DOI: https://doi.org/10.20870/oeno-one.2025.59.1.8305

OPEN ACCESS

ORIGINAL RESEARCH ARTICLES

Evaluation of the influence of rootstock cane characteristics on grafting success rate

Ana Villa-Llop[1, 2, 7*], Sara Crespo-Martínez[1, 2], Diana Marín[3], Nazareth Torres[1, 2], Sarah Jane Cookson[4], Grégoire Loupit[4, 5], Pierre-Olivier Bonhomme[4], Duyên Prodhomme[4], David Gramaje[6], Rebeca Bujanda[6], Javier Eraso[7], Luis Gonzaga Santesteban[1, 2]

[1] Department of Agronomy, Biotechnology and Food Science, Univ. Pública de Navarra UPNA, Pamplona, Navarra, Spain
[2] Institute for Multidisciplinary Research in Applied Biology (IMAB-UPNA), Public University of Navarre, Campus Arrosadia 31006 Pamplona, Spain
[3] Negociado de sanidad vegetal–Departamento de desarrollo rural y medio ambiente–Gobierno de Navarra, C/ González tablas 7, 31005 Pamplona, Spain
[4] EGFV, Univ. Bordeaux, Bordeaux Sciences Agro, INRAE, ISVV, F-33882, Villenave d'Ornon, Bordeaux France
[5] Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés, CNRS, Université Lyon 1, France
[6] Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas (CSIC)–Univ. de La Rioja–Gobierno de La Rioja, Ctra. LO-20 S 13, Finca La Grajera, 26071 Logroño, Spain
[7] Vitis Navarra S.A.T. Nursery, Larraga, Navarra, Spain

Article number: 8305

*correspondence:
ana.villa@unavarra.es

Associate editor:
Laurent Jean-Marie Torregrosa

Received:
14 September 2024

Accepted:
12 February 2025

Published:
10 March 2025

This article is published under the **Creative Commons licence** (CC BY 4.0).

Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.

ABSTRACT

The success of grafting in grapevine nurseries can vary significantly depending on the wood used, even if the same scion and rootstock are used, yet the underlying causes of this variation remain poorly understood. To elucidate the factors influencing grafting success, rootstock wood (*Vitis berlandieri* x *V. rupestris* cv. 110 Richter) from ten production fields–each representing different rootstock origins–in Spain and France was characterised and grafted to cv. Tempranillo, and grafting success rates were subsequently determined. Histological analysis of the rootstock canes was carried out to evaluate tissue dimensions (pith, xylem, and phloem), number of xylem vessels, and hydraulic conductivity, revealing significant differences, particularly in the size and presence of large vessels. Metabolite analysis (starch, sucrose, glucose, fructose, and proteins) and isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) further differentiated the batches. Grafting success rates were recorded after uprooting and showed positive associations with parameters of water status ($\delta^{13}C$), nitrogen assimilation ($\delta^{15}N$), and anatomical characteristics of the rootstock canes. These findings underscore the complex interaction of physiological, anatomical, and environmental factors influencing grafting outcomes in grapevines.

KEYWORDS: xylem hydraulic conductivity, carbohydrates content, graft, success rate

INTRODUCTION

The grafting of grape (*Vitis vinifera L.*) varieties onto rootstocks of other *Vitis* species became mandatory in European wine growing regions in the second half of the nineteenth century following the accidental introduction of phylloxera (*Daktulosphaira vitifoliae* Fitch) from North America. Nowadays, it is estimated that 80-85 % of vineyards worldwide use rootstocks (Smith, 2004). Successful grafting requires the establishment of effective connections between the rootstock and the scion; it is a complex process that involves the differentiation and regeneration of the tissue cells of the rootstock and the scion (Pina *et al.,* 2017), changes in the morphological and structural characteristics of the vascular system, biochemical changes (Loupit *et al.,* 2023), and even the exchange of genetic material between the rootstock and the scion (Feng *et al.,* 2024; Wang *et al.,* 2017). As a consequence, satisfactory grafting relies on skilful execution and favourable conditions (Hartmann *et al.,* 2011).

Grapevine grafting was initially carried out manually in the field, but bench grafting of dormant plants has gradually gained popularity and has become the main way of propagating grapevine worldwide. Among the existing types of bench grafting, the omega graft accounts for 95 % of total grafted grapevine plants (Mary *et al.,* 2017), and is carried out in specialised nurseries that are usually devoted solely to the production of plants of this species. The graft production process has been remarkably improved in nurseries, resulting in better quality plants and higher rates of grafting success. Remarkable progress has been made in recent years, including the improvement in hygiene and the storage conditions of plant material (Fourie & Halleen, 2004; Parisi, 2014; Waite *et al.,* 2018), the introduction of antifungal solutions at critical points of the production chain (Becker & Hiller, 1977; OIV, 2016; Rego *et al.,* 2009), and a better parametrisation of temperature and humidity conditions at the callus-formation stage (Gramaje & Armengol, 2011). However, despite all these improvements, it is common for success rates to be below expected levels and be uneven among different wood batches, leading to considerable annual economic losses for nurseries (Hunter *et al.,* 2003). The reasons for such unsatisfactory success rates are diverse and they involve interactions between several factors, such as the quality of the wood, storage conditions, inadequate callus development conditions, poor sanitary conditions (Stamp, 2001; Waite *et al.,* 2015), presence of virus and fungal trunk diseases (Fourie & Halleen, 2006; Martelli, 2017), misalignment of the grafted cane segments (Marín *et al.,* 2022), and the partial incompatibility of scion and rootstock (Canas *et al.,* 2015).

So-called "wood quality"–one of the aforementioned interacting factors–is considered by grafters and viticulturists as key to defining grafting success. Although wood quality is not an easily definable term, textbooks and practitioners agree on the fact that the cuttings selected for grapevine propagation should come from moderately vigorous canes, and canes that have been water-stressed, over-cropped, defoliated by insects, or subjected to frost or disease before the wood has fully matured to a satisfactory carbohydrate status should be avoided, as cane reserves are hypothesised to be crucial for the production of callus, root, and new tissues (Nicholas *et al.,* 1992; Vrši & Valdhuber, 2009). However, there is very little research in this regard. García (2019) reported a slightly negative correlation between wood starch content and the mortality of grafted plants in the nursery, and, in an experiment conducted in a nursery in Canada, Phillips *et al*. (2015) observed that soluble sugars in the scion cane were more closely associated to successful healing than total carbohydrates. In other woody species such as macadamia nut, grafting healing ability has also been found to be influenced by scion carbohydrate content (Almansa *et al.,* 2018). Thus, to the best of our knowledge, no research has yet evaluated the role of the characteristics of rootstock canes on grapevine grafting success, despite the rootstock representing a much higher proportion of the total biomass of grafted grapevines than the scion. Therefore, it can be hypothesised that, being the carbohydrate source, rootstock contributes more to the generation of callus and the growth of new roots and shoots. Within this context, the aim of this study was to (i) evaluate any variability in the characteristics of batches of cane from different origins, and (ii) evaluate the contribution of rootstock cane composition to grafting success. The originality of the approach used in the study lies in the fact that while the cane batches were of remarkably different origins, they all underwent grafting under the same nursery conditions.

MATERIALS AND METHODS

1. Plant material: rootstock cane batches

1.1. Origin of rootstock cane batches

Ten batches of 110 Richter rootstock (*V. berlandieri* x *V. rupestris* clone 237) woody canes were obtained from 10 mother plant production fields in 2019. Six of the ten mother fields were located in Spain and four in France (Figure 1). The cultivation system of the rootstock mother plant production fields was spread over the soil surface, and the plants were pruned as a low head-trained system ("tête de saule") at a space of 3 × 3 m. Irrigation was carried out to achieve maximum vegetative growth following the common practices used in the area. Weed management of the vine plantation was conducted by applying herbicide (glyphosate 36 %) in winter. Located in limestone-calcareous soils, the mother fields differ highly in their climate conditions, as summarised in Table 1. The Huglin Index (HI) classifies Vendée and Camiran as temperate (1800<HI<2100); Chaborra, Sansuañe-1, Sansuañe-2, Aubignan and Carcassonne as warm-temperate (2100<HI<2400); and Villena, Montesa and Albaida as warm regions (2400<HI<3000). Annual rainfall varies from 336 mm in Villena to 950 mm in Camiran. These ten cane production areas were grouped according to their agroclimatic characteristics: North Atl (N Atl), South Atl (S Atl), North Med (N Med) and South Med (S Med). These four groups are hereafter referred to as "production origins".

TABLE 1. Location of the mother fields and weather conditions at each production origin in 2019.

		Weather conditions[1]		
Production origins	Batch	Huglin Index[2]	Annual rainfall (mm)	Season rainfall[3] (mm)
N Atl	Vendée (FR)	1792	903	434
	Camiran (FR)	2155	950	469
S Atl	Chaborra (SP)	2221	535	277
	Sansuañe-1 (SP)	2307	414	227
	Sansuañe-2 (SP)	2307	414	227
N Med	Aubignan (FR)	2286	660	297
	Carcassonne (FR)	2313	551	313
S Med	Villena (SP)	2625	336	276
	Montesa (SP)	2817	602	425
	Albaida (SP)	2822	583	392

[1] Data retrieved from French (www.meteo60.fr) and Spanish (https://eportal.mapa.gob.es/websiar) national climatic websites. [2] Heliothermal index calculated according to Huglin (1978) [3] Growing season is considered as being from 1 April to 31 October.



FIGURE 1. Location of rootstock cane production origins in France and Spain (Google Inc., 2015).

1.2. Gathering and characterisation of rootstock cane batches

Rootstock cane batches were collected in all the mother fields following the same sampling protocol: ten vines arranged diagonally within the production field were selected and, from each vine, six shoots were chosen at intervals of ± 60 degrees, with the aim of covering all possible orientations and thus avoiding discrimination between shoots more or less exposed to light. In December 2019, once pruned, the material was cut to form 1 to 2 m-long canes and sent to the facilities of Vitis Navarra (Larraga, Spain). Upon reception, this material was cut to form 38-40 cm-long rootstock canes (from which buds had been manually removed), sprayed with fungicide and kept in cold storage at 4 °C (TARRE, Noain, Spain) until grafting. At the time of grafting, 15 rootstock canes from each batch were randomly sampled and used for cane size and anatomical characteristics, nutritional status and carbon and nitrogen stable isotopes ratio.

As a preliminary step, an RT-PCR analysis was carried out to evaluate the presence of the five major grapevine viruses in Europe (ArMV, GFLV, GFkV, GLRaV-1 and GLRaV -3), following the procedure detailed in Crespo-Martínez *et al*. (2023). Similarly, an analysis for the prevalence of pathogens associated with fungal trunk diseases (Petri and Black foot diseases, and *Botryosphaeria* dieback) was carried out applying the method described in Ramsing *et al*. (2021). All batches of rootstock canes proved to be free of the viruses mentioned above and only a sporadic presence of the fungal species *Cadophora luteo-olivacea* (6 %) was detected in the canes from the Montesa (S Med) and Vendée (N Atl) mother fields. Therefore, the influence of viruses or trunk disease-causing pathogens can be considered negligible. The methods for characterising the canes are described in the following subsections.

1.2.1. Cane size and anatomical characteristics

Cane size was assessed by measuring the weight (CW) of the canes with a digital scale (Sartorius BSA 822-CW, Goettingen, Germany). The cane cross-sectional area (CCSA) was estimated by measuring the diameters of both phyllotaxis planes in the cane middle-zone internode with a digital calliper (Mitutoyo CD67-S15PP, Kanagawa, Japan) (at approximately 25 cm from the base of the rootstock cane). In a first step, 5 cm segments of each rootstock cane sample were fixed in ethanol (95 %) /acetic acid 3:1 (v/v) over 24 h, and then transferred to ethanol (70 %) at 4 ˚C for conservation (Irisarri *et al.,* 2019). Subsequently, semi-thin free-hand sections of 1 mm thickness were cut with a blade and transferred into a Petri dish where they were stained for 30 seconds with 0.2 % toluidine blue O solution. Anatomical observation was carried out with a stereoscopic microscope equipped with a digital camera (Stemi 305/Axiocam ICc 3, Carl Zeiss). For each segment, xylem total area (XA) and conductive area (CA) and the distribution of xylem vessels according to their diameter, in 40 µm increments, were determined. Counts and measurements were performed using ImageJ image analysis software (v. 1.53k, National Institutes of Health, USA) and Microsoft Excel® analysis software. Image contrast and saturation were adapted to maximise the differentiation between occluded and non-occluded xylem vessels. The regions of interest were then measured (number, surface areas and diameters) using the 'Analyse Particles' command in ImageJ. Anatomical information was used to estimate the theoretical hydraulic conductivity (K_h) based on the Hagen-Poiseuille law (Scholz *et al.,* 2013).

1.2.2. Cane nutritional status

A subsample of each rootstock cane was dried and ground to assess cane nutritional status by determining starch, glucose, sucrose, and fructose. Ethanolic extraction was carried out as described by Hendriks *et al*. (2003), and sucrose, glucose and fructose were measured in the ethanolic supernatant and by HPLC (Hendriks *et al.,* 2003; Prodhomme *et al.,* 2019). Starch content was determined as follows: the ethanol extract was dissolved by heating it to 95 °C in 0.1 M NaOH for 30 min; after acidification to pH 4.9 using HCl/sodium-acetate, part of the suspension was digested overnight with amyloglucosidase and α-amylase, and the glucose content of the supernatant was then used to assess the starch content of the sample (Hendriks *et al.,* 2003). Finally, total protein content was measured using the method described in Bradford (1976).

1.2.3. Cane carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratio

Another subsample of each rootstock cane was oven-dried at 60 °C for 72 h and ground to a homogeneous fine powder. Then 2 mg of the powder was analysed for $\delta^{15}N$ and $\delta^{13}C$ using an elemental analyser (NC2500, Carlo Erba Reagents, Rodano, Italy) coupled to an isotopic mass spectrometer (Thermoquest Delta Plus, ThermoFinnigan, Bremen, Germany). The isotope ratios were expressed as parts per thousand deviations from that standard using the equations included in Santesteban *et al*. (2015).

2. Plant material: grafting procedure and evaluation of success rate

2.1. Grafting procedure

The woody canes obtained from the batches of different geographic origin were transferred to Vitis Navarra SA.T. Nursery in December 2019 and grafted on 15 April 2020. For each batch, 100 plants were grafted, using *V. Vinifera* cv. Tempranillo (clone VN69) as the scion. The grafting process was carried out following the commercial protocol used in Vitis Navarra SA.T. Nursery. In brief, all the plant material was kept in cold until grafting; then it was soaked for 24 h in water at room temperature, and one-bud dormant hardwood scions were omega-grafted to the de-budded rootstock canes. The grafted area was immediately waxed and placed in the stratification chamber at 20-25 °C and 90-95 % relative humidity for ± 20 days. Upon successful callusing, the bases of the grafted canes were soaked with rooting hormone and re-waxed before planting in the nursery on 28 April 2020. The plants grew for one season in the nursery and were dug up after leaf fall (on 23 December 2020). The climatic conditions during nursery production are detailed in Figure S1.

2.2. Grafting success rate evaluation and plant characterization

At the end of the season, the grafted plants were mechanically uprooted and transported to the nursery for evaluation. To evaluate success rate (SR) per batch of wood, all plants were individually inspected, and those not fulfilling the technical features of a successful grafted plant were discarded. The technical features considered were (i) lack of vegetative development, (ii) poor root development, and (iii) lack of resistance to the "thumb test", a manual test used to evaluate the mechanical strength of the union (Real Decreto 208/2003, 2003). Success rate was calculated as the ratio between the number of grafts fulfilling the technical criteria and the total number of grafts produced. Successful grafted plants were characterised measuring the cross-sectional area of the rootstock (RCSA) and the shoot (SCSA) using a Mitutoyo CD67-S15PP digital calliper, and the plant weight (PW) was calculated with a digital scale (Sartorius BSA 822-CW, Goettingen, Germany).

3. Data Analysis

Statistical analysis was carried out with R version 4.1.0. (R Core Team, 2020) and RStudio software (version 1.4.1103) (RStudio Team, 2021). For all trials, outliers were removed using the "identify outliers" tool from the rstatix package (Kassambara, 2022). The number of outliers removed never exceeded 5 % of the values. The effect of the cane origin on the measurements was analysed using one-way analysis of variance (ANOVA), after assessing the normality of the data with the origin as the main factor. Means ± standard errors (SE) were calculated and, when the F ratio was significant ($P \leq 0.05$), a Tukey's honest significance difference (HSD) test was executed using "agricolae" 1.2–8 R package (Mendiburu, 2021). Finally, a principal component analysis (PCA) was performed and visualised with the same software, using the "factoextra" package (Kassambara & Mundt, 2020). Pearson's correlation matrix was created with the same software using the "corrplot" package (Wei *et al.,* 2021).

RESULTS AND DISCUSSION

1. Rootstock cane characteristics

1.1. Cutting weight and cane anatomical characteristics

Three parameters related to the anatomic characteristics of the cane were measured: cutting cross-sectional area, total xylem area and conducting area. Despite the significant variability observed in the cutting cross-sectional area (55.2 to 87.5 mm^2), the weights of the canes from the ten wood production fields were similar, ranging from 23.2 to 35.2 g (Table 2). These values correspond to the standards established by European regulations for the production of grapevine plants in nurseries (Real Decreto 208/2003, 2003) and, therefore, are comparable to standard production practices in nurseries. The area of the xylem varied from 33.5 mm^2 to 55.8 mm^2, while the conducting area ranged from 6.8 mm^2 to 11.3 mm^2 and was calculated by measuring the diameter of the xylem vessels. In relative terms, the area of the xylem represented a minimum of 60 % and a maximum of 63 % of the total cross-sectional area of the cane. In light of these results, we consider the cuttings to be mature since, according to Dardeniz *et al*. (2007), well-grown and mature canes are well lignified when their piths are narrow and the xylem tissues are wide. Conducting vessels accounted for between 8 % and 20 % of the total surface area and between 13 % and 33 % of the xylem area, in agreement with the value range observed in Carlquist (1985).

The batches differed markedly in terms of the canes' anatomic characteristics: the biggest cuttings were those in the batches from Vendée and Sansuañe-1, and the smallest were those from Villena, Montesa and Albaida. Within the conductive area, Montesa and Vendée batches exhibited the highest values (11.3 and 9.7 mm^2, respectively). Nonetheless, the canes in the batch from Sansuañe-1 displayed a reduced surface area of conducting vessels (7 mm^2–13 % of the xylem area), while those from the Albaida field exhibited lower values (6.8 mm^2–20 % of the xylem area). This result highlights disparities in hydraulic conductivity, as it varied by several orders of magnitude from the minimum value obtained in cuttings from Albaida (39.3 mm^4/MPa/s) to the value reached in the cuttings from Vendée ($142.9 \cdot 10^3$ mm^4/MPa/s). Cuttings from Vendée notably exceeded all other production fields in this regard, compared to Sansuañe-1 which, despite having the highest xylem area-to-CCSA ratio (63.7), had smaller vessels and thus a lower hydraulic conductivity value. This significant effect is associated with differences in the density and diameter of the xylem vessels. The fact that vessels developed under different environmental conditions and practices would explain this difference in xylem anatomy traits (Pouzoulet *et al.,* 2020).

1.2. Cane hydraulic characteristics

The counting and analysis of all the vessels revealed a significantly different vessel distribution between the batches from the production fields considered (Figure 2). The diameters measured in our work ranged from 0-40 µm to 280 µm.

Although for all the batches > 35 % of the xylem vessels were found in the range of 0-40 µm, the canes from Albaida stood out with 48.5 % within this range, while in those from Chaborra it was 38 % (Figure 2A). This size distribution agrees with the general anatomical structure of many vines and climbers, which exhibit a significant presence of narrow vessel elements alongside some vessels with large diameters (Carlquist, 1985; Peccoux, 2011; Schultz & Matthews, 1993). When the hydraulic conductivity values were estimated from vessel number and diameters (Figure 2B), the batches with the highest proportion of large vessels were found to also have the highest conductivity (Sperry *et al.,* 2006): although most of the vessels (85.4 %) were small, they only contributed to 28 % of total hydraulic conductivity; meanwhile, the largest diameter categories (> 160 µm) were responsible for 70 % of conductivity, despite numerically representing only 12 % of the total vessels. A similar phenomenon of dominance by wide vessels in terms of total hydraulic conductivity has also

TABLE 2. Cutting weight and cane anatomical characteristics of 110R rootstock batches of ten different geographical origins. Values represent means (n=15).

Batch	Cutting weight (g)	Cane cross-sectional area (mm^2)	Xylem area (mm^2)	Conductive area (mm^2)	X/CCSA	Hydraulic conductivity ($mm^4/MPa/s$)
Vendée	32.7 a	76.7	48.3	9.7 ab	63.0 ab	142.9 · 10^3 a
Camiran	26.4 bcd	59.0	36.1	8.1 bc	61.2 ab	54.9 c
Chaborra	28.8 b	66.8	41.5	7.9 bc	62.1 ab	56.6 bc
Sansuañe-1	35.2 a	87.5	55.8	7.0 c	63.7 a	43.3 c
Sansuañe-2	25.5 cdef	58.2	35.6	7.2 c	61.1 ab	45.8 c
Aubignan	27.0 bc	62.8	38.7	8.7 bc	61.7 ab	56.5 bc
Carcassonne	26.1 cde	64.4	39.9	8.3 bc	61.9 ab	62.3 bc
Villena	24.1 def	56.3	34.3	8.5 bc	60.9 b	56.8 bc
Montesa	23.2 f	56.2	34.2	11.3 a	60.9 b	101.2 b
Albaida	23.8 ef	55.2	33.5	6.8 c	60.7 b	39.3 c
P. value	< 0.001	n.s.	n.s.	< 0.001	0.0152	< 0.001

Within each column, different letters indicate significant differences (Tukey test; $p > 0.05$).

been recorded in other species and in stems of grapevine and *V. riparia* (Hargrave *et al.,* 1994; Lobos-Catalán & Jiménez-Castillo, 2023; Munitz *et al.,* 2018; Tibbetts & Ewers, 2000).

Regarding the diameter and frequency of the xylem vessels (Figure 2A), the canes from Sansuañe-1 and Albaida showed a higher proportion of small vessels (91.2 and 90.6 % of 0-160 µm, respectively) than the batch from Vendée (78.2 %). It is important to note that, except for Vendée, the rootstock canes analysed here did not exhibit the bimodal distribution of vessel size that is typical of vines and climbers (Carlquist, 1985), and is characterised by numerous small vessels and numerous large vessels, with a decrease in the frequency of medium-sized vessels (60-140 µm).

Regarding the distribution of conductivity by xylem vessel size (Figure 2B), the Sansuae-1 and Albaida batches showed a higher proportion of conductivity associated to the small vessels (37.1 and 45.7 % respectively for vessel diameter in the 0-160 µm range) than the batch from Vendée (12.7 %). By contrast, the conductivity associated to the large vessels was lower in the former than in the latter batches (59 and 54 % vs. 87.2 % for 160 to 400 µm-diameter vessels). On average, conductivity peaked at a single point between the diameters of 160 and 200 µm. By contrast, the canes from Vendée reached their maximum percentage of conductivity at around 200 to 240 µm, which was due to their higher percentage of vessels within that diameter range compared to the other batches. It should be noted that cuttings from the same *V. berlandieri* x *V. rupestris* cross acquire very different structural and physiological traits when grown under different weather conditions and soils. The proportions of the different xylem vessel sizes can affect the sap flow pattern (McElrone *et al.,* 2021; Tyree & Zimmermann, 2002) and can be explained by factors such as salinity (Quintana-Pulido *et al.,* 2018), different growing conditions (Olson & Rosell, 2013), radiation intensity, shoot orientation, and water stress or water deficit (Lovisolo *et al.,* 2010), the latter being related to a considerable reduction in the average vessel size (Pire *et al.,* 2007). However, while stressful habitats have been reported to induce changes in xylem development in roots (Rajaei *et al.,* 2013) and stems (Lovisolo & Schubert, 1998) of *V. vinifera,* to date, no such data is available for *Vitis* sp. rootstocks. As already mentioned, the batches of cuttings from the "drier" climatic zones (*i.e.*, Albaida) had a higher percentage of narrow vessels than those from the "wetter" areas (*i.e.*, Vendée). Additionally, the high-water availability at the beginning of the growing season in Vendée may have led to vigorous vegetative growth with wider vessels and higher hydraulic conductivity, as observed by Munitz *et al*. (2018) and Netzer *et al*. (2019) for Merlot and Cabernet-Sauvignon.

1.3. Cane nutritional status and isotope composition

Starch accounted for the majority of carbohydrate reserve compounds measured in the canes in all the analysed batches, followed by sucrose, glucose, and fructose in that order (Table 3). Starch concentration values varied from 159 mg/g to over 200 mg/g depending on the sample. These values are within the range reported by Phillips *et al*. (2015), and correspond to a slightly broader range than the ones observed by Hunter *et al*. (2003) and Somkuwar *et al*. (2017). Total soluble sugar concentrations ranged from 32.6 to 52.1 mg/g and accounted for an average of around 20 % of the total CHO, which is very similar to the percentage reported in Phillips *et al*. (2015). The different soluble sugar concentrations were as follows: 27.8 mg/g sucrose, 8.4 mg/g glucose, and 8.2 mg/g fructose. Sucrose represented, on average, 69 % of the soluble sugars. The total CHOs content in the samples varied from 194 to 264 mg/g. Consistent with the findings of Phillips *et al.* (2015); Wample and Bary (1992); Winkler and Williams (1945), while high levels of total

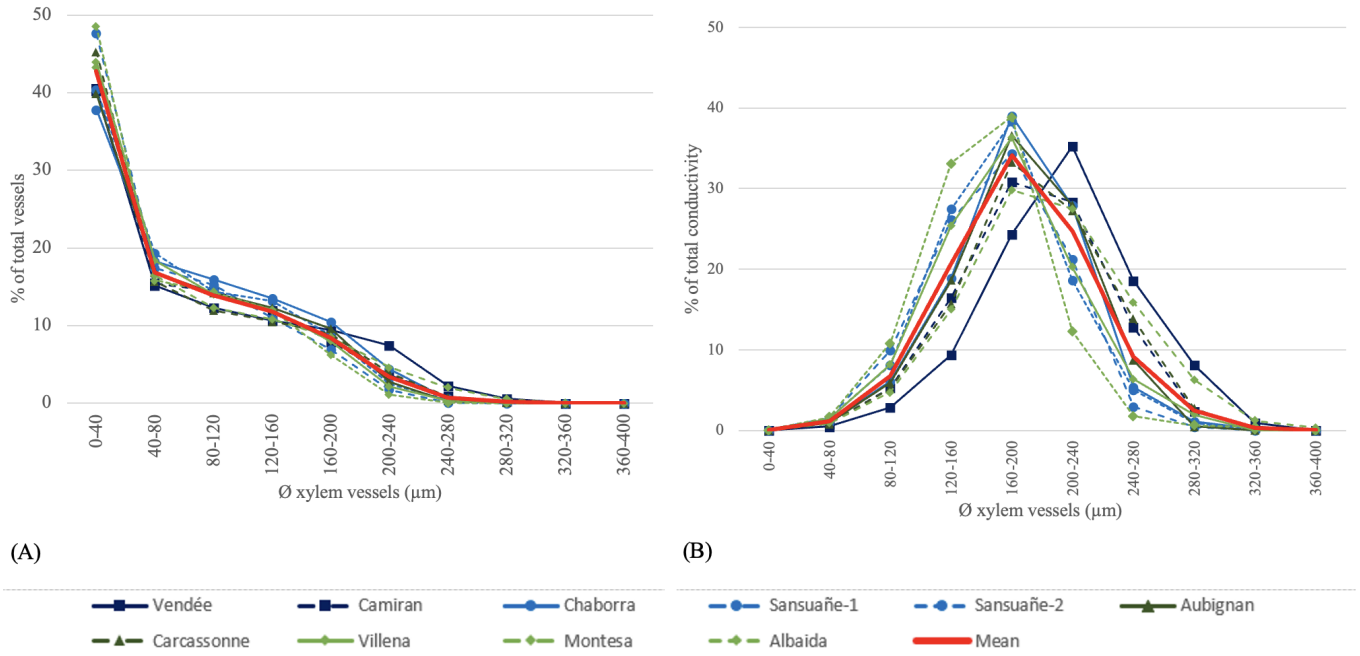


FIGURE 2. Hydraulic characteristics of 110Richter rootstock batches from ten different geographical origins. Distribution of cane (A) xylem vessels and (B) hydraulic conductivity according to diameter classes (µm).

CHO were associated with high starch concentrations, there was a weak relationship between soluble sugars and total CHO. Significant differences in carbohydrate concentrations were observed among production fields. The highest starch concentration was found in the batch from Villena, followed by those from Sansuañe-1 and Montesa, all of which exhibited above-average starch values (172.4 mg/g). By contrast, the batches from Aubignan and Albaida had notably low starch content, with values of 159.1 and 160.4 mg/g, respectively. This trend was consistent for the other analyzed sugars, except in Sansuañe-1, which showed values below the average for glucose, fructose, and sucrose. In contrast, Albaida had above-average values for these parameters.

The range of the soluble sugars to starch ratio was between 0.19 and 0.37, which was lower than that reported by Todic *et al*. (2005) (0.30 to 0.55), and therefore corresponds to high cane maturity and conservation status. The sucrose concentration positively affected this parameter, with higher for the batch from Albaida (0.37) and lower ratios for Sansuañe-1 and Sansuañe-2 (0.19 and 0.21, respectively). This indicates that the Sansuañe-1 and Sansuañe-2 rootstock canes exhibited a higher degree of wood maturation than Albaida, as their soluble sugar content was 1.5 times higher, especially the sucrose content.

The protein content differed among the batches, ranging from 4.8 to 8.6 mg/g (Villena and Sansuañe-2, respectively). The protein content of each of the batches from Villena, Albaida and Aubignan was below the average value, while those of the other batches exceeded a mean of 7.13 mg/g. These values are much lower than those reported by Somkuwar *et al*. (2017) for 110 Richter rootstock canes in India, but the reason for this difference is unknown to us.

The variability of the nitrogen isotope ratio was also high, with values ranging between -0.6 and 5.2 ‰ and with a mean of 2.1 ‰, which are similar to those achieved in Tempranillo canes (Santesteban *et al.,* 2024). Interpreting $\delta\ ^{15}N$ values is complex, because plant $\delta\ ^{15}N$ values reflect various interactions between nitrogen sources and isotope fractionations that vary over time and across different compartments (Stamatiadis *et al.,* 2007). A likely explanation for the high $\delta\ ^{15}N$ values in the canes of the Chaborra, Camiran and Aubignan batches (5.22, 4.84 and 4.62 ‰, respectively) was that the plants had relied more on organic sources of nitrogen for their nutrition (Bateman & Kelly, 2007). In contrast to the latter canes, the values of those from the Villena, Carcassonne, Albaida, and Sansuañe-2 batches were below 0 ‰. This may indicate that an increase in available inorganic nitrogen made plants more dependent on inorganic sources, resulting in lower $\delta\ ^{15}N$ values. Additionally, the soils in these regions may have had low organic matter content, limiting the availability of organic nitrogen (Santesteban *et al.,* 2024).

Canes are not usually considered a sensitive organ to detect differences in water status though the measurement of the carbon isotope ratio, since they are majorly formed before water deficit arises (Santesteban *et al.,* 2014). Thus, samples from vegetative tissues (roots and wood) typically exhibit a ^{13}C isotope content lower than that in berries (Taskos *et al.,* 2020; Zyakun *et al.,* 2013). In our study, there were relevant differences in the isotope ratio of ^{13}C among the production fields, for which the values ranged between -25.3 and -27.6 ‰ (Table 3), which represent a significant range of variation that should correspond, in our case, to differences in the water status of the production fields. ^{13}C isotope values of close to and above -26 ‰ were reported for the batches

TABLE 3. Starch, soluble sugars (mg/g of dry material), total carbohydrates (CHOs), soluble sugar-to-starch ratio, proteins (mg/g of dry material) and nitrogen and carbon isotope ratios (‰) of 110 Richter rootstock batches from ten different geographical origins. Values represent means (n=15) ± SEM.

Batch	Starch	Glucose	Fructose	Sucrose	Soluble sugars	Total CHOs	S.S./Starch	Proteins	$\delta^{15}N$	$\delta^{13}C$
Vendée	168.5 ab	7.1 b	9.24	27.5	37.1	205.6 b	0.23 ab	7.54 ab	2.36 d	-27.6 e
Camiran	167.0 ab	8.7 ab	8.73	32.8	50.2	217.3 ab	0.32 ab	7.37 ab	4.84 ab	-25.2 a
Chaborra	163.7 ab	9.2 ab	8.09	24.3	38.4	202.1 b	0.25 ab	7.39 ab	5.22 a	-26.5 bc
Sansuañe-1	181.8 ab	6.3 b	4.54	25.2	33.3	215.1 ab	0.19 b	7.32 ab	3.68 c	-26.6 bcd
Sansuañe-2	167.0 ab	7.7 ab	8.97	22.6	32.6	199.6 b	0.21 b	8.60 a	-0.58 g	-27.3 de
Aubignan	159.1 b	6.4 b	6.64	24.7	34.8	193.9 b	0.24 ab	6.61 abc	4.62 b	-26.0 b
Carcassonne	167.0 ab	7.9 ab	12.26	22.8	33.7	200.7 b	0.22 ab	7.74 ab	-0.07 fg	-26.1 b
Villena	213.8 a	9.8 ab	7.40	33.9	50.1	263.9 a	0.24 ab	4.98 c	0.02 f	-26.9 cde
Montesa	176.4 ab	12.7 a	5.37	26.5	38.8	215.3 ab	0.23 ab	7.54 ab	1.38 e	-27.2 cde
Albaida	160.4 b	8.9 ab	11.09	38.0	52.1	212.5 ab	0.37 a	6.18 bc	-0.30 fg	-27.5 e
P. value	0.0365	0.0175	n.s.	n.s.	n.s.	0.0068	0.083	< 0.001	< 0.001	< 0.001

Within each column, the different letters indicate significant differences (Tukey test; $p > 0.05$).

from Camiran, Aubignan and Carcassonne, which indicates lower water availability during the growing season, while the values of the batches from Vendée, Albaida, Sansuañe-2 and Montesa (< -27 ‰) correspond to conditions of less water stress (Taskos *et al.,* 2020). These differences in water status probably resulted from a combination of weather conditions and the use of irrigation, since the least stressful conditions are not only associated with the rainier area of Vendée, but also with areas with the driest climates, where irrigation is systematically used to ensure wood production.

On the whole, the range of the cane nutritional status is relatively broad. The reasons behind this diversity cannot be determined with our dataset, but they may be partly due to the variability in meteorological conditions of the mother field sites or to differences in soil composition, fertilisation and irrigation management (Bianchi *et al.,* 2020). The differences observed for both carbon and nitrogen isotope ratios support the hypothesis of relevant differences in the growing conditions of the mother vines.

1.4. Correlation between rootstock cane characteristics

Correlations between rootstock cane characteristics were assessed using a correlation heatmap (Figure 3). Regarding the relationships between meteorological parameters (Huglin Index (HI) and rainfall) and cane characteristics, HI showed a significant positive correlation with cane glucose content. Albeit not statistically significant, the same trend was found for the other measured sugars, except for fructose. By contrast, a negative significant relationship was observed between HI and the cutting size, estimated as cutting weight (CW). Our dataset did not allow us to find a relationship between rainfall and cane characteristics. Although wetter conditions can lead to a higher sugar concentration and a higher sugar-to-starch ratio (Liu *et al.,* 2018) and promote the formation of wider vessels (Tyree & Zimmermann, 2002), the interaction of irrigation with rainfall probably prevented us from finding any such relationship.

Regarding the cane anatomical characteristics, the cutting weight (CW), cane cross-sectional area (CCSA), and xylem area (XA) were positively correlated. As expected, given that vessel diameter is a key determinant in the Hagen-Poiseuille equation for assessing theoretical hydraulic conductance (Tombesi *et al.,* 2010), high conductivity values were associated with a high proportion of wider vessels (> 120 µm).

When cane nutritional variables were compared, significant positive correlations were observed between starch and total carbohydrates. A similar trend was observed between sucrose and soluble sugar content, as well as between sucrose and the ratio of soluble sugars to starch. Cane protein content exhibited a negative correlation with all measured carbohydrates, notably with sucrose content, soluble sugars, and total carbohydrates. Regarding nitrogen and carbon isotopic ratios, no significant correlation was observed.

An overall comparison of all rootstock cane traits revealed that a superior conductive area (CA) is conducive to the development of larger conducting vessels (VD > 120 µm) and improves hydraulic conductivity. Lastly, improvements in anatomical characteristics seem to reduce the glucose content in the propagation wood, with a significant interaction with the cutting weight (CW).

2. Grafting success and vegetative growth after uprooting

An average success rate of 85 % was obtained in the trial, the highest graft success being recorded for the material from Chaborra (91.5 %), and the lowest for the batch from Albaida (76.3 %) (Table 4). The high graft survival rate observed in this study, which surpasses the typical success rate of 61 % in

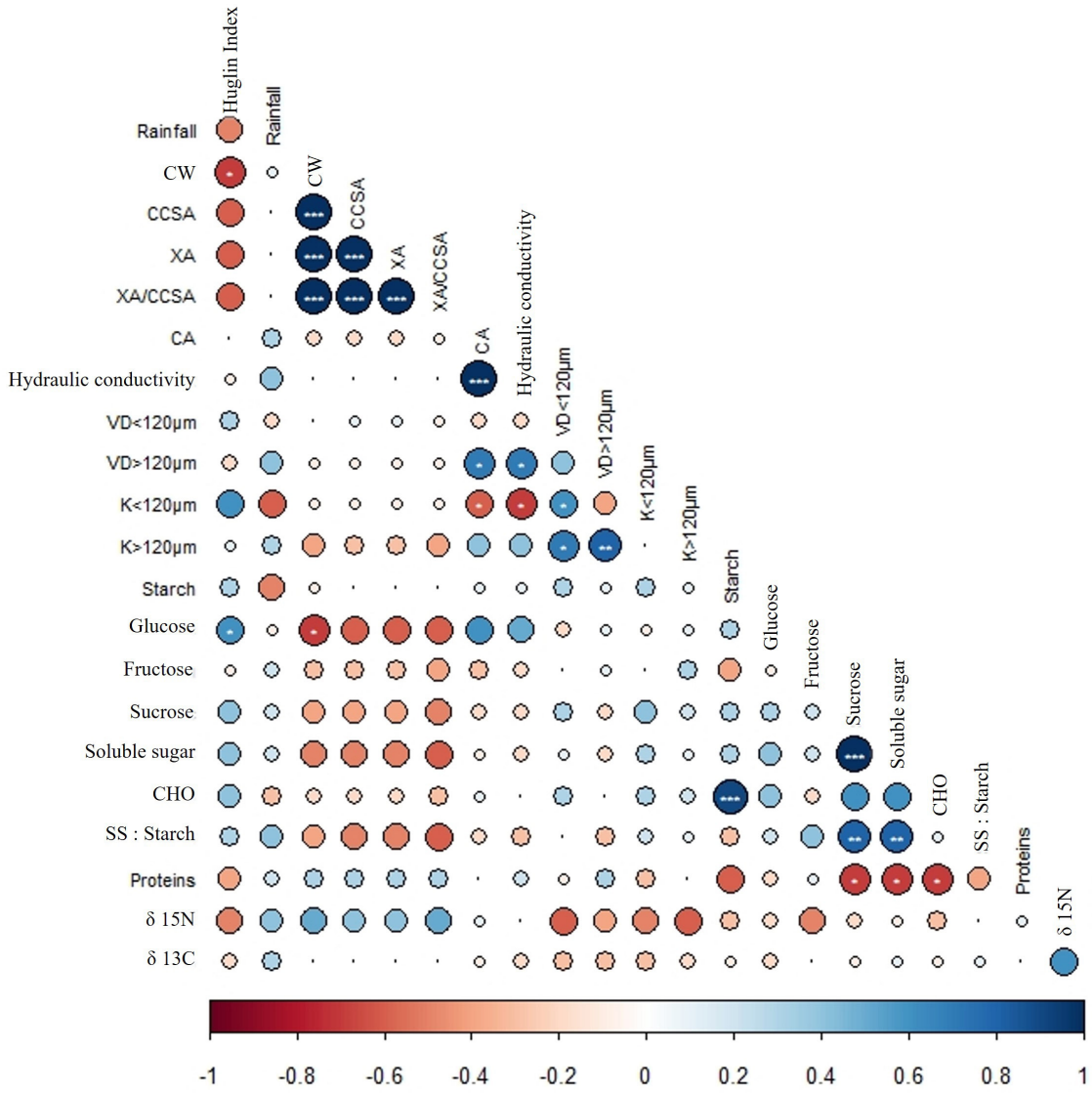


FIGURE 3. Correlation matrix of all the variables measured in 110R rootstock batches from ten different geographical origins. Circle size and colour represent R values for Pearson's correlation analysis *, ** and *** indicate significance at probability levels of 0.05, 0.01 and 0.001, respectively.

a commercial nursery (Waite *et al.,* 2015), can be attributed to the meticulous execution of the entire production process of the grafted plants. This careful approach likely facilitated superior and earlier graft union formation, potentially due to enhanced contact between the cambial layers of the rootstock and the scion (Verma *et al.,* 2018). The geographic origin of the rootstock canes appeared to affect the nursery success rate. The canes of northern and southern Atlantic origins showed notably high success rates, exceeding 87 %. Conversely, the canes of northern and southern Mediterranean origins exhibited lower average success rates of 83 % and 81 %, respectively.

Plant weight (PW) was significantly higher in the batches from Sansuañe-1, Sansuañe-2, Chaborra, and Vendée, all exceeding an average weight of 69 g, while that of Villena was the lowest at 61 g. As expected, plant weight was directly influenced by initial cutting weight (CW), as well as by rootstock growth (RCSA). RCSA values were above the average of 82.2 mm^2 in Sansuañe-1, Chaborra, and Vendée, while in Villena they fell below this average, being 68.6 mm^2. However, this pattern was not reflected in shoot development (SCSA), except in Vendée. The grafted plants from the Albaida, Montesa, and Vendée batches showed higher vegetative areas (26.1, 24.7 and 24.6 mm^2, respectively) than those developed from the batches from Aubignan and Carcassonne (19.6 and 18.5 mm^2, respectively).

3. Evaluation of the influence of rootstock cane characteristics on grafting success and growth

The representation of the grafting success ratio, rootstock cross-sectional area, shoot development, and plant weight, along with the principal component projection of all variables measured in the cane samples, facilitated the identification of relationships between them (Figure 4). Two main components

TABLE 4. Evaluation in the nursery of the success rate and vegetative growth variables measured in Tempranillo cv. plants grafted onto the rootstock 110 R from ten different geographical origins. Values represent means ± SEM.

Production origins	Batch	Succes rate (%)	Plant weight (g)	Rootstock cross-sectional area (mm^2)	Shoot cross-sectional area (mm^2)
N Atl	Vendée	85.6 ab	72.8 ab	98.8 ab	24.6 ab
	Camiran	89.1 ab	65.7 bc	89.6 bc	20.4 bc
S Atl	Chaborra	91.5 a	73.4 ab	87.3 c	20.2 c
	Sansuañe-1	86.0 ab	76.4 a	101.5 a	21.7 abc
	Sansuañe-2	87.1 ab	74.5 ab	75.8 def	22.9 abc
N Med	Aubignan	83.0 ab	68.5 abc	81.0 cd	19.6 c
	Carcassonne	83.7 ab	70.0 abc	78.0 de	18.5 c
S Med	Villena	87.9 ab	61.4 c	68.6 f	22.7 abc
	Montesa	80.1 ab	66.3 abc	69.2 ef	24.7 ab
	Albaida	76.3 b	67.4 abc	72.3 def	26.1 a
P. value		< 0.001	< 0.001	< 0.001	< 0.001

Within each column, different letters indicate significant differences (Tukey test; $p > 0.05$).

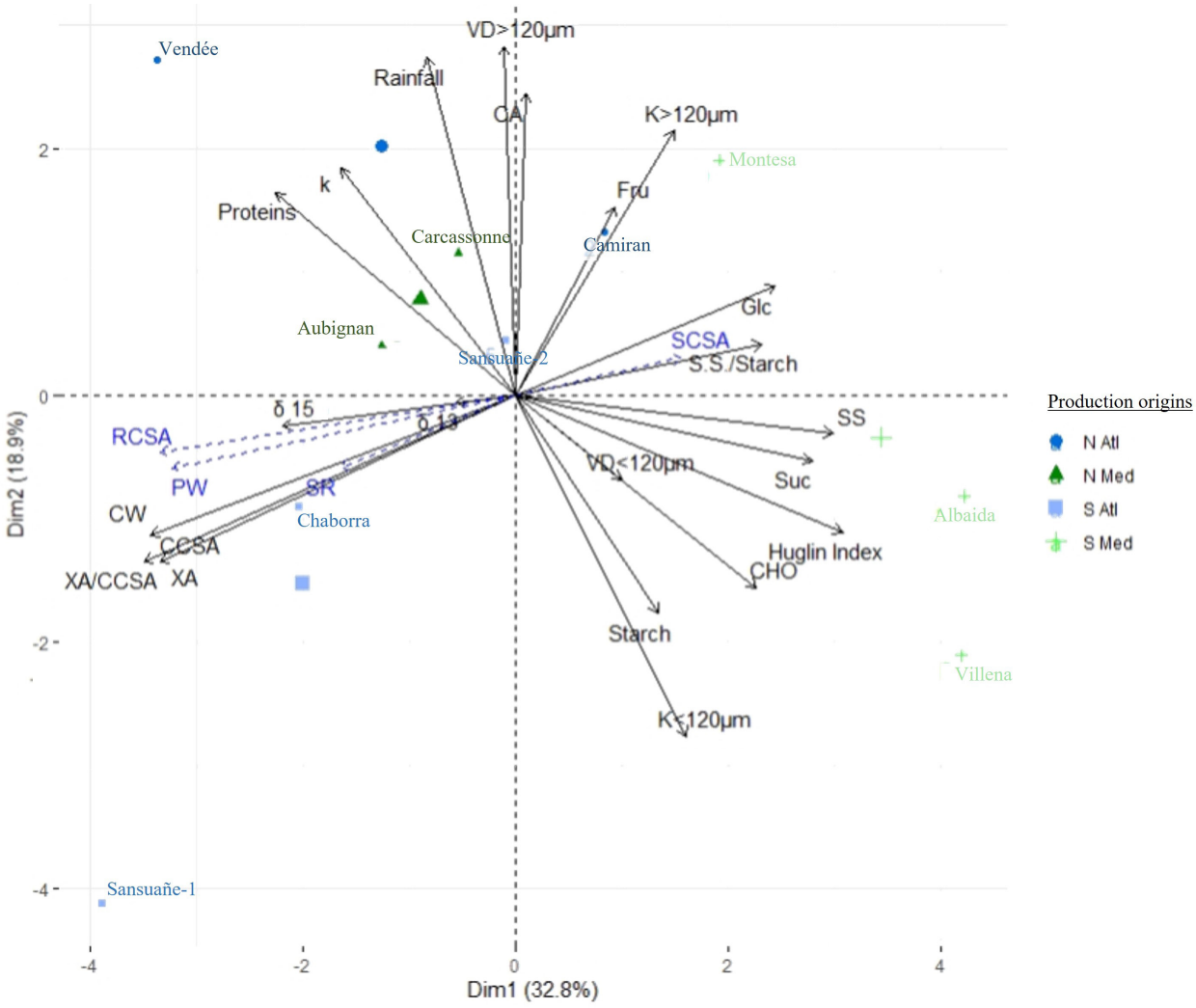


FIGURE 4. Principle component (PC) analysis of the variables measured in the canes of 110R rootstock of the ten different production origins grafted in the 2019 season.

were identified, representing ~51 % of the total variance. The first principal component (PC1) accounted for 32.8 % of the variance, and was strongly associated with the Huglin Index and greater cane nutritional status, as well as with the development of smaller vessels and their conductivity. The second principal component (PC2) accounted for 18.9 % of the variance and was related to rainfall, protein content, hydraulic conductivity, conductive area, and the development of larger vessels and their conductivity.

The geographic origin of the samples appeared to affect cane characteristics, since the batches were grouped according to geographic origin in the first component of the analysis (PC1) (*i.e*., Northern Atlantic (N Atl) and Northern Mediterranean (N Med) close together, followed by Southern Atlantic (S Atl) on the negative side of PC1 and opposite Southern Mediterranean (S Med)). The first group (N Atl and N Med) exhibited a higher protein content and a greater hydraulic conductivity due to the formation of wide vessels. S Atl aligned with higher values of rootstock cane anatomical parameters (CCSA, CW and XA) and had the highest success rate, with higher nitrogen and carbon isotope ratios. However, S Med batches, located in the warmer climatic zone, were associated with higher carbohydrate and soluble sugar content and to the development of narrower vessels. Furthermore, their soluble sugar-to-starch ratio was higher than that of the other analysed batches; *i.e*., they had a lower "wood maturation".

When comparing the projection of the success rate (SR) of the grafting process to the two first components of the PCA, it is observed to be projected centrally, near the origin, indicating that it is not strongly associated with either PC1 or PC2. Therefore, the variables considered globally were unable to predict variations in the success rate. Nevertheless, certain associations were observed between SR and several anatomical parameters of the rootstock canes (CW, CCSA, XA, and XA-to-CCSA ratio). Furthermore, SR is shown to be linked to some extent to nitrogen and carbon isotope ratios, suggesting that optimal plant water status and the application of an organic fertiliser or improved soil composition with higher organic matter content could contribute to increasing the percentage of grafted canes that successfully complete the nursery process.

Therefore, the results of the study did not show the expected relationship between success rate and starch and/or soluble sugar content in the rootstock canes. Nonetheless, the soluble sugar-to-starch ratio was opposite the SR projection in the PCA, suggesting that improved wood maturation could potentially enhance grafting success. The lack of a clear association is probably due to the fact that all the batches of rootstock cane met the necessary quality standards, and the grafting process was carried out efficiently, minimising the impact of the variables analysed. However, this does not exclude the possibility that if cane characteristics or grafting conditions had been more limiting, these variables could have had a significant or even critical impact on the success rate. Similarly, Treeby and Considine (1982) found in their study of *V. champini* cv. Ramsey cuttings that neither starch content per se nor total carbohydrate content were statistically associated with the proportion of propagated cuttings or with subsequent growth of shoots and roots. The cane diameter range in our study (8-10 mm) falls within the ranges considered by Rustem and Etker (2018) as optimal for achieving a satisfactory grafting success rate in grafted plants.

CONCLUSIONS

Despite there being many wine-growing regions in Europe, rootstock cutting production is limited to Spain, France, and Italy (87 % of the total rootstock acreage), and the most widely planted cultivar in Europe is 110 Richter (Zavaglia *et al.,* 2016). In recent years, the quality of rootstock cane has been assessed from an anatomical and nutritional point of view, taking into account the time of harvesting of the wood, the node of the cane used for propagation, the storage quality of the material and the de-budding of the cuttings (Hunter *et al.,* 1996; Hunter *et al.,* 2003; Reed *et al.,* 2004; Somkuwar *et al.,* 2017). However, the origin of the propagation material and its effect on the quality of the grafted plant, as well as the plastic responses (xylem development) of the same genotypes of *Vitis* spp. in multiple environments have rarely been evaluated.

Here, anatomical and nutritional measurements did not have a significant impact on final success rate. Nonetheless, thicker cuttings with a lower soluble sugar-to-starch ratio and those grown in production fields with higher ^{15}N and ^{13}C ratios–indicating increased organic fertilisation and reduced water stress–showed a slight increase in success rates. It is worth noting that all measurements were conducted on rootstock material of sufficiently high quality, which prevented the observation of low success rates. Beyond anatomical and nutritional parameters, further research from biochemical and physiological perspectives is needed to fully understand the impact of propagation wood on grapevine development and grafting success.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the expert assistance of the technical staff in Vitis Navarra nursery in this study, especially Rafael García and Javier Eraso.

FUNDING

This work received the financial support of the projects EFA 324/19–VITES QUALITAS and EFA 033/01 VITRES, 65 % co-funded by the European Regional Development Fund (ERDF) through the Interreg Program V-A Spain-France-Andorra (POCTEFA 2014-2020) and the Interreg Program VI-A Spain-France-Andorra (POCTEFA 2021-2027), whose aim is to reinforce the economic and social integration of the Spain-France-Andorra border area. A. Villa-Llop is the beneficiary of an Industrial pre-doctoral contract of the Government of Navarra (Ref. 283E/2020). N Torres is a beneficiary of a Ramón y Cajal Grant RYC2021–034586-I

funded by MCIN/AEI/10.13039/501100011033 and by "European Union NextGeneration EU/PRTR". D. Marín was beneficiary of postgraduate scholarship funded by Universidad Publica de Navarra (FPI-UPNA-2016).

REFERENCES

Almansa, E. M., Chica, R. M., & Lao, M. T. (2018). Influence of the quality of artificial light on grafting tomato. *Australian Journal of Crop Science*, *12*(2), 318–325. https://doi.org/10.3316/informit.561143320447414

Bateman, A. S., & Kelly, S. D. (2007). Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health Studies*, *43*(3), 237–247. https://doi.org/10.1080/10256010701550732

Becker, H., & Hiller, M. H. (1977). Hygiene in Modern Bench-Grafting. *American Journal of Enology and Viticulture*, *28*(2), 113–118. https://doi.org/10.5344/ajev.1974.28.2.113

Bianchi, D., Grossi, D., Simone Di Lorenzo, G., Zi Ying, Y., Rustioni, L., & Brancadoro, L. (2020). Phenotyping of the "G series" Vitis hybrids: First screening of the mineral composition. *Scientia Horticulturae*, *264*, 109155. https://doi.org/10.1016/J.SCIENTA.2019.109155

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Canas, S., Assunção, M., Brazão, J., Zanol, G., & Eiras-Dias, J. E. (2015). Phenolic compounds involved in grafting incompatibility of Vitis spp: Development and validation of an analytical method for their quantification. *Phytochemical Analysis: PCA*, *26*(1), 1–7. https://doi.org/10.1002/pca.2526

Carlquist, S. (1985). Observations on Functional Wood Histology of Vines and Lianas. *Aliso*, *11*(2), 139–157. https://doi.org/10.5642/aliso.19851102.03

Crespo-Martínez, S., Ramírez-Lacunza, A., Miranda, C., Urrestarazu, J., & Santesteban, L. G. (2023). Dynamics of GFLV, GFkV, GLRaV-1, and GLRaV -3 grapevine viruses transport toward developing tissues. *European Journal of Plant Pathology*. https://doi.org/10.1007/s10658-023-02703-1

Dardeniz, A., Müftüoğlu, N. M., Gökbayrak, Z., & Fırat, M. (2007). Assessment of morphological changes and determination of best cane collection time for 140 RU and 5 BB. *Scientia Horticulturae*, *113*(1), 87–91. https://doi.org/10.1016/j.scienta.2007.01.029

Feng, M., Augstein, F., Kareem, A., & Melnyk, C. W. (2024). Plant grafting: Molecular mechanisms and applications. *Molecular Plant*, *17*(1), 75–91. https://doi.org/10.1016/j.molp.2023.12.006

Fourie, P. H., & Halleen, F. (2004). Proactive Control of Petri Disease of Grapevine Through Treatment of Propagation Material. *Plant Disease*, *88*(11), 1241–1245. https://doi.org/10.1094/PDIS.2004.88.11.1241

Fourie, P. H., & Halleen, F. (2006). Chemical and biological protection of grapevine propagation material from trunk disease pathogens. *European Journal of Plant Pathology*, *116*(4), 255–265. https://doi.org/10.1007/s10658-006-9057-9

García, M. S. (2019). *Niveles de almidón en injertos de vid y su influencia en la tasa de mortalidad en vivero*. Universidad de León.

Gramaje, D., & Armengol, J. (2011). Fungal Trunk Pathogens in the Grapevine Propagation Process: Potential Inoculum Sources, Detection, Identification, and Management Strategies. *Plant Disease*, *95*(9), 1040–1055. https://doi.org/10.1094/PDIS-01-11-0025

Hargrave, K. R., Kolb, K. J., Ewers, F. W., & Davis, S. D. (1994). Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). *New Phytologist*, *126*(4), 695–705. https://doi.org/10.1111/j.1469-8137.1994.tb02964.x

Hartmann, H. T., Kester, D. E., Davies, F. T., & Geneve, R. L. (2011). Principles of Grafting and Budding. In *Plant Propagation: Principles and Practices* (8th ed., pp. 415–463). Prentice Hall. https://tree.worldagroforestry.org/article?id=118&category=9

Hendriks, J. H. M., Kolbe, A., Gibon, Y., Stitt, M., & Geigenberger, P. (2003). ADP-Glucose Pyrophosphorylase Is Activated by Posttranslational Redox-Modification in Response to Light and to Sugars in Leaves of Arabidopsis and Other Plant Species. *Plant Physiology*, *133*(2), 838–849. https://doi.org/10.1104/pp.103.024513

Huglin, M. (1978). Nouveau mode d'évaluation des possibilités héliothermiques d'un milieu viticole. *Comptes Rendus de l'Académie d'Agriculture de France*, *64*, 1117–1126.

Hunter, J. J., Le Roux, D. J., & Volschenk, C. G. (1996). *Reserve nutrient status of Vitis graft material as related to callus success and growth during the callus period.* Fourth Int. Symp. on Cool Climate Viticulture and Enology, Rochester, New York State, USA.

Hunter, J. J., Volschenk, G. C., Roux, D. J., Fouche, G. W., & Adams, L. (2003). *The Physiological and Morphological Quality of Plant Material—A compilation of research*.

Irisarri, P., Zhebentyayeva, T., Errea, P., & Pina, A. (2019). Inheritance of self- And graft-incompatibility traits in an F 1 apricot progeny. *PLoS ONE*, *14*(5), 1–14. https://doi.org/10.1371/journal.pone.0216371

Kassambara, A. (2022). *fviz_pca: Visualize Principal Component Analysis*. Https://Rdrr.Io/Github/Kassambara/Factoextra/Man/Fviz_pca.Html.

Kassambara, A., & Mundt, F. (2020). *factoextra: Extract and Visualize the Results of Multivariate Data Analyses* (Version 1.0.7) [Computer software]. https://CRAN.R-project.org/package=factoextra

Liu, W., Su, J., Li, S., Lang, X., & Huang, X. (2018). Non-structural carbohydrates regulated by season and species in the subtropical monsoon broad-leaved evergreen forest of Yunnan Province, China. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-19271-8

Lobos-Catalán, P., & Jiménez-Castillo, M. (2023). The functional mechanism behind the latitudinal pattern of liana diversity: Freeze–thaw embolism reduces the ecological performance of liana species. *Ecology and Evolution*, *13*(9), e10486. https://doi.org/10.1002/ece3.10486

Loupit, G., Brocard, L., Ollat, N., & Cookson, S. J. (2023). Grafting in plants: Recent discoveries and new applications. *Journal of Experimental Botany*, *74*(8), 2433–2447. https://doi.org/10.1093/jxb/erad061

Lovisolo, C., & Schubert, A. (1998). Effects of water stress on vessel size and xylem hydraulic conductivity in Vitis vinifera L. *Journal of Experimental Botany*, *49*(321), 693–700. https://doi.org/10.1093/jexbot/49.321.693

Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., & Schubert, A. (2010). Drought-induced changes in development and function of grapevine (Vitis spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: A physiological and molecular update. *Functional Plant Biology*, *37*, 98–116. https://doi.org/10.1071/FP09191

Marín, D., Santesteban, L. g., Dayer, S., Villa-Llop, A., Abad, F. j., Gambetta, G. a., Torres-Ruiz, J. m., & Torres, N. (2022). Connection matters: Exploring the implications of scion–rootstock alignment in grafted grapevines. *Australian Journal of Grape and Wine Research*, *28*(4), 561–571. https://doi.org/10.1111/ajgw.12559

Martelli, G. P. (2017). An overview on grapevine viruses, viroids, and the diseases they cause. In *Grapevine Viruses: Molecular Biology, Diagnostics and Management* (pp. 31–46). Springer International Publishing. https://doi.org/10.1007/978-3-319-57706-7_2

Mary, S., Laveau, C., Lecomte, P., Birebent, M., & Roby, J.-P. (2017). Impact of grafting type on Esca foliar symptoms. *OENO One*, *51*(2). https://doi.org/10.20870/oeno-one.2016.50.4.1408

McElrone, A. J., Manuck, C. M., Brodersen, C. R., Patakas, A., Pearsall, K. R., & Williams, L. E. (2021). Functional hydraulic sectoring in grapevines as evidenced by sap flow, dye infusion, leaf removal and micro-computed tomography. *AoB PLANTS*, *13*(2). https://doi.org/10.1093/aobpla/plab003

Mendiburu, F. de. (2021). *agricolae: Statistical Procedures for Agricultural Research* (Version 1.3-5) [Computer software]. https://CRAN.R-project.org/package=agricolae

Munitz, S., Netzer, Y., Shtein, I., & Schwartz, A. (2018). Water availability dynamics have long-term effects on mature stem structure in *Vitis vinifera*. *American Journal of Botany*, *105*(9), 1443–1452. https://doi.org/10.1002/ajb2.1148

Netzer, Y., Munitz, S., Shtein, I., & Schwartz, A. (2019). Structural memory in grapevines: Early season water availability affects late season drought stress severity. *European Journal of Agronomy*, *105*, 96–103. https://doi.org/10.1016/j.eja.2019.02.008

Nicholas, P., Chapman, A., & Cirami, R. (1992). Grapevine propagation. In B. Coombe & P. Dry (Eds.), *Viticulture* (Vol. 2, pp. 1–22).

OIV. (2016). *Grapevine Trunk Diseases. A review* (pp. 1–24). International Organisation of Vine and Wine. https://www.oiv.int/public/medias/4650/trunk-diseases-oiv-2016.pdf

Olson, M. E., & Rosell, J. A. (2013). Vessel diameter–stem diameter scaling across woody angiosperms and the ecological causes of xylem vessel diameter variation. *New Phytologist*, *197*(4), 1204–1213. https://doi.org/10.1111/NPH.12097

Parisi, M. D. (2014). *Process and product innovations in the Sicilian vine nursery*. ISA / UL.

Peccoux, A. (2011). *Molecular and physiological characterization of grapevine rootstock adaptation to drought*. Université Bordeaux Segalen.

Phillips, N., Reynolds, A., & Profio, F. D. (2015). Nonstructural Carbohydrate Concentrations in Dormant Grapevine Scionwood and Rootstock Impact Propagation Success and Vine Growth. *HortTechnology*, *25*(4), 536–550. https://doi.org/10.21273/HORTTECH.25.4.536

Pina, A., Cookson, S. J., Calatayud, A., Trinchera, A., & Errea, P. (2017). Physiological and molecular mechanisms underlying graft compatibility. *Vegetable Grafting: Principles and Practices*, 132–154. https://doi.org/10.1079/9781780648972.0132

Pire, R., Sanabria, M. E., Pereira, A., & Díez, J. (2007). Conductividad hidráulica y grosor de los vasos del xilema en cinco materiales de vid sometidos a déficit hídrico. *Interciencia*, *32*(1), 35–40.

Pouzoulet, J., Rolshausen, P. E., Charbois, R., Chen, J., Guillaumie, S., Ollat, N., Gambetta, G. A., & Delmas, C. E. L. (2020). Behind the curtain of the compartmentalization process: Exploring how xylem vessel diameter impacts vascular pathogen resistance. *Plant, Cell & Environment*, *43*(11), 2782–2796. https://doi.org/10.1111/pce.13848

Prodhomme, D., Valls Fonayet, J., Hévin, C., Franc, C., Hilbert, G., de Revel, G., Richard, T., Ollat, N., & Cookson, S. J. (2019). Metabolite profiling during graft union formation reveals the reprogramming of primary metabolism and the induction of stilbene synthesis at the graft interface in grapevine. *BMC Plant Biology*, *19*(1), 599. https://doi.org/10.1186/s12870-019-2055-9

Quintana-Pulido, C., Villalobos-González, L., Muñoz, M., Franck, N., & Pastenes, C. (2018). Xylem structure and function in three grapevine varieties. *CHILEAN JOURNAL OF AGRICULTURAL RESEARCH*, *78*(3). https://doi.org/10.4067/S0718-58392018000300419

R Core Team. (2020). *R: A language and environment for statistical computing* [Computer software]. R Foundation for Statistical Computing. https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing

Real Decreto 208/2003 (2003). Real Decreto 208/2003, 48, BOE, 7576. https://www.boe.es/eli/es/rd/2003/02/21/208

Rajaei, H., Yazdanpanah, P., Dadbin, M., Haghighi, A. A. K., Sepaskhah, A. R., & Eslamzadeh, T. (2013). Comparison of root anatomy and xylem vessel structure in rain-fed and supplementary irrigated 'Yaghooti-Syah Shiraz' grapevine (Vitis vinifera L.). *Horticulture, Environment, and Biotechnology*, *54*(4), 297–302. https://doi.org/10.1007/s13580-013-0079-x

Ramsing, C. K., Gramaje, D., Mocholí, S., Agustí, J., Cabello Sáenz de Santa María, F., Armengol, J., & Berbegal, M. (2021). Relationship Between the Xylem Anatomy of Grapevine Rootstocks and Their Susceptibility to Phaeoacremonium minimum and Phaeomoniella chlamydospora. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.726461

Reed, A. B., O'Connor, C. J., Melton, L. D., & Smith, B. G. (2004). Determination of Sugar Composition in Grapevine Rootstock Cuttings Used for Propagation. *American Journal of Enology and Viticulture*, *55*(2), 181–186. https://doi.org/10.5344/ajev.2004.55.2.181

Rego, C., Nascimento, T., Cabral, A., Silva, M. J., & Oliveira, H. (2009). Control of grapevine wood fungi in commercial nurseries. *Phytopathologia Mediterranea*, *48*(1), Article 1. https://doi.org/10.14601/Phytopathol_Mediterr-2881

RStudio Team. (2021). *RStudio: Integrated development for R* [Computer software]. RStudio. http://www.rstudio.com/

Rustem, C., & Etker, M. (2018). The effects of rootstock cutting thickness on final take, quality of potted grapevine saplings. *Yüzüncü Yil Universitesi Journal of Agricultural Sciences (YYU J Agr Sci)*, *28*(Special Issue), 50–56.

Santesteban, L. G., Jiménez, C. M., Urretavizcaya, I., & Díaz, J. B. R. (2014). El ratio isotópico del carbono (δ13C) como método de integración a posteriori del estado hídrico del viñedo. *I Jornada del Grupo de Viticultura y Enología: Comunicaciones, Logroño, 19 y 20 de noviembre, 2014, 2014, ISBN 978-84-8125-675-8, págs. 146-152*, 146–152. https://dialnet.unirioja.es/servlet/articulo?codigo=6005324

Santesteban, L. G., Loidi, M., Urretavizcaya, I., Galar, M., Crespo-Martínez, S., Royo, J. B., & Miranda, C. (2024). Nitrogen isotope ratio (δ15N): A nearly unexplored indicator that provides useful information in viticulture. *OENO One*, *58*(2). https://doi.org/10.20870/oeno-one.2024.58.2.7961

Santesteban, L. G., Miranda, C., Barbarin, I., & Royo, J. B. (2015). Application of the measurement of the natural abundance of stable isotopes in viticulture: A review. *Australian Journal of Grape and Wine Research*, *21*(2), 157–167. https://doi.org/10.1111/ajgw.12124

Scholz, A., Klepsch, M., Karimi, Z., & Jansen, S. (2013). How to quantify conduits in wood? *Frontiers in Plant Science*, *4*. https://doi.org/10.3389/fpls.2013.00056

Schultz, H., & Matthews, M. (1993). Xylem development and hydraulic conductance in sun and shade shoots of grapevine (Vitis vinifera L.): Evidence that low light uncouples water transport capacity from leaf area. *Planta*, *190*, 393–406. https://doi.org/10.1007/BF00196969

Smith, J. P. (2004). *Investigations into the mechanisms underlying grapevine rootstock effects on scion growth and yield*. Charles Sturt University.

Somkuwar, R. G., Bhange, M., Sharma, J., Upadhyay, A. K., & Khan, I. (2017). Interaction of biochemical and nutritional status of nodal sections with rooting success in grape rootstocks. *Journal of Environmental Biology*, *38*(January), 115–121. https://doi.org/10.22438/jeb/38/1/MRN-757

Sperry, J. S., Hacke, U. G., & Pittermann, J. (2006). Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany*, *93*(10), 1490–1500. https://doi.org/10.3732/ajb.93.10.1490

Stamatiadis, S., Christofides, C., Tsadila, E., Taskos, D., Tsadilas, C., & Schepers, J. S. (2007). Relationship of Leaf Stable Isotopes (δ13C and δ15N) to Biomass Production in Two Fertilized Merlot Vineyards. *American Journal of Enology and Viticulture*, *58*(1), 67–74. https://doi.org/10.5344/ajev.2007.58.1.67

Stamp, J. A. (2001). The contribution of imperfections in nursery stock to the decline of young vines in California. *Phytopathologia Mediterranea*, *40*(3), 369–375. https://doi.org/10.14601/Phytopathol_Mediterr-1640

Taskos, D., Zioziou, E., Nikolaou, N., Doupis, G., & Koundouras, S. (2020). Carbon isotope natural abundance ($\delta^{13}C$) in grapevine organs is modulated by both water and nitrogen supply. *OENO One*, *54*(4), Article 4. https://doi.org/10.20870/oeno-one.2020.54.4.3600

Tibbetts, T. J., & Ewers, F. W. (2000). Root pressure and specific conductivity in temperate lianas: Exotic *Celastrus orbiculatus* (Celastraceae) vs. native *Vitis riparia* (Vitaceae). *American Journal of Botany*, *87*(9), 1272–1278. https://doi.org/10.2307/2656720

Todic, S., Tesic, D., & Beslic, Z. (2005). The effect of certain exogenous growth regulators on quality of grafted grapevine rootlings. *Plant Growth Regulation*, *45*(2), 121–126. https://doi.org/10.1007/s10725-005-1784-y

Tombesi, S., Johnson, R. S., Day, K. R., & DeJong, T. M. (2010). Relationships between xylem vessel characteristics, calculated axial hydraulic conductance and size-controlling capacity of peach rootstocks. *Annals of Botany*, *105*(2), 327–331. https://doi.org/10.1093/aob/mcp281

Treeby, M. T., & Considine, J. A. (1982). Propagation of *Vitis Champini* Planchon CV. Ramsey: Relationship between Carbohydrate Metabolism during Storage and Cutting Performance. *American Journal of Enology and Viticulture*, *33*(1), 53–56. https://doi.org/10.5344/ajev.1982.33.1.53

Tyree, M. T., & Zimmermann, M. H. (2002). *Xylem Structure and the Ascent of Sap*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-04931-0

Verma, S. K., Singh, S. K., & Kumar, A. (2018). Evaluation of different grafting methods in Grapes cv. Pusa Urvashi. *International Journal of Agricultural Invention*, *3*(01), 105–107. https://doi.org/10.46492/IJAI/2018.3.1.22

Vrši, S., & Valdhuber, J. (2009). Influence of Defoliation on Carbohydrate Reserves of Young Grape-vines in the Nursery. *Europ.J.Hort.Sci*, *74*(5), 1611–4426.

Waite, H., Armengol, J., Billones-Baaijens, R., Gramaje, D., Halleen, F., Di marco, S., & Smart, R. (2018). A protocol for the management of grapevine rootstock mother vines to reduce latent infections by grapevine trunk pathogens in cuttings. *Phytopathologia Mediterranea*, *57*(3), 384–398. https://doi.org/10.14601/Phytopathol_Mediterr-22772

Waite, H., Whitelaw-Weckert, M., & Torley, P. (2015). Grapevine propagation: Principles and methods for the production of high-quality grapevine planting material. *New Zealand Journal of Crop and Horticultural Science*, *43*(2), 144–161. https://doi.org/10.1080/01140671.2014.978340

Wample, R. L., & Bary, A. (1992). Harvest Date as a Factor in Carbohydrate Storage and Cold Hardiness of Cabernet Sauvignon Grapevines. *Journal of the American Society for Horticultural Science*, *117*(1), 32–36. https://doi.org/10.21273/JASHS.117.1.32

Wang, J., Jiang, L., & Wu, R. (2017). Plant grafting: How genetic exchange promotes vascular reconnection. *New Phytologist*, *214*(1), 56–65. https://doi.org/10.1111/nph.14383

Wei, T., Simko, V., Levy, M., & Xie, Y. (2021). *Visualization of a Correlation Matrix. Version 0.90*. https://cran.r-project.org/web/packages/corrplot/corrplot.pdf

Winkler, A. J., & Williams, W. O. (1945). STARCH AND SUGARS OF *VITIS VINIFERA*. *Plant Physiology*, *20*(3), 412–432. https://doi.org/10.1104/pp.20.3.412

Zavaglia, C., Pecile, M., Gardiman, M., & Bavaresco, L. (2016). Production of propagating material of grapevine rootstocks in the EU and Italy. *Acta Horticulturae*, *1136*, 57–62. https://doi.org/10.17660/ActaHortic.2016.1136.9

Zyakun, A. M., Oganesyants, L. A., Panasyuk, A. L., Kuz'mina, E. I., Shilkin, A. A., Baskunov, B. P., Zakharchenko, V. N., & Peshenko, V. P. (2013). Mass spectrometric analysis of the 13C/12C abundance ratios in vine plants and wines depending on regional climate factors (Krasnodar krai and Rostov oblast, Russia). *Journal of Analytical Chemistry*, *68*(13), 1136–1141. https://doi.org/10.1134/S106193481313011X