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Exploring how graft length shapes root system architecture and morphology in grapevine rootstocks

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ABSTRACT

The success of grafting and the sustained growth of newly grafted grapevines depend on the ability of hardwood cuttings taken from parent plants to rapidly initiate new adventitious roots (ARs) and to establish a functional, well-distributed root system in the soil. There is a paucity of literature on the plasticity and diversity of grapevine root systems due to the difficulty of phenotyping the hidden part of the plant. This study aimed to investigate whether varying rootstock cutting length (8, 20, 30 and 50 cm), and as a consequence the initial pool of woody reserves, influences root system architecture (RSA) and root morphological traits. The analysis focused on two scion/rootstock combinations, *Vitis riparia* cv. Riparia Gloire de Montpellier (RGM) and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P), both grafted with scions of *V. vinifera* cv. Cabernet-Sauvignon (CS). Two experiments were carried out: one in 2022 involving 240 plants grown in pots, and another in 2023 with 32 plants grown in rhizotrons. The latter allowed for non-invasive, time-lapse 2D imaging of early root system development providing quantitative data on adventitious and lateral roots, including their number, length, diameter and branching patterns. The length of the rootstock significantly influenced the recovery and early-stage growth of the grafted grapevines, impacting both shoot and root development. Longer rootstocks led to accelerated emergence of ARs and increased their number, irrespective of rootstock genotype, as well as promoted greater total root system length and mass. The maximum number of ARs was positively correlated with graft fresh mass, suggesting a link between initial reserves and root development. While CS/1103P produced 1.3 times more ARs and had longer first-order roots compared to CS/RGM, both combinations produced similar total root biomass. However, they displayed distinct RSA patterns: 1103P ARs grew at more acute angles, facilitating deeper soil penetration, while RGM roots spread more horizontally, promoting surface soil exploration. These contrasting growth patterns suggest the two rootstocks may have different mechanisms for accessing water and nutrients. Although certain root morphological traits (*i.e.*, inter-branch distance) remained unaffected by rootstock length, RGM showed greater plasticity in response to reduced initial reserves, as indicated by a smaller apical diameter with shorter grafts than that in CS. Overall, this study emphasises the significance of considering rootstock length and RSA in viticultural propagation practices. The insights gathered here contribute to a deeper understanding of the mechanisms governing root development in grafted grapevines, thereby informing strategies to enhance grafting techniques and optimise grapevine cultivation.

KEYWORDS: adventitious root, rooting, root traits, phenotyping, 2D imaging, rootstock, *Vitis*

INTRODUCTION

Many perennial fruit crops are commercially grafted, an ancient practice in which a scion selected for desirable fruit-producing traits is joined to a rootstock chosen for its resistance to biotic and abiotic stresses or its ability to modify scion phenotypes (Warschesky *et al.*, 2016). Grafting is essential in viticulture worldwide, particularly since the phylloxera crisis in the late 19th century. Most cultivated grapevines are *Vitis vinifera* scions grafted onto rootstocks derived from North American *Vitis* spp. and their hybrids. The production of a bench-grafted grapevine is a complex process, starting with winter harvesting of the canes that will be grafted and ending with the selection of viable plants in the nursery and their planting in the vineyard (Waite *et al.*, 2015). Successful establishment and long-term growth of grafted plants depends primarily on the compatibility between the two genotypes and their ability to form robust vascular connections during the formation of the graft union (Tedesco *et al.*, 2022; Loupit *et al.*, 2023). It also depends on the ability of (i) the scion to develop leafy shoots from dormant buds and (ii) the rootstock to initiate roots and develop a functional, well-structured root system.

The geometrical, topological and morphological traits of roots, including the apical diameter, specific length, tropism, branching angle, branching density and lateral root expansion, determine the spatial configuration of the root system (*i.e.*, the root system architecture, RSA) and influence plant performance and resilience during soil environmental stresses situations (Bardgett *et al.*, 2014). The spatial configuration of the root system develops to ensure anchorage in the soil, to promote interactions with beneficial microbial communities in the rhizosphere, and to uptake and transport water and nutrients from the soil, especially under heterogeneous or limited resource availability (Lynch, 2007). The RSA is shaped by a complex set of individual root developmental processes, including initiation, emergence, growth (axial and radial), branching, tropism, senescence and decay. These processes are organised in space and time and are genetically determined, resulting in a great phenotypic diversity in form and structure between species, populations or clones (Hodge *et al.*, 2009). The expression of these root processes is influenced by both endogenous signals and exogenous factors, such as the physico-chemical properties of the soil, temperature or humidity, which fluctuate greatly over the lifetime of the plant (Rellan-Alvarez *et al.*, 2016). As a result, the number, location, and direction of growth of each root in the system is highly variable, even among genetically identical plants (*i.e.,* root plasticity).

In contrast to seed propagation, where primary roots emerge from the seed and give rise to secondary lateral roots, vegetative propagation results in a root system composed entirely of adventitious roots (ARs) from which lateral roots branch off (Albrecht *et al.*, 2017). In hardwood cuttings, AR rhizogenesis and root system development are strongly influenced not only by their genetic origin but also by the physiological and biochemical state of the parent plant from which the cuttings were taken (Smart *et al.*, 2003). Endogenous hormones are key regulators of AR formation, with auxin acting as the primary signal to trigger rhizogenesis from non-root tissues (Druege *et al.*, 2019). Recent studies, mainly in model plants, have demonstrated that other plant growth regulators including nitric oxide, ethylene, polyamines, jasmonic acid and strigolactones may also be involved in the process of root primordia induction (reviewed by Bannoud & Bellini, 2021; Altamura *et al.*, 2023). In addition to the aforementioned growth regulators, the initial levels of endogenous carbohydrates, mineral nutrients and other biochemical components, such as phenolic compounds, that may act as rooting cofactors or modulators of auxin metabolism and transport, also impact AR formation and the subsequent root development in cuttings (Da Costa *et al.*, 2013; Bannoud & Bellini, 2021). In grapevine, as early as 1927, Winkler observed a positive correlation between the degree of iodine staining of the starch present in the mother canes and the AR formation in cuttings (Winkler, 1927). More recently, the concentration of carbohydrates in the wood used for grafting (originating from different geographical areas) has been linked to grafted plant survival and development in grapevine (Bartolini *et al.*, 1996). In poplar, carbohydrate availability in the base of cuttings was demonstrated to be higher in easy-to-root cultivars than in difficult-to-root cultivars, and soluble sugars were found to be more influential than starch in initiating root primordia (Denaxa *et al.*, 2012). Rooting also tends to improve with increasing cutting length, as observed in woody species such as poplar (Desrocher & Thomas, 2003) and olive (Porfírio *et al.*, 2016), possibly due to the presence of higher amounts of carbohydrate resources and/or higher enzymatic activity to remobilise and transport them to the base of the cutting. In grapevine, the results obtained are not always consistent, as they are highly dependent on the cultivars and cutting type (Boeno & Zuffellato-Ribas, 2022). Moreover, the role of carbohydrates in AR formation and early root growth remains unclear (Phillips *et al.*, 2015). Finally, in the majority of studies investigating adventitious rooting in hardwood cuttings, root system characterisation is limited to a few traits such as the number of roots emitted after a few days or the length of the longest root. This is likely due to the difficulty and time-consuming nature of phenotyping roots *in situ*, even on young plants at an early stage of development (Vielba *et al.*, 2020). To date, no comprehensive studies have been conducted to characterise the morphological and architectural features of the grapevine root system in relation to the initial state of the hardwoods used for grafting.

The objectives of this study were to investigate in young, grafted grapevines (i) the relationship between the quantity of reserves and architectural and morphological traits of the roots, and (ii) the genetic variation of these architectural and morphological traits. The quantity of reserves was manipulated by grafting scions with rootstock hardwoods of varying lengths. Genetic variation was examined using two commercial rootstocks, selected for their agronomic performance and contrasting root system patterns in vineyards. A deeper understanding of the mechanisms underlying AR formation and early RSA development is essential for improving propagation strategies in viticulture, particularly for hard-to-root rootstocks like *V. berlandieri* hybrids. This knowledge can help nurseries and grape growers to produce uniform, high-quality plants with greater consistency, thereby enhancing the success and long-term viability of vineyard plantings.

MATERIALS AND METHODS

1. Plant material

Two grapevine rootstocks were used: *V. riparia* cv. Riparia Gloire de Montpellier (RGM), which is considered to be susceptible to drought and clay soils, confers low scion vigour and develops a shallow root system, and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P), which is considered to be tolerant to drought and clay soils, confers high scion vigour and develops a deep root system (Archer & Saayman, 2018). Both rootstocks were grafted with the scion variety *V. vinifera* cv. Cabernet-Sauvignon. The hardwoods were collected in December from a vineyard of INRAE near Bordeaux (France) and stored in a cold room (4 °C) until grafting in the first week of April. The grafting procedures were repeated in 2022 and 2023. One-bud scions were grafted onto de-budded rootstocks of approximately the same diameter (about 10 mm); four lengths of rootstock hardwood were selected (8, 20, 30 and 50 cm). The day before grafting, the hardwoods were removed from

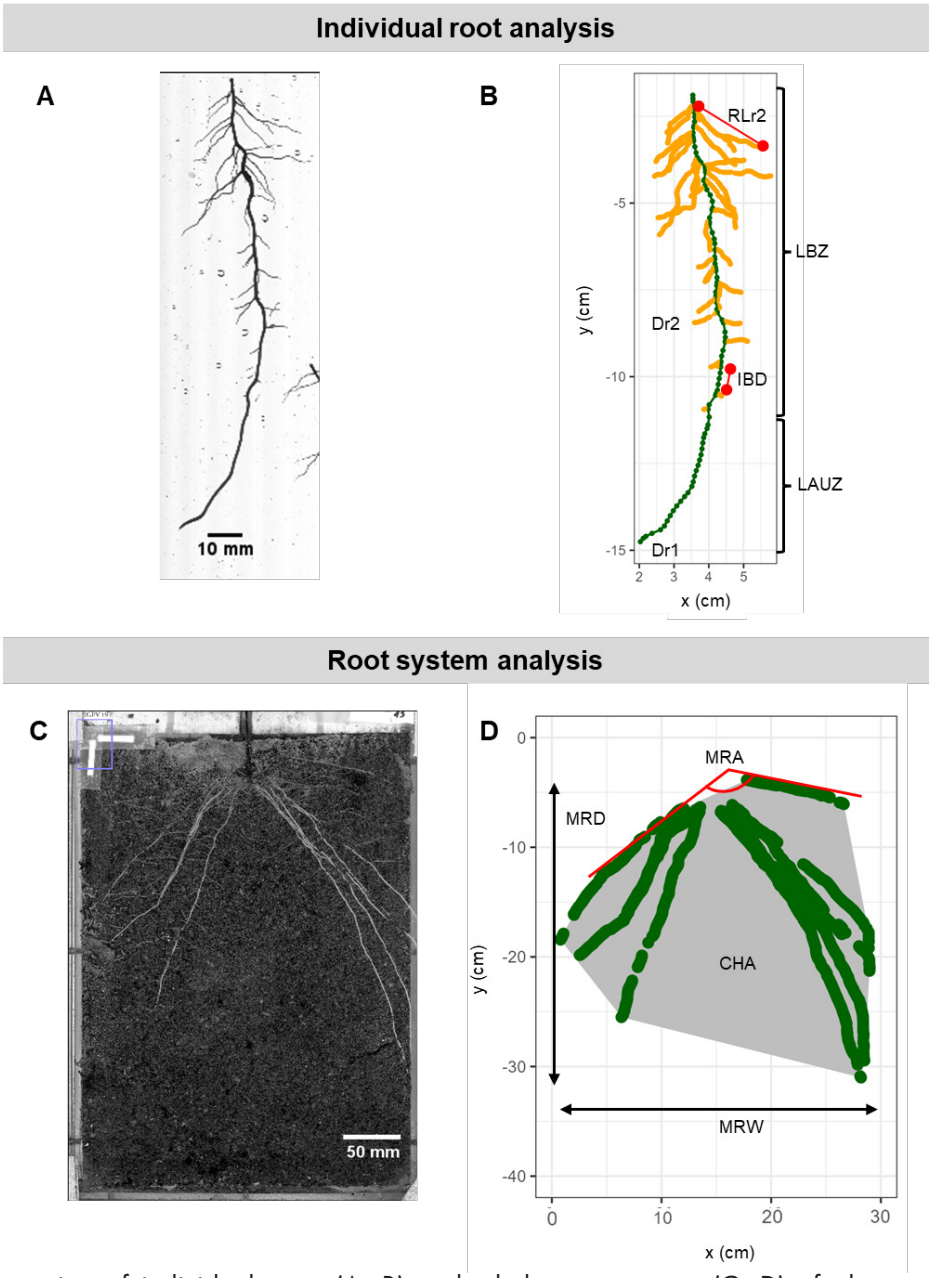


FIGURE 1. Phenotyping of individual roots (A, B) and whole root systems (C, D) of plants grown for 30 d in rhizotrons (Exp. B).

(A) High-resolution (1200 dpi) grayscale image of an individual root after harvest; (B) SmartRoot vectorized image and measured morphological traits: length of the branching zone (LBZ), length of the apical unbranched zone (LAUZ), inter-branch distance (IBD), the apical diameter of a first-order adventitious root (Dr1), apical diameter of a lateral root (Dr2), length of a lateral root (RLr2); (C) Grayscale image with a 600 dpi resolution of a root system in rhizotron; (D) SmartRoot vectorized image and measured architectural traits: maximal first-order root spreading angle (MRA), maximal horizontal extension of the root system (MRW), maximal vertical extension of the root system (MRD), convex hull area (CHA). First-order roots (green), second-order roots (orange).

cold storage and rehydrated in tap water. The wood was mechanically omega-grafted. The grafts were briefly dipped in melted wax (Staehler Rebwachs pro with 0.0035 % of dichlorobenzoic acid, Chauvin) and then placed in a plastic container filled with 3 cm of water and stored in a room at 28 °C and 85 % humidity to promote callus formation. When root tips emerged at the base of the cuttings (approximately three weeks later), uniform grafts were transplanted either in pots (Experiment A in 2022) or in rhizotrons (Experiment B in 2023).

2. Growing conditions and experimental design

Two independent experiments were conducted at the Institute of Vine and Wine Sciences (ISVV) in Bordeaux, France (44° 47 N, 0° 34 W).

2.1. Experiment A

In 2022, 30 plants per rootstock length and scion/rootstock combination were grown on an outdoor platform in 7 L pots with a 2:1 (v:v) mixture of sand and compost sieved to 2 mm. A total of 240 pots, each containing one plant, were irrigated three times a day with 40 mL of a nutrient solution (KNO_3: 0.55 mM; $(NH_4)_2SO_4$: 0.20 mM; K_2HPO_4: 0.60 mM; K_2SO_4: 0.40 mM; $Ca(N0_3)_2$: 0.35 mM; $CaCl_2$: 1.27 mM; $MgSO_4$: 0.69 mM). Each batch of 30 plants was randomly divided into 3 batches of 10 plants each. The first batch of plants was grown for one month, the second for 3 months, and the third for 4 months.

2.2. Experiment B

In 2023, four plants per rootstock length and scion/rootstock combination were grown in a rhizotron phenotyping system in a greenhouse (Figure 1C). A total of 32 rhizotrons (L × W × D: 40 × 30 × 5 cm) were filled with a 2:1 (v:v) mixture of sand and compost sieved to 2 mm. The rhizotrons were wrapped with white-black sheeting (to insulate against radiative heating and keep the roots in the dark) and positioned at a 45° angle to encourage the roots to grow against the transparent underside. The plants were micro-irrigated three times a day with 40 mL of the nutrient solution used in Exp. A, using a pump and two nozzles placed at the top of the rhizotrons. Holes are drilled in the bottom spacer to permit drainage of excess solution. Plants were grown for one month and harvested before the roots had colonised the entire rhizotron.

3. Growth characterisation and root system imaging

In both experiments, the plants were harvested to characterise several shoot and root traits (Table 1). The root systems were completely extracted and the substrate was removed by gentle washing with tap water. The length of the longest root (MLr1, cm) was measured with a ruler, and the number of ARs and leaves was counted. Subsequently, individual roots (three chosen at random in Exp. A and all roots were selected in Exp. B) were scanned with a flatbed scanner (Epson Perfection V850 Pro, America, Inc., USA) by placing them in a Plexiglas tray (22 cm × 30 cm, 1.5 cm) (Figure 1A). The Plexiglas tray was filled with a few millimetres of water, which was covered with a rigid transparent plastic sheet to disentangle the roots and minimise overlapping. Images were captured in TIFF format with a resolution of 1200 dots per inch (dpi) using the transparent mode. Finally, shoot and root samples were dried (in an oven at 60 °C until reaching a constant mass) and weighed. This allowed the calculation of the root biomass fraction (RMF, g g^{-1}) and the root-to-shoot mass fraction (RSR, g g^{-1}) (Table 1).

In Exp. B, the visible root systems of the rhizotrons were scanned periodically with an A3 flatbed scanner (Epson Expression 1640xl, America, Inc., USA) three times a week from 7 d after planting until harvest (day 30). Black and white images were captured in TIFF format at a resolution of 600 dpi (Figure 1C).

4. Image analysis and root trait estimation

Rhizotron images obtained in Exp. B were semi-automatically vectorised in an RSML format using the Smartroot v4.21 plugin (https://smartroot.github.io/; Lobet *et al.*, 2011) of the ImageJ v1.52 software (https://imagej.net/ij/). Each vectorised root was defined by a set of nodes characterised by 2D spatial coordinates, diameters, root orders and parental nodes (Figure 1B). For time-lapse images of root systems in rhizotrons, only first-order ARs emerging from the base of the rootstock cutting were analysed (Figure 1D). For each date, the vectorised root system from the previous date was superimposed on the image, and the new root tips were incorporated into the RSML files (Lobet *et al.*, 2015). An in-house R script, adapted from the ArchiDART package (Delory *et al.*, 2016), was then used to calculate, for each date, the number (Nr) and total length (TRLr1, cm) of ARs visible on the transparent side of the rhizotron, as well as the maximum depth (MRD, cm) and horizontal width (MRW, cm) reached by the root system and the surface area of the convex hull (CHA, cm^2). To quantify the maximum spreading angle of a root system (*i.e.*, the insertion angle between the two outermost roots) (MRA, °deg), a slightly modified version of the protocol described in Dayoub *et al.* (2021) was applied to the most recent image of the time series (Figure 1D).

Additional root morphological and topological information was also collected from 2D images of individual roots sampled after one month in rhizotrons. Four individual roots per plant in Exp. B, for a total of 128 roots, were fully vectorised using SmartRoot software as described above. Several traits were determined for each AR of order 1 to 3 (Table 1), including (i) the apical diameter (Dr1 and Dr2, mm), (ii) the length of the apical unramified zone (LAUZ, mm), which is the length between the root tip and the nearest lateral root, (iii) the inter-branching distance (IBD, mm), which is the average length between two consecutive lateral roots, (iv) the total root length by root order (RL, cm), (v) the average length of one daughter root per parent root (meanRLr2, mm) and (vi) the length of the first-order root branching zone (LBZ, cm). Branching density was quantified using IBD (its reciprocal) because this variable could be measured for each lateral root. Root length, mass and volume allow the calculation of specific root length (SRL, m g^{-1}) and root tissue density (RTD, g cm^{-3}).

TABLE 1. Statistics describing shoot and root growth, and individual root morphological traits of grapevine grafts cultivated for one month in rhizotrons.

Traits	Unit	Abbreviation	Descriptive statistics				ANOVA (p values)		
			Mean	Min	Max	CV (%)	Rootstock	Size	R x S
Stem dry weight	g	SDW	1.15	0.14	2.79	66.5	0.740	<0.001***	0.984
Leaf mass fraction	g g-1	LMF	51.4	45.3	66.3	9.4	0.206	0.044*	0.498
Number of leaves		LN	13.6	8.0	20.0	20.0	0.127	<0.001***	0.510
Global traits (at the root system level)									
Root dry weight	g	RDW	0.56	0.13	1.01	43.2	0.423	<0.001***	0.385
Root mass fraction	g g-1	RMF	0.178	0.122	0.354	25.8	0.646	0.035*	0.251
Root-to-shoot mass ratio	g g-1	RSR	0.220	0.139	0.548	35.1	0.613	0.067	0.276
Number of adventitious roots		Nr	20.6	6.0	74.0	64.0	0.003**	<0.001***	0.066
Total first-order root length	cm	TRLr1	5.43	1.52	15.10	59.1	<0.001***	<0.001***	0.02*
Specific root length	cm g-1	SRL	46.4	27.2	61.6	16.7	0.211	0.512	0.118
Root tissue density	g cm-3	RTD	0.065	0.052	0.080	7.9	0.317	0.031*	0.659
Maximal first-order root length	cm	MRLr1	38.1	31.0	45.0	10.0	<0.001***	<0.001***	0.75
Maximal spreading first-order root angle	°deg	MRA	110.0	86.2	132.0	12.3	0.114	0.083	0.421
Root system convex hull area	cm2	CHA	505	107	809	33.5	0.136	<0.001***	0.591
Maximal horizontal extension of the root system (width)	cm	MRW	23.5	7.7	29.5	23.1	<0.001***	0.002**	0.562
Maximal vertical extension of the root system (depth)	cm	MRD	31.1	8.5	37.0	18.0	0.057	0.016*	0.685
Morphological traits (at the individual root level)									
First-order root apical diameter	mm	Dr1	0.820	0.230	1.960	40.2	0.121	0.047*	0.420
Lateral root apical diameter	mm	Dr2	0.25	0.13	0.33	13.7	<0.001***	0.017**	0.083
Individual root length	cm	RL	131.0	9.5	489.0	76.1	0.006**	0.071	0.175
First-order individual root length	cm	RLr1	20.8	1.5	44.6	52.1	0.172	<0.001***	0.212
Total length of all lateral roots per adventitious root	cm	RLr2	88.7	2.3	304.0	78.8	0.002**	0.12	0.152
Mean lateral root length	cm	meanRLr2	1.8	0.4	3.9	45.5	<0.001***	0.017*	0.083
Number of first-order lateral roots per adventitious root		Nr2	109	3	388	73.8	0.782	0.499	0.527
Inter-branch distance	mm	IBD	4.69	1.96	14.60	46.4	0.007**	0.528	0.421
Length of the apical unbranching zone	cm	LAUZ	8.1	1.2	19.9	37.4	0.367	0.098	0.835
Length of the branching zone	cm	LBZ	18.08	0.87	37.34	47.4	0.988	0.011*	0.240

Vitis vinifera cv. Cabernet-Sauvignon was grafted onto varying lengths of *V. riparia* cv. Riparia Gloire de Montpellier (RGM) and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P). Results of a two-way ANOVA (with rootstock length and genotype as factors) are provided. ANOVA significance, ns: p-value < 0.05, * : 0.05 > p-value > 0.01, ** : 0.01 > p-value > 0.001, *** : p-value < 0.001

Finally, all individual roots were also analysed using the Rhizovision Analyser software (https://www.rhizovision.com/; Seethepalli *et al.*, 2021) to measure the total length of the roots on scans and root volume that allow us to calculate the integrated traits SRL and RTD on a large batch of samples. A detailed description of the traits is given in Table 1.

5. Data analysis

All graphs, data calculations and analyses were performed using R software (R Development Core Team 2023; R version 4.1.1; http://www.r-project.org/). A home-made R script using the 'XML' and 'dplyr' libraries was used to read RSML files and calculate root traits. Analyses of variance to compare rootstock

length and genotype effects were performed using the 'aov' function in the 'stat' library and post-hoc Student–Newman–Keuls analyses using the 'SNK.test' function in the 'agricolae' library. Prior to analysis, the normality and homoscedasticity of the data were assessed using the Shapiro–Wilk test in the 'stat' library and the Levene test in the 'rstatix' library, respectively. Principal component analyses were performed using the PCA function in the 'FactoMineR' library. Plotting was done using the 'ggplot2' library. To compare the morphological root traits in Figure 6, the data were normalised between 0 and 1 using the normalisation method: (data-min)/(max-min).

RESULTS

1. Phenotypic variation of plant growth and root traits

Plants grew for 112 d in pots (Exp. A) or 30 d in rhizotrons (Exp. B) and showed no visual signs of stress or mineral deficiency. A 1:1 linear correlation was observed for each scion/rootstock combination between the number of ARs or root mass production in plants grown in pots and those grown in rhizotrons, highlighting comparable root system development across both containers (Figure S1). Rootstock length had a significant effect on 100 % of shoot traits, 75 % of global root traits and 40 % of root morphological traits (Table 1). In Exp. A, plant survival was not significantly affected by rootstock length, but there was a trend towards more viable grafts with a 50 cm rootstock than with an 8 cm rootstock (Figure 2A). Grafts with 50 cm rootstocks produced twice as much shoot and root biomass as those with 20 or 30 cm rootstocks and four times as much as those with 8 cm rootstocks throughout the time course; these growth differences occurred from the first time point measured, 28 d after planting (Figure 2B). Root biomass increased dramatically over time, multiplying by 5 to 10 times between the first and the fourth month after planting, while shoot

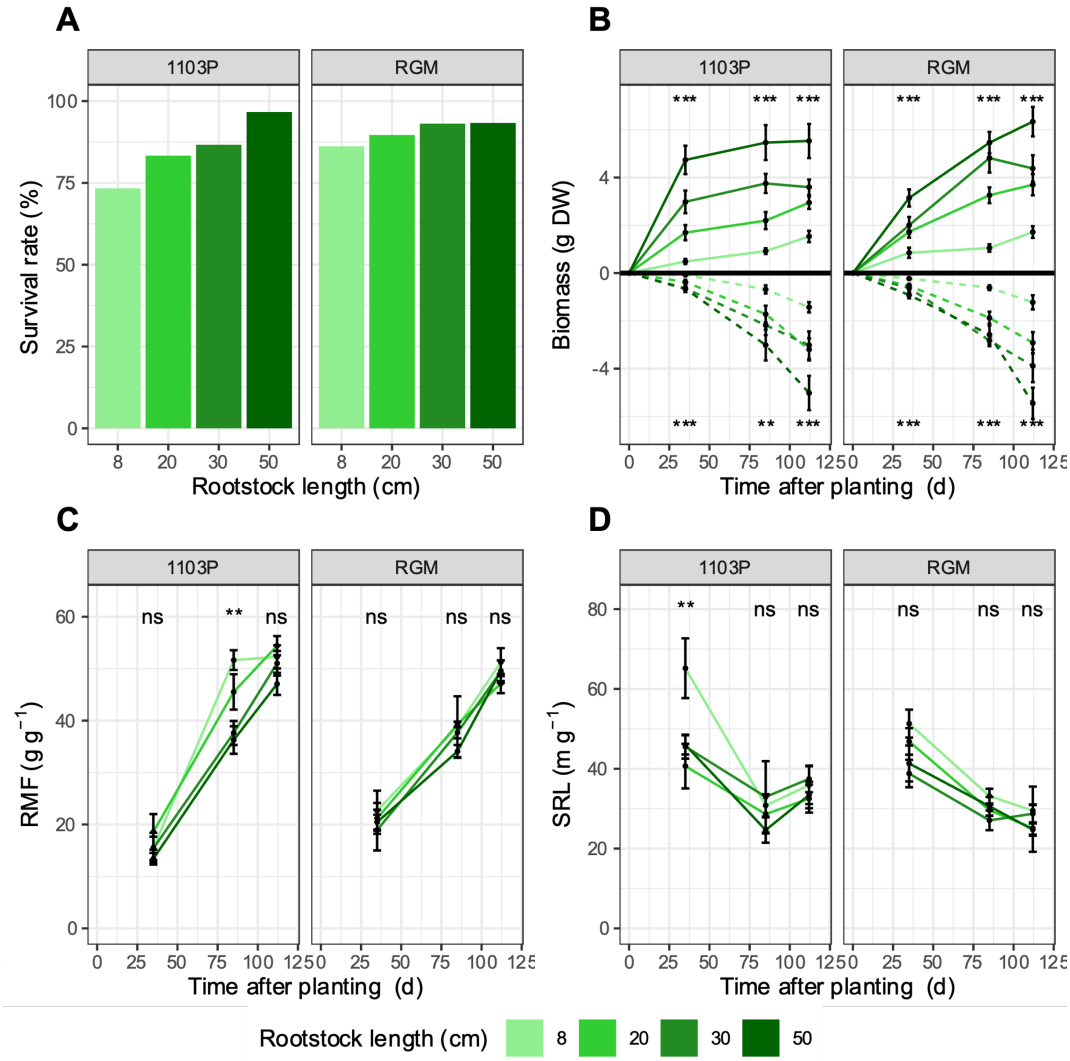


FIGURE 2. Effects of rootstock length on (A) plant survival rate, (B) shoot (solid line) and root (dotted line) dry mass accumulation, (C) root mass fraction (RMF) and (D) specific root length (SRL) in two scion/rootstocks combinations.

Vitis vinifera cv. Cabernet Sauvignon was grafted onto four lengths of *V. riparia* cv. Riparia Gloire de Montpellier (RGM) and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P) and grown for 112 days in pots (Exp. A). (A) rootstock length does not affect grafted plant survival (Chi² test). (B-D) ANOVA results: not significant, ns; 0.05 > p-value > 0.01, *; 0.01 > p-value > 0.001, **; p-value < 0.001, ***.

biomass increased by only 1.5 to 2 times during the same period. Consequently, the root mass fraction (RMF) doubled over this time (Figure 2C). However, the increase in root biomass was not proportional to the increase in root length, resulting in a 50 % decrease in specific root length (SRL) from one to four months after planting (Figure 2D).

Principal component analysis (PCA) was used to summarise the differences in RSA traits between CS/1103P and CS/RGM and between the four rootstock lengths for each experiment (Figure 3). For the pot experiment (Exp. A), principal components (PCs) 1 and 2 explained 44.8 % and 25.9 % of the total variability, respectively (Figure 3A, 3B and 3C). Principal component 1 separated the samples based on rootstock length (Figure 3B) and PC2 separated the samples based on rootstock genotype (Figure 3C). Rootstock length was positively correlated with shoot and root biomass, and negatively correlated with SRL, and rootstock genotypes were correlated with differences in RMF and RTD (Figure 3A). For the rhizotron experiment (Exp. B), PC1 and 2 explained 31.1 % and 22.1 % of the total variability, respectively (Figure 3D, 3E, 3F), and PC1 and 2 also separated the samples based on rootstock length (Figure 3E) and genotype (Figure 3F). Principal component 1 might be positively associated with variation in maximum and total primary root length and CHA, and negatively with the RTD and RMF. Principal component 2 was positively correlated with variation in mean lateral root length and diameter and negatively correlated with the IBD (Figure 3D).

2. Adventitious root rhizogenesis

Rhizotrons allowed precise measurement of AR numbers, showing a linear correlation ($R^2 = 0.47$ for CS/1103P and $R^2 = 0.88$ for CS/RGM, respectively) between ARs observed on the rhizotron wall and those counted during destructive harvesting one month after planting (Figure S2). The number of ARs initiated at the basal part of the graft was significantly influenced by both rootstock genotype and length, with no interaction between these factors (Table 1). In experiment B, the highest rate of root emergence occurred within the first 15 days and increased with rootstock length (Figure 4A). After one month, the 50 cm grafts produced on average three times more roots than the 8 cm grafts, and CS/1103P had 1.3 times more roots than CS/RGM. For both rootstocks, the number of ARs one month after planting was positively correlated with the initial fresh biomass of the graft (Figure 4B).

3. Root system architecture dynamics

The temporal dynamics of the two-dimensional root system distribution within the rhizotron were assessed by time series measurements of the total root length of first-order roots (TRLr1) and the convex hull area of soil explored by roots (CHA) (Figure 5, Figure S3). The measure of TRLr1 observed on the rhizotron wall correlated with TRLr1 measured on individual Ars after destructive sampling for both combinations (CS/1103P $R^2 = 0.95$, CS/RGM $R^2 = 0.78$; Figure S4). The total root length of first-order root values differed significantly between rootstock lengths at each time point (Figure 5A), with 50 cm rootstocks showing higher TRLr1. The combination CS/1103P had approximately twice the TRLr1 than CS/RGM for the same rootstock length.

However, this TRLr1 difference did not correspond to similar soil exploration patterns. Roots of RGM extended more vertically along the rhizotron wall, whereas 1103P roots grew in a narrow pattern (Figure S3), as reflected in the differences in mean root width (MRW) values (Table 1). Convex hull area increased during the first month for all treatments (Figure 5). Rootstock length significantly affected CHA; 8 cm rootstocks explored 2 to 3 times less area than longer rootstocks. The root system of CS/RGM occupied 1.2 times more area than CS/1103P in the case of the longest rootstocks, during the last three days before harvest (Figure 5B). Convex hull area coordinates were used to calculate maximum root depth (MRD) and width (MRW) (Figure 5C and 5D). At 20 days after planting, 30 and 50 cm CS/RGM root systems reached the side walls of the rhizotron, whereas 30 and 50 cm CS/1103P root systems only covered two-thirds of the width (Figure 5D). Maximum root width also increased with rootstock length for both genotypes, with 8 cm rootstocks being 1.5 to 2 times narrower than the longer rootstocks (Figure 5D). Maximal root depth was similar between CS/1103P and CS/RGM for the same rootstock length and increased with rootstock length (Figure 5C).

4. Individual root morphology

Significant differences in root morphological traits were observed between CS/1103P and CS/RGM and between different rootstock lengths, particularly in the diameter and mean length of lateral roots (Table 1). Traits related to the first-order axis (Dr1 and LAUZ) did not vary significantly with rootstock length for CS/1103P, but Dr1 was affected by rootstock length for CS/RGM. For both combinations, lateral root traits were not affected by rootstock length (Figure 6). The SmartRoot data showed that the mean inter-branch distance (IBD) increased along the length of the branching zone (LBZ) of the first-order root axis for both rootstock genotypes (Figure 7A, 7B and 7C). In the basal, oldest parts of the first-order roots, the mean IBD was 2.5 mm, increasing to over 5 mm in the youngest parts (Figure 7A). This increase was consistent along the first-order axis for all CS/RGM rootstock lengths (Figure 7C), but less so for CS/1103P, which showed elevated IBD values in the mid-root zone (Figure 7B). The mean length of lateral roots decreased progressively along the LBZ, from 3 cm to 0.5 cm in CS/RGM and from 4 cm to 0.5 cm in CS/1103P (Figure 7D). This decrease was more pronounced in CS/1103P than in CS/RGM, irrespective of the length of the rootstock (Figure 7E and 7F). The apical diameter of lateral roots remained constant at 0.3 mm in CS/RGM, but decreased from 0.3 mm to 0.2 mm along the first-order root axis in CS/1103P, being significantly smaller in the distal LBZ (Figure 7G). In addition, the shorter hardwood resulted in a smaller Dr2 for CS/RGM (Figure 7H and 7I).

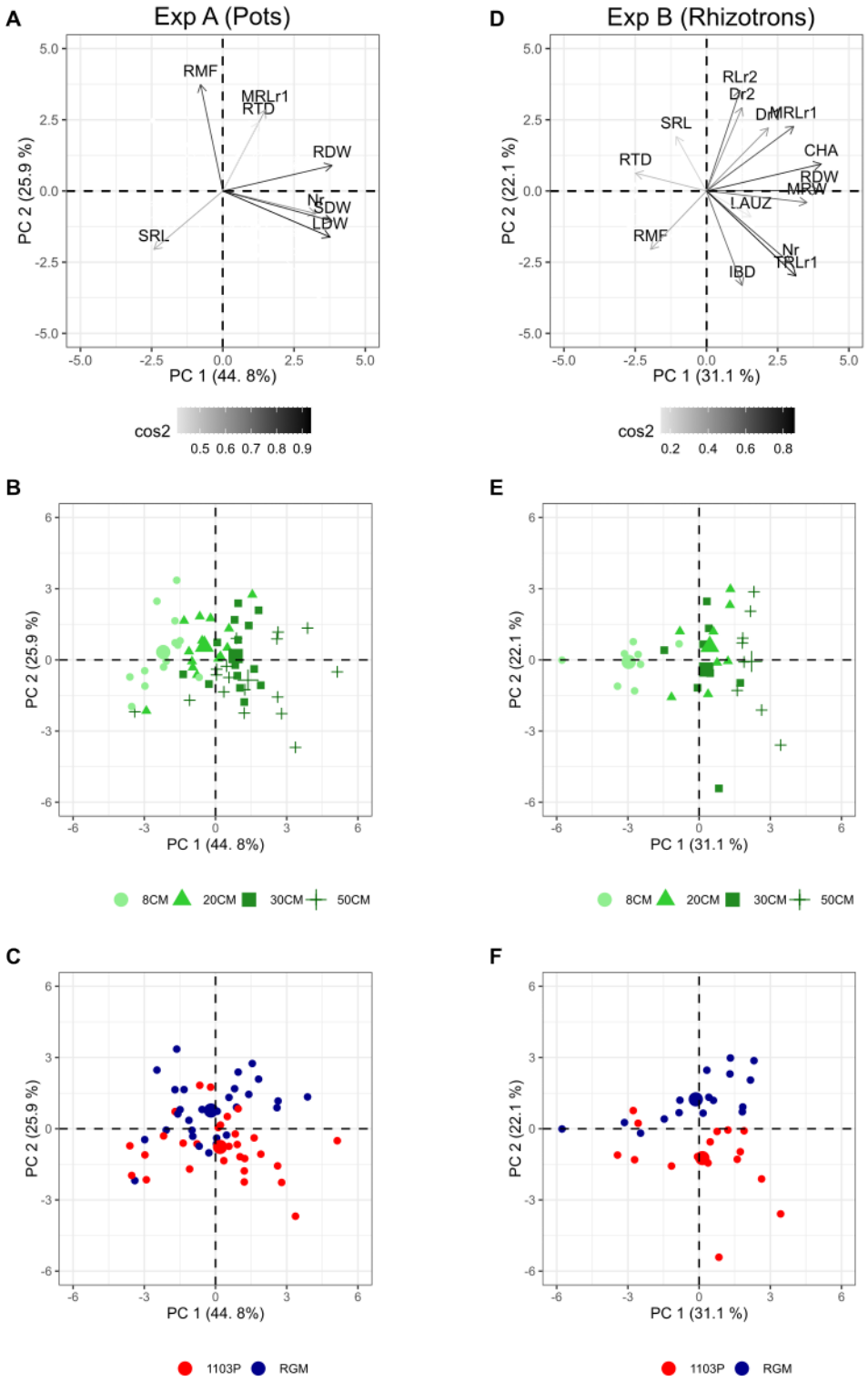


FIGURE 3. Principal component analysis of growth traits measured on (A–C) pot- and (D–F) rhizotron-grown *Vitis vinifera* cv. Cabernet-Sauvignon grafted onto four lengths of *V. riparia* cv. Riparia Gloire de Montpellier (RGM) and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P).

(A, D) Distribution of variables represented as vectors; (B, E) Distribution of individual observations labelled by rootstock length: 8 cm (circle), 20 cm (triangle), 30 cm (square), and 50 cm (cross); (C,F) Distribution of individual observations labelled by rootstock genotype: RGM (blue) and 1103P (red). Traits included are leaf dry weight (LDW), stem dry weight (SDW), root mass fraction (RMF), root tissue density (RTD), maximal length of a first-order root (MRLr1), number of first-order adventitious roots (Nr), root dry weight (RDW), specific root length (SRL), maximal adventitious root spreading angle (MRA), maximal horizontal extension of the root system (MRW), maximal vertical extension of the root system (MRD), convex hull area (CHA), total length of first-order roots (TRLr1), length of the branching zone (LBZ), length of the apical unbranched zone (LAUZ), inter-branch distance (IBD), apical diameter of a first-order root (Dr1), apical diameter of a lateral root (Dr2) and length of a lateral root (RLr2).

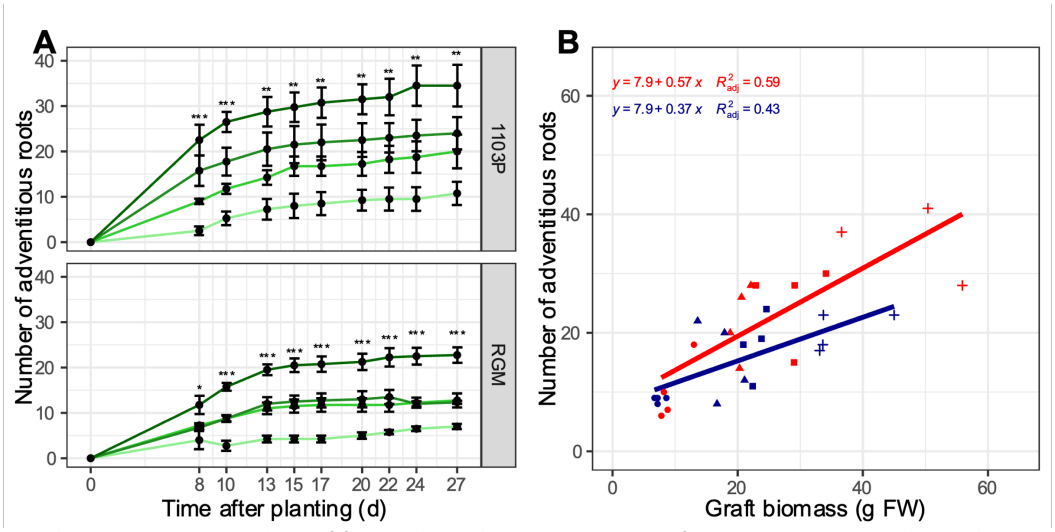


FIGURE 4. (A) Changes in the number of first-order adventitious roots of two scion/rootstock combinations grown in rhizotrons for 30 d as a function of rootstock length and (B) the relationship between the fresh mass of the grafted plant at grafting and the number of first-order adventitious roots of the two combinations after 30 d growth in rhizotrons.

Vitis vinifera cv. Cabernet Sauvignon was grafted onto four lengths of *V. riparia* cv. Riparia Gloire de Montpellier (RGM, blue) and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P, red). Rootstock length: 8 (circles), 20 (triangles), 30 (squares) and 50 cm (crosses). Results of ANOVA: not significant, ns; 0.05 > p-value > 0.01, *; 0.01 > p-value > 0.001, **: p-value < 0.001, ***. The results of the post-hoc test, highlighting group differences, are provided in Table S1.

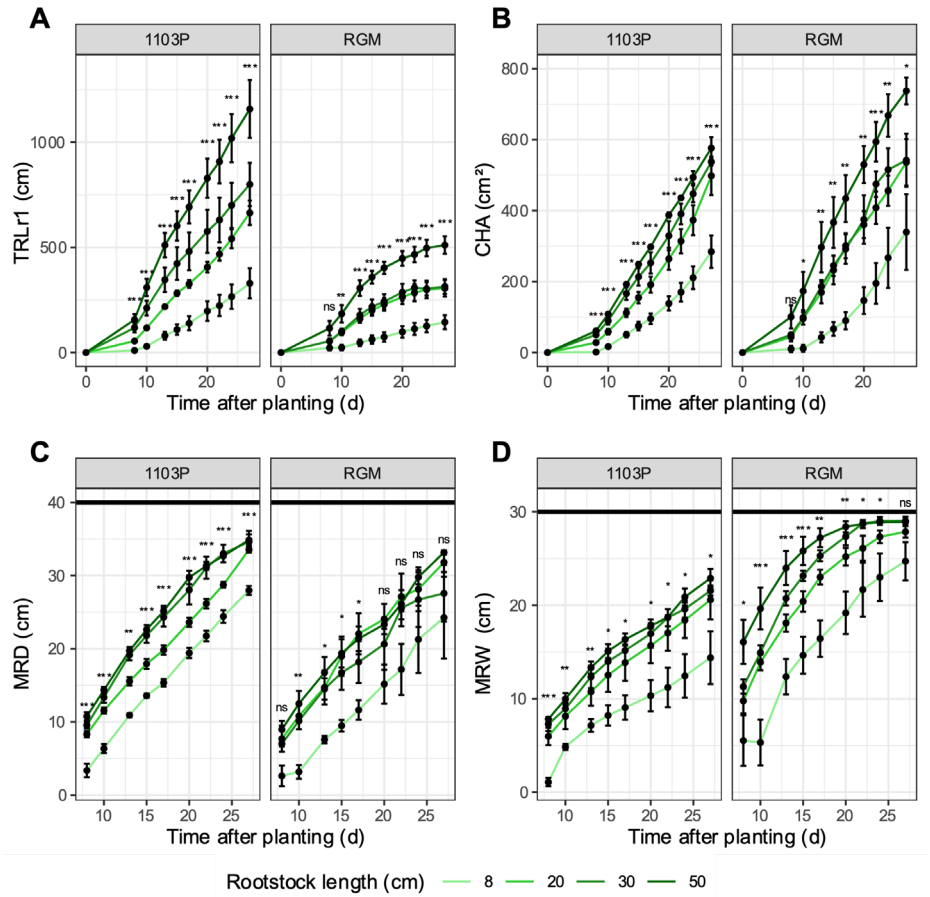


FIGURE 5. Changes in four global root system traits of two scion/rootstock combinations grown in rhizotrons for 30 d as a function of rootstock length.

(A) total first-order root length (TRLr1), (B) convex hull area (CHA), (C) maximal vertical extension of the root system (MRD) and (D) maximal horizontal extension of the root system (MRW). *Vitis vinifera* cv. Cabernet Sauvignon was grafted onto four lengths of *V. riparia* cv. Riparia Gloire de Montpellier (RGM) and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P). Results of ANOVA given: not significant, ns; 0.05 > p-value > 0.01, *; 0.01 > p-value > 0.001, **; p-value < 0.001, ***. The results of the post-hoc test, highlighting group differences, are provided in Table S1. The thick line shows the limit of the rhizotron (40 cm in C, 30 cm in D).

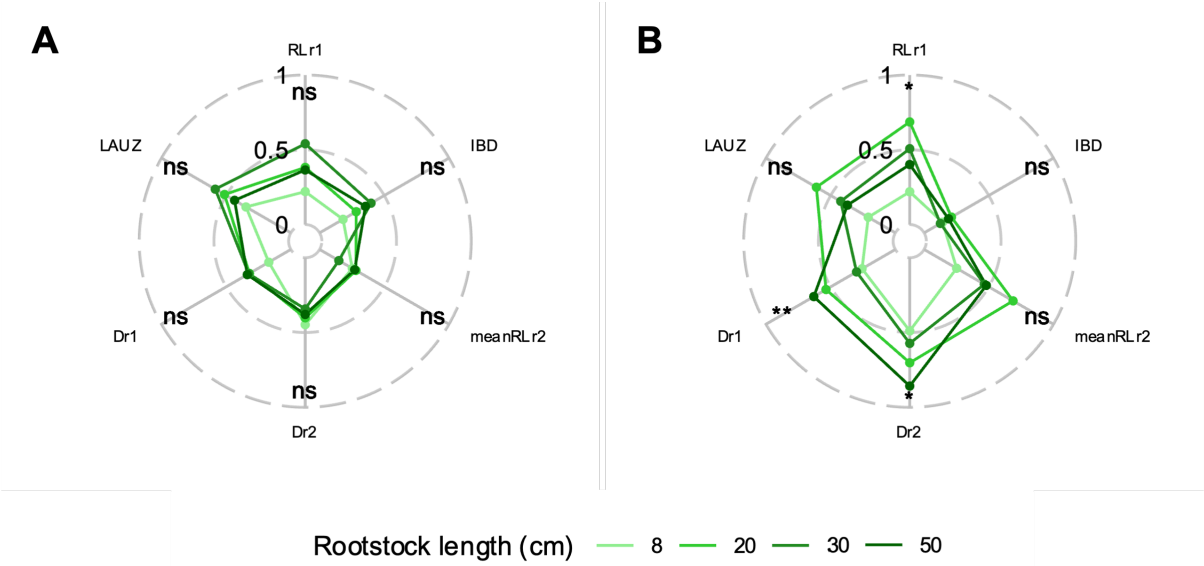


FIGURE 6. Effects of rootstock length on six morphological traits of individual roots of *Vitis vinifera* cv. Cabernet Sauvignon grafted onto four lengths of (A) the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P) and (B) *V. riparia* cv. Riparia Gloire de Montpellier (RGM) after 30 d of growth in rhizotrons.

Traits: First-order root length (RLr1), inter-branch distance (IBD), mean lateral root length (meanRLr2), lateral root apical diameter (Dr2), first-order root apical diameter (Dr1) and length of the apical unramified zone (LAUZ). Data are normalised between 0 and 1. Results of ANOVA: not significant, ns; 0.05 > p-value > 0.01, *; 0.01 > p-value > 0.001, **; p-value < 0.001, ***.

DISCUSSION

1. Rhizotron-based phenotyping system can accurately quantify grapevine RSA traits

One of the advantages of using rhizotrons for plant cultivation is the non-invasive observation and phenotyping of root systems over time, even in perennial species (Krzyzaniak *et al.*, 2021; Lesmes-Vesga *et al.*, 2022). However, rhizotrons can also result in a limited volume of growing medium, leading to physical confinement of the root system, which can potentially affect both the morphological and physiological function of the roots (Herrera *et al.*, 2021). To mitigate this issue, our study focused on analysing root architectural and morphological traits within a 30-day period after planting. Beyond this timeframe, root tips would have reached the rhizotron boundaries, complicating the accurate estimation of the projected root system area (Figure S3). In addition, root biomass density may have exceeded 1 g/L, the threshold reported by Poorter *et al.* (2012), above which the container size is considered limiting for the proper root system development. Despite these limitations, our observations showed vigorous shoot and root growth in the rhizotrons, comparable to similar plants grown in pots with the same substrate (Figure S1), consistent with previous studies (Dumont *et al.*, 2016; Maskova *et al.*, 2020). Two-dimensional images obtained from the rhizotrons provided quantitative phenotypic data on the number, length, and branching of adventitious and lateral roots, as well as their spatial distribution over time. A significant correlation was observed between the number of roots visible on the rhizotron wall on the last day before harvest and the manually counted ARs at harvest (Figure S2). The number of ARs estimated from the 2D images was slightly overestimated for CS/1103P, but not for CS/RGM. This discrepancy may be due to errors in counting either ARs that hit the wall several times in a discontinuous manner or large-diameter lateral roots that are misclassified as first-order roots. In contrast to previous literature (Nagel *et al.*, 2012), the total length of first-order roots correlated well with the length observed at the rhizotron wall. These results validate the 2D rhizotron imaging method for quantifying root characteristics in young grapevines.

2. Rootstock length is positively correlated with plant growth and adventitious rhizogenesis

The length of rootstock material used for grafting significantly impacted the recovery and early-stage growth of young grafted grapevine during the first month after planting, encompassing both shoot and root development. This phenomenon is well-documented among grape growers (Waite *et al.*, 2015) and has been observed in other woody species such as poplar (Desrocher & Thomas, 2003) and olive (Porfírio *et al.*, 2016). Longer hardwood cuttings accelerated AR emergence and increased the maximum number of ARs, regardless of rootstock genotype. Moreover, longer grafts enhanced total root system length and mass, facilitating rapid anchoring in the soil and enhancing water and mineral uptake to support shoot development (Lynch, 2013).

The variation in rhizogenesis among genotypes in response to graft length can be attributed to the physiological and biochemical characteristics of the hardwood cuttings taken from the mother plants. Smart *et al.* (2003) showed that cuttings from the base of the shoot had a higher rooting capacity than those from the apex, due to their larger diameter and greater maturity, characterised by more advanced wood

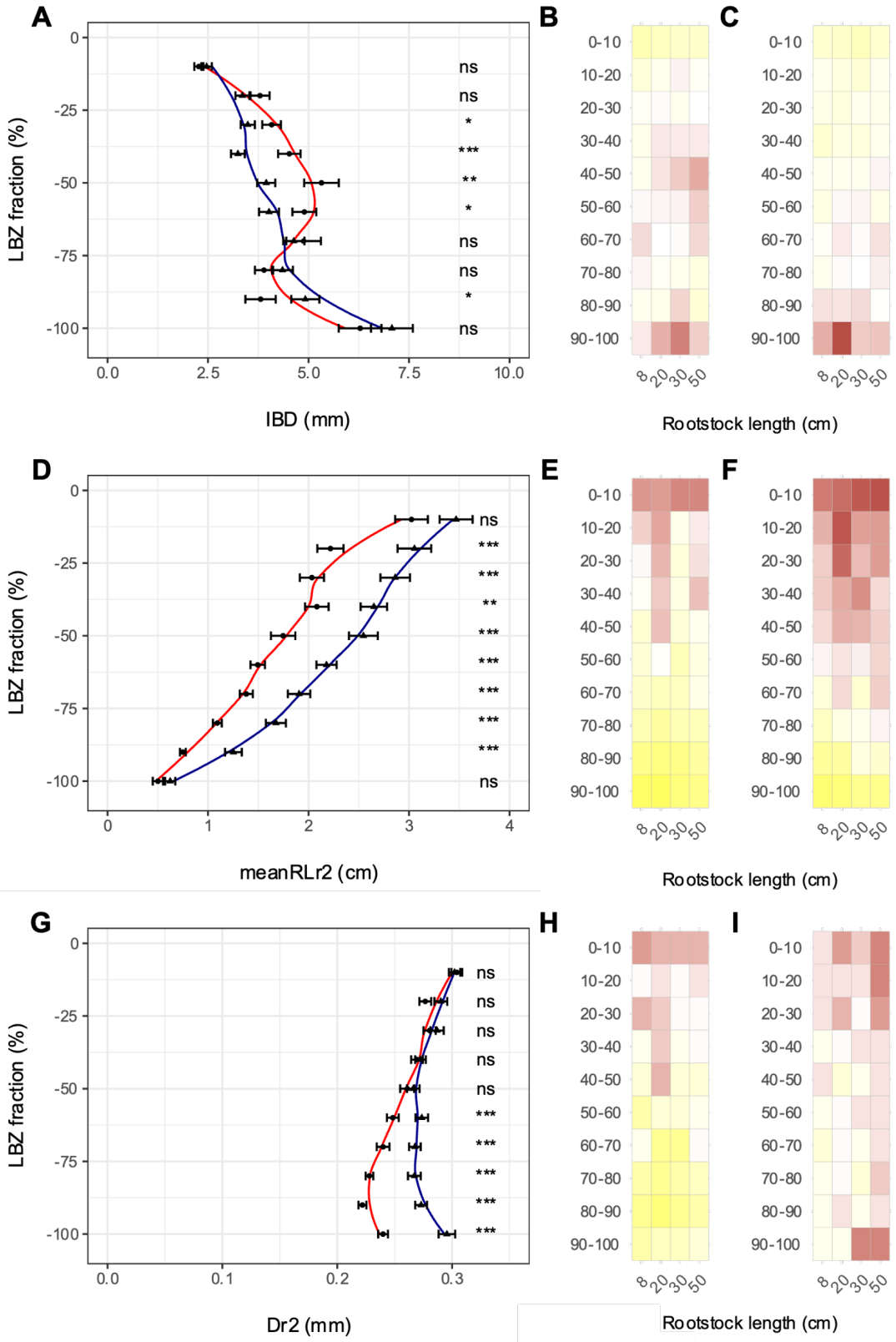


FIGURE 7. Distribution of the lateral root traits along the relative position on the first-order adventitious root branching zone (LBZ fraction) of *Vitis vinifera* cv. Cabernet-Sauvignon grafted onto four lengths of *V. riparia* cv. Riparia Gloire de Montpellier (RGM, blue) and the *V. berlandieri* × *V. rupestris* hybrid cv. 1103 Paulsen (1103P, red) after 30 d growth in rhizotrons.

(A-C) Inter-branch distance (IBD), (D-F) mean length of lateral root (meanRLr2) and (G-I) lateral root apical diameter (Dr2). (A, D, G) Means for each genotype, (B-I) heatmaps of the scale of variation (low yellow; high brown) for (B, E, H) 1103P and (C, F, I) RGM. The range from 0 to −100 corresponds to the basal to apical LBZ fraction. ANOVA significance, ns: p-value < 0.05, * : 0.05 > p-value > 0.01, ** : 0.01 > p-value > 0.001, *** : p-value < 0.001.

lignification. Therefore, to ensure consistent rooting potential, all cuttings in this study were standardised according to their position on the mother plant and their diameter.

Longer grafts are also more likely to root easily than shorter ones due to potentially higher levels of auxins, carbohydrates, mineral nutrients, or other biochemical components such as phenolic compounds (Bannoud & Bellini, 2021). Carbohydrates play a crucial role, not only in providing energy and carbon chains for biosynthetic processes in new root meristems but also in influencing gene expression in conjunction with auxin (Druege *et al.*, 2019). The positive correlation observed between initial graft biomass and the number of roots produced suggests that greater carbohydrate allocation at the root formation site may have favoured AR formation in longer grafts. Additionally, the maximum number of ARs was achieved approximately 15 d after planting under each condition, possibly due to a progressive shift in phytohormonal balance from promoting to repressing adventitious rooting (Da Costa *et al.*, 2013). The positive influence of graft length on rooting capacity exhibited significant variation among rootstock genotypes. Notably, CS/1103P had a higher maximum number of ARs than CS/RGM for equivalent graft lengths, contrary to existing literature suggesting lower rooting efficiency in 1103P compared to RGM (Gautier *et al.*, 2018). Although CS/1103P produced a greater number of roots than CS/RGM, there was no significant difference in total root mass between the combinations. This indicates a decoupling in the mechanisms of AR emergence and subsequent growth.

3. Rootstock length modified few individual root morphological traits

A number of early root morphological traits were quantified at the individual root level, with only a few influenced by rootstock length or genotype (Figure 1B, Figure 6 and Table 1). For both CS/1103P and CS/RGM, the average length of the first-order roots (RLr1) was slightly greater in the 50 cm grafts compared to the 8 cm grafts. This outcome is consistent with the hypothesis proposed by Thaler and Pagès (1998), which suggests that individual root elongation rates are partially regulated by assimilate availability. However, traits such as the length of the unbranched apical zone (LAUZ) and the apical diameter of the first-order axis (Dr1), which are often considered predictive indicators of root elongation rate (Pagès *et al.*, 2010), were not significantly affected by rootstock length in our study. Interestingly, only CS/RGM exhibited a smaller Dr1 with the shortest rootstocks. In general, the development of very fine roots in response to limited assimilates is considered a strategy to increase soil–root exchange surface area at minimal cost (Eissenstat *et al.*, 2000). In this context, RGM appears to display greater plasticity compared to 1103P.

Similarly, root traits associated with branching, such as the number of lateral roots (Nr2), the total length of lateral roots (RLr2), and the inter-branch distance (IBD), were minimally impacted by rootstock length. Notably, only the lateral roots of the CS/RGM displayed a larger apical diameter (Dr2) in longer grafts compared to shorter ones, consistent with the apical diameter of their parent root (Dr1). This finding is consistent with previous non-grapevine studies, which have shown that root traits related to emission and elongation are more influenced by the physiological state of the plant, whereas traits related to root branching are more influenced by the surrounding soil environment (Bui *et al.*, 2015).

Another consistent trend observed using Smartroot root image segmentation was the decrease in lateral root length, apical diameter, and to some extent, branching density along first-order roots, regardless of rootstock length (Figure 7). Typically, older roots near the base are longer than those recently formed at the apex (Pagès *et al.*, 1993). However, for CS/1103P, the reduction in lateral root length and apical diameter along the primary axis may also result from reduced carbon assimilate availability during plant development or reduced sink capacity of apical root meristems to attract these assimilates. Furthermore, IBD increased progressively from the basal to the distal part of first-order roots for CS/RGM, while IBD was more variable for CS/1103P. Similar increases or longitudinal variations in branching density have been documented in several species (Pagès, 2014). While soil gradients, especially nutrient availability, often account for such fluctuations, however, the relatively homogeneous substrate and consistent nutrient solution watering in this study suggest otherwise. Instead, differences in root elongation rate between genotypes during the first 30 d might explain these variations.

4. Distinct root system architectures differentiate RGM and 1103P rootstocks

The 2D RSA varied significantly between the two scion/rootstock combinations. For the same graft length, CS/1103P produced more ARs and had a greater total length of primary roots compared to CS/RGM, even though both genotypes had similar total root biomass. Additionally, while both rootstocks had similar branching densities, the lateral roots of CS/1103P were significantly shorter and thinner. The roots of CS/1103P grew at narrower angles from the cutting, indicating a stronger gravitropic response and a strategy giving priority to the exploration of deeper soil layers. In contrast, CS/RGM spread its larger ARs more horizontally. These distinct root system patterns reflect different strategies for soil exploration and resource use (Dayoub *et al.*, 2021). The narrower angles and deeper roots of CS/1103P suggest a strategy optimised for water uptake, while the horizontal root spread of CS/RGM might be better suited for accessing immobile nutrients in the upper soil layers. These findings highlight the importance of considering RSA in rootstock selection to optimise grapevine performance under diverse environmental conditions.

CONCLUSION

Here, we present an original study characterising the architectural and morphological traits of young grafted grapevine root systems in response to variations in rootstock length and genotype. The results indicate a direct correlation between rootstock length and increased adventitious

rhizogenesis and overall root system length, which could enhance soil exploration. Despite variations in total root number, individual root morphology remained unchanged in response to rootstock length, suggesting in particular that root branching was likely insensitive to carbon availability. These findings are consistent across two distinct rootstock genotypes with different RSA patterns and no common genetic background. Future research should focus on the long-term effects of rootstock length on grapevine development, using molecular and biochemical analyses to elucidate the underlying mechanisms. This knowledge will help to refine rootstock selection protocol and optimise planting practices, thereby contributing to more efficient and sustainable grapevine-growing strategies.

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