Effects of graft quality on growth and grapevine-water relations.

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Abstract

Climate change is challenging viticulture worldwide compromising its sustainability. Grafting *Vitis vinifera* L. (wine traditional cultivars) onto North American grapevine species or hybrids is routinely used in most grape growing areas to combat phylloxera (*Daktulosphaira vitifoliae*), since many of the American *Vitis* species are tolerant to this soil born pest. Decline of vineyard longevity might be partially explained by a decline in grafting quality in the nurseries. Omega grafting stands out as the most popular grafting method given its higher success rate in nurseries. However, the high pace of the grafting production leads to a poor-quality union of the graft point. We hypothesized that performing omega grafts of higher or lower technical quality could have implications on grapevine physiology, especially in terms of water relations. Results showed that completely aligned scion and rootstock cuttings plants had a higher rate of vegetative growth and higher gas exchange performance compared to those with partial alignments. These trends were not explained by increased hydraulic conductivity. Therefore, this study highlights the relevance of exploring the effect of the grafting quality on the grapevine water relations to identify how the changes in plant performance could help on achieving resilient plants to water stress or drought.

Introduction

Climate change is threatening viticulture worldwide and compromising its sustainability due to warmer temperatures and the increased frequency of extreme events (Jones et al., 2022). Grafting *Vitis vinifera* L. (traditional cultivars for wine production) onto North American grapevine species or hybrids is routinely used in most grape growing areas accounting for about the 80% of vineyards globally (Ollat et al., 2016). Grapevine grafting started at the end of the 19th century to fight against phylloxera (*Daktulosphaira vitifoliae*), since many of the American *Vitis* species are tolerant to this soil born pest. In nurseries, the most popular grafting method is the Omega grafting due to its higher success rate (Mary et al., 2017). However, the high pace of the grafting production sometimes leads to a poor-quality union of the graft point, with a smaller contact surface and presumably a worse connection area, compromising the phloem and xylem formation. Decline of vineyard longevity might be partially explained by a decline in grafting quality in the nurseries (Gramaje & Armengol 2011, Waite et al. 2015). We identified two levels of technical quality frequently found in nurseries (Figure 1): CA, completely aligned scion and rootstock cuttings where the scion and the cane had the same diameter and PA, partially aligned scion and rootstock cuttings where the scion and rootstock had different diameters and alignment was obtained in one of the sides. Finally, the aims of this study were to assess the implications that the connection between the scion and rootstock hardwood cuttings had on: (i) the success rate and the vegetative growth in the nursery, and (ii) the water use and hydraulic behaviour of potted vines.

Materials and methods

Plant material and grafting conditions

CA plants were obtained after a careful selection prior to grafting, so that the scion and the rootstock cane were of similar diameter, while for PA plants, one of their sides was aligned to guarantee a complete contact between the cambium of both canes with a moderate lack of diameter match intentionally sought (Figure 1). Grafting tasks were performed in Vitis Navarra nursery facilities, following the commercial protocol established in the nursery and described in Marín et al. (2022a). Briefly, one-bud (approx. 4-5 cm) hardwood dormant cuttings of Tempranillo were grafted onto 30 cm manually de-budded canes of RG8 (41 B × 110 R) rootstock, a newly developed rootstock obtained by the collaborating nursery, which has recently been authorized as commercial rootstock (Marín et al., 2022b). Then, grafted plants were placed in a callogenesis chamber for 22 days, and planted in the rooting field following a randomized complete block design after their uprooting for evaluation.

Evaluation of the success rate and growth after uprooting

Grafting success rate (SR) was determined by individually evaluating first the resistance and robustness of the graft union (Waite et al. 2015), and then the general aspect of the plant and the characteristics of their root system. First, each graft was manually subjected to the thumb test, by firmly pushing the scion with the thumb finger, successful plants were visually evaluated, classifying as unsuccessful when they had bad aspect and/or less than 3 well-developed and well-distributed roots. Finally, success rate (%) was calculated as the ratio of successful plants which were used to perform different growth measurements and part of these plants were planted in pots for three years.

Evaluation of hydraulic performance and water use

During dormancy, three-year-old CA and PA plants were uprooted and placed in 12 L pots as described in Marín et al. (2022a) for five months until two main shoots grew on each plant. After that, plants were transported to the high through-phenotyping platform at the ISVV-INRAE Bordeaux (France) to evaluate the hydraulic behaviour and water use. Plants were irrigated up to their pot capacity, allowed to drain overnight and monitored in an automated mini-lysimeter greenhouse phenotyping platform, following the procedures detailed in Dayer et al. (2020). Pots were continuously weighed on individual scales (CH15R11, OHAUS type CHAMP, Nänikon, Switzerland) and watered daily based on the plant weight loss by transpiration. Air temperature, relative humidity, and radiation conditions were automatically monitored to avoid any heat stress.

Grapevine growth, water balance data analysis and xylem hydraulic conductivity

Leaf area (LA) was estimated through the relationship obtained between the leaf midrib length and the leaf area (measured with a leaf area meter Model LI-3000, LI-COR, Lincoln, NE, USA) using to ca. 150 leaves of Tempranillo. The total leaf area per plant was estimated by measuring the leaf midrib length weekly. Daily water consumption (gH_2O vine^{-1}), transpiration per leaf area (E, mmol m^{-2} s^{-1}) and the canopy stomatal conductance (G_c, mmol m^{-2} s^{-1}) were calculated as described in Dayer et al. (2020). Water status was estimated by measuring the pre-dawn leaf water potential (Ψ_{PD}) on three plants per treatment in a basal fully expanded leaf with a Scholander pressure chamber (Precis 2000, Gradignan, France).

Plants were maintained under controlled conditions and after two months, hydraulic conductivity was measured by applying the gravimetric method proposed by Torres-Ruiz et al. (2012) in a plant portion containing the graft union. Plants were cut under water to avoid the entry of air to discard the root system and allowing a proper length in the shoots. Then, both shoots were connected via a tubing system to a tank containing a 20 mM KCl solution, and a flow was allowed to pass through the sample to a precision electronic balance (CPA225D, Sartorius, Germany) recording the weight every 5 seconds through WinWedge v3 5.0 Standard Edition TAL Technologies, at seven increasing pressures. Hydraulic conductance, k (g· s^{-1}·MPa^{-1}) was obtained by the linear coefficient of the slope generated by flux and the corresponding pressure gradient. For all the samples, the linear relationship between flux and pressure obtained were characterized by R^2>0.95. In order to standardized data, lengths of rootstock and shoot portions were measured with an electronic calliper and hydraulic conductivity (K_h, g·m·s^{-1}·MPa^{-1}) was calculated as the product between the k and L (sum of the lengths of the rootstock and the two shoots).

Statistical analysis

Statistical analysis was carried out with Rstudio (version 1.4.1103) software (RStudio Team 2021). For each data point, normality of data was confirmed and T- Student test to assess the statistical differences ($p \leq 0.05$) between the two levels of connection was applied.

Results and discussion

Figure 2a shows that CA grafts had higher success rate values, presumably because the election of material before grafting according to their diameter improved grafting productivity through the higher contact between the cambial tissues of both individuals (Cangi & Etker, 2018).

Results also showed that CA plants had a higher rate of vegetative growth (Fig. 2b) which could be explained by differences in xylem vessel development that arise when rootstock and scion cuttings are not properly aligned at the moment of grafting (Milien et al. 2012). Thus, these authors showed that a proper alignment accounted for a 3-fold increase compared to the xylem area at grafting, whereas the misalignment led to a smaller developed xylem area after using X-ray tomography observations on a single plant in the nursery stage. CA plants also had higher gas exchange performance in terms of transpiration and canopy stomatal conductance and water status (Fig. 2c-f). Similarly, Torres et al. (2021) found that increased transpiration, stomatal conductance and stem water potential were associated with the increment of leaf biomass of adult Cabernet sauvignon grapevines accounting for a higher carbon assimilation rate and intrinsic water use efficiency.

These trends were not explained by increased hydraulic conductivity at the scion level, thus, results suggested an effect of the grafting quality on the phloem formation. Thus, Gautier et al. (2019) recently pointed out that the graft interface itself is very unlikely to affect the scion development directly when connections are totally formed given that the graft union offers little resistance to water movement. Likewise, previous studies found that changes in hydraulic conductance were not explained by the xylem anatomy (Alsina et al., 2010) given that it was observed a well-integrated xylem network across the graft union despite the presence of shorter open-conduit lengths in grafted vines (Gambetta et al., 2009).

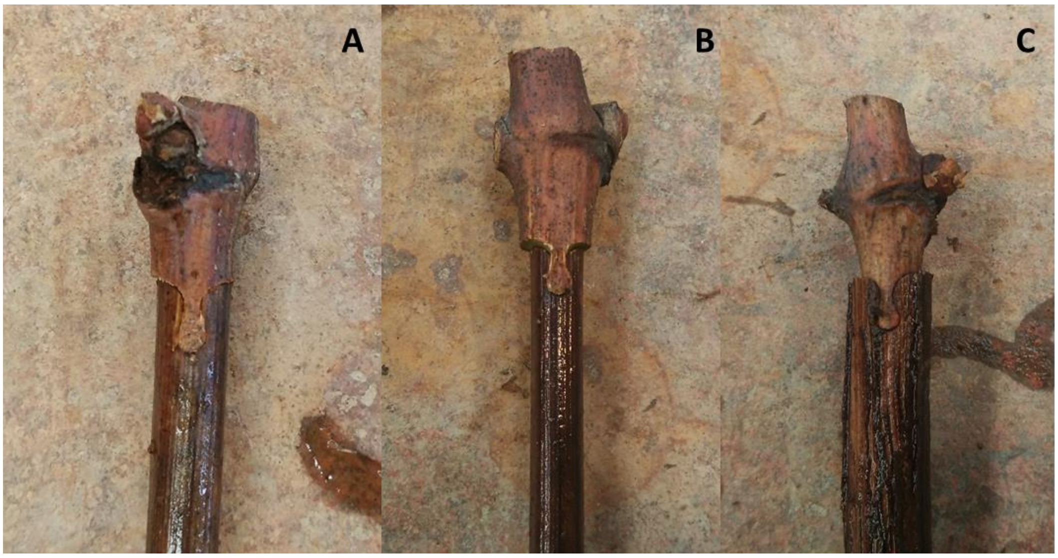

Figure 1. Examples of completely aligned scion and rootstock cuttings (CA, A), and partially aligned scion and rootstock cuttings (PA, B and C).

Conclusion

This study highlights the relevance of exploring the effect of the grafting quality on the grapevine water relations to identify how the changes in plant performance could help on achieving resilient plants to water stress or drought. Our results show that the alignment of the connection at the grafting point has a clear effect on the success rate in the nursery, which may have other negative consequences on future plant development. However, we need a better understanding of the role of vascular connections in different graft types and qualities given that the lack of alignment between the two individuals may be associated with the development of necrotic wood in the graft area. Therefore, from the growers' point of view, it would be particularly relevant to evaluate the effect of alignment the incidence of grapevine trunk diseases and vineyard decline.

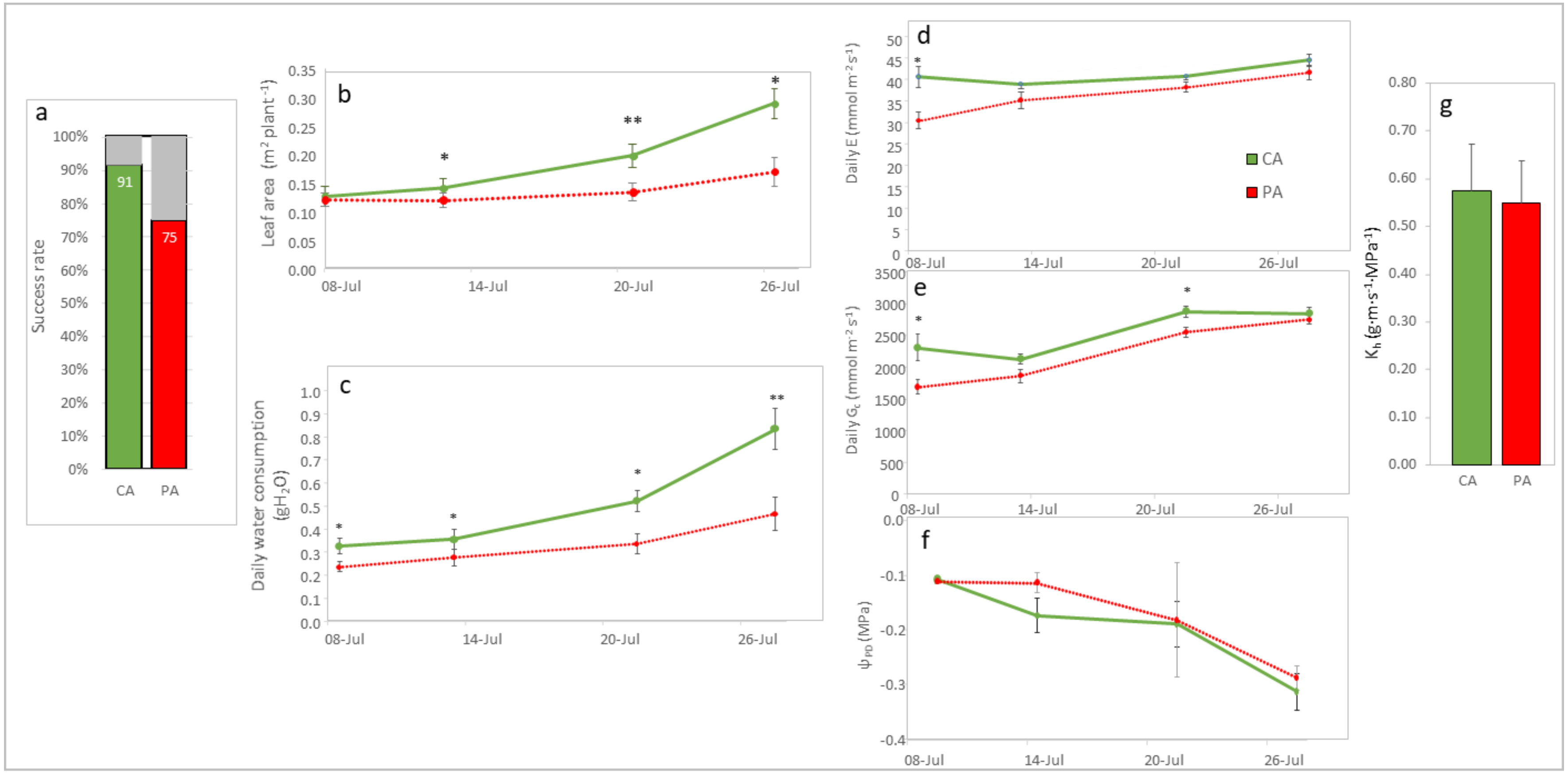

Figure 2. Success rate (%, a), Leaf area (b), Daily water consumption (c), Daily evapotranspiration (E, d), Daily canopy gas conductance (G_c, e), Water potential (ψ_{PD}, f) and Hydraulic conductivity (K_h, g) of completely aligned scion and rootstock cuttings (CA), and partially aligned scion and rootstock cuttings (PA). Values represent means ± SE (n = 5). *, and ** indicate significance at 5, and 0.5% probability levels, respectively.

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