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ORIGINAL RESEARCH ARTICLE

The role of rootstocks for grape growing adaptation to climate change. Meta-analysis of the research conducted in Spanish viticulture

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ABSTRACT

Rootstock election is one of the key decisions when designing a vineyard. Although the research performed to determine the effect they induce in the behaviour of scion varieties is not scarce, it is not easy to have a global idea, as results are usually published scattered. In this work, we re-examine previous research conducted in Spain on rootstock implications on vine agronomic performance through the performance of a meta-analysis (MA). As a result, we were able to integrate the information reported in 20 articles that included rootstock experimentation conducted with 36 different varieties and 47 different rootstocks, totalling 764 individual records. However, when the information was filtered before the meta-analysis, this number decreased to 312 records, for which rootstock Response Ratios (RR) were calculated.

The characteristics conferred by the rootstock were more closely related to the rootstock itself, rather than to the characteristics of the *Vitis* sp. crossing used to create the rootstock. Several rootstocks were identified as more suitable for adapting to future climate change conditions, as far as they were able to moderate sugar accumulation and pH (161-49 C, 41-B MGt and 420A MGt). Meanwhile, 140 Ru and 5-BB were observed to provide high pH and sugar contents despite their high yield. In conclusion, despite being based on data from a single country, the meta-analysis was shown to be a useful tool for enhancing the value of previous research on rootstocks. Combining articles from both peer-reviewed and technical journals helped in the assessment of the implications of different rootstocks, although further steps should be taken to facilitate data integration (harmonisation of measurement and reporting procedures, open data repositories, etc).

KEYWORDS: meta-analysis, rootstock, yield, vegetative development, grape composition, climate change

INTRODUCTION

Meta-analysis (MA) is a method that allows the statistical synthesis of the results of several similar individual studies (Figure 1). This term was introduced in the 1970s as a useful tool for the scientific community to pool and summarise the enormous amount of information collected in the literature (Glass, 1976). However, the use of MA extends beyond quantifying the results of different trials; it also enables the identification of the specific characteristics in which variability lies and potential areas for future research focus. Additionally, by presenting new hypotheses in response to conflicting results, MA can serve also as a tool to facilitate further investigation.

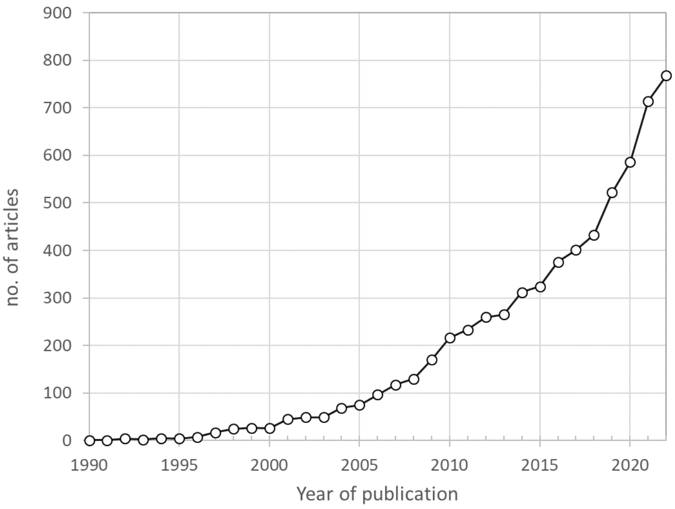

FIGURE 1. Evolution in the number of articles included in the Scopus database within the agronomy category where "meta-analysis" is included in the title or in the abstract.

Agronomy, like any other science, needs to compile the enormous amount of data and information collected in research work. For this reason, the MA is a methodology of great interest for this science, which researchers are increasingly using. For example, it is remarkable the number of papers in the agronomy field that mention the word "meta-analysis" in their title or abstract when consulting the Scopus database. There has been an exponential increase in their use, from nearly no records in the 1990s to around 50 articles per year in the early 2000s, rising to 250 articles per year in the early 2010s, and currently reaching 750 articles per year (Figure 1). In the case of viticulture, some relevant meta-analyses have also been performed in recent years, providing comprehensive and insightful results for the evaluation of agronomical practices on vineyard performance (González-Domínguez *et al*., 2019; Previtali *et al*., 2022; VanderWeide *et al*., 2021) as well as on vineyard biodiversity and ecosystemic services (Karimi *et al*., 2020; Katayama *et al*., 2019; Payen *et al*., 2021; Winter *et al*., 2018), or either for answering methodological or physiological questions (Brillante *et al*., 2018; Lavoie-Lamoureux *et al*., 2017; Santesteban *et al*., 2019).

Viticulture is nowadays facing emerging challenges due to the effect of climate change on grape yield and composition, and also due to the social demand for environmental-friendly agricultural management, and in this context the use of soil-and-climate-adapted rootstocks is crucial (Marín *et al*., 2020). In Spain, the country with the greatest vineyard acreage in the world, a remarkable number of rootstock trials has been carried out during the last 50 years, providing results that may be influenced by local soil and climate conditions. However, to disentangle the rootstock effect from other environmental effects, it is necessary to jointly re-examine all these previous works. Therefore, the inherent effects on vine performance will be better characterised, and the behaviour expected for each rootstock under climate change conditions could be better anticipated. The importance of this re-examination cannot be overemphasized, considering that rootstock performance can only be evaluated after time and space-consuming experiments and that any decision made when establishing a vineyard will determine grape and wine characteristics throughout its entire lifetime. The joint statistical analysis of the results of previous scientific studies (i.e., their meta-analysis) can serve as a guide to new field experiments for the evaluation of rootstock suitability to new conditions.

The objective of this work is to contribute to understanding the effect of the rootstock and parental rootstock crossings on grape berry and plant responses through meta-analysis of the previous research conducted in Spain. Unlike most meta-analyses, we constructed a complete database that included research works published in peer-reviewed international journals but also in technical national journals, since this source can be considered useful for such global analysis considering the specificities of experimentation in viticulture.

MATERIALS AND METHODS

1. Document search and creation of a database

Document searches were carried out by several methods, including multiple search strategies for the characteristics of the information sought. The databases consulted were Scopus, Google Scholar, Dialnet (a database of works published in Spanish) and Sirius (a search tool for Spanish library catalogues). The search was carried out, using combinations of the following keywords: "rootstock", "grapevine", "vine", "and "vinifera", in English or Spanish depending on the database. No limit was imposed on the date of the study. Additionally, the tables of contents of the volumes published in the last 15 years in the most relevant transfer technical journals in Spain (Enoviticultura, Viticultura y Enología Profesional, and Vida Rural) were also checked.

Once all the documents were available, the information provided by each was transferred to an Excel spreadsheet. On the one hand, the metadata of the trial (e.g., region, rootstocks and varieties used, climatic data, soil characteristics) were compiled after reading the details provided in the Material and Methods sections, and used to avoid the presence of duplicate results published in different journals. On the other hand, the results presented were converted into standardised tabular form. Where data were not presented in tabular form in the original articles, WebPlotDigitizer, a web-based tool for extracting data from plots and images, was used (Rohatgi, 2022). All the data obtained were maintained in the database, although subsequent analysis only focused on the articles providing, at least, yearly disaggregated yield and sugar concentration.

2. Statistical Analysis

Response ratios (RR) and 95 confidence intervals were calculated to analyse rootstock effects and significance levels. Data were processed as described by Hedges *et al*. (1999). Briefly, the RR value for each response value was calculated to quantify the effect of each rootstock compared to the average response on each study according to Eq. 1:

(Eq. 1) $RR = \ln X_r - \ln X_{ta}$

where X_r is the mean of value for each rootstock and X_{ta} is the mean of the response variable in the study. Among publications used for analysis, few reported the standard error for all the parameters included in this study. Given this, the variable errors within each experiment were not accounted for. A weight factor ω was estimated instead for each study using the root of the number of years evaluated in the study. The mean effect of the rootstock of each given variable was calculated using Eq. 2:

(Eq. 2) $RR_p = \frac{\sum_{i=1}^{j} \omega_i \times RR_i}{\sum_{i=1}^{j} \omega_i}$

where i represents the i[th] study and j is the total number of studies for a given variable. The per cent changes (C%) of the investigated variables induced by the rootstock were calculated with Eq. 3:

(Eq. 3) $C\% = (e^{RR} - 1) \times 100$

The effect of the rootstocks was unitless, and the mean rootstock effect and bias correction (95 % per cent interval) was calculated using R v4.2.1 statistic package (R Core Team., 2014) with RStudio. Numerical data were plotted using RStudio using the package forestplotter 1.0.0 (Dayimu, 2022). The principal component analysis (PCA) displaying the relationship between the per cent change of the investigated variables in response to the rootstock was plotted using FactoMineR (Lê *et al*., 2008). Euclidean distances were calculated among the rootstocks to determine a neighbour-joining (NJ) tree based on the RRs for the response variables using the R stats package and plotted using ggplot2 (Wickham, 2016).

RESULTS AND DISCUSSION

1. Database creation

The search process allowed the compilation of a diversity of documents that were deemed appropriate for inclusion in the database and, thus, for the ensuing meta-analysis. Altogether, we had access to 20 technical documents that included rootstock experimentation conducted on 36 different varieties, 47 different rootstocks and at 59 different sites throughout Spain during 1989–2021, all of which were incorporated into the database. Considering each rootstock-variety-site combination as a single observation, a total of 764 individual records were considered at this stage. Nevertheless, once the information was filtered previous to the meta-analysis, to consider only documents providing data disaggregated yearly that included yield and sugar concentration, the total number decreased to 312 records. The information on the documents included in the database is provided in Table S1, and the climatic characteristics of each site are summarised in Table S2. Although a good deal of information (metadata and results) could be gathered from the documents reviewed, the meta-analysis focused on the five variables that appeared more often, namely, yield, pruning wood weight, yield to pruning weight ratio (i.e., the Ravaz index), sugar concentration and pH.

2. Single effects of crossings and rootstocks

The calculation of Response Ratios and the construction of forest plots provided a clear overview of the general effect of each rootstock, and of *Vitis* sp. crossings, on the studied variables featuring vineyard agronomic performance (Figure 2). Vegetative growth was significantly affected by the rootstock (Figure 2a). In detail, the crossings of *V. berlandieri* × *V. riparia* and *V. berlandieri* × *V. vinifera* showed lower pruning weight response ratios, and those of *V. berlandieri* × *V. rupestris* showed, on average, higher pruning weight response ratios. However, within each crossing, there was not a uniform response and, therefore, the behaviour of each rootstock should be considered separately. Thus, within the *V. berlandieri* × *V. riparia* crossings, 161-49 C and 420-A MGt significantly decreased vegetative growth, whereas SO4 increased it, and no significant changes were observed for 5-BB. Within the *V. berlandieri* × *V. rupestris* crossings, 110 R showed a pruning weight response ratio slightly below zero, while the highest increase was observed for 140 Ru, and no significant changes were observed for 1103 P and 99 R. Other rootstocks showing a pruning weight over average were R. du Lot and 333 EM, although for the latter, data were obtained from a single experiment, and 41-B MGt showed a lower pruning weight. A similar trend was observed for 196-17 Cl, although it was not statistically significant.

Regarding yield, the rootstock affected the average response ratio (Figure 2b). As already observed for pruning weight, the effect was more closely associated with the rootstock than with the crossing itself. Within the *V. berlandieri* × *V. riparia* crossings, SO4 and 161-49 were more productive than the average, whereas 420-A MGt showed a lower yield, although the great variability observed for the yield response ratio indicates that this response ratio was very dependent on the experimental conditions. Within the *V. berlandieri* × *V. rupestris* crossings, 110 R, and 140 Ru were shown to be slightly more productive than the average, whereas 1103 P and 99 R were less productive.

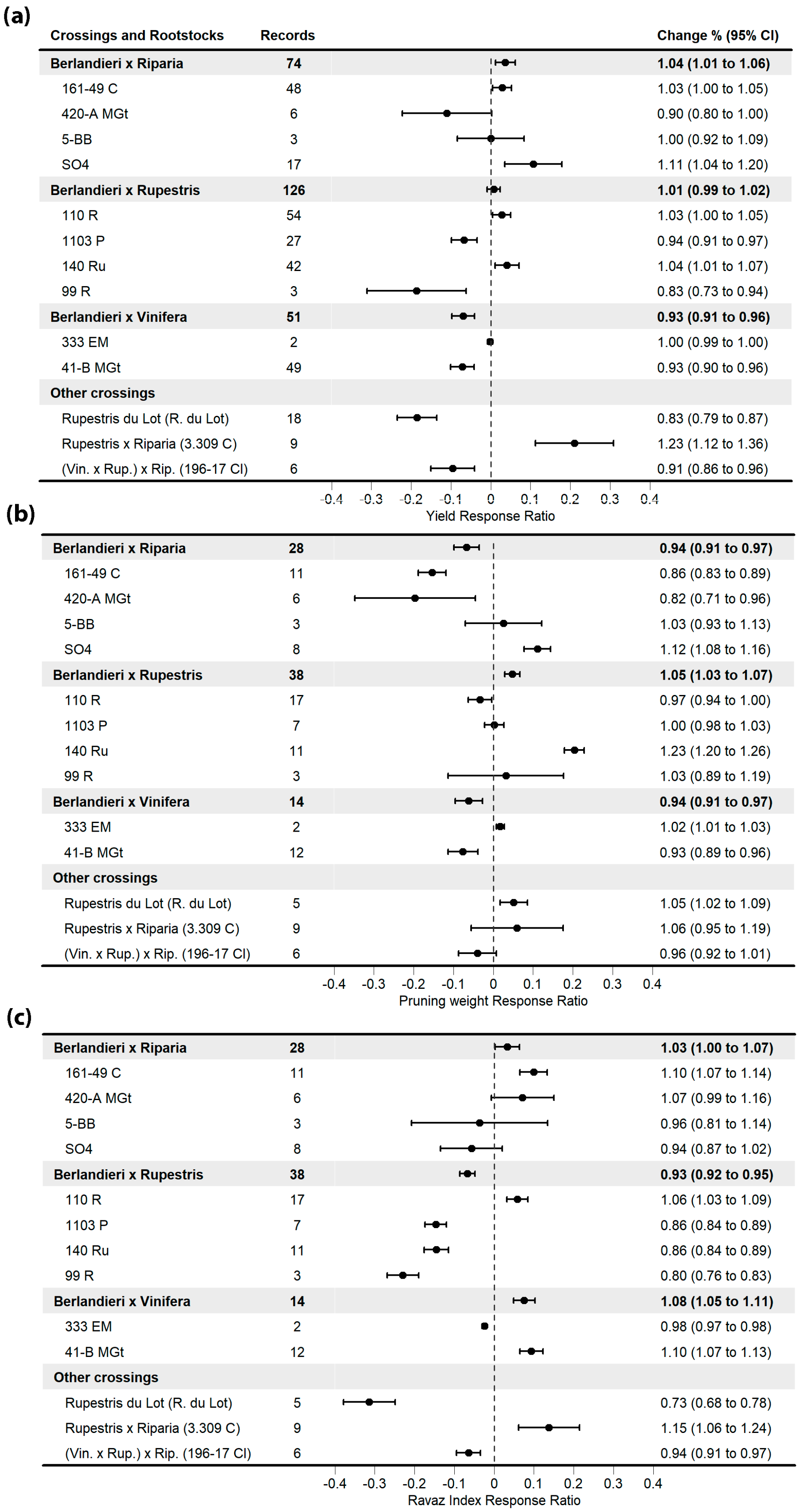

FIGURE 2. Forest plot showing the Response Ratio observed for (a) yield, (b) pruning weight and (c) Ravaz Index for different *Vitis* sp. crossings and rootstocks. Horizontal bars stand for the 95 % confidence intervals (CI), thus, effects are significant at p = 0.05 when bars do not cross the zero-response ratio vertical dashed line.

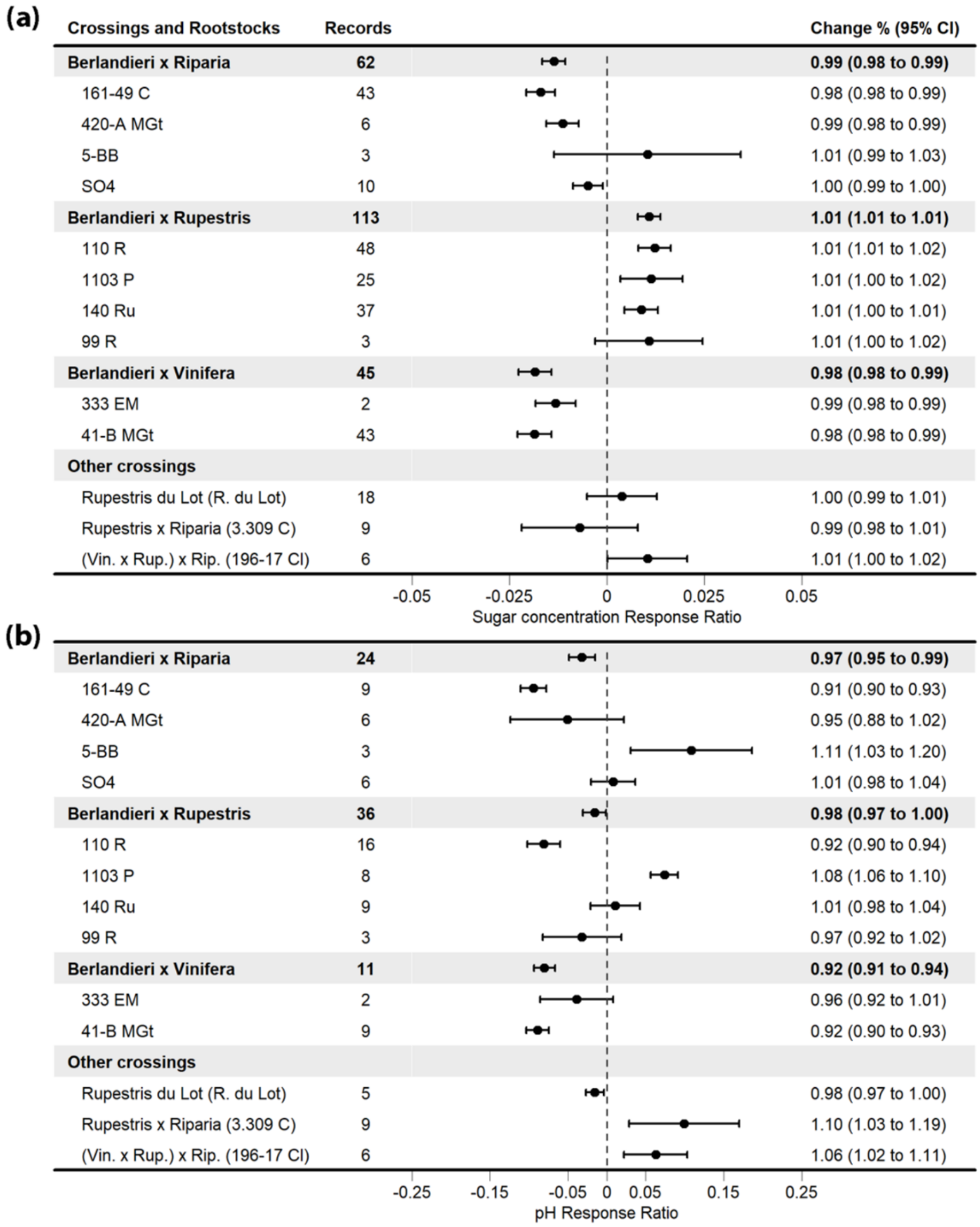

FIGURE 3. Forest plot showing the Response Ratio observed for (a) sugar concentration and (b) pH for different *Vitis* sp. crossings and rootstocks. Horizontal bars stand for the 95 % confidence intervals (CI), thus, effects are significant at p = 0.05 when bars do not cross the zero-response ratio vertical dashed line.

Among the remaining crosses, 3.309 C was observed to be more productive than average, while 41-B MGt, R. du Lot and 196-17 Cl provided lower yields.

The yield-to-pruning weight ratio (Ravaz Index) was also clearly affected by the crossing conducive to the rootstock (Figure 2c). Specifically, the *V. berlandieri* × *V. riparia* crossings produced, in general, more grape yield per vegetative growth unit, while *V. berlandieri* × *V. rupestris* showed the opposite trend. However, as already observed for pruning weight and yield, the Ravaz index depended more on the rootstock itself than on the crossing leading to it. For instance, 161-49 C, 110 R, 41-B MGt, and 3.309 C showed Ravaz Index response ratios significantly higher than zero. On the contrary, 1103 P, 140 Ru, 99 R, 333 EM, R. du Lot and 196-17 Cl showed response ratios significantly below zero.

Contrastingly, sugar concentration within each crossing was relatively stable and uniform (Figure 3a), depending more on the crossing than on the specific rootstock. Thus, both *V. berlandieri* × *V. riparia* and *V. berlandieri* × *V. vinifera* crossings showed a clear trend to decrease must sugar content, whereas the *V. berlandieri* × *V. rupestris* crossings resulted in higher sugar concentrations. Among the remaining crossings, 196-17 C also showed a slight increase in sugar concentration. Lastly, similarly to the vine production variables, must pH varied mainly due to the rootstock itself and not to the crossing combination (Figure 3b). This way, 161-49 C, 110 R, 41-B and R. du Lot led to lower pH, while 5-BB, 1103 P, 3.309 C and 196-17 Cl led to higher pH values.

3. Joint effects of crossings and rootstocks: climate change prospectives

The different vine production and grape quality variables analysed separately in the previous section are known to depend on each other. This was exemplified by the fact that when the response ratios were taken globally, the classically well-known negative correlation between sugar content and yield, and the positive correlation between sugar content and pH were also observed (Figure 4). Additionally, a positive association between changes in pH and pruning weight was observed, i.e., the rootstocks which caused bigger canopy development were also the ones leading to higher must pH (Figure 4a). Therefore, to extract informative results on the suitability of the different rootstocks in terms of adaptation to future climate change conditions, the meta-analysis should go beyond the response ratios of single traits, and rootstocks should be analysed in groups of similar behaviour.

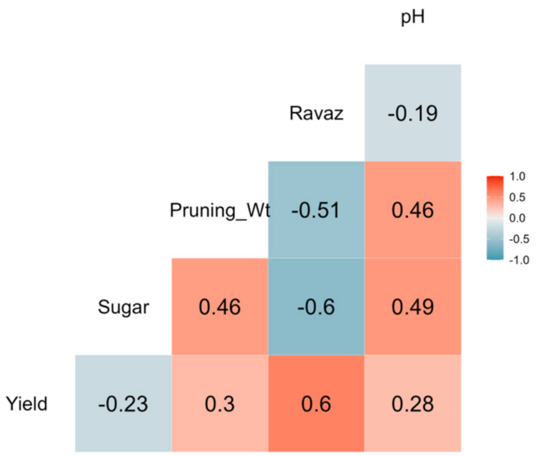

FIGURE 4. Correlation heatmap (Pearson's r coefficients) for the Response Ratios of the variables included in the meta-analysis.

The hierarchical clustering (Figure 5) showed that rootstock behaviour was not particularly associated with the *Vitis* sp. crossing, but with the rootstock itself, as already observed for the vine production variables and must pH. In this respect, two of the *V. berlandieri* × *V. riparia* (161-49 C and 420-A MGt) crosses performed similarly, but very differently than the other two considered (SO4 and 5-BB). Similarly, *V. berlandieri* × *V. rupestris* crosses (110 R, 1103 P, 140 Ru and 99 R) were not grouped together. These results confirm that, notwithstanding the interest in including other *Vitis* sp. to provide specific features in rootstock breeding programs (Marín *et al*., 2020), new crossings of classically considered species can provide a broad range of variation for agronomic behaviour of grapevines (Bianchi *et al*., 2020; Marín *et al*., 2022).

The Principal Component Analysis (PCA) scores obtained for each rootstock (Figure 6) allowed the detection of several relevant trends that could be informative regarding the suitability of the different rootstocks in terms of adaptation to future climate change conditions. The first two components explained nearly 80 % of the data variability, with the first component (47 % var. explained) being positively associated with sugar content, pruning weight and pH, and the second component (32 % var. explained) being positively associated to yield, pruning weight, Ravaz Index and pH. It is noteworthy that, although partially related, response ratios for yield, sugar content and pH were not aligned, which indicates that rootstock is a tool to modulate the relationships between them. Although the choice of rootstock is a complex decision that has to take into account a large number of factors, in most wine-growing regions there is a need for rootstocks that, on the one hand, have a moderate sugar accumulation for a given yield and, on the other hand, have a lower pH for a given sugar concentration (Gutiérrez-Gamboa *et al*., 2021; Previtali *et al*., 2022).

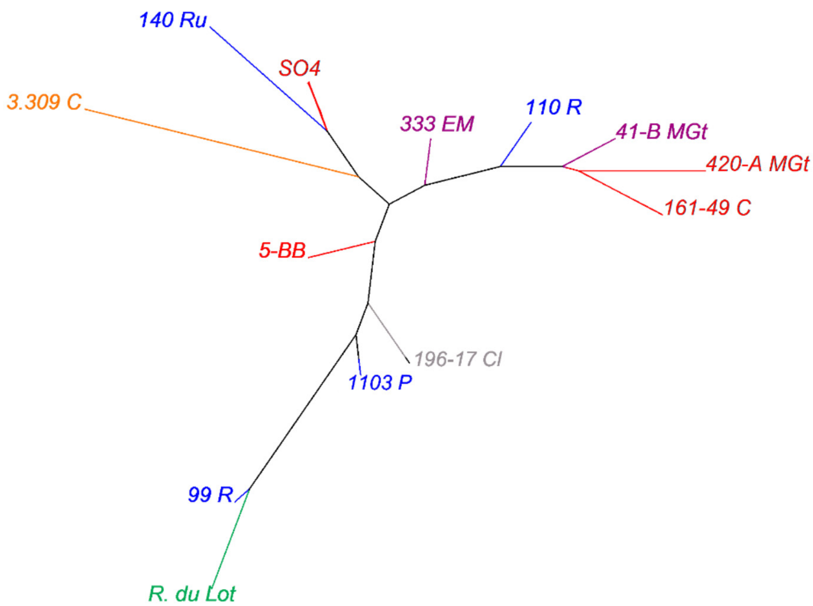

FIGURE 5. Clustering of rootstocks according to the Response Ratios (RR) observed. Colours correspond to the different *Vitis* sp. crossings, as detailed in Figure 6.

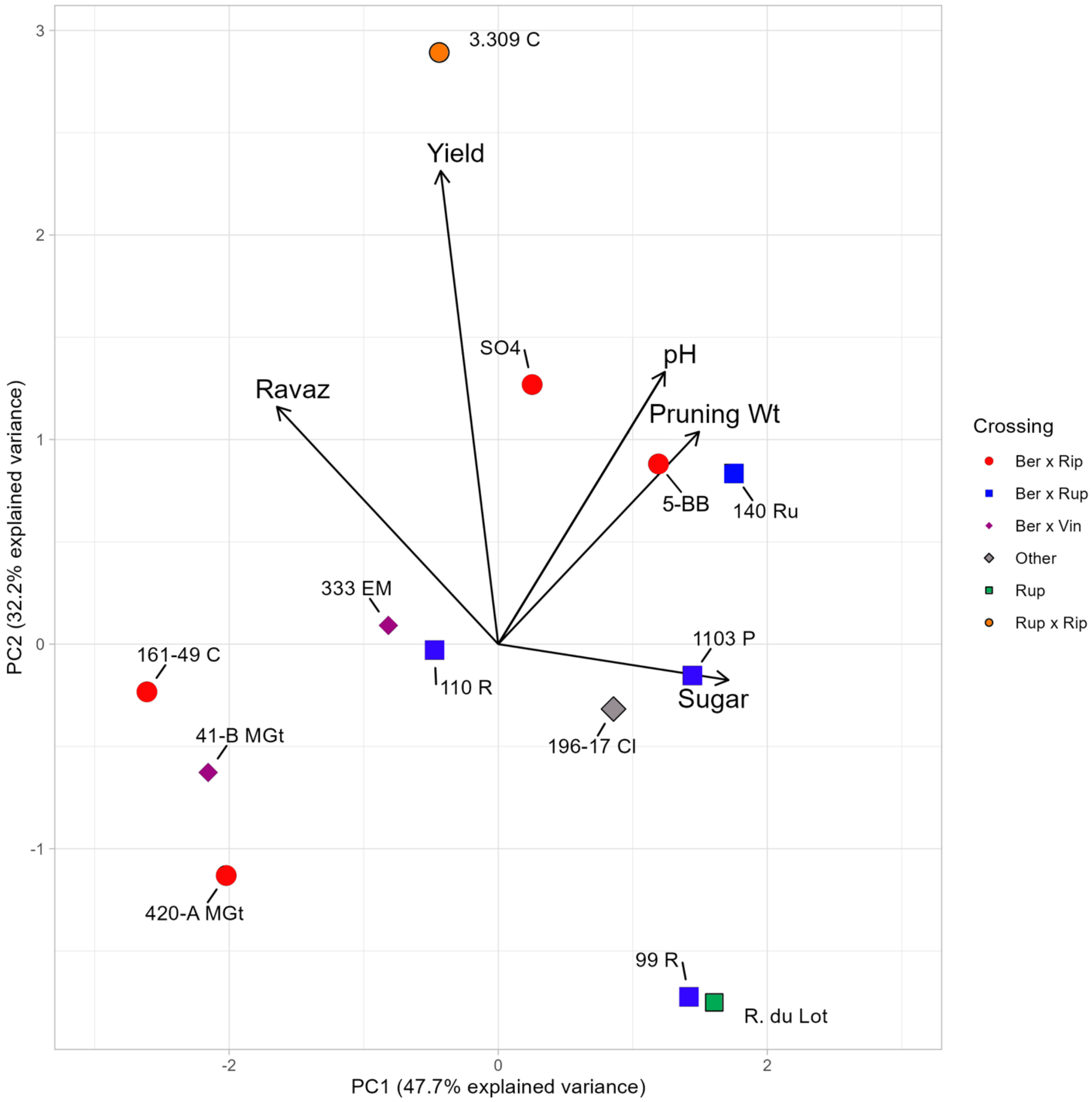

FIGURE 6. Principal component analysis (PCA) of the response ratios observed for yield, pruning weight, Ravaz index, sugar concentration and pH (expressed as H+ concentration).

In this regard, the group formed by 161-49 C, 41-B MGt and 420A MGt appears to be able to moderate sugar accumulation and pH the most, being relatively independent of yield for 161-49 C and 41-B MGt. On the other hand, 140 Ru and 5-BB were generally observed to result in higher pH and sugar content despite their high yield.

It is important to note that the results presented in the meta-analysis should be considered general trends rather than specific predictions and that they are subject to many relevant factors such as soil and climate characteristics, as well as the variety being studied. Therefore, it is crucial to acknowledge that our meta-analysis reflects global tendencies in the literature but might not anticipate the effects of specific circumstances on the results.

As a prospective conclusion, we can highlight that the meta-analysis carried out, despite having the limitation of being based on data from a single country, proved to be a useful tool to enhance the value of the data collected in the research work carried out over the years on a specific topic, thus, making it possible to have a general idea of all the information available and to integrate it. Bringing together articles published in both peer-reviewed and technical journals proved to be a useful tool for assessing the global implications of the use of different rootstocks. In any case, any meta-analysis requires a very heavy workload to compile and standardise the results of previous trials, which limits its generalisability. To facilitate this task, it would be of great interest to encourage the harmonisation of measurement and reporting procedures and the creation and improvement of open data repositories that would allow access to and re-analysis of the results.

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