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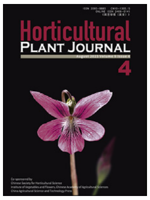

Agronomic evaluation of eight 41 B × 110 richter grapevine genotypes as rootstock candidates for mediterranean viticulture

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ABSTRACT

Choosing the most appropriate rootstock(s) is a key decision for the profitability of vineyards; therefore, there must be a sufficient range of rootstocks in the market adapted to different environmental conditions and production objectives. However, rootstock-breeding programs have been scarce in recent decades, and most of the rootstocks used today were bred a century ago, when the needs of the sector were very different from today. In this work, we aimed to evaluate new rootstock candidates before their introduction in the market. An agronomic evaluation was conducted on eight novel rootstock genotypes obtained from the first generation of the cross-pollination of 41 B Millardet et de Grasset (41 B) and 110 Richter (110 R) grafted with 'Syrah' and 'Tempranillo' and planted in a typical vineyard of the Ebro Valley in Spain. During the four consecutive growing seasons (2016–2019), growth, yield and berry composition parameters at harvest were collected. A linear mixed-effects model was constructed, considering year and block as random effects. Multiple factor analysis and hierarchical clustering on principal components were performed to establish clusters of genotypes with similar behaviour. The rootstock candidates showed a very wide performance range compared to their parents. The trial allowed us to identify two very promising candidates (RG8 and RG10), whose registration as commercial rootstocks is already in progress.

Keywords: *Vitis vinifera*; Graft; Phenolic quality; Vigour; Yield

1. Introduction

Grafting *Vitis vinifera* onto North American grapevine species, or hybrids including at least one phylloxera (*Daktulosphaira vitifoliae*) tolerant parent, is routinely performed in most grape-growing areas worldwide, and more than 80% of vineyards are grafted globally (Ollat et al., 2016). This practice was adopted at the end of the 19th century as the best solution to combat phylloxera, since many of the American *Vitis* species were tolerant to the presence of this pest in the soils, to which *V. vinifera* was very sensitive. This solution allowed the persistence of traditional *V. vinifera* cultivars, whose grapes are desirable for wine production.

At first, breeders were only focused on that purpose, using individuals of *V. riparia* and *V. rupestris* species as rootstocks due to

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their high resistance to the pest and their good ability to graft (Cousins, 2005; Ollat et al., 2016). However, these two species showed poor adaptation to limestone soils, characteristic of many Mediterranean areas, so *V. berlandieri* (synonymous *V. cinerea* 'Helleri') and *V. vinifera* species were included in the early 1880s into breeding programs because of their good behaviour in this kind of soil (Cousins, 2005; Bavaresco et al., 2015). Rootstocks also have a strong influence on the growth and vegetative cycle of vines, bud fertility, yield and berry composition (May, 1994; Morris et al., 2007; Pulko et al., 2012; Miele and Rizzon, 2017; Marín et al., 2019a).

Due to the formidable work done by breeders at the end of the 19th century and the beginning of the 20th century, a large number of rootstocks with different characteristics have been available on the market since then (Marín et al., 2020), although only a few have been widely used by the grape industry due to their good reputation (de Andrés et al., 2007; Bavaresco et al., 2015; Zavaglia et al., 2016; Marín et al., 2019b). However, the requirements of the sector in traditionally cultivated Mediterranean areas are changing, mainly due to the effects of climate change (Santos et al., 2020). Climate change causes longer drought events (Van Leeuwen et al., 2019) and critical periods of water stress when irrigation is not available (Costa et al., 2016; Fraga et al., 2018). It also implies warmer conditions for plants (Jones et al., 2005), which advances the ripening cycle and may cause decoupling between sugar and phenolic accumulation (Gutiérrez-Gamboa et al., 2021), resulting in an excessive sugar content and low acidity, which are negative characteristics for the quality of the wine (Martínez de Toda and Balda, 2015; Van Leeuwen et al., 2019). Additionally, climate change could also favour the pressure exerted by certain pests in some areas (Caffarra et al., 2012), such as the spread of soilborne fungal diseases (Larignon et al., 2009). Within this scenario, rootstock election emerges as an essential adaptation tool to overcome these negative aspects related to climate change (Neethling et al., 2017; Van Leeuwen et al., 2019; Marín et al., 2020; Santos et al., 2020).

Nevertheless, hardly any new rootstock better adapted to current requirements has been developed since the beginning of the 20th century. To the best of our knowledge, only eight organizations have released new successful rootstocks to the market during the 21st century. Detailed information on the different breeding programs can be found in Marín et al. (2020). Thus, the need to develop new rootstocks seems to be a matter of real concern.

Therefore, the aim of this study was to evaluate the agronomic performance of eight rootstock candidates (RG2, RG3, RG4, RG6, RG7, RG8, RG9 and RG10) in comparison to their parents 41 B (*V. vinifera* × *V. berlandieri*, clone V14D) as female and 110 R (*V. rupestris* × *V. berlandieri*, clone 1D) as male, grafted with 'Syrah' and 'Tempranillo' when planted in a typical vineyard in the Ebro Valley in Northern Spain over four consecutive growing seasons.

2. Materials and methods

2.1. Cross-breeding program

The Vitis Navarra nursery, in collaboration with the team led by Dr. José Bernardo Royo from the Public University of Navarre (UPNA), initiated a rootstock-breeding program in Northern Spain in the 1990s. Their main goal was the development of a new series of rootstocks better adapted to Mediterranean conditions. With this purpose, they carried out hybridization via cross-pollination between 41 B (*V. vinifera* × *V. berlandieri*, clone V14D) and 110 R (*V. rupestris* × *V. berlandieri*, clone 1D). The former was chosen for its well performance in limestone soils, and the latter for its good tolerance to drought, two very common characteristics in Mediterranean soils. Cross pollination was conducted according with a simple protocol developed in the nursery. Briefly, anthers were removed from the 110 R flowers without breaking them and pollen grains were collected into 10 mL falcon tubes in spring. Then, these pollens grains were used for pollinize the 41 B inflorescences by using laboratory pincers and a magnifying glass. Seeds were collected at the end of the growing season and planted in pots. After initial tests to evaluate performance in terms of cane productivity and compatibility, eight virus-free genotypes were selected from the progeny and named RG2, RG3, RG4, RG6, RG7, RG8, RG9 and RG10 (breeder personal communication). The genetic background of the new genotypes was confirmed through 25 SSR markers (Table S1).

2.2. Plant material and growing conditions

An experimental vineyard located in Miranda de Arga (42°27′50.6″ N 1°48′10.6″W, 308 MASL, Navarra, Spain) was established with plants bench grafted by omega technique described in Reynier (1989), using the eight novel candidates (RG2, RG3, RG4, RG6, RG7, RG8, RG9 and RG10) and their parents (41 B and 110 R) as rootstocks. The vineyard was planted with 'Tempranillo' in Spring (2011) and with 'Syrah' in Spring (2012), following a randomized complete block design with three replicates of 10 vines per rootstock and cultivar. The vines were trellised to a unilateral cordon Royat (Reynier, 1989), pruned to five two-node spurs per vine, with no shoot trimming during the seasons to minimize its interference in the evaluation of rootstock effects on vegetative growth. Plants were spaced 3 m between rows and 1 m within rows (0.3333 vines $\cdot$ m^{-2}). The climate in this area is continental-Mediterranean, with an average rainfall of 350–400 mm $\cdot$ year^{-1}. Within the years of study (2016–2019), the values of total annual rainfall were higher than the global average (530 mm in 2016, 457 mm in 2017, 515 mm in 2018, and 415 mm in 2019), with differences in the distribution of rainfall (Fig. 1). At specific times from July to September, the vineyard was drip irrigated through 4 L $\cdot$ h^{-1} pressure-compensated emitters (AZUD PRO, AZUD, Spain) placed 0.5 m along a single drip line hanging under the vines. The total amount of water applied by irrigation accounted for approximately 36 mm in 2016 and 2018, and 48 mm in 2017 and 2019. The soil was maintained with spontaneous permanent inter-row cover, whereas the crop line was maintained free of vegetation with an herbicide (Flazasulfuron 25%). The vineyard is located in a Quaternary sedimentary soil (main characteristics measured before planting are summarized in Table 1) with a loamy texture and highly calcareous (Ca/Mg ratio 27.2, total carbonates 41%) but moderate active lime content (8%), similar to many soils in the Mediterranean area but not extreme.

2.3. Agronomic evaluation

The agronomic evaluation was carried out over four consecutive seasons (2016–2019). A total of 12 parameters were

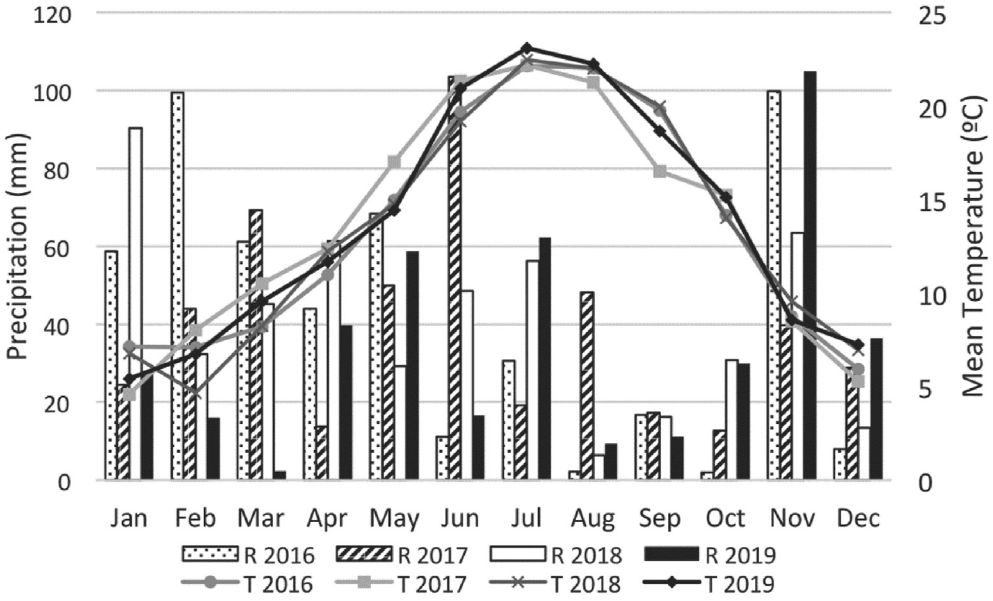

Fig. 1 Monthly rainfall (R) and mean temperature (T) in 2016–2019 in the experimental vineyard located in Miranda de Arga (Navarra, Spain)

evaluated: winter pruning weight, yield, bunch number, bunch weight, Ravaz index, berry weight, total acidity, pH, L-malic acid, total soluble solids, potential tannins index and total extractable anthocyanin content. Table 2 summarizes the main information of all the parameters measured. Growth and production measurements were made on a per vine basis in the 10 plants comprising each of the three replicates (i.e., a total of 30 plants were evaluated for each rootstock, and the average value per block was then calculated). Pruning weight was measured in winter, whereas production, yield and grape quality parameters were evaluated at harvest time. For each season, the harvest was carried out the same day for all rootstocks of the same cultivar, determining the harvest day regarding grape composition evolution. The day before harvest, a sample of 200 berries was collected from each replicate. Each sample consisted of 20 berries picked from four different bunches per vine; two of them picked berries from the outer side, and the remaining two picked berries from the inner side. These five berries were taken following the same pattern, two from the shoulders, two from the middle and one from the tip of the cluster. The samples were weighed immediately to determine the mean berry weight and then delivered in a cold box with ice (4 °C) to the nearby Excell Iberica company laboratory (La Rioja, España) for analysis. Samples were homogenized with a Classic 8-Speed Blender (Oster, Wisconsin, USA) at full speed, and part of this homogenate (100 g) was macerated for 1 h at room temperature (22 °C) and then centrifuged at 10 000 r · min^{-1} with a Hettich MIKRO 200/200 R centrifuge (Hettich, Massachusetts, USA) at 4 °C. The supernatant was used to measure grape composition following standard procedures described in Table 2.

2.4. *Statistical analysis*

Statistical analysis was carried out in R version 3.6.1 (R Core Team, 2019) with Rstudio (version 1.2.2019) software (RStudio Team, 2020). For each cultivar, analysis of variance (ANOVA) was carried out with a linear mixed-effects model (lmer function from lme 4 package) (Bates et al., 2015), considering "rootstock" as a fixed effect and "year" and "block" as random effects. We used the plot of the standardized residuals *vs*. the fitted values to check the model assumptions. The cld function from the multcomp package (Hothorn et al., 2008) was used to set up the compact letter display of all pairwise comparisons. Multiple factor analysis [MFA from factoextra (Kassambara and Mundt, 2019)] was

Table 1 Main characteristics of the vineyard soil analysed in 2019

Parameter/Unit	Value	Interpretation proposed by the laboratory
Water pH	8.6	Slightly alkaline
Organic matter/(g·kg^{-1})	20.3	High, but 'fossilised'
Assimilable P/(mg·kg^{-1})	28.8	High
Assimilable K/(mg·kg^{-1})	145.2	Appropriate
Assimilable Mg/(mg·kg^{-1})	61.7	Correct
Carbonates/(g·kg^{-1})	407.4	High. Could limit the assimilation of other minerals
Active lime/(g·kg^{-1})	75.4	Low. No lack of trace elements is expected
Cation exchange capacity/[cmol (+)/Kg]	12.35	Medium nutrient retention
Electrical conductivity/(dS·m^{-1})	0.4	No salinity risk

Table 2 Main information of the measured parameters

Category	Parameter	Method
Vigor	Winter pruning weight/(kg·vine^{-1})	Total pruning weight of 10 plants/10
Production and Yield	Yield/(kg·vine^{-1})	Weighing total yield of 10 plants/10
	Bunch number	Counting total bunch number of 10 plants/10
	Bunch weight/g	Yield/Number of bunches
Balance	Ravaz index	Yield/pruning weight (Ravaz, 1911)
Industrial maturity	Berry weight/g	Total weight of 200 berries/200
	Titratable acidity/(g·L^{-1})	Titrimetric. (European Commission, 2009)
	pH	Digital pH meter. (European Commission, 2009)
	L-Malic acid/(g·L^{-1})	Enzymatic analysis. Method OIV-MA-AS313-11 (OIV, 2009)
	Total soluble solids/°Brix	High precision temperature compensating refractometer. (European Commission, 2009)
Phenolic maturity	Potential Tannins index	A higher index means a higher tannin content in grapes$^{¥}$
	Total extractable anthocyanin content/(mg·L^{-1})	Ribéreau-Gayon and Stonestreet (1965)

conducted on all the evaluated parameters grouped by year using the whole dataset of both cultivars. Hierarchical clustering on principal components [HCPC from *FactoMineR* (Lê et al., 2008)] was performed to establish clusters of rootstocks with similar behaviour. The data were standardized within each parameter and year before carrying out the MFA and the HCPC. Outliers were initially eliminated before the different analyses using the identify_outliers tool from the rstatix package (Kassambara, 2020).

3. Results

Tables 3 and 4 summarize the effect of the rootstock candidates and their parents (41 B and 110 R) on the growth, yield and berry composition parameters for the scion genotypes 'Syrah' and 'Tempranillo', respectively. The values presented in both tables are the average effect over the four years of evaluation, since no significant interaction between rootstock and year was observed, and the study of vintage was secondary in this study. Below, we detail the most relevant results obtained.

3.1. *Vegetative expression and yield components*

A significant effect of "rootstock" was observed in all the parameters related to growth and yield for both varieties (Tables 3 and 4). In relation to the vigour conferred by their parents, the novel hybrids showed a broader range of responses in both directions. Specifically, in 'Syrah' (Table 3), both parents showed medium–high vigour in terms of winter pruning weight. RG8 outperformed the vigour of both parents by 40% and RG10 by 5%. On the other hand, RG6 and RG7 were the least vigorous (50% lower with respect to 110 R). For 'Tempranillo' (Table 4), 41 B conferred the lowest vigour to the scion, and 110 R conferred a similar medium–high vigour. RG8 was equally the most vigorous hybrid (40% more than the parents), and RG9 and RG2 were less vigorous than the parents (40% and 80% lower, respectively).

Regarding productivity, 41 B showed intermediate yield, whereas 110 R was one of the least productive genotypes for 'Syrah'. This difference was due both to differences in the number of bunches per vine (14.53 in 41 B compared to 11.88) and to differences in bunch weight (144 g *vs.* 108 g). The new RG genotypes showed a highly variable behaviour, and some of them again exceeded the yield values shown by their parents in both directions. RG8 and RG4 were the most productive novel hybrids in 'Syrah', showing production 80% and 60% higher than that of 110 R, respectively. RG2 was the least productive, with 40% lower production than 41 B. In 'Tempranillo', 41 B and 110 R had intermediate to high yields (2.15 kg·vine^{-1} and 1.67 kg·vine^{-1}, respectively) with intermediate to high bunch numbers (10.79 bunches·vine^{-1} and 9.10 bunches·vine^{-1}, respectively). RG2 was the least productive, as it showed hardly any bunch production, which was also related to the very low growth described above. Its differences from the parents were significant both in terms of yield (90% lower than 41 B and 87% lower than 110 R) and bunch number (75% lower than 41 B and 72% lower than 110 R). RG9 also showed scarce productivity compared to 41 B (yield 59% less). RG10 showed similar behaviour to 110 R in terms of both yield and bunch number. Finally, RG3, RG4, RG6, RG7 and RG8 provided medium to high yields within the parameters of the trial.

The Ravaz index allows us to evaluate the balance between vegetative growth and yield. Among the 'Syrah' scions, 41 B showed an increase in this ratio towards yield relative to 110 R (3.83 and 2.20, respectively), and the latter was the rootstock with the lowest Ravaz index within this cultivar. RG3, RG4, RG6, RG7 and RG9 also resulted in Ravaz index values higher than those of 110 R (63%, 127%, 95%, 107% and 69% more, respectively), whereas RG4 was the only novel hybrid that showed a Ravaz index 30% higher than 41 B. Among the 'Tempranillo' scions, the differences between 41 B (5.02) and 110 R (3.57) were not significant, and RG4 was again the candidate with the highest Ravaz values, along with RG7.

3.2. *Berry composition at harvest*

Regarding industrial maturity parameters, the 'rootstock' effect was somewhat significant for berry weight and was highly significant for L-Malic acid and total soluble solids in both 'Syrah' and 'Tempranillo', whereas no significant effect was observed on titratable acidity and pH for any of the cultivars.

The berries were smaller in RG9 than in 110 R for 'Syrah' (13% less) (Table 3). In 'Tempranillo', RG9 was also the rootstock with the smallest berries but only in comparison with RG7 and RG10 (Table 4).

A strong effect of "rootstock" on sugar concentration at harvest was found in 'Syrah', 110 R (24.31 °Brix) which was one of the sweeter rootstocks, along with RG2, compared to 41 B (22.59 °Brix). On the other hand, RG10 showed a general decrease in

Table 3 Effect of "rootstock" on growth, yield components and maturity parameters of 'Syrah'

Rootstock	'Syrah' growth and yield										'Syrah' juice composition											
	Pruning weight/ (kg·vine^{-1})		Yield/ (kg·vine^{-1})		Bunch		Bunch weight/g		Ravaz index[c]		Berry weight/g		Titra-table acidity[d]	pH	L-Malic acid/(g·L^{-1})		Total soluble solids/°Brix		Potential tannins index		Antho-cyanins/ (mg·L^{-1})[e]	
41 B	0.59	ab[a]	1.98	bcd	14.53	bcd	144.00	bc	3.83	bcd	1.47	ab	5.09	3.62	1.52	ab	22.59	ab	38.75	ab	556.30	a
RG2	0.38	ab	1.15	a	10.95	a	92.65	a	2.78	ab	1.33	ab	5.40	3.57	1.53	ab	24.56	c	44.75	b	748.00	b
RG3	0.59	ab	2.10	bcd	15.23	bcd	135.16	bc	3.59	bc	1.45	ab	5.24	3.65	1.57	ab	23.28	abc	39.25	ab	705.00	ab
RG4	0.45	ab	2.29	cd	16.13	cd	137.87	bc	5.00	e	1.43	ab	5.40	3.61	1.25	a	23.18	abc	38.75	ab	703.50	ab
RG6	0.35	a	1.50	abc	13.13	abc	113.11	ab	4.31	cde	1.38	ab	5.41	3.56	1.34	a	23.89	bc	39.75	ab	672.80	ab
RG7	0.33	a	1.77	abc	13.87	bcd	119.21	ab	4.57	de	1.39	ab	5.22	3.63	1.31	a	23.78	bc	41.00	ab	734.50	b
RG8	0.86	c	2.63	d	16.82	d	156.59	c	3.21	abc	1.49	ab	5.60	3.68	2.11	c	23.22	abc	40.00	ab	644.50	ab
RG9	0.45	ab	1.56	abc	13.57	abcd	114.19	ab	3.72	bc	1.31	a	5.70	3.60	1.54	ab	24.10	bc	43.75	b	689.80	ab
RG10	0.64	bc	1.74	abc	14.51	bcd	120.67	abc	3.10	abc	1.40	ab	5.42	3.63	2.03	bc	21.89	a	39.50	ab	563.80	a
110 R	0.63	bc	1.41	ab	11.88	ab	108.33	ab	2.20	a	1.51	b	5.00	3.72	1.87	abc	24.31	c	36.50	a	660.50	ab
Significance[b]		***		***		***		***		***		*	NS	NS		***		***		**		**

Note: Values are the average effect over the years evaluated.

[a] Different letter denote significant differences in all-pairwise comparisons.

[b] Significance (*P* value) in Analysis of Variance on a linear mixed-effects model are indicated by asterisks symbols: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant.

[c] Ravaz index = Yield/Pruning weight.

[d] Total Acidity expressed as g Tartaric·L^{-1}.

[e] Anthocyanins = Total extractable anthocyanin content.

Table 4 Effect of "rootstock" on growth, yield components and maturity parameters of 'Tempranillo'

Rootstock	'Tempranillo' growth and yield										'Tempranillo' juice composition											
	Pruning weight/ (kg·vine^{-1})		Yield/ (kg·vine^{-1})		Bunch number		Bunch weight/g		Ravaz index[c]		Berry weight/g		Titratable acidity[d]	pH	L-Malic acid/ (g·L^{-1})		Total soluble Solids/ °Brix		Potential tannins index		Antho -cyanins/ (mg·L^{-1})[e]	
41 B	0.45	cd[a]	2.15	c	10.79	cde	177.62	bc	5.02	bcd	1.55	ab	4.47	3.67	1.30	ab	22.72	a	39.50	a	778.00	b
RG2	0.08	a	0.23	a	2.62	a	70.87	a	2.28	a	1.46	ab	4.18	3.73	1.48	ab	25.37	c	54.67	b	763.67	ab
RG3	0.41	bcd	2.13	c	10.17	cd	181.66	c	4.56	bcd	1.57	ab	4.46	3.64	1.33	ab	23.95	abc	43.75	ab	666.00	ab
RG4	0.42	bcd	2.46	c	13.05	de	176.79	bc	6.03	d	1.54	ab	4.34	3.69	1.29	ab	23.71	ab	43.25	ab	679.25	ab
RG6	0.39	bc	1.96	c	10.40	cde	182.49	c	5.18	cd	1.54	ab	4.43	3.60	1.19	a	23.30	ab	38.25	a	632.00	a
RG7	0.45	cd	2.50	c	13.25	e	181.72	c	5.80	d	1.62	b	4.40	3.64	1.24	a	23.00	a	43.25	ab	693.50	ab
RG8	0.58	d	2.42	c	10.38	cde	208.25	c	4.23	bcd	1.58	ab	4.61	3.66	1.61	ab	23.26	ab	45.50	ab	719.50	ab
RG9	0.27	b	0.89	ab	6.31	b	116.90	ab	3.09	ab	1.39	a	4.32	3.72	1.24	a	24.35	bc	57.50	b	786.75	b
RG10	0.45	cd	1.75	bc	8.68	bc	173.77	bc	3.44	abc	1.66	b	4.85	3.70	1.72	b	22.99	a	47.00	ab	774.75	b
110 R	0.44	cd	1.67	bc	9.10	bc	161.98	bc	3.57	abc	1.57	ab	4.71	3.67	1.28	ab	24.00	abc	48.25	ab	787.50	b
Significance[b]		***		***		***		***		***		*	NS	NS		**		***		**		*

Note: Values are the average effect over the years evaluated.

[a] Different letter denote significant differences in all-pairwise comparisons.

[b] Significance (*P* value) in Analysis of Variance on a linear mixed-effects model are indicated by asterisks symbols: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; NS, not significant.

[c] Ravaz index = Yield/Pruning weight.

[d] Total Acidity expressed as g Tartaric·L^{-1}.

[e] Anthocyanins = Total extractable anthocyanin content.

sugar content over the different seasons in ‘Syrah’, obtaining on average a total soluble solids value 10% lower than 110 R. For ‘Tempranillo’, 110 R (24.00 °Brix) showed generally higher values than 41 B (22.72 °Brix). RG2 and RG9 were the candidates with the highest values (10% and 7% more than 41 B, respectively). RG10 and RG7 again showed a trend of lower total soluble solids values than 110 R.

Concerning L-Malic acid in ‘Syrah’, 41 B (1.52 g · L^{-1}) showed a trend of lower values than 110 R (1.87 g · L^{-1}). RG8 was the novel hybrid with the highest values, surpassing 41 B by 38%. In ‘Tempranillo’, both parents showed similar values (1.30 for 41 B and 1.28 for 110 R), and no significant differences were seen between them and any of the novel hybrids.

Regarding phenolic maturity parameters, a significant effect was observed on potential tannins index and total extractable anthocyanin content for both cultivars. For ‘Syrah’ (Table 3), 41 B (38.75) and 110 R (36.50) obtained relatively low values of potential tannins index, although this trend was generally observed for all the genotypes evaluated. RG2 and RG9 showed a trend to present 20% higher values of potential tannins index than 110 R. Regarding total extractable anthocyanin content, 41 B (556.30 mg · L^{-1}) had lower values on average than 110 R (660.50 mg · L^{-1}). RG2 and RG7 were the novel hybrids with the highest values, approximately 30% higher than that of 41 B.

Contrary to ‘Syrah’, 41 B presented on average a lower potential tannins index content than 110 R in ‘Tempranillo’ (Table 4). RG2 and RG9 were again the candidates with the highest potential tannins index, 40% higher than 41 B. Regarding total extractable anthocyanin content, 41 B (778.00 mg · L^{-1}) and 110 R (787.50 mg · L^{-1}) presented the highest values, together with RG9 and RG10.

3.3. *Overall evaluation through multiple factor analysis (MFA) and hierarchical clustering on principal components (HCPC)*

To evaluate and summarize the implications of using a novel rootstock series, MFA was conducted considering the mean value of the three blocks for each rootstock, year and cultivar. The initial data were standardized within each year before performing the analysis.

The first two principal components (PC1 and PC2) explained approximately 35% and 23% of the total variance, respectively (Fig. 2). PC1 was highly positively associated with some of the growth and yield parameters, mainly bunch number, winter pruning weight and yield (Fig. 2, a), while some of the grape composition parameters, such as potential tannins index, total soluble solids and pH, were negatively associated with PC1. Additionally, the separation of production variables (bunch weight, berry weight and Ravaz index) was mainly linked to PC2. Regarding the distribution of individuals, Fig. 2, b shows a great effect of the cultivar, with the ‘Syrah’ individuals scattered mainly within the fourth quadrant, while ‘Tempranillo’ individuals were distributed between the second and third quadrants.

To improve the visualization and understanding of the relationships between genotypes, HCPC was performed on the first two PCs. The results were plotted in a phylogenetic tree (Fig. 3), where clear differentiation between both cultivars and novel rootstock candidates was also observed. The different genotypes were grouped into three clusters for each cultivar. In ‘Syrah’, 41 B, RG3, RG4, RG8 and RG10 were grouped together, and they were highly correlated with PC1 [i.e.: medium to high vigour (winter pruning weight), and medium to high productivity in terms of yield and bunch number]; these genotypes were also the

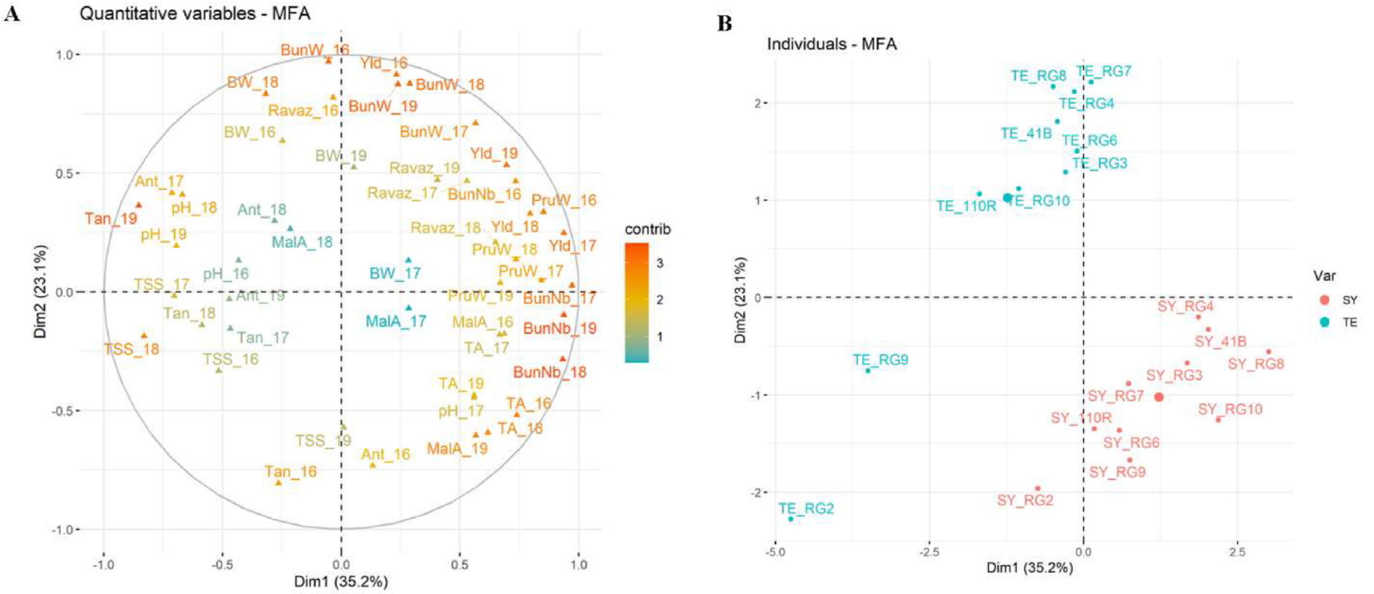

Fig. 2 Plot of quantitative variables (a) and individuals (b) resulting from the multiple factor analysis

Different colours indicate the contribution of each variable to the dimensions (a) or different cultivars (b). Quantitative variables have been defined in Table 2. SY: Syrah; TE: Tempranillo; PruW = winter pruning weight; Yld = yield; BunNb = bunch number; BunW = bunch weight; Ravaz = Ravaz index; BW = berry weight; TA = titratable acidity; pH = pH; MalA = L-Malic acid; TSS = total soluble solids; Tan = potential tannins index; Ant = total extractable anthocyanin content.

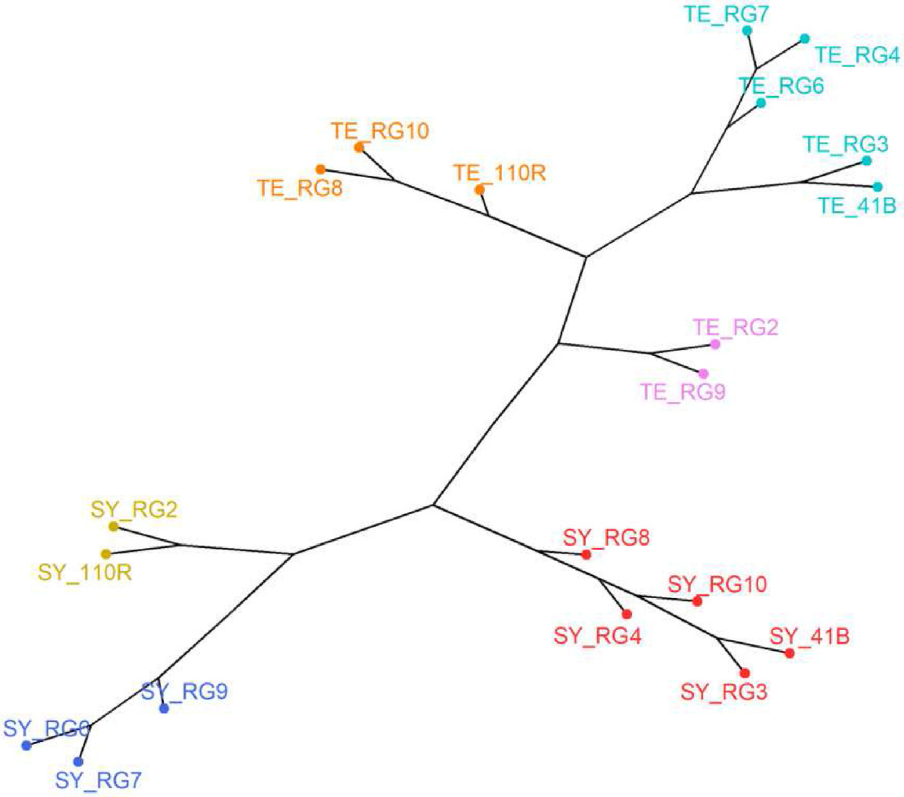

Fig. 3 Phylogenetic tree showing the grouping of the rootstocks as a result of the hierarchical clustering performed on the first two components of the principal components analysis

SY: Syrah; TE: Tempranillo.

genotypes with the lowest total soluble solids values, among which RG10 notably showed 10% lower total soluble solids values than 110 R (Table 3). In the opposite cluster, we found RG2 and 110 R, which were characterized by low yields; in terms of both bunch number and bunch weight, and probably due to that low yield, they stood out in presenting higher total soluble solids values. Finally, genotypes RG6, RG7 and RG9 were grouped together and showed intermediate behaviour in terms of yield, despite their relatively low vigour.

In ‘Tempranillo’, two out of three clusters were located in the second quadrant, while the cluster that contained the RG2 and RG9 genotypes was clearly separated in the third quadrant. This last cluster was characterized by its opposite relationship with PC1 (i.e., low vigour and low yield), especially related to the low number of bunches per vine and high values of potential tannins index and total soluble solids. On the other hand, RG3, RG4, RG6 and RG7 were grouped with the parent 41 B. This cluster was characterized by being highly correlated with PC2 (i.e., high yields due to high average bunch weight) and not at all by PC1. Finally, RG8 and RG10 were grouped together with the parent 110 R in ‘Tempranillo’. This cluster was mainly characterized by high vigour with a medium to high yield and intermediate behaviour in terms of grape quality parameters. Within this group, RG8 stood out for being more vigorous and more productive than the others, and RG10 again stood out for its low sugar content and high acidity values.

4. Discussion

In this paper, we present the evaluation of the agronomic behaviour of a new series of rootstock candidates developed in Spain. Under the specific conditions of the trial, rootstock genotype was shown to have a substantial influence on the performance of both cultivars, not only in terms of vigour but also in terms of production and berry composition parameters. For most of the measured parameters, the novel rootstock candidates provided a range of variation exceeding that provided by their parents. This point is noteworthy since it demonstrates the great genetic variability that can be obtained from even simple cross-breeding programs.

Within this century, important changes in grape ripening in relation to the sugar/acid ratio have been observed due to the impact of climate change (Santos et al., 2020). In the Mediterranean area, the loss of acidity during ripening and the high sugar levels obtained result in wines that lack freshness and have excessive alcohol contents, which is currently an important problem (Jones et al., 2005; Lopez-Bustins et al., 2014; Van Leeuwen et al., 2019). Throughout the four years of evaluation, RG8 stood out as the most vigorous and one of the most productive candidates in the series for both cultivars, with intermediate oenological performance but preservation of acidity during maturation. Similarly, RG10 stood out as an interesting rootstock for overcoming climate change-related effects.

For both cultivars, RG10 showed adequate behaviour in terms of vigour and production and produced a lower sugar content than the other genotypes evaluated throughout the years of the trial. In addition, it also seemed to show some delay in terms of phenolic maturity. These results suggested that RG10 may delay the growing cycle, which could be interesting in the context of climate change. This good performance shown by RG10 is especially relevant if we compare it with its parent 110 R in 'Syrah'. According to a report published by the International Organization of Vine and Wine (OIV, 2017), 'Syrah' is the eighth most cultivated variety in the world, covering approximately 190 000 ha and showing an upwards trend in 2015. The countries where this cultivar is one of the most cultivated cultivars are Australia, South Africa, Argentina, Chile, France and the United States. However, decay symptoms have been observed in many 'Syrah' vineyards for several decades (Renault-Spilmont and Boursiquot, 2002). This disorder is known as "Syrah decline", and it causes specific symptoms such as swelling, cracking and grooving at the graft union and leads to early reddening (Beuve et al., 2013). To date, the causes of this disorder are still unknown, but some rootstocks are observed to be more susceptible to this pathology, among which 110 R is not recommended for 'Syrah'. Thus, this study provides evidence that the RG10 genotype may be an alternative for grafting with 'Syrah' given the good results in terms of vigour, production and sugar content.

Within the pool of new candidates, we also found some with inadequate behaviour. This was the case for RG2 in both cultivars and RG9 in 'Tempranillo'. Both genotypes showed a very poor vigour and production in 'Tempranillo', which caused considerable improvement in phenolic compounds at harvest. The same occurred with RG2 in 'Syrah', which was grouped together with 110 R, showing some signs of incompatibility with 'Syrah', as we have mentioned previously. Thus, we found that the low growth and production that we observed in plants grafted with RG2 may be explained by a smaller root system resulting in less vigorous plants, as other studies have previously reported in other hybrid populations (Filler et al., 1994a, 1994b; Guillaumie et al., 2020). However, 'Syrah' plants grafted onto the RG2 genotype also showed early leaf reddening, which became more visible in the last two years of the trial. These results suggested a certain degree of incompatibility between RG2 and both cultivars. This highlights the fact that when we perform crossbreeding generating genetic variability, we can also find different levels of incompatibility between the cultivar and the rootstock that need to be specifically evaluated.

The influence that a rootstock has on a scion is very complex and depends on the interaction with the environment and with the cultivar itself. Therefore, it is difficult to extrapolate the results obtained in a single field trial in a given location to other conditions. However, the fact that, in general terms, the behaviour of the candidates evaluated herein was relatively similar for the two studied varieties may indicate that the characterization performed over these four years can be sound. In any case, it is necessary to keep in mind that hybrid evaluation before introduction to the market requires further investigation under different soil, climate and management conditions (Cibriáin et al., 2013). Currently, the nursery where the breeding program was established continues to perform adaptation and behavioural trials in the main Spanish appellations of origin.

5. Conclusions

To the best of our knowledge, little information is available about the rootstock breeding programs carried out by nurseries. Our results showed the high potential of rootstock breeding programs for adaptation to grape growing challenges but also showed the complexity of this approach. Moreover, the present study is an example of a private company's effort to take some steps in this direction. This trial allowed us to select two of the candidates, RG8 and RG10, based on the interesting characteristics (high vigour and yield and a good balance between acidity and sugars) they presented when growing in a typical Mediterranean vineyard compared to their parents. Therefore, the company initiated the bureaucratic process for authorization as commercial rootstocks, and the Community Plant Variety Office recently granted Community plant variety right to the RG8 candidate. This newly registered rootstock has already started to undergo multiplication in the nursery, and it will probably soon be available in the market within the European territory.

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Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hpj.2022.10.002.

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