successful. Thus, alternative simpler and easier to mechanize types of graft were developed, such as the *V* graft or the *omega* graft, which allow faster grafting and therefore a greater productivity in the nursery. Among them, the *omega* graft is the most successful as, contrary to *V*, it does not require the grafts to be tied one by one. These reasons have made the omega graft the most used grafting technique in the grapevine nursery sector, especially in Europe (Birebent, 2017).

At present, no one doubts the benefits and necessity of grafting. However, the *industrialization* and increase in productivity that this process has undergone in recent decades has been associated to the fact that it is usually performed by inexperienced workers, at a high pace and, sometimes, without taking care of the proper adjustment of the diameters of the rootstock and the scion, which can lead to lower quality plant production. Indeed, some experts, such as Marc Birebent, affirms that the practice of grafting leads to a decrease in longevity of the vineyards, and highlights the limitations of the omega graft (Birebent, 2017). In fact, some winegrowers are returning to field grafting upon this assumption.

Therefore, there is a growing concern in the sector about the reduced longevity of the vineyards and, among other factors, it could be related to the low quality of graft-plants produced in the nurseries. But, what does a "good quality grafted plant" mean? Despite the fact that grafting is a widely used practice all over the world, little is known about the underlying mechanisms associated to it (Pisciotta *et al.,* 2017) and there are only few scientific studies in viticulture that explored this issue (some preliminary studies can be found in Milien *et al.*, 2012; Cookson *et al.*, 2013; Canas *et al.,* 2015; Assunção *et al.,* 2016; Pisciotta *et al.,* 2017). García (2017), points out that when a nurseryman speaks about "good quality plant", he refers to three dimensions: genetic, sanitary and physiological quality. Genetic quality refers to a good choice of plant material (scion, rootstock and clone). Sanitary quality refers to the absence of harmful pathogens in the plant. And, finally, he refers to the physiological quality as the most difficult to achieve, especially considering nurseries are a self-taught sector. Physiological quality could be defined as a solid graft, a uniform callus, aligned vascular vessels, a vigorous scion shoot and a sufficient number of roots. However, achieving all these aspects is not an easy task. Indeed, evaluating the physiological quality of a grafted plant is also complex since there is no standardized procedures yet. Normally, the only quality assessment performed on grafted plants is the "thumb" test which is based on exerting pressure on the graft and discard those plants that break, and it is not always done. In view of the above, it seems evident that it is necessary to deepen our knowledge of the mechanisms underlying grafting to standardize protocols which allow us to evaluate the physiological quality of a grafted-plant.

In this sense, the main aim of this study was to evaluate the physiological quality of grapevine grafted plants based on their vascular connections by determining the xylem hydraulic conductivity measurements at the graft junction. For this purpose, two specific trials were carried out: (1) the physiological quality based on the vascular connections in one-year-old dormant plants belonging to six different scion/rootstock combination, whose subjective quality was previously known from the nursery's experience was evaluated, and (2) the vascular connections between the rootstock and the scion were evaluated on growing plants of Airén grafted onto 110 R according to five different grafting techniques.

Materials and Methods

In both trials the XYL´EM conductivity meter (Bronkhorst, Montigny-les-Cormeilles, France) was used to measure the hydraulic conductivity (K) in grafted plants. In the first one, plants from six different scion/rootstock combination were used, whose quality was subjectively defined from the nurseryman experience in relation to the success rate obtained over the years in the nursery. Measurements were made on one-year-old dormant plants, which were kept in a cold chamber since leaving the nursery until the measurements were made. The day before measurements, samples were cleaned, cut trying to make L_1, L_2 and L_3 constant between different samples

(Figure 1), buds were sealed to prevent water leaks and the embolisms removed by vacuum infiltration in order to measure the maximum hydraulic conductivity of each sample.

To perform the measurements, each sample was connected to XYL´EM and a flow of a $KCl+CaCL_2$ degassed solution was injected into the sample from above, driven only by gravity force. The average maximum hydraulic conductance (k) was recorded once the flow stabilized. Three different measurements were carried out in each sample: k_{max} of the entire samples, k_{max} of the rootstock and k_{max} of the graft junction (Figure 1 and Figure 2). To standardize the measurements, the length (L), xylem diameter, and pith diameter were measured for each part with an electronic caliper. Hydraulic conductivity was calculated as K = k * L, expressed in $Kg·m·s^{-1}·MPa^{-1}$. With external diameter and pit diameter values, Xylem Area (A_{xyl}) was calculated. Finally, specific hydraulic conductivity was obtained by: $K_s = K / A_{xyl}$, and expressed in $kg·m^{-1}·s^{-1}·MPa^{-1}$.

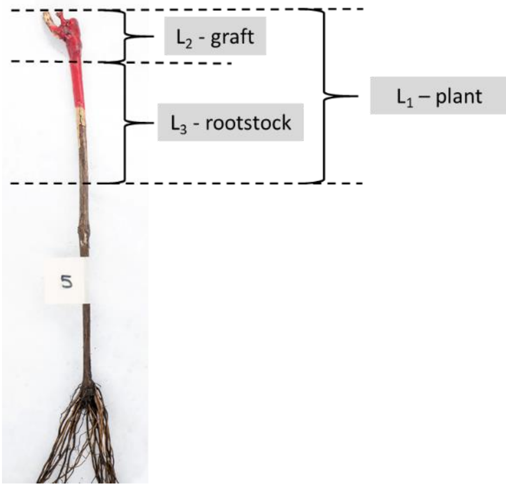

Figure 1: Diagram of the different areas of the sample where K was measured.

Figure 2: Images of the connection of each measurement zone with the XYL'EM system. From left to right: plant, rootstock, graft.

The second trial was carried out on growing plants of Airén grafted onto 110 R according to five different grafting techniques: 'omega' (OM), 'full cleft' (FC), 'manual whip and tongue' (maWT), 'mechanical whip and tongue' (meWT), and 'V' (V). Those plants were produced in a nursery *ad-hoc* for this and other trials, we could track them throughout all the production process. For this specific trial, 20 plants grafted according to each grafting technique were planted in pots in June 2019. Two shoots per plant were left, and measurements began when both shoots reached a length of at least 20 cm. Unlike the first experience, this time K_s measurements were only carried out on the whole plant by determining the water flow from the shoots to the rootstock (Figure 3).

Figure 3: Image of the connection between both shoots and the XYL'EM system.

On the day of measurements, the plant was taken to the laboratory avoiding any stress, and it was carefully cut under water to prevent air entrance into the vascular system according to Torres-Ruiz *et al.* (2015). All samples were chosen to be of similar length to minimize sample heterogeneity. Later, both shoots were connected to the XYL´EM for determining their hydraulic conductance gravimetrically. Again, the average maximum hydraulic conductance (k) was recorded once the flow stabilized. This time we measured the three lengths and the three sections (first shoot, second shoot and rootstock), and used them to estimate K_s.

Results and Discussion

Results of the first experiment (Figure 4) showed that two of the six batches evaluated (batches 4 and 6) had significantly lower values of K_s when measured in the whole plant and at the graft junction. These results agree with the empirical information offered by the nurseryman, who considered these batches of poorer quality due to the low quality of the wood prior to grafting and to the success rates obtained over the years in the nursery (empirical information provided by the nurseryman). Batches 4 and 6 also showed lower K_s values when measured at the graft junction, although there was not such a clear differentiation. Figure 4 also shows that K_s values in the whole plant and graft junction were of similar magnitude, whereas K_s values were much higher when measured only in the rootstock since there is hardly any resistance for the water to flow in that part. Therefore, as expected, the graft is the part of the plant with the greatest resistance to the passage of water, so good vascular connections in the graft area will lead to a greater movement of water from the soil.

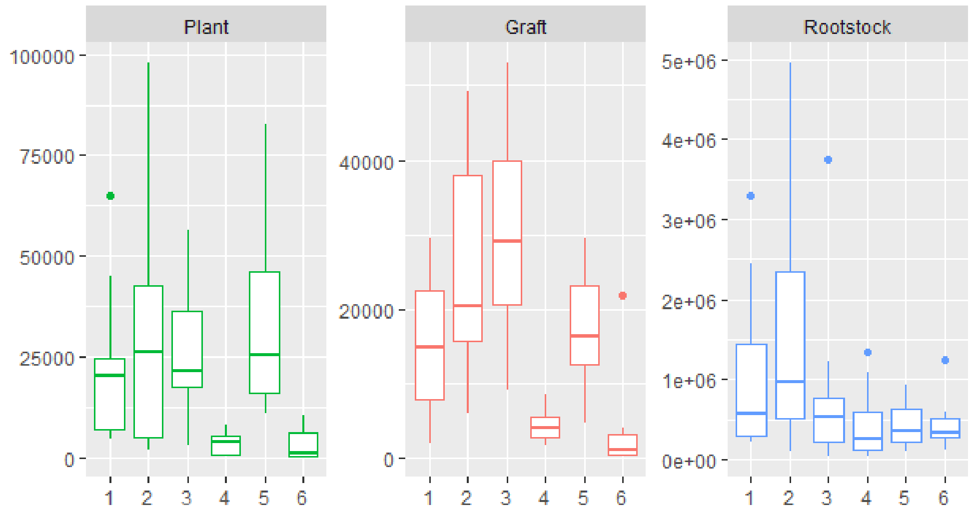

Figure 4: Boxplot with all the K_s values measured over the three different measurement areas (whole plant, graft junction and rootstock) for batches 1 to 6.

Regarding the second experience (Figure 5), results show a tendency that the *maWT, meWT* and *V* grafts had higher K_s values than *FC* or *OM* grafts. These results correspond with higher growth rates according to results previously collected on a field trial performed with plants of the same batches (data not shown in the present manuscript). Results of both experiences are preliminary as they require further statistical analysis.

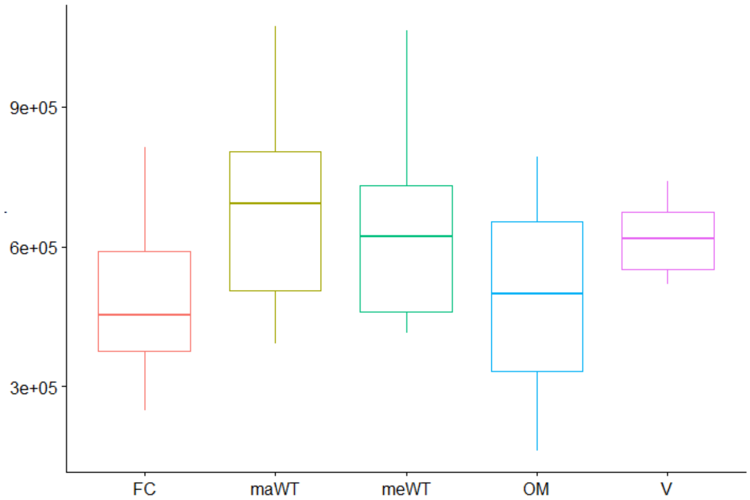

Figure 5: Boxplot of K_s values measured on the whole plant for each different grafting technique (FC = Full cleft, maWT = manual whip and tongue, meWT = mechanical whip and tongue, OM = omega, V= V).

Conclusions

The experiments described here are, to the best of our knowledge, the first approach made to compare hydraulics measurements with grafting techniques. Preliminary results suggest that the hydraulic functioning at the graft junction could be an interesting tool to measure the quality/quantity of vascular connections on grafted plants. These findings encourage us to continue work in this area, in order to be able to use this measure to characterize batches in experimentation or, even, to evaluate batches from the nurseries for quality control.

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