

## Article

# Effects of Rootstocks on Blade Nutritional Content of Two Minority Grapevine Varieties Cultivated under Hyper-Arid Conditions in Northern Chile

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**Abstract:** In the 90s, as in other countries, transformation of Chilean viticulture brought about the introduction and spread of European grapevine varieties which has resulted in a massive loss of minor local and autochthonous grapevine varieties traditionally grown in several wine growing regions. Fortunately, in recent years, autochthonous and minority varieties have been revalued due to their high tolerance to pests and diseases and because of their adaptation to thermal and water stress triggered by global warming. In this study, we assessed the nutritional status of two autochthonous grapevines grafted onto four different rootstocks under the hyper-arid climatic conditions of Northern Chile over three consecutive seasons. The results showed that R32 rootstock induced high N, P, Ca, Mg and Mn levels in blades compared to Harmony rootstock. R32 rootstock and to a lesser extent, 1103 Paulsen and 140 Ruggeri rootstocks kept balanced levels of nutrients in blades collected from Moscatel Amarilla and Moscatel Negra grapevine varieties. Additionally, Harmony presented slight nutritional imbalance compared to the rest of studied rootstocks due to its low absorption of Mg, Mn, Ca and P, and its high K absorption, which was exacerbated under warm weather and salinity soil conditions. These results may provide a basis for specific cultivar/rootstock/site combinations, a nutritional guide for the viticulturists of Northern Chile, and options to diversify their production favoring the use of minority and autochthonous varieties that adapt well to hyper-arid conditions of Northern Chile.



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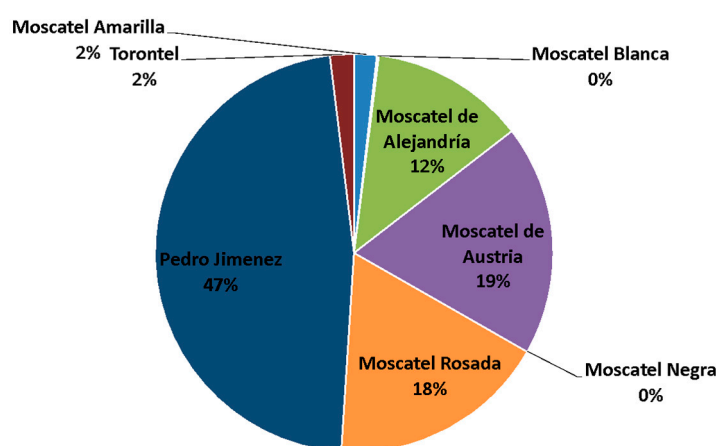
**Keywords:** *Vitis vinifera*; *V. berlandieri*; autochthonous; Moscatel Amarilla; Moscatel Negra; *V. rupestris*

## 1. Introduction

The role of grape varieties is increasingly important in worldwide wine products, both wines and spirits [1]. The strategy of new world wine countries has appealed to the relevance of varietal wines as the axis of their production and marketing strategy, while European wine industry emphasize their terroir and their thousand-year wine-making history [1,2]. Currently, Chile has around 137,000 ha of vine cultivation surface distributed between the Coquimbo (30.0° SL) and Araucanía (38.7° SL) regions, although there are some experiences that are expanding the wine-growing areas in Southern Araucanía and Northern Coquimbo [3]. The Chilean wine industry is rather concentrated and strongly export oriented. In this fashion, wine exports represent more than 1800 million dollars and represent more than 80% of wine produced, reaching more than 130 countries [3]. The most cultivated grapevine varieties in Chile are Cabernet Sauvignon (30.0%), Sauvignon Blanc (11.2%), Merlot (8.6%), Chardonnay (8.2%), Carmeénère (7.8%) and Listán Prieto (7.5%), mainly intended for wine elaboration [4].

Pisco is a brandy produced in wine-growing regions of Chile and Peru that is produced by distilling fermented grape juice [5]. In Chile, Pisco has been recognized, delimited

and protected by the State as Denomination of Origin since 1931, and has managed to sustain itself as an emblematic national beverage until today [1]. Pisco-oriented grape production is restricted to northern Chile from the desert fringe to the agricultural valleys between Copiapó and Choapa rivers [6]. Today, 9150 ha of Pisco vineyards are planted in Atacama (5.8%) and Coquimbo (94.2) regions, representing 6.3% of the country's total vineyards, about 1.3% less than a decade ago [1,6]. The Muscat grape yield in Northern Chile is dedicated almost exclusively to Pisco production [6,7] as is shown in Figure 1. Pszczółkowski and Lacoste [1] reported that the varieties used for Pisco production in Chile correspond to Moscatel de Alejandría and native varieties, with low percentages of European varieties. Based upon these findings, these authors proposed to define the Chilean Pisco as an Andean spirit made from native and historical varieties from Spanish colony, which would position the Chilean pisco to the Andean territory by traditional and genetic material.



**Figure 1.** Varieties cultivated in Northern Chile intended for Pisco elaboration (in percentage). Moscatel Amarilla: 166.0 ha; Moscatel Blanca: 15.9 ha; Moscatel de Alejandría: 1711.2 ha; Moscatel Negra: 0.68 ha; Moscatel Rosada: 1630.3 ha; Pedro Jiménez: 4298.9 ha; Torontel: 179.41 ha. Figure elaborated based onto the data provided by SAG [4].

The introduction and spread of recognized European varieties over the last few decades have caused a massive loss of autochthonous and minor locally grown grapes traditionally grown in different wine regions [8–12]. The current varietal homogenization of the vineyards results in an increase in genetic vulnerability in relation to pathogen spread against which some cultivars are not resistant [10]. Fortunately, in recent years there is a significant reevaluation of autochthonous and minor locally grown varieties worldwide because they can become a potential source of natural adaptation to current challenges of climate change in viticulture [13–15]. This opens up an interesting possibility of evaluating autochthonous and minor locally grown varieties for the viticultural development of each productive zone.

Chilean viticulture has been developed for centuries with ungrafted vineyards because there is no presence of phylloxera (*Daktulosphaira vitifoliae*) [16–18]. Due to this, there is limited local experience in the use of rootstocks in viticulture for wine grapes [16,19–22]. However, during the last years, plant-parasitic nematodes have become a main productive constraint in the Chilean viticulture [23]. Rootstock adoption in Chile is justified not only by the resistance against nematodes or the need to replace old or unproductive vineyards, but also by the need to overcome soil and water restraints, especially in the arid Northern Chile [16,19,21,24].

To our knowledge, there are no published reports regarding the vegetative behaviour of minor locally grown or autochthonous varieties grafted onto different rootstocks in hyper-arid conditions to date, much less about their nutritional status. Grapevine nutritional management is a fundamental issue for winegrowers in Northern Chile, since soils

are saline and the absorption of Mg, Ca and other nutrients may be affected. The varieties such as Moscatel Amarilla and Moscatel Negra are minority and autochthonous varieties distributed only in Northern Chile that deserve to be investigated. An early study reported that Moscatel Amarilla variety produces grapes with high levels of free terpenes levels and it could become a great alternative for the Chilean Pisco production [6]. In addition, to date there are no published studies concerning Moscatel Negra variety. Therefore, the aim of this field trial was to evaluate nutritional status of Moscatel Amarilla and Moscatel Negra grapevines grafted onto four different rootstocks under hyper-arid climatic conditions over three consecutive seasons.

## 2. Materials and Methods

### 2.1. Characterization of Study Site, Plant Material and Experimental Design

The field trial was conducted in an experimental vineyard located at the Vicuña Experimental Center belonging to the Instituto de Investigaciones Agropecuarias (INIA) (30°02' S, 70°41' W, 630 m above sea level; Coquimbo region, Chile) in three consecutive seasons (2017–18, 2018–19 and 2019–20). The climate of the area is classified as hyper-arid, with an average daily temperature of 16.1 °C and a mean annual rainfall of 100 mm that concentrate in winter (June–September). The vineyard soil is a sandy loam alluvial Entisol and has a flat topography (<1%). The soil holds moderate depth (>50 cm), field capacity of 11.2% (v v<sup>-1</sup>), permanent wilting point of 5.2% (v v<sup>-1</sup>), pH value of 7.3 (calcareous soil), a 1.5% percentage of organic matter, an electrical conductivity of 2.3 dS m<sup>-1</sup> in saturated paste.

Two Chilean autochthonous and minority grapevine varieties cv. Moscatel Amarilla (synonyms: Torrontés and Torrontés Riojano) and cv. Moscatel Negra (synonym: Canela) (Figure 2) were grafted onto three commercial rootstocks (1103 Paulsen, 140 Ruggeri and Harmony) and one naturalized genotype (R32) selected in Northern Chile due to their tolerance to water deficit [19,25,26]. The vineyard was established in the winter of 2016 in a replanting soil previously planted with *Vitis vinifera* grapevines. The grapevines were grafted using the Omega technique following the procedure described by Ibacache and Sierra [20]. The grapevines were planted at spacing of 3 m × 3 m, trained on an overhead trellis system and cane pruned leaving 4 to 5 nodes. Due to the low rainfall that is recorded during the season (less than 100 mm) it is necessary to apply water through irrigation. In this regard, the grapevines were drip irrigated using one irrigation line per row with emitters supplying water at a rate of 4 l h<sup>-1</sup> spaced at 1 m (3 emitters per plant). The reference evapotranspiration during the three seasons varied between 1127 and 1162 mm (September–April). Field trial received a standard agronomic management used in commercial vineyards in terms of irrigation, fertilization, pruning, pest and disease management in each growing season. The fertilization program consisted of applications of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (90, 50, 70 kg ha<sup>-1</sup> respectively) by means of fertigation in the spring and early summer. Nutritional content of the soil at the beginning of the study was 40 mg kg<sup>-1</sup> of available N, 8 mg kg<sup>-1</sup> of available P and 105 mg kg<sup>-1</sup> of available K.

Both varieties grafted onto four rootstocks were assigned in a completely randomized design at planting. The experimental design consisted in four treatments per varieties with three replicates (blocks) of five grapevines each to cope for soil variability along the vineyard. The description of the rootstocks under study is shown in Table 1.

To characterize the vineyard climatic conditions in terms of temperature and precipitation during the seasons, an automatic weather station (AWS) located at 100 m from the experimental vineyard was utilized. Based on the data provided by the AWS, different bioclimatic indices, such as Growing Season Temperature (GST), Cool Night index (CI), Heliothermal index (HI), Growing Degree Days (GDD), Mean Spring Temperature Summation (SON Mean), Maximum Spring Temperature Summation (SON Max) and the accumulated precipitation from May (year n) to April (year n + 1), were calculated as is shown in Table 2.



**Figure 2.** Clusters of autochthonous varieties not used commercially and authorized for the production of Pisco in accordance with Chilean alcohol regulation. (a) Moscatel Amarilla. (b) Moscatel Negra.

**Table 1.** Description and abbreviations of the rootstocks selected in this field trial.

Rootstock	Abbreviation	Pedigree <sup>1</sup>	Origin
1103 Paulsen	1103 P	<i>V. berlandieri</i> × <i>V. rupestris</i>	Italy
140 Ruggeri	140 Ru	<i>V. berlandieri</i> × <i>V. rupestris</i>	Italy
Harmony	Harmony	Couderc 1613 × <i>V. champinii</i> (Dog Ridge)	USA
R32	R32	<i>V. vinifera</i>	Chile

<sup>1</sup> V: *Vitis*.

**Table 2.** Bioclimatic indices calculated each season under study.

Season	GST (°C)	CI (°C)	HI (Heat Units)	GDD (Heat Units)	SON Mean (Heat Units)	SON Max (Heat Units)	PP May-Apr (mm)
2017–18	18.1	8.6	2488	1727.0	1442.9	2391.3	236.3
2018–19	18.4	8.8	2573	1780.2	1533.4	2505.6	36.2
2019–20	18.8	10.5	2608	1855.6	1443.3	2402.2	7.9
30-years (mean) <sup>a</sup>	18.5	10.0	2409.7	1808.2	1493.2	2310.7	93.1

GST: Growing Season Temperature [27]; CI: Cool Night Index [28]; HI: Heliothermal Index [29]; GDD: Growing Degree Days [30]; SON Mean: Mean Spring Temperature Summation [31]; SON Max: Maximum Spring Temperature Summation [31]. PP May-Apr: Accumulated precipitation from May (year n) to April (year n + 1). <sup>a</sup> Mean of 1985–2015 years.

## 2.2. Measurements

Forty leaf blades per replicates were collected at veraison stage of each studied season. The leaf blades were located on the opposite side to the bunches, fully expanded, healthy, and without any symptom of nutritional deficiency. The samples were dried in an oven at 65 °C until a constant mass was achieved, then samples were milled and sieved through a 1 mm mesh. Then, concentration of macro (N, P, K, Ca and Mg) and micro (Zn, Mn and Cu) nutrients were analysed at the Foliar Analysis Laboratory of Vicuña Experimental Centre. The Kjeldahl method was used to analyse N content of leaf blades according to methodology described by Nikolaou et al. [32]. The Olsen colorimetric method was utilized to analyse P content in leaf blades using a Spectronic 21 spectrophotometer (Spectronic Instruments, Garforth, UK) at 440 nm. K was determined by atomic absorption

spectrophotometry (Unicam 929, Unicam Ltd., Cambridge, UK) according to the method described by Garcia et al. [33]. Ca and Mg were determined by atomic absorption. Zn, Mn and Cu concentrations were analysed by sample calcination and atomic absorption spectrophotometry. Macronutrient concentration was expressed in terms of percentage ( $w w^{-1}$ ), while micronutrients were expressed in ppm. In order to determine the effect of rootstocks on the relationship between different macronutrients, the following relationships were calculated: (i) K to Ca ratio (K/Ca), (ii) K to Mg ratio (K/Mg) and (iii) K to Ca+Mg ratio [K/(Ca + Mg)].

### 2.3. Statistical Analysis

The variables were analysed considering a completely randomized design with factorial arrangement, accounting two varieties, four rootstocks by three study seasons. Variables were subjected to an analysis of variance (ANOVA). The significance of the differences was determined by Tukey's test ( $p \leq 0.05$ ). Interactions between varieties-rootstocks and rootstocks-season were examined. Additionally, a principal component analysis (PCA) was performed to determine relationships among variables according to rootstocks. Both analyses were performed using the Xlstat Software version 2020.3.1 (Addinsoft SARL, Paris, France).

## 3. Results

### 3.1. Weather Conditions

Among seasons, 2017–18 displayed lower Growing Season Temperature (GST) than the rest of studied seasons (2018–19 and 2019–20) and the 30-year average, calculated from September 1st to end of March (Table 2). This caused a lower heat accumulation for the 2017–18 season than other seasons in terms of Heliothermal Index (HI), Growing Degree Days (GDD), Mean Spring Temperature Summation (SON Mean) and Maximum Spring Temperature Summation (SON Max). Cool Night index (CI) reached values below 12 in all seasons allowing to classify them as very cool nights [28]. On the contrary, 2019–20 presented higher GST, CI and HI than 2017–18 and 2018–19 seasons. Concerning HI, all seasons under analysis were classified as warm, including 30-year average [29]. The sum of daily temperature during spring (SON Mean) and the sum of daily maximum temperature (SON Max) were higher in 2018–19 than the rest of seasons and the 30-year average. Regarding precipitations, 2019–20 was an extremely dry season reaching a precipitation of only 7.9 mm from May (year  $n$ ) to April (year  $n + 1$ ), whereas the maximum level of rainfall was reached during the 2017–18 season, quantifying 236.3 mm.

### 3.2. Blade Nutrient Content

#### 3.2.1. Macronutrients

The Variety factor significantly influenced N, P and Mg blade content, whereas Rootstock and Season factors determined the content of all macronutrients in blades of Moscatel Amarilla and Moscatel Negra grapevines growing under hyper-arid conditions (Table 3). The variety and rootstock interaction influenced both N and K blade content while rootstock and season factor influenced Ca and Mg blade content (Table 3). Moscatel Amarilla blades presented lower content of N and P, and higher Mg content than Moscatel Negra samples (Table 3). Harmony induced lower N and P content in grapevine blades than R32 rootstock (Table 3). In addition, Harmony determined the highest K and the lowest Mg levels in blades in both varieties. R32 rootstock induced lower K content in blades than 140 Ru and Harmony but stimulated the highest Ca and Mg content in blades in both varieties. Blade samples in the 2017–18 season exhibited the lowest N, P and K content, and the highest Ca and Mg levels in blades (Table 3). Moscatel Negra grafted onto R32 showed higher N blade contents than most of the variety and rootstock interactions with the exception of the blade samples obtained from Moscatel Negra grapevines grafted onto 140 Ru and 1103 P (Supplementary Table S1). Blades from Moscatel Negra grapevines grafted onto R32 showed the lowest K content. Blades from Moscatel Negra and Moscatel

Amarilla grapevines grafted onto Harmony showed higher K content than most of the variety and rootstock interactions, with the exception of blades collected from Moscatel Negra grafted onto 140 Ru. Normally, R32 rootstock in the 2017–18 season induced the highest Ca and Mg content in blades. As a whole, Harmony rootstock in 2019–20 season induced lowest Ca content in blades, whereas in 2018–19 and 2019–20 season Harmony promoted lower Mg content in blades in both varieties than R32 in all seasons, 1103 P in 2017–18 and 2018–19 seasons and 140 Ru in 2017–18 season (Supplementary Table S2).

**Table 3.** Effect of varieties and rootstocks on leaf blade macronutrient content growing under hyper-arid conditions over three consecutive seasons.

Factor	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Variety (V)					
Moscatel Amarilla	1.96 b	0.14 b	1.07	2.00	0.28 a
Moscatel Negra	2.22 a	0.17 a	1.07	2.01	0.25 b
Rootstock (R)					
1103 P	2.10 ab	0.15 ab	0.99 c	2.01 b	0.28 b
140 Ru	2.08 ab	0.15 ab	1.08 b	1.87 bc	0.27 b
Harmony	2.04 b	0.14 b	1.29 a	1.77 c	0.20 c
R32	2.15 a	0.16 a	0.91 c	2.36 a	0.32 a
Season (S)					
2017–18	1.93 b	0.14 b	0.96 c	2.25 a	0.31 a
2018–19	2.15 a	0.15 a	1.06 b	2.08 b	0.25 b
2019–20	2.18 a	0.16 a	1.19 a	1.68 c	0.24 b
Signif <sup>1</sup>					
V	0.0001 <sup>2</sup>	0.0001	0.74	0.83	0.001
R	0.012	0.041	0.0001	0.0001	0.0001
S	0.00001	0.0001	0.0001	0.0001	0.0001
V × R	0.033	0.45	0.000	0.55	0.31
R × S	0.11	0.30	0.33	0.019	0.017

<sup>1</sup> Significance (*p*-value) for Variety (V), Rootstock (R), Season (S), V × R and R × S interactions. For a given factor and significance *p* < 0.05, different letters within a column represent significant differences (Tukey's test, *p* < 0.05). <sup>2</sup> In red, *p*-value lower than 0.05.

### 3.2.2. Micronutrients

Considering micronutrients uptake and accumulation, Variety factor significantly affected Zn blade content only, where blades from Moscatel Amarilla showed lower Zn content than the samples from Moscatel Negra (Table 4). In turn, rootstock factor influenced Mn content in Moscatel Amarilla and Moscatel Negra blades collected from grapevines growing under hyper-arid conditions (Table 4). Harmony rootstock induced lower Mn content in blades than 140 Ru and R32 rootstocks, and this latter promoted higher Mn levels in blades than 1103 P (Table 4). Season factor significantly affected Zn and Mn blade content. Blades in the 2017–18 season showed the highest Zn and the lowest Mn content (Table 4). Rootstock and season interaction affected Mn content only (Table 4). 140 Ru rootstock in the 2019–20 season promoted higher levels of Mn in blades than 1103 P in 2017–18, Harmony in 2017–18 and in 2019–20, and 140 Ru in 2017–18 (Supplementary Table S2). In general, Harmony rootstock managed to induce lower Mn content in blades over the seasons compared to the rest of rootstocks and season interactions.

**Table 4.** Effect of varieties and rootstocks on leaf blade micronutrient content growing under hyper-arid conditions over three consecutive seasons.

Factor	Zn (ppm)	Mn (ppm)	Cu (ppm)
Variety (V)			
Moscatel Amarilla	36.5 b	113.4	6.9
Moscatel Negra	42.4 a	118.2	6.5
Rootstock (R)			
1103 P	35.8	109.6 bc	6.4
140 Ru	38.1	123.1 ab	6.8
Harmony	40.7	98.6 c	6.5
R32	43.3	131.8 a	7.1
Season (S)			
2017–18	54.3 a	96.9 b	6.2
2018–19	19.0 c	126.1 a	7.0
2019–20	45.0 b	124.3 a	7.0
Signif <sup>1</sup>			
V	0.022 <sup>2</sup>	0.30	0.24
R	0.19	0.0001	0.51
S	0.0001	0.0001	0.16
V × R	0.87	0.09	0.18
R × S	0.19	0.007	0.7

<sup>1</sup> Significance (*p*-value) for Variety (V), Rootstock (R), Season (S), V × R and R × S interactions. For a given factor and significance *p* < 0.05, different letters within a column represent significant differences (Tukey's test, *p* < 0.05).  
<sup>2</sup> In red, *p*-value lower than 0.05.

### 3.2.3. Relationship between Macronutrients

Variety factor did not influence any of the ratios between blade macronutrients, whereas rootstock and season factors significantly affected all the relationships among nutrients (Table 5). However, Moscatel Negra blades presented higher K to Mg ratio than the samples collected from Moscatel Amarilla grapevines (Table 5). Harmony rootstock induced the highest K/Ca, K/Mg and K to Ca+Mg ratios in blades of both varieties, whilst R32 rootstock showed the opposite effect (Table 5). 1103 P and 140 Ru rootstocks displayed similar and intermediate levels of these calculated relationships in blades compared to Harmony and R32 rootstocks. Blades in the 2017–18 season presented the lowest K/Ca, K/Mg and K to Ca+Mg ratios, while blades collected in the 2019–20 season exhibited the opposite effects on the calculated ratios (Table 5). The interaction between variety and rootstock factors affected K/Mg ratio, as blades obtained from Moscatel Negra grapevines grafted onto Harmony rootstocks presented the highest K/Mg ratio (Supplementary Table S1). Moscatel Negra grapevines grafted onto R32 grapevines presented lower K/Mg ratio compared to most of variety and rootstock combinations with the exception of blade samples collected from Moscatel Amarilla grapevines grafted onto R32 and 1103 P rootstocks (Supplementary Table S1). The interaction between rootstock and season factors considerably affected K/Ca ratio and K to Ca+Mg ratio (Table 5). Harmony rootstock in the 2019–20 season induced the highest K/Ca and K to Ca+Mg ratio in blades of both varieties. Mostly, R32 rootstock in 2017–18 season promoted lower K/Ca and K to Ca+Mg ratio in blades of both varieties than most of the rootstock and season interactions, with the exception of R32 rootstock in 2018–19 and 1103 P in 2017–2018 season (Supplementary Table S2).

**Table 5.** Effect of varieties and rootstocks on relationship between leaf blade macronutrient content growing under hyper-arid conditions over three consecutive seasons.

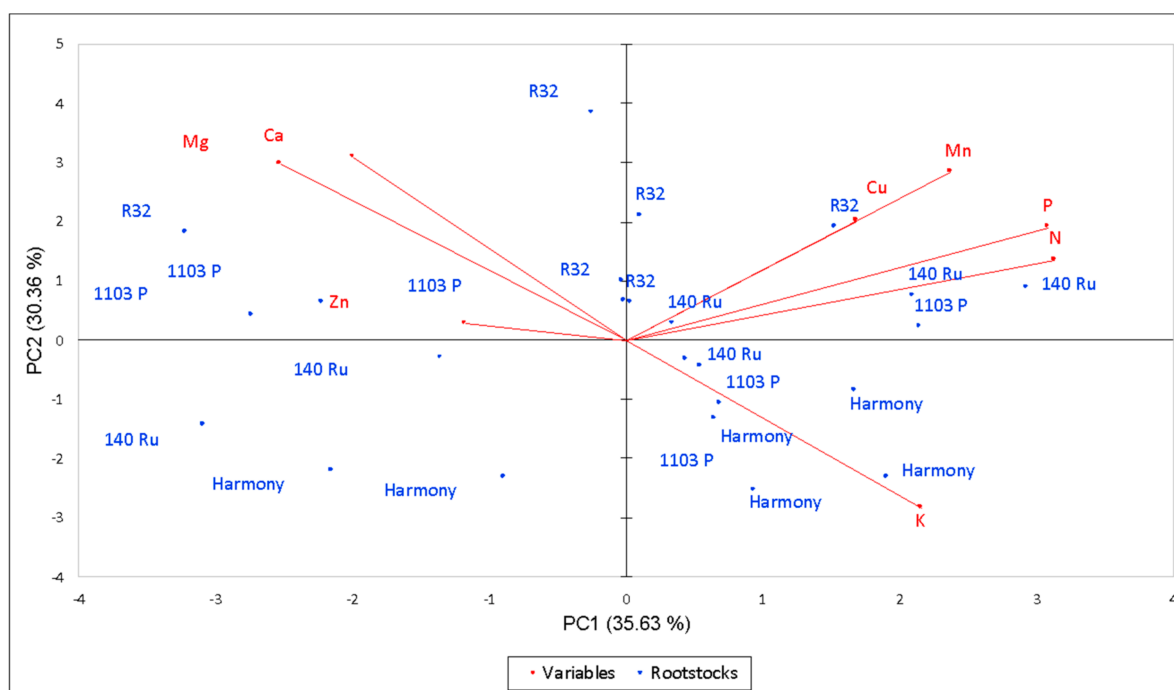
Factor	K/Ca	K/Mg	K/(Ca+Mg)
Variety (V)			
Moscatel Amarilla	0.59	4.21 b	0.51
Moscatel Negra	0.56	4.63 a	0.50
Rootstock (R)			
1103 Pa	0.52 c	3.87 b	0.46 b
140 Ru	0.60 b	4.18 b	0.52 b
Harmony	0.77 a	6.64 a	0.69 a
R32	0.40 d	3.00 c	0.35 c
Season (S)			
2017–18	0.44 c	3.42 c	0.39 c
2018–19	0.52 b	4.54 b	0.46 b
2019–20	0.76 a	5.31 a	0.66 a
Signif <sup>1</sup>			
V	0.15 <sup>2</sup>	0.007	0.34
R	0.0001	0.0001	0.0001
S	0.0001	0.0001	0.0001
V × R	0.75	0.02	0.56
R × S	0.001	0.19	0.001

<sup>1</sup> Significance (*p*-value) for Variety (V), Rootstock (R), Season (S), V × R and R × S interactions. For a given factor and significance *p* < 0.05, different letters within a column represent significant differences (Tukey's test, *p* < 0.05).  
<sup>2</sup> In red, *p*-value lower than 0.05.

### 3.3. Principal Component Analysis

In order to classify the different rootstocks and assess their influence on blade nutrient content in both varieties, a principal component analysis (PCA) was performed (Figure 3). Principal component 1 (PC 1) explained 35.63% of the variance and principal component 2 (PC2) explained 30.36%, representing 65.99% of all the analysed variance. PC 1 was correlated (−) with Mg (and Ca), and (+) N, P and Mn, whereas PC 2 was (−) correlated with K, and (+) Ca, Mg and Mn. Depending upon season and variety, Harmony and 1103 Paulsen rootstocks were (+) correlated with K, and (−) correlated with Mg and Ca blade content. Conversely, 140 Ru and 1103 P were (+) correlated to P and N blade content and (−) correlated to Harmony rootstock respectively. Similar behaviour regarding season was observed for R32 and 1103 P rootstocks, which were (+) correlated to Zn, Mg and Ca blade content. Pearson correlations confirmed some relationships (Supplementary Table S3), such as N blade content that was (+) related to P (*r* = 0.87) and Mn, (*r* = 0.58). P blade content was (+) related to Mn (*r* = 0.63). K blade content was (−) related to Ca (*r* = −0.68) and Mg (*r* = −0.77). Ca blade content was (+) correlated with Mg (*r* = 0.75). Mn blade content was (+) correlated with Cu (*r* = 0.62).





**Figure 3.** Principal component analysis (PCA) performed with nutritional variables obtained from Moscatel Amarilla and Moscatel Negra grapevines grafted onto Paulsen 1103, Ruggeri 140, Harmony and R32, during three seasons. *Footnote:* The distribution of variables (red lines) and individual observations according to rootstocks (blue dots) on PC1 and PC2 are shown.

#### 4. Discussion

Irrigation in arid and semiarid regions over prolonged periods can lead to a build-up of salt near the soil surface. Most table and raisin grapes are grown in rather dry and warm climatic regions, such as southwestern Asia, California, Chile, or Australia, and are thus especially threatened by salinity [34]. Indeed, the soils of Northern Chile are alkaline due to the presence of  $\text{CaCO}_3$  resulting in a high pH, which affects the availability of essential nutrients for crop development [20,35]. These types of soils display an accumulation of soluble salts and a high content of exchangeable Na and boron leading to micronutrient deficiencies such as Fe, Zn, Mn and Cu in crops [36,37]. Based upon the nutritional standards for viticultural management [38], both grafted grapevine varieties under study exhibited adequate levels in all nutrients analysed in blades. However, blades collected in the 2018–19 season presented deficiencies in Zn levels (Table 4). Zn is an essential micronutrient for plants that plays a key role in photosynthetic redox reactions, and it is an essential cofactor for many enzymes involved in nitrogen metabolism and protein synthesis [39,40]. Zn solubility is strongly dependent on pH, and similar to Fe, Zn availability is low in calcareous soils with a  $\text{pH} > 7$  and high bicarbonate content [40]. Zn deficiency may alter the expression and function of proteins at metabolic level that results in different physiological symptoms characterized by root apex necrosis, and in a decline in starch production and sugar accumulation in leaves [40,41]. Severe Zn deficiency in grapevines may result in the production of clusters with few berries that also vary in size from normal to very small [42,43]. Interestingly, Gainza-Cortés et al. [41] reported that *VvZIP3* encodes a putative plasma membrane Zn transporter protein member of the ZIP gene family that might play a role in Zn uptake and distribution during the early reproductive development in grapevines. Based on our results, the season that ensued a lower accumulation of Zn in the leaves had a higher thermal accumulation during spring and low accumulated rainfall, which could result in a certain degree of thermal and drought stress during the reproductive stages of the grapevine. Zn applications in grapevines cultivated under drought conditions enhance acquisition of many plant nutrients, pro-

moting the vegetative and generative developments related to shoot and leaf growth, greener leaves, enhancing berry development and vine yield [44]. Furthermore, abscisic acid alleviates uptake and accumulation of Zn in grapevines by inducing expression of ZIP and detoxification-related genes [40]. Thereby, Zn absorption and accumulation in blades may be affected by high temperatures and drought conditions in grapevines cultivated under the hyper-arid conditions of Northern Chile. Zn and Cu are integral parts of one form of the antioxidant enzyme superoxide dismutase which may also help protect plant tissues against the production of reactive oxygen species that lead to oxidative stress that accompanies many environmental stresses [40]. Pearson's correlations did not show a relationship between Zn and Cu under the edaphoclimatic conditions of the present study. Since solubility is strongly dependent on pH, Zn availability is low in calcareous soils with a pH >7 and high carbonate content [40].

Moscatel Amarilla tended to accumulate lesser amounts of N, P and Zn, and more Mg content than Moscatel Negra blade leaves (Tables 3–5). This resulted in the K to Mg ratio being higher in the Moscatel Negra variety than Moscatel Amarilla blades and mostly if these grapevine varieties were grafted onto Harmony rootstock (Table 3 to Table 5). The natural crossing between Listán Prieto and Moscatel de Alejandría occurred in the XVII century originated Moscatel Amarilla and probably Moscatel Negra [1,26,45]. Local experience carried out in recent years has shown that Moscatel Amarilla presents a medium to large cluster, grapevines can produce between 30 to 50 tons ha<sup>-1</sup> and technological maturity occurs in the second week of February [46]. Moscatel Negra presents a small to medium cluster, the grapevines can produce between 25 to 35 tons ha<sup>-1</sup> and technological maturity occurs in the first week of February [46]. Thereby, the differences in productivity and cluster size between both varieties can affect the uptake and accumulation of nutrients in the blades in veraison. Recently, Verdenal et al. [47] reported that fertilizer N uptake and its assimilation in Chasselas (*Vitis vinifera*) grapevines appeared to be strongly stimulated by high-yielding conditions. Interestingly, grapevines were able to modulate root N reserve mobilization and fertilizer N uptake in function of the crop load, thus maintaining a uniform N concentration in fruits [47]. K/Mg ratio is an important factor in determining Mg uptake and high K/Mg ratio determines low Mg uptake [48]. As a whole, Harmony rootstock induced high accumulation of K in leaf tissues in different grapevine varieties cultivated in Northern Chile [20]. Our results confirm these findings since Harmony induced a high K accumulation in blades that was exacerbated when Moscatel Negra grapevines were grafted onto Harmony rootstock (Table 3 and Table S1). In addition, Harmony accumulated less amount of Mg in the leaves independent of the climatic conditions of study season (Table S2). Recently, Gautier et al. [49] reported that rootstocks with a *V. riparia* parent conferred a lower petiolar concentration of P, Mg, B and Al, but a higher petiolar concentration of S, whereas rootstocks with a *V. rupestris* genetic background conferred a higher petiolar concentration of P, B and Fe. Mg deficiency may reduce chlorophyll content in leaves and changes the chlorophyll a to b ratio in favour of chlorophyll b [48,50]. In fact, Mg-deficient grapevines are extremely light-sensitive which accelerates the appearance of the characteristic interveinal chlorosis [40]. This issue can become quite a serious problem for the vineyards grown in Northern Chile since the grapevines in this area are cultivated at higher altitudes than in other Chilean viticultural regions and receive higher UV radiation compared to more Southern locations. Bascañán-Godoy et al. [51] reported that the yield rise of Red Globe grapevines grafted onto Harmony and Salt Creek was correlated with the increase in light capture, greater leaf area, photosynthetic rate, light absorption capacity, production and mobilization of reserve carbohydrates on these rootstocks. However, Harmony in spite of having parents in common with Freedom, is recognized in California because it induces lower vigour to the canopy and also reduces uptake of nitrogen [52]. Pearson's correlation showed a negative relationship between K and Mg ( $r = -0.77$ ) (Table S3). Toumi et al. [48] explained that the negative relationship observed between the K and Mg in leaves may be explained due that the Mg decreases by K to Mg antagonism. However, in this study the high K supply was accompanied by low Mg supply and it could

have merely been caused by shortage of Mg rather than abundance of K. As regards our results, Mg and K contents in Moscatel Amarilla and Moscatel Negra grapevines grafted onto Harmony should be strictly monitored during grape ripening to avoid and correct Mg deficiencies on grapevines. In this fashion, rootstocks that harbour a *V. berlandieri* genetic background could be an interesting alternative for these autochthonous grapevines varieties since they confer a higher of scion vigour and yield than rootstocks with a *V. riparia* genetic background what is an attribute of interest for the Pisco production. In parallel, R32 rootstock induced low accumulation of K in Moscatel Negra grapevines and induced a high accumulation of Mg in both varieties. Low K supply to grapevines strongly reduces xylem sap flow and limits shoot and fruit growth and greatly increases the risk of drought stress [53]. K deficiency in grapevines also suppresses sugar transport in the phloem and can result in sucrose accumulation in the leaves to substitute for the missing K as osmoticum [40,54].

R32 (*Vitis vinifera*) is a native rootstock that corresponds to a naturalized genotype selected for its tolerance to drought conditions of Northern Chile [19,26]. R32 rootstock induced a higher accumulation of N, P, K and Mn than Harmony. Mn availability is highly pH dependent and is minimal when soil pH reaches close to 7 [40]. Mn is required for the function of enzymes such as glucosyltransferases, which attach a glucose molecule to phenolics and other compounds [55]. Mn deficiency may increase tissue sensitivity to oxidative stress, which can be caused by different environmental stresses [40]. Deficiency symptoms are consequently more severe on sun-exposed leaves [40]. Contrary to this, our results pointed that Mn accumulation in blades was lower in the coldest season that was in 2017–18 than in 2018–19 and 2019–20 seasons which were the warmest. The occurrence of chlorotic leaves in response to insufficient Mn availability occurs first in the basal portion of the shoots, soon after budbreak [56]. Thus, high temperatures early in the grapevine growing season could induce Mn deficiencies in the grapevines. Pearson's correlation showed that Mn blade content was correlated with N ( $r = 0.58$ ), P ( $r = 0.63$ ) and Cu ( $r = 0.62$ ) (Table S3). N depending on its form, i.e., as  $\text{NO}_3^-$  or  $\text{NH}_4^+$ , can affect Mn soil solubility and shoot Mn uptake by altering rhizosphere pH [57]. The solubilization of higher Mn-oxides in soil is also facilitated by its organic matter content, and extreme heating and drying [57]. Mn absorption also could be affected by other microelements due to a close interaction of Mn nutrition and antioxidant metabolism in plants. This is because cytosolic CuZn-SOD and mitochondrial Mn-SOD activities increase under conditions of Mn-excess as well as Mn-starvation [58,59]. The main factor determining P and Mn availability and solubility to plants is soil pH and P inactivation may occur also in calcareous soils due to high concentration of Ca ions and high soil pH [60]. Our results confirm this notion since P contents in the blade samples were below the nutritional optimum but did not reach deficiency levels. Despite this, Mn levels in blades were above the optimal nutritional content without reaching toxic levels for the plant. Both P and Mn ions are rather immobile in soils implying that factors such as root length and root architecture, as well as rhizosphere processes, have a major impact on their availability for plants [61]. High soil P resulted in elevated plant P status which interfered in the uptake and/or translocation of Mn [62]. Similar to this, a negative effect of high P levels on Mn accumulation was shown and it is suggested that P interferes directly with Mn at the uptake and/or translocation level [63]. However, despite these findings, it is likely that under the edaphoclimatic conditions of our study, there is a positive relationship between P and Mn accumulation due to the high soil pH that limit P availability and absorption, triggering a greater absorption of Mn by grapevines. R32 rootstock may be suitable under these conditions since it promotes a high P and Mn accumulation compared to rest of studied rootstocks independent of the weather conditions of the season. In addition, rootstocks that presents *V. rupestris* and *V. berlandieri* genetic background may have acquired efficient mechanisms to increase P acquisition or use in response to the limited-P environment since they are native from the south of the USA, particularly where soils are calcareous and often deficient in P due to precipitation

of calcium phosphate [49]. Thereby, 140 Ru and 1103 P rootstocks can also be interesting alternatives to both grapevines varieties under study to be grafted.

The highest accumulation of Ca and Mg was determined in R32 rootstocks that was heightened in the 2017–18 season. Ca accumulation could have a key role in the osmotic adjustment of grape leaves as other inorganic ions such as K [64,65]. Some authors show an increase in calcium oxalate crystals in leaves of grapevines cultivated under drought conditions, suggesting that these structures in the mesophyll could either play a functional role in water stress and Ca regulation, or represent an unintended result of increased Ca accumulation [64,66]. Our results showed that during the warmest season, rootstocks showed lower Ca accumulation with the exception of R32, than the rest of the rootstock and season interactions (Table 3). During the cooler season, R32 rootstock showed the highest accumulation of Ca and Mg (Table 3). This caused a lower K/Ca and K/Ca+Mg ratios in the R32 rootstock, and that Harmony reached the highest values of the exposed ratios in the warmer season (Table S2). The nutrient antagonism is produced when an excessive concentration of one nutrient inhibits the uptake of another [67]. Because K, Ca and Mg have similar charged ionic forms, and are taken up in a similar way, the absorption of high amounts of one nutrient may inhibit the uptake of another nutrient. García et al. [33] showed that K to Ca and K to Mg antagonisms were well expressed in Négrette (*V. vinifera*) varieties grafted onto different rootstocks. In their study, 3309 C (*Riparia tomentosa* × *Rupestris martin*) induced the lowest K level in leaves and SO4 (*V. berlandieri* × *V. riparia*) absorbed K more readily than 101–14 Mgt (*V. riparia* × *V. rupestris*), suggesting that 3309 C appears to be the most appropriate rootstock for Négrette variety [33]. Therefore, based upon our results, for both autochthonous varieties under study, R32 rootstock and to a lesser extent, the 1103 P and 140 Ru rootstocks can be interesting alternatives to keep balanced levels of nutrients under the hyper-arid conditions of Northern Chile. On the other hand, the use of Harmony rootstock displayed noticeable nutritional imbalances, especially due to its low absorption of Mg, Mn, Ca and P and its high absorption of K, which is exacerbated under warm weather and soil salinity conditions. Moreover, Ibacache et al. [21] reported that Harmony accumulated more chloride than 1613 Couderc, Freedom, 1103 Paulsen, 110 Richter, 99 Richter, 140 Ruggeri, SO4, Salt Creek and Saint George in blade petioles. The higher chloride concentration in petioles at full flowering stage of Flame Seedless and Muscat of Alexandria growing on their own roots compared to grafted grapevines may reflect the poor capacity of *V. vinifera* grapevines for chloride exclusion [21]. Consequently, monitoring of these nutrients is critical to nutritional management of grapevines grafted onto Harmony rootstock. Novel determinations regarding nutritional requirements of grapevine, will rise to integrated sustainable practices, considering developmental periods in which grapevine needs more P, such as the flowering stage, or more N at the first berry expansion stage, less nutrient at the seed stone hardening stage, and more P and K at the second berry expansion stage and veraison stage [68]. Also, roots are still able to respond to temperature and hypoxia in the absence of the known perception mechanisms for the corresponding stress, and some authors hypothesize that this is also the case for sodium sensing leading to sodium-specific growth away from high salinity, and root directional growth is caused by asymmetrical PIN2 distribution during hypoxia and halotropism [69].

Water scarcity and salinity, along with high temperature will impose more frequent and severe drought and stress events. Under this perspective, water management will play a major role, but taking care of avoiding eventual salinity problems. In areas with water scarcity such as Northern Chile, more efficient rootstocks and scions will be the best option, always combined with appropriate soil and canopy management [70]. Improved knowledge of structure and function of grapevine roots and rhizosphere in different soils, climates and under diverse agronomical practices may provide a wider range of solutions to cope with the challenges associated to global change. In this regard, the genetic diversity hosted in *Vitis* ssp. can provide new functional abilities [70,71], necessary to match specific clone/cultivar/rootstock/site combinations. Finally, our results may provide a nutritional guide for the viticulturists and growers of Northern Chile and to bring the possibility to

diversify their production using these minority and autochthonous varieties that adapt well to the edaphoclimatic conditions of hyper-arid Northern Chile.

**Supplementary Materials:** The following materials are available online at <https://www.mdpi.com/2073-4395/11/2/327/s1>, Table S1: Interactions between varieties (V) and rootstocks (R), Table S2: Interactions between rootstocks (R) and seasons (S), Table S3: Pearson’s correlation obtained from Principal Component Analysis (Figure 3).

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