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ROOTSTOCK REGULATION OF SCION PHENOTYPES: THE RELATIONSHIP BETWEEN ROOTSTOCK PARENTAGE AND PETIOLE MINERAL CONCENTRATION

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Abstract:

Context and purpose of the study – Grapevine is grown grafted in most of the world largely because of Phylloxera. Rootstocks not only provide tolerance to Phylloxera, but also ensure the supply of water and mineral nutrients to the scion. Rootstocks are an important means of adaptation to environmental conditions if we want to conserve the typical features of the currently used scion genotypes. To aid this adaptation, we can exploit the large diversity of rootstocks used worldwide. To fully explore this existing rootstock diversity, this work benefits from the unique GreffAdapt vineyard, in which four scion genotypes were studied onto 55 commercial rootstocks in three blocks. The aim of this study was to characterise rootstock regulation of scion mineral status and how it relates to scion development.

Material and methods – *Vitis vinifera* cvs. Cabernet-Sauvignon, Pinot noir, Syrah and Ugni blanc were grafted onto 55 different rootstock genotypes and planted in a vineyard as three replicates of five plants. The experimental vineyard is located on a sandy gravelly soil at la Grande Ferrade near Bordeaux, France (44°47'26.7"N 0°34'26.5"W). In 2020 and 2021, petioles were collected in the cluster zone with six replicates per combination. Petiolar concentrations of 13 mineral elements (N, P, K, S, Mg, Ca, Na, B, Zn, Mn, Fe, Cu, Al) were determined at veraison. In 2020 and 2021, winter cane pruning weight, vigour, leaf chlorophyll content, fertility and yield were measured. In 2021, Mg deficiency severity was visually scored on each plant; these observations were qualitative and a score between 0 and 3 was assigned. Rootstocks were also grouped according to their parentage when at least 50 % of a *Vitis species* was present. Data were analysed according to these genetic groups in order to determine whether the petiole mineral composition could be related to the genetic parentage of the rootstock.

Results – Scion, rootstock and their interaction explained the same proportion of the phenotypic variance for most mineral elements. Rootstock genotype showed a significant influence on the petiole mineral element composition. Rootstock effect explained from 8 % for Al to 42 % for S of the variance and an important part for Mg with 35 %. The genetic background *V. riparia* seems to increase the probability of low P and Mg content. Petiole Mg measurements were related to Mg deficiency symptoms. Severity of Mg deficiency symptoms varied depending on the scion cultivar. The differences in mineral status conferred by rootstocks did not show significant correlations with vigour or fertility.

Significance of the study – This unique experimental design has shown that the rootstock effect is higher than the scion effect on the concentration of large majority of mineral elements in the petiole. The evaluation of Mg levels by petiole analysis and observations of the intensity of deficiency symptoms shows for the first time the variability of the thresholds of satisfactory mineral nutrition between grapevine cultivars. Therefore, fertilization management have to take the rootstock into account.

Keywords: *Vitis*, grapevine, plant material, mineral status, genetic background, rootstock × scion interaction, magnesium

1. Introduction

Since the late 19th century, grapevine is grown grafted in most of the world largely because of the pest Phylloxera (*Daktulosphaira vitifoliae*). Rootstocks allow tolerance to Phylloxera, but also play a dominant role in water and mineral nutrients absorption (Ollat et al., 2016). They strongly interact with the scion genotypes and modify the whole plant development through the modification of yield and vigour in an environmentally dependent manner. The differences in rootstock behaviour have strong impacts on the grape growers choices for soil adaptation, yield, fertilizer requirements and canopy management (Ibacache et al., 2020). Rootstocks are an important means of adaptation to environmental conditions if we want to conserve the typical features of the currently used scion genotypes.
Plants absorb elements from the soil and the atmosphere, and incorporate them into their tissues. Mineral elements are classified into two categories: macroelements and microelement. Macroelements such nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), calcium (Ca) represent the major requirements of the plant in terms of quantity (99,5% of plant tissues). Microelements as boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu) and aluminium (Al) are essential in plant development, but required at low quantity (<1% of plant tissues). They are catalytic elements involved in metabolism reactions as enzymes or cofactors.

The aim of the study is 1) to characterize the relative of the effects of the scion and the rootstock, 2) to evaluate the role of rootstock genetic backgrounds on scion mineral content and 3) to determine the relationship between petiole concentrations and mineral deficiency symptoms by suggesting a new classification of rootstocks.

2. Material and methods

Plant materials – V*itis vinifera* cvs. Cabernet-Sauvignon clone 169, Pinot noir clone 113, Syrah clone 524 and Ugni blanc clone 481 were grafted onto 55 different rootstock genotypes. This panel includes 25 rootstocks commercially used in France and 30 rootstocks used in other countries selected for relevant characteristics about lime-induced iron deficiency chlorosis, drought and conferred vigour. Rootstocks were grouped according to their parentage in order to reduce the number of modalities. The parentage was assigned when at least 50 % of a genetic background was present (Maul et al., 2023; Riaz et al., 2019). Some rootstocks (Nemadex AB, Georgikon 121, Georgikon 251, 1616C, Freedom, Dog Ridge, Harmony and Ramsey) were removed from the study because they have complex parentages, this was to avoid that a single genotype was used to represent a parentage category. To study the effect of genetic background a panel of seven parentages was formed with 47 rootstocks (Figure 1).

Experimental design – The experimental vineyard Greffadapt is 0.8 ha, planted in 2015 in three blocks of five vines and is located at la Grande Ferrade near Bordeaux, France (44°47'26.7"N 0°34'26.5"W). The density of the vineyard is 6230 vines/ha with a row spacing of 1.6 × 1 m. In 2020 and 2021, during the experiment, the vines were respectively 5 and 6 years old.
On average, in the different blocks, there is a disequilibrium between soil Mg and K ratio (K/Mg >>1) which leads to Mg deficiency through an excess of K. In this vineyard, the first organomineral horizon (0-35cm) is composed of about 2 % of organic matter. The cation exchange capacity (CEC) of the soil is low (<7) and the pH is in classic range between 6 and 7.

Petiole sampling and analysis – In 2020 and 2021, eight petioles were collected with six replicates per combination at veraison. The samples were harvested in the cluster zone between the fourth and the sixth node and dried in oven at 60°C. Petiolar concentrations of 13 mineral elements at veraison were analysed by Waypoint Analytical Virginia, that included N, P, K, S, Mg, Ca, Na, B, Zn, Mn, Fe, Cu and Al. Mineral elements concentrations were determined by an inductively coupled plasma mass spectrometer (ICP-OES MS 730-ES), except for N concentration, which was determined by Leco FP-528 instrument, an N determinator. Concentrations were expressed in terms of percentage (weight/weight) for macroelements and parts per million (ppm) for microelements.

Vine physiology – In 2021, Mg deficiency severity was visually scored on each plant in the GreffAdapt vineyard. The observations were qualitative and a score between 0 and 3 was assigned. The null score was assigned if no Mg deficiency was observed; 1 when lower leaves expressed the deficiency; 2 when the bottom half of the canopy expressed deficiency, and 3 when all leaves expressed Mg deficiency (Figure 1). Annual growth of each vine was evaluated by winter cane pruning weight. The number of shoots of each vine was counted and the vigour was calculated by dividing the winter cane pruning weight per number of shoots. Each year there is 15 replicates of each measurement for each scion/rootstock combination.
At harvest, the number of bunches per vine was counted and the bunch weight of each vine of the vineyard was measured. The fertility of each plant was calculated by dividing the number of clusters by the number of shoots.

Statistical analysis – A three-way analysis of variance with interaction effect (ANOVA) was performed to characterize the importance of the different factors (scion, rootstock and block). The effect of each factor on each petiolar nutrient content was determined by the percentage of variance explained (PVE). Each year was analysed independently because the experiment and values were not independent, samples were collected on the same vines but at different years.

Linear mixed-effects analyses models (LMM) were used to estimate only the effect of the rootstock factor on each element, in order to study multiple responses from the same plant across the two years. Phenotypic values were corrected for environment variability using genotype replicates for year, scion and block. Linear mixed-effects model were fitted by maximum likelihood (REML method). Each mineral was fitted with a model, where block, scion and year were used as fixed effects and rootstock was chosen as a random effect: " Mineral element∼ Block + Scion + Year + (1|Rootstock) + ε ". The best model was selected by the smallest Bayesian Information Criterion (BIC) and was applied on all the mineral variables for the sake of uniformity. An ANOVA was applied to each linear mixed model created to estimate only the effect of the rootstock.

To study the effect of genetic backgrounds, the mineral content values for N, P, K and Mg were grouped by status: excess, optimum and deficiency (Delas, 2011). The dependence between the genetic background and the mineral status was determined using a chi-square test (significance level p-value <0.05) on the contingency tables established for each mineral element. The sign of the standardised residuals measures if the difference between what we would expect (the prediction) in a category if the variables were truly independent and what we really obtain is higher
(sign +) or lower (sign -) (Agresti, 2019). For the significance of the relation, the error rate, alpha, was set at 0.05(*), 0.01(**) or 0.001(***), which respectively translates into z-critical values |1.96|, |2.58|and |3.09|.

3. Results and discussion

3.1. *The effect of the rootstock is stable from year to year and generally stronger than the other factors*

The rootstock effect is significant and stable from year to year for most macroelements except for N in 2021 (Table 1). However, we demonstrated that the two dominant factors in our study are the rootstock and the scion. Rootstock effect can be equivalent of the scion and sometimes even stronger. Harris et al. (2021) also showed that for the majority of macroelements studied, the rootstock effect is significant as a single fixed-effect.
After characterising the ranking of the factors, we focused on the rootstock factor by using LMM. All mineral petiolar contents of the scion were highly significantly (p-value < 0.001) affected by the rootstock genotype with the LMM method. If we consider the main macroelements, rootstock effect explained 35 % for Mg, 33 % for K, 27 % for P and 23 % for N of variation in mineral content.

3.2. *Genetic background influences the mineral status of most mineral macroelements*

There is a significant effect of the genetic background on N, P, K, Mg and Ca status. For these mineral elements, the genetic background influences significantly the proportion of plants that are in deficiency, optimum or excess for at least one year. Among the studied elements, we focused on Mg and P because there is a significant effect of the genetic background on theses elements over the two years. To understand the relationship between genetic background and the Mg status, we checked the significant effect of the genetic background. The sign of standardized residuals measures whether a genetic backgrounds modality induces more or fewer plants in a mineral status modality for a given element. Table 2 shows the stable influence of the genetic backgrounds on Mg status over the two-year period studied. The rootstocks with a dominant *V. riparia* genetic background have a significantly higher proportion of plants in Mg or P deficiency and a significantly lower proportion of plants in Mg or P excess, these results confirms what was observed in Gautier et al. (2020).

3.3. *The relationship between petiole content and vine physiology define a mineral nutrient classification of the rootstocks*

The experimental design allowed us to explore the correlations between Mg petiole concentration and Mg symptoms. An exhaustive classification of all the rootstocks studied according to the assessment of Mg petiole concentration and deficiency observations was created (Table 3). The Mg deficiency expressed by plant did not show significant effects on vigour, pruning weight, yield or fertility. The current satisfactory mineral nutrition thresholds are accurate for the majority of the rootstocks, but for the first time, we had highlighted the variability of satisfactory mineral nutrition thresholds depending on rootstocks and scions. Consequently, the work confirmed that fertilization choices have to take the rootstock into account (Cordeau, 1998).

4. Conclusions

The study of such an experimental design has shown that the rootstock effect is stronger than the scion and block for most mineral elements. Rootstock effect explained about 30 % of variation in mineral content for the main elements with the LMM method. The joint evaluation of Mg levels by petiole analysis and observations of the intensity of Mg deficiency symptoms underlines for the first time the variability of the thresholds of satisfactory mineral nutrition. This work shows that Mg deficiency sensitivity conferred by rootstocks depends on the scion genotype studied.

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7. Figures

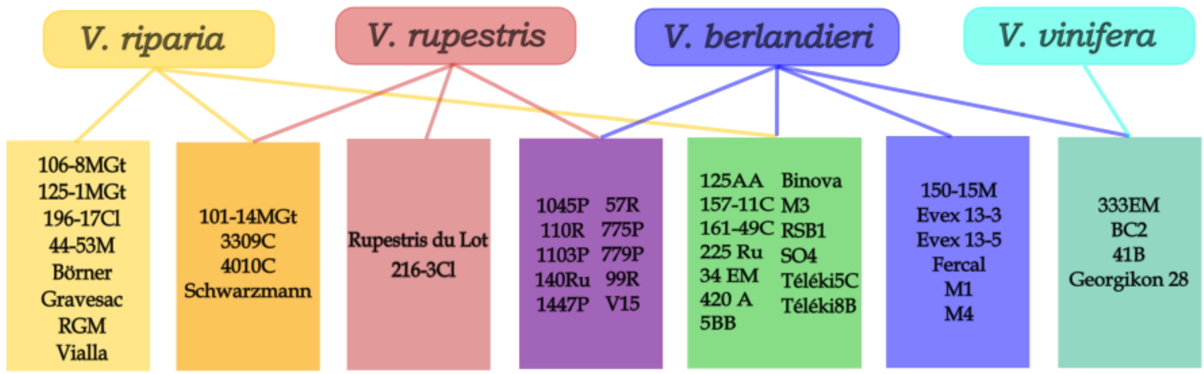

Figure 1: Rootstock classification according to their dominant parentage for the genetic background study, which could be *Vitis riparia, V. rupestris, V.berlandieri, V.vinifera* or a combination of these genotypes.

Table 2: Percentages of variance explained by the factors block, rootstock and scion and their interactions obtained by ANOVA-test of macroelement concentration in 2021. White cases are not significant, coloured cases are highly significant (p-value <0.001).

Elements	Block	Rootstock	Scion	Block × Rootstock	Block × Scion	Rootstock × Scion	Block × Rootstock × Scion
N	ns	5-10	< 5	10-15	< 5	10-15	>20
P	< 5	15-20	5-10	10-15	ns	15-20	>20
K	5-10	>20	>20	5-10	< 5	10-15	15-20
S	< 5	>20	< 5	5-10	< 5	10-15	10-15
Mg	5-10	>20	10-15	10-15	ns	10-15	15-20
Ca	< 5	15-20	>20	5-10	< 5	15-20	>20

PVE (%)

ns | < 5 | 5-10 | 10-15 | 15-20 | >20

Figure 1: Pictures of the Mg deficiency rating scale; 0 no symptoms; 1 first lower leaves expressed Mg deficiency; 2 at least the bottom half of the canopy expressed Mg deficiency, and 3 all leaf levels expressed Mg deficiency symptoms.

Table 2 : Summary of the stable significant effects of genetic backgrounds on magnesium status over two years

		Magnesium		
		deficiency	optimum	excess
Genetic backgrounds	*Ber*			
	Ber×Rip			
	Ber×Rup			more
	Ber×Vinif	less		more
	Rip	more	less	less
	Rip×Rup			
	Rup			

Table 3 : Rootstock classification based on magnesium symptoms and validated by petiole analysis

	Magnesium deficiency sensitivity		
Rootstocks	low	medium	high
1045P			x
106-8MGt		x	
1103P	x		
125-1MGt		x	
125AA			x
140Ru	x		
157-11C			x
1616C			x
196-17Cl			x
216-3Cl			x
225Ru			x
333EM	x		
41B	x		
44-53M			x
57Richter		x	
779Paulsen	x		
Binova			x
Börner			x
Dog Ridge	x		
Evex13-5		x	
Freedom		x	
Georgikon121	x		
Georgikon251	x		
Harmony			x
M3	x		
M4	x		
RSB1		x	
Schwarzmann			x
SO4			x
Téléki5C		x	