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Modifications induced by rootstocks on yield, vigor and nutritional status on *Vitis vinifera* cv Syrah under hyper-arid conditions in Northern Chile

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Abstract: Hyper-arid regions are characterized by extreme conditions for growing and lack of water (<100 mm annual rainfall average), where desertification turns human activities almost impossible. In addition to the use of irrigation, different viticultural strategies should be taken into account to face the adverse effects of these conditions, in which rootstocks may play a crucial role. Research aim was to evaluate the effects of the rootstock on yield, vigor and petiole nutrient content in Syrah grapevines growing under hyper-arid conditions during five seasons and compare them to ungrafted ones. St. George induced lower yield than 1103 Paulsen. Salt Creek induced higher plant growth vigor and Cu petiole content than ungrafted vines in Syrah, which was correlated to P petiole content. However, Salt Creek and St. George rootstocks induced an excess of vigor in Syrah according to Ravaz Index. Rootstocks with *V. berlandieri* genetic background should be used in Syrah growing under hyper-arid conditions to maintain adequate levels of plant vigor and to avoid deficiencies or toxicity of macro and micronutrients.

Keywords: vine; rootstocks; *V. berlandieri*; *V. rupestris*; *V. riparia*; bioclimatic indices

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1. Introduction

Chilean viticulture has been developed for many years with ungrafted (own-rooted) vineyards unlike what occurs in other viticultural countries with long winemaking tradition, such as Portugal, Spain, Italy or France that have been forced to use rootstocks mainly due to the presence of phylloxera (*Daktulosphaira vitifoliae*) [1–3]. Since this root parasite has not yet been reported in Chile, there is limited local experience in the use of rootstocks in the national viticulture for wine grapes [4–6]. However, plant-parasitic nematodes are a main constraint in the national viticulture, affecting vine root development [7]. These nematodes may cause several damages that are typically reflected in lower production and, in some cases, total crop loss [7]. Rootstock adoption in Chile is justified not only by the need for resistance against nematodes or the need to replace old or unproductive vineyards, but also by the need to overcome soil limitations, especially in the arid northern region of the country [4–6].

Grafting is a millennial horticultural technique performed in perennial and annual crops, which is used to join scion and root from two plants, allowing to enable the desirable traits of these different genotypes to be combined in a single plant [8]. Grafting allows

some grape grower control over important agronomic traits and provides flexibility in growing a particular scion or cultivar across diverse soil and environmental conditions [9]. Vine rootstocks are commonly characterized according to the vigor attributes conferred to the scion, and are able to modify scion gas exchange, water use efficiency and by consequence, crop productivity [10,11]. Recent reports have shown significant effects on technological parameters, organic acids, yeast assimilable nitrogen, phenolic and volatile compounds in grapes and wines obtained from grafted and ungrafted vines [2,12–14]. On the other hand, some researchers have reported that rootstocks modified mineral composition of scion petioles and blades [5,8,15]. In this regard, Gautier et al. [8] determined that the genetic background of a rootstock is related to the ability to modify accumulation and concentrations of phosphorus, magnesium and sulfur in the scion petioles. Thus, the rootstock confers vigor to the scion, which is related to nitrogen concentration in petioles, where higher vigor is related to higher concentrations [5].

Scion-rootstock relationships are extremely specific and depends on affinity and compatibility of the grafting combination, and the soil and climatic adaptation [16]. Indeed, Carrasco-Quiroz et al. [2] reported that wines produced from ungrafted vines had lower ester concentration compared to wines elaborated from vines grafted onto 99 Richter, 140 Ruggeri, 110 Richter, 1103 Paulsen and Gravesac. Diverging to this, Olarte Mantilla et al. [13] reported that most of esters were higher in the wines made from vines on their own-roots. These contradictory results could have occurred due to the differences in vigor caused by the rootstock, since in the report published by Olarte Mantilla [13], the ungrafted vines had a higher pruning mass compared to the grafted vines, contrary to the results published by Carrasco-Quiroz et al. [2].

Recent studies suggest that rootstocks could be useful to mitigate the negative effects of climate change, enhancing resistance against temperature, salinity and drought stress [17–19]. Rootstock could be an interesting tool for the viticulture management of hyper-arid zones that are characterized by the shortage of water, limited resources, salinity and the permanent uncertainty of the desert climate. To our knowledge, there is insufficient information in Chile about the use of rootstocks under hyper-arid climatic conditions. Therefore, the aim of this field trial was to evaluate yield, vigor and nutritional status of Syrah grapevines own-rooted and grafted onto eight rootstocks under hyper-arid climatic conditions over five consecutive seasons.

2. Materials and Methods

2.1. Study site and plant material

The field trial was conducted in an experimental vineyard located at the Vicuña Experimental Center belonging to the Instituto de Investigaciones Agropecuarias (INIA) (30º 02'S, 70º41'W, 630 meter above sea level; Coquimbo region, Chile) in five consecutive seasons (2003-04, 2004-05, 2005-06, 2006-07 and 2007-08). Syrah grapevines own-rooted and grafted onto 1103 Paulsen, 140 Ruggeri, 99 Richter, Freedom, Harmony, Salt Creek, SO4 (Selection Oppenheim 4) and St. George (Rupestris du Lot) were established in 2001 in a replanting soil previously planted with avocado (*Persea americana*). The vines were grafted using the Omega technique following the procedure stated by Ibacache and Sierra (2009). The vines were planted at a distance of 1.5 m × 2.5 m, formed in bilateral cordon, trained on a vertical shoot position trellis system and spur pruned to two buds. Syrah grapevines were managed according to the standard viticultural practices used in commercial vineyard in terms of fertilization, pruning, pest and disease in each growing season. Briefly, fertilization program consisted of applications of N, P_2O_5 and K_2O (90, 50, 70 kg ha^{-1}) by means of fertigation in the spring and early summer.

Soil conditions were previously reported by Ibacache and Sierra [20]. The vineyard soil is a loamy alluvial Entisol and has a flat topography (< 1 % slope). The soil has moderate depth (> 50 cm), alkaline pH (7.9), a 2.4 % of organic matter, an electrical conductivity of 1.0 dS m^{-1} in saturated paste. The mineral N, P Olsen and exchangeable K levels was 9, 12 and 237 mg kg^{-1}, respectively. Before the vineyard establishment, a nematological

analysis was performed into the vineyard soil, reporting a presence of *Pratylenchus*, *Meloidogyne*, *Criconemoides*, *Pratylenchus* and *Tylenchulus semipenetrans* species lower than 18 individuals per 250 g of soil.

To characterize the vineyard climatic conditions in terms of temperature and precipitation during the seasons, an automatic weather station (AWS) located at 100 meters 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), Biologically Effective Degree Days (BEDD), 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 are shown in Table 1.

Table 1. Bioclimatic indices calculated each season of study.

Season	GST (ºC)	CI (ºC)	HI (heat units)	GDD (heat units)	BEDD (heat units)	SON Mean (heat units)	SON Max (heat units)	PP May-Apr (mm)
2003-04	18.6	9.6	2385.0	1822.9	1557.3	1561.5	2371.7	93.3
2004-05	18.7	10.1	2455.9	1854.4	1555.8	1537.1	2396.5	83.9
2005-06	18.5	8.7	2416.3	1801.2	1533.8	1439.7	2273.2	66.4
2006-07	18.3	9.0	2388.7	1756.2	1542.6	1552.2	2431.6	51.4
2007-08	17.9	8.8	2311.5	1681.5	1473.0	1377.6	2209.3	17.8
30-years (mean)[a]	18.5	10.0	2409.7	1808.2	1528.7	1493.2	2310.7	93.1

GST: Growing Season Temperature [21]; CI: Cool Night Index [22]; HI: Heliothermal Index [23]; GDD: Growing Degree Days [24]; BEDD: Biologically Effective Degree Days [25]; SON Mean: Mean Spring Temperature Summation [26]; SON Max: Maximum Spring Temperature Summation [26]. PP May-Apr: Accumulated precipitation from May (year n) to April (year n+1). [a]Mean of 1985-2015 years.

The zone under study is classified as hyper-arid (Dry Index = 0.05) based on the United Nations Environmental Program [27]. Due to the low rainfall that is recorded during the season and the high vapor pressure deficit, it is necessary to apply water through irrigation. In this regard, the vines were drip irrigated using one irrigation line per row with emitters supplying water at a rate of 4 l h^{-1} spaced at 1 m. The reference evapotranspiration (ET_0) during the five seasons varied between 1127 and 1162 mm (September-April). Annual ET_0 ranged between 1368 and 1420 mm (January-December).

2.2. Experimental design

Syrah grapevines own-rooted and grafted onto 1103 Paulsen, 140 Ruggeri, 99 Richter, Freedom, Harmony, Salt Creek, SO4 and St. George rootstocks were assigned in a completely randomized design at planting. The experimental design consisted in nine treatments (one: own-rooted and eight: grafted vines) and four replicates of four adjacent vines each to cope for soil variability along the vineyard. The description of the rootstocks under study is shown in Table 2.

Table 2. Description and abbreviations of the rootstocks under study.

Rootstock	Abbreviations	Pedigree	Origin	Rootstock ~~feature~~

1103 Paulsen	1103 P	*V. berlandieri* x *V. rupestris*	Italy	Intermediate drought tolerance, poor resistance to nematodes, intermediate scion vigor
140 Ruggeri	140 Ru	*V. berlandieri* x *V. rupestris*	Italy	Intermediate drought tolerance, poor resistance to nematodes, intermediate scion vigor
99 Richter	99 Ri	*V. berlandieri* x *V. rupestris*	France	Intermediate drought tolerance, poor resistance to nematodes, high scion vigor
Freedom	Freedom	Couderc 1613 x *V. champinii*	USA	Poor drought tolerance, resistance to nematodes and scion vigor
Harmony	Harmony	Couderc 1613 x *V. champinii*	USA	Poor drought tolerance, resistance to nematodes and scion vigor
Salt Creek	Salt Creek	*V. champinii*	USA	Poor drought tolerance and resistance to nematodes, and high scion vigor
SO4	SO4	*V. berlandieri* x *V. riparia*	France	Poor drought tolerance, low resistance to nematodes and intermediate scion vigor
Saint George	St. George	*V. rupestris*	USA	Poor drought tolerance and resistance to nematodes, and high scion vigor

Rootstock feature information was obtained from ~~the chapter published by~~ Ibacache et al. [5].

2.3. Measurements

2.3.1. Yield and yield components

Harvest was carried out when berries reached a total soluble solids content between 22 and 23 ºBrix. All bunches of the replicates were manually harvested and weighed in a digital weight scale, recording yield by vine (kg vine^{-1}) and the number of bunches per vine. The bunch weight (g) was obtained, dividing the yield by the number of bunches per plant. During spring of each season, budburst percentage (ratio of number of buds that burst vs total number of buds left at pruning) and fruitfulness percentage (ratio of number of bunches vs total number of buds left at pruning) were measured on each plant.

2.3.2. Vine vigor and balance

Vines of each replicate were manually pruned in winter and the pruning weight (kg vine^{-1}) was determined. Based on yield and pruning weight obtained, the Ravaz Index was calculated as ratio between yield and pruning weight, representing the balance between vine reproductive and vegetative activity. Scion trunk circumference (cm) was measured at end of each season (April) at 30 cm above the ground using a metric tine.

2.3.3. Petiole nutrient content

Petiole of leaves opposite to the bunches were collected at flowering stage of each study season. Forty petioles per treatment and per replicates were sampled. The samples were dried in an oven at 65 ºC 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 analyzed at the Foliar Analysis Laboratory of Vicuña Experimental Center. The Kjeldahl method was used to analyze N content of leaf petioles according to methodology described by Nikolaou et al. [28]. The Olsen colorimetric method was utilized to analyze P content in leaf petioles using a Spectronic 21 spectrophotometer (Spectronic Instruments, Garforth, United Kingdom) at 440 nm. K was determined by atomic absorption spectrophotometry (Unicam 929, Unicam Ltd., Cambridge, UK) according to method described by Garcia et al. [29]. Ca and Mg were determined by atomic absorption. Zn, Mn and Cu concentrations were analyzed by sample calcination and atomic absorption spectrophotometry. Macronutrient concentration was expressed in terms of percentage (w w^{-1}), while micronutrients were expressed in ppm.

2.4. Statistical analysis

The variables were analyzed considering a completely randomized design with factorial arrangement, accounting nine treatments (eight rootstocks plus own-rooted) by five study seasons. Variables were subjected to an analysis of variance (ANOVA). The significance of the differences was determined by Tukey's test ($p \leq 0.05$). Additionally, a principal component analysis (PCA) was performed to determine relationships among variables according to treatment (rootstocks) and season, using the average data in each case. Both analyses were performed using the Xlstat Software version 2020.3.1 (Addinsoft SARL, Paris, France).

3. Results

3.1. Weather conditions

Among seasons, 2007-08 displayed lower Growing Season Temperature (GST) calculated from 1 September to 31 March than rest of studied seasons (2003-04, 2004-05, 2005-06, 2006-07) and the 30-year average (Table 1). This resulted in a lower heat accumulation for the 2007-08 season than the rest of the seasons in terms of Heliothermal Index (HI), Growing Degree Days (GDD), Biologically Effective Degree Days (BEDD), Mean Spring Temperature Summation (SON Mean) and Maximum Spring Temperature Summation (SON Max). According HI, 2004-05, 2005-06 and 30-year average were classified as hot seasons, whereas 2003-04, 2006-07 and 2007-08 were classified as warm seasons [23]. The sum of daily temperature during spring (SON Mean) and the sum of daily maximum temperature (SON Max) were higher in 2003-04, 2004-05 and 2006-07 compared to the rest of the seasons and the 30-year average. These indices were calculated for months of September, October, and November, with no upper or lower temperature thresholds imposed [26]. The Cool Night Index (CI) indicated that all seasons presented very cool nights [22]. In this way, 2005-06 and 2007-08 presented lower CI than the rest of the seasons, while 2004-05 season showed the highest CI. Regarding precipitations, 2007-08 was a very dry season having a precipitation of 17.8 mm from May (year n) to April (year n+1), while the maximum level of rainfall in this study was reached in 2003-04 season, quantifying 93.3 mm, close to the 30-year average (93.1 mm).

3.2. Yield and its components

Season factor significantly affected yield, number of bunches per vine, bunch weight, and percentage of budburst and fruitfulness, while rootstock factor only affected yield in Syrah grapevines growing under hyper-arid conditions (Table 3). Syrah grapevines grafted onto 1103 Paulsen reached a higher yield (7.9 kg) than vines grafted onto St. George (5.9 kg). Vine yield and bunch weight decreased as seasons passed with the exception of the last year of study, which exhibited an intermediate level of these variables. The number of bunches per vine was higher in 2004-05 than 2003-04, 2006-07 and 2007-08 seasons, while 2006-07 displayed lower number of bunches per vines than rest of the seasons with exception of 2007-08. Normally, percentage of budburst and fruitfulness were higher in 2004-05 and 2006-07 seasons and lower in 2005-06 than other seasons. The interaction of factors did not affect yield nor any of its components in Syrah grapevines cultivated under hyper-arid conditions.

Table 3. Effect of rootstock on yield and its components of Syrah grapevines growing under hyper-arid conditions over five consecutive seasons.

Factor	Yield (kg vine)	Nº bunches per vine	Bunch weight (g)	Budburst (%)	Fruitfulness (%)
Rootstock (R)					
Own Roots	7.7ab	47.7	160.0	94.2	173.1
1103 Paulsen	7.9 a	47.1	166.1	95.0	168.0
140 Ruggeri	7.1ab	42.8	168.5	94.1	170.0

99 Richter	7.4ab	45.4	164.4	95.3	171.3
Freedom	7.4ab	44.1	166.8	93.8	168.9
Harmony	6.6ab	41.8	159.1	93.0	156.2
Salt Creek	7.6ab	45.7	163.2	93.4	171.1
SO4	7.4 ab	46.0	158.7	92.1	167.3
St. George	5.9 b	38.7	149.1	91.6	164.3
Season (S)					
2003-04	10.086 a	45.2 bc	222.0 a	94.6 b	167.6 ab
2004-05	8.049 b	54.4 a	150.0 b	97.3 a	174.8 a
2005-06	7.583 b	48.3 ab	157.4 b	87.5 d	156.9 b
2006-07	4.099 d	34.1 d	120.1 c	97.3 a	171.7 a
2007-08	6.184 c	39.7 cd	159.2 b	91.4 c	167.9 ab
Signif[a]					
R	0.025[b]	0.12	0.67	0.053	0.13
S	<0.0001	<0.0001	<0.0001	<0.0001	0.001
R × S	0.86	0.98	0.88	0.64	0.93

[a]Significance (p-value) of Rootstock (R), Season (S) and R × S interactions. For a given factor and significance $p < 0.05$, different letters within a column represent significant differences (Tukey's test, $p < 0.05$). [b]In red, *p*-value lower than 0.05.

3.3. Vine vigor and balance

Both rootstock and season factors affected pruning weight, Ravaz index and scion trunk circumference in Syrah grapevines cultivated under hyper-arid conditions (Table 4). Syrah grapevines grafted onto Salt Creek (2.5 kg), SO4 (2.2 kg) and St. George (2.3 kg) rootstocks showed higher pruning weight than the ungrafted ones (1.6 kg) vines. Ungrafted Syrah grapevines reached higher Ravaz index than vines grafted onto Salt Creek and St. George rootstocks, and higher scion trunk circumference than vines grafted onto Freedom. Concerning the season factor, 2005-06 resulted in ines with lower pruning weight than 2004-05 and 2006-07 seasons, while the latter showed the lowest Ravaz index. This trait was higher in 2003-04 and 2005-06 than in the rest of the seasons. Factor interactions did not affect none of measured parameters of this section in Syrah grapevines cultivated under hyper-arid conditions.

Table 4. Effect of rootstock on vine vigor, vine balance and scion trunk circumference of Syrah grapevines growing under hyper-arid conditions over five consecutive seasons.

Factor	Pruning weight (kg per vine)	Ravaz Index	Scion Trunk circumference (cm)
Rootstock (R)			
Own Roots	1.57 c	5.14 a	13.98 a
1103 Paulsen	2.02 abc	4.34 ab	13.54 ab
140 Ruggeri	2.05 abc	3.79 abc	13.71 ab
99 Richter	1.85 bc	4.22 ab	12.96 ab
Freedom	1.96 abc	3.89 abc	12.74 b
Harmony	1.88 bc	3.86 abc	13.90 ab
Salt Creek	2.45 a	3.22 bc	13.24 ab
SO4	2.18 ab	3.85 abc	13.24 ab
St. George	2.33 ab	2.73 c	12.98 ab
Season (S)			
2003-04	1.94 ab	5.47 a	7.48 d
2004-05	2.20 a	3.88 b	13.23 c
2005-06	1.74 b	4.99 a	13.43 c

2006-07	2.20 a	1.96 c	15.73 b
2007-08	2.07 ab	3.16 b	16.95 a
Signif[a]			
R	<0.0001[b]	0.000	0.011
S	0.001	<0.0001	<0.0001
R × S	0.97	0.98	0.93

[a]Significance (p-value) of Rootstock (R), Season (S) and R × S interactions. For a given factor and significance $p < 0.05$, different letters within a column represent significant differences (Tukey's test, $p < 0.05$). [b]In red, *p*-value lower than 0.05.

3.4. Petiole nutrient concentration

The nutrient content varied between 0.8 and 1.2 % for total N (ungrafted and St. George, respectively), 0.14 and 0.43 % for P (ungrafted and St. George, respectively), 2.2 and 3.5 % for K (SO4 and Harmony, respectively), 1.4 and 1.9 % for Ca (ungrafted and Harmony, respectively), 0.3 and 0.6 % for Mg (SO4 and ungrafted, respectively), 35 and 61 ppm for Zn (Harmony and Salt Creek, respectively), 38 and 82 ppm for Mn (Harmony and 140 Ruggeri, respectively), and 6 and 10 ppm for Cu (Harmony and Salt Creek, respectively).

Rootstock affected significantly N, P, K, Ca, Mg, Zn, Mn and Cu petiole content. Syrah ungrafted grapevines presented lower N petiole concentration than Harmony and St. George. Syrah ungrafted grapevines presented lower P petiole content than most of the rootstocks with the exception of Harmony. Ca petiole content was lower in Syrah ungrafted grapevines than in most of the rootstocks, with the exception of 99 Richter, Freedom and Salt Creek (Table 5). Syrah grapevines grafted onto Harmony presented highest K petiole content, and lower Zn petiole content than most of studied rootstocks with the exception of 1103 Paulsen and 140 Ruggeri. The rootstocks induced lower Mg petiole content compared to ungrafted vines in Syrah. Vines grafted onto Harmony presented lower Mn petiole content than vines grafted onto 140 Ruggeri and St. George, whereas vines grafted onto Salt Creek showed higher Cu petiole content than vines grafted onto 99 Richter, Freedom, Harmony, SO4 and St. George rootstocks.

Season factor also affected N, P, K, Ca, Mg, Mn and Cu petiole content in Syrah grapevines. Zn petiole content in Syrah grapevines was not affected by season. N petiole content in Syrah grapevines was higher in 2003-04, 2005-06 and 2006-07 than in the 2004-05 season, while P petiole content in vines was higher in the last three study seasons than in the first two ones. K petiole content was higher in 2003-04 and 2005-06 than in the 2006-07 season in Syrah grapevines, while the latter exhibited highest Ca petiole content in Syrah vines. Mg petiole content was lower in 2007-08 than in the 2006-07 and 2003-04 seasons, while Mn petiole content in this latter season was higher than in the 2004-05 and 2007-08 seasons in Syrah grapevines. Cu petiole content was higher in 2005-06 and 2006-07 than in the 2004-05 and 2007-08 seasons in Syrah grapevines. Factor interaction only influenced Mg petiole content in Syrah grapevines cultivated under hyper-arid conditions. Ungrafted vines growing in 2003-04 season presented the highest Mg petiole content. In addition, Syrah grapevines grafted onto St George (2007-08), Salt Creek (all seasons, except 2006-07), Freedom (all seasons) and SO4 (all seasons) showed lower Mg petiole content than those corresponding to other rootstock-year interaction for Mg petiole concentration (Supplementary Table 1).

Table 5. Effect of rootstock on petiole nutrient content of Syrah grapevines growing under hyper-arid conditions over five consecutive seasons.

Factor	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)	Mn (ppm)	Cu (ppm)
Rootstock (R)								
Own Roots	0.84 c	0.14 d	2.62 bc	1.44 d	0.55 a	48.9 bc	45.9 bc	8.00 ab
1103 Paulsen	0.95 bc	0.28 bc	2.45 c	1.64 c	0.47 b	43.6 cd	60.0 abc	7.75 ab

140 Ruggeri	0.95 bc	0.33 b	2.28 c	1.68 bc	0.47 b	44.5 cd	82.0 a	8.05 ab
99 Richter	0.93 bc	0.27 bc	2.31 c	1.46 d	0.46 b	49.0 bc	53.4 bc	6.60 b
Freedom	0.93 bc	0.23 c	2.34 c	1.54 cd	0.35 c	49.1 bc	40.2 bc	7.30 b
Harmony	1.09 ab	0.21 cd	3.54 a	1.92 a	0.49 b	35.3 d	38.0 c	6.30 b
Salt Creek	1.01 abc	0.32 b	2.45 c	1.57 cd	0.37 c	60.8 a	47.5 bc	9.60 a
SO4	0.91 bc	0.25 bc	2.24 c	1.63 c	0.32 c	56.5 ab	44.6 bc	6.50 b
St. George	1.18 a	0.43 a	3.01 b	1.82 ab	0.46 b	51.9 abc	61.8 ab	7.45 b
Season (S)								
2003-04	1.01 ab	0.21 b	2.43 b	1.63 b	0.45 ab	46.1	66.5 a	7.19 bc
2004-05	0.87 c	0.22 b	2.51 ab	1.58 b	0.43 bc	47.4	48.2 b	6.39 c
2005-06	1.04 a	0.34 a	2.49 b	1.63 b	0.43 bc	51.7	53.6 ab	7.92 b
2006-07	1.08 a	0.28 a	2.79 a	1.73 a	0.47 a	49.8	52.7 ab	9.81 a
2007-08	0.89 bc	0.32 a	2.68 ab	1.60 b	0.41 c	49.1	41.8 b	6.22 c
Signif[a]								
R	<0.0001[b]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S	<0.0001	<0.0001	0.005	0.000	<0.0001	0.26	0.000	<0.0001
R x S	0.35	0.25	0.97	0.17	<0.0001	0.76	0.95	0.15

[a]Significance (p-value) of Rootstock (R), Season (S) and R × S interactions. For a given factor and significance $p < 0.05$, different letters within a column represent significant differences (Tukey's test, $p < 0.05$). [b]In red, *p*-value lower than 0.05.

3.5. Principal Component Analysis (PCA)

In order to classify the different rootstocks assessing their influence on productivity, viticultural parameters and petiole nutrient content, a Principal Component Analysis (PCA) was performed (Figure 1). Principal component 1 (PC 1) explained 43.13 % of the variance and principal component 2 (PC2) explained 25.44 %, representing 68.57 % of all the variance. PC 1 was strongly correlated (-) with N, Ca, and (+) to number of bunches per vine and yield, while PC 2 was strongly (+) correlated with Zn. Ungrafted vines were inversely correlated with P petiole content and pruning weight, and these variables were inversely correlated with Ravaz index. According to PC 1, St. George and Harmony rootstocks were positively correlated with N, Ca and K petiole content, and these variables were negatively correlated with the number of bunches per vine, yield, Ravaz index and the percentage of fruitfulness. According to PC 2, Salt Creek rootstock was inversely correlated with Mg petiole content and scion trunk circumference. Pearson correlations confirmed some relationships (Supplementary Table 2), such as N petiole content that was positively related to K (r=0.70) and Ca, (r=0.82) and negatively related to number of bunches per vine (r=-0.85), yield (r=-0.85), Ravaz index (r=-0.80) and percentage of fruitfulness (r=-0.70). P petiole content was directly related to pruning weight (r=0.76) and inversely to Ravaz index (r=-0.84). K petiole content was positively related to Ca (r=0.76) and inversely related to yield (r=-0.67) and percentage of fruitfulness (r=-0.84). Ca was inversely correlated with number of bunches per vines (r=-0.75), yield (r=-0.76) and percentage of fruitfulness (r=-0.91). Mg petiole content was positively correlated with scion trunk circumference (r=0.67).

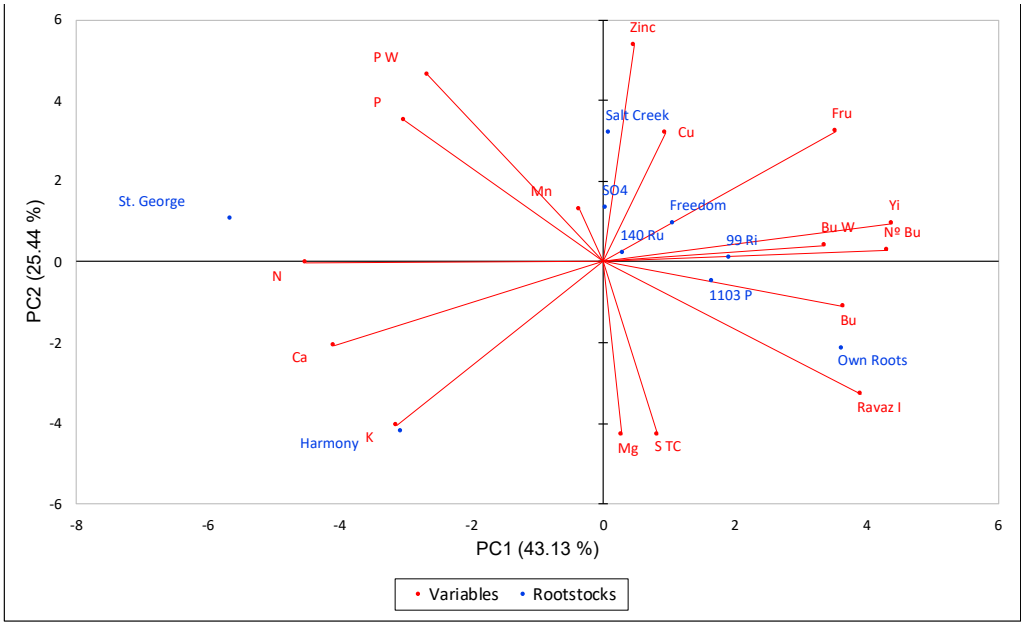


Figure 1. Principal component analysis (PCA) performed with yield, vigor and nutritional variables obtained from Syrah grapevines grown ungrafted and grafted onto 1103 Paulsen, 140 Ruggeri, 99 Richter, Freedom, Harmony, Salt Creek, SO4 (Selection Oppenheim 4) and St. George (Rupestris du Lot). Footnote: The distribution of variables (red lines) and individual observations according to rootstocks (blue dots) on PC1 and PC2 are shown. Yi: Yield, NºBu: Number of bunches per plant, Bu W: Bunch weight, Bu: Budburst, Fru: Fruitfulness, P W: Pruning weight, Ravaz I: Ravaz Index, S TC: Scion trunk circumference.

In order to classify the different seasons and to assess their influence in viticultural productivity parameters and petiole nutrient content, a PCA was also performed (Figure 2). PC 1 explained 43.42 % of the variance and principal component 2 (PC2) explained 26.78 %, representing 70.20 % of all the variance. PC 1 was strongly correlated (-) with K and (+) to yield, number of bunches per vine and Ravaz index, while PC 2 was strongly (+) correlated with P and Zn, and (-) to percentage of budburst and fruitfulness. The 2007-08 season was positively correlated with N, whereas P petiole content was correlated positively with Zn petiole content. The 2006-07 season was directly correlated especially to K and according to PC1, it was inversely correlated to yield, Ravaz index and 2003-04 season. The 2005-06 season was inversely correlated with percentage of budburst and fruitfulness, pruning weight and at a lesser extent to Mg. The 2004-05 season was directly correlated to Mn and was inversely correlated to scion trunk circumference. Indeed, Pearson correlations confirmed some relationships (Supplementary Table 3). N petiole content was positively related to Cu (r=0.88). P petiole content was directly related to Zn (r=0.93). K petiole content was inversely related to yield (r=-0.96) and Ravaz index (r=-0.96). Ca petiole content was directly related to Cu (r=0.97). Mn petiole content was inversely related to scion trunk circumference (r=-0.90).

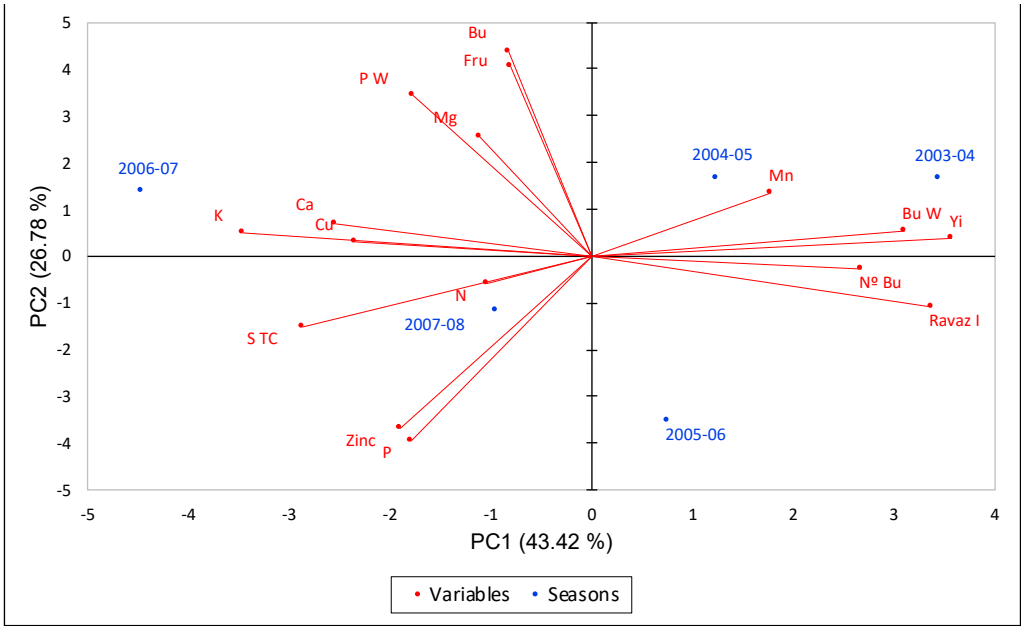


Figure 2. Principal component analysis (PCA) performed with yield, vigor and nutritional variables obtained from Syrah grapevines according to the study seasons. Footnote: The distribution of variables (red lines) and individual observations according to seasons (blue dots) on PC1 and PC2 are shown. Yi: Yield, NºBu: Number of bunches per plant, Bu W: Bunch weight, Bu: Budburst, Fru: Fruitfulness, P W: Pruning weight, Ravaz I: Ravaz Index, S TC: Scion trunk circumference.

4. Discussion

This five-year study evaluated scion performance and petiole nutrient content effects of ungrafted Syrah young grapevines in comparison to eight rootstocks in a hyper-arid irrigated vineyard. St. George induced lower yield than 1103 Paulsen rootstock in Syrah and there were no statistical differences among the rest of the rootstocks and ungrafted vines. Previous studies had shown that rootstocks significantly affected scion yield [30–34]. Bascuñán-Godoy et al. [32] reported that yield was related to number and weight of bunches and pruning weight in vines grafted onto different rootstocks growing under hyper-arid conditions. These results partially coincided to those presented here, since yield was positively related to number (r=0.96) and weight (r=0.73) of bunch (Supplementary Table 2 and 3), but these components were not affected by rootstock factor and were not related to pruning weight (Table 3). Recently, Clingeleffer et al. [35] showed that scion × rootstock interactions affected yield, yield components, pruning weight and Ravaz index in vines grafted onto different rootstocks. These findings indicate that specific rootstocks will be required to optimize scion variety performance under our studied conditions. Agreeing to our results, some reports have evidenced that rootstocks did not affect scion yield components in different grapevine varieties growing under cold and tropical climate conditions [36,37]. In this way, season was the most important factor influencing yield components in Syrah grapevines growing under hyper-arid climatic conditions (Table 3). As expected, annual precipitation was correlated to vine yield (r=0.71), whereas Mean (SON Mean) and Maximum (SON Max) Spring Temperature Summation was correlated to budburst percentage (r=0.75 and r=0.82, respectively) (data not shown). These last bioclimatic indices have been elaborated based on heat summation method during bud break and flowering [26]. Mean and maximum springtime temperatures modulate timing of bud break as well as growth rate throughout the season [26,38,39]. Number of bunches per vines was correlated to different bioclimatic indices such as Growing Season Temperature (GST) (r=0.74), Heliothermal Index (HI) (r=0.69) and Growing Degree Days (GDD) (r=0.75) (data not shown). Recently, it was reported that bud fruitfulness was mostly influenced by bud light interception, while inflorescence primordia size was positively correlated to shoot growth capacity and carbohydrate level of buds [40].

Rootstocks have the ability to modulate yield by modifying several plant traits [41,42]. In this fashion, water and nutrient uptake have been identified as two key processes that differ between own-rooted and grafted plants [8,20,41]. Indeed, Salt Creek induced higher plant growth vigor than ungrafted vines in Syrah measured as pruning weight (Table 4), which was also correlated to P petiole content (r=0.79) (Supplementary Table 2). A previous study showed that Salt Creek induced an increment in N and P concentrations in aerial portion of Red Globe vines, which could be positively related to plant growth vigor [20]. Phosphorus is an essential element for plant growth and is involved in many fundamental processes, including photosynthesis, biosynthesis and respiration, because of its role in energy generation via adenosine triphosphate [8,43]. Low P availability in vines may limit cell division, restricting leaf initiation of shoot apical meristem, expansion of newly developed leaves and lateral shoot growth [44,45]. P deficiency may decrease root hydraulic conductance so that reduction in water supply to growing organs restricts cell expansion, which in turn strongly limits leaf expansion [45,46]. Despite the above, ungrafted vines presented highest Ravaz index, defined as the ratio yield: pruning wood weight, and that value indicate that these vines were vegetatively balanced. In this way, Syrah grapevines grafted onto Salt Creek and St. George rootstocks held an excess of vigor according to their Ravaz index (Table 4), confirming previous rationale. K petiole content was inversely correlated to yield and Ravaz index according to Fig. 2. Based on our results, the vines that reached high K levels in their leaves tissues presented lower yield (Table 4 and 5). In addition, in most of the samples, K content in petioles was above the optimal nutritional content (> 1.8 %) [45] (Table 5). K is the most abundant cation in grape berry [47]. Plant growth and development require large K fluxes to provide this ion to growing tissues and deficiencies in K may reduce xylem sap flow, limiting shoot and fruit growth and by consequence affecting yield [45,47]. Nevertheless, according to our results, K management should be considered a priority in the nutritional management program by vine growers of the Northern Chile to avoid problems of excesses of this nutrient. Scion trunk circumference (STC) was higher in ungrafted vines than in vines grafted onto Freedom rootstock and STC increased as seasons passed (Table 4). The vines under study were young so STC was strongly influenced by the season since vines were actively growing season to season. Most of published studies have shown that STC allows to characterize vine vigor, which was strongly related to pruning weight [48,49]. Nevertheless, in our study vines were young and STC was not related to pruning weight (r=0.14) or yield (r=0.46) and possibly other variables may affect their changes such as Mg petiole content (r=0.67). In this regard, graft union may play a key role in water transport [41] and thus in vigor, especially in the first years of vine development. A successful graft union has to differentiate functional phloem and xylem connections across graft surface with the aim to uptake and transport of water, nutrients and photo-assimilates [41,45]. Grafting can have a negative impact on hydraulic conductivity and therefore on development and lifespan of the scion [41,50]. Therefore, it is possible that during first years of development, ungrafted vines do not possess these limitations and can grow quickly compared to grafted vines.

Normally, the region under study presents a significant amount of alkaline soils that have a high pH and presence of $CaCO_3$, which affects essential nutrient availability for crop development [51]. These soils can display an accumulation of soluble salts and a high content of exchangeable Na resulting in micronutrient deficiencies such as Fe, Zn, Mn and Cu in crops [52–54]. However, according to nutritional standards for viticultural management [55], Syrah ungrafted and grafted grapevines under study exhibited adequate levels in all nutrients analyzed in petioles with the exception of ungrafted vines for P (deficient), St. George for N (excess) and K (excess), and Harmony for K (excess). St. George and Harmony rootstocks induced higher petiole N concentration than ungrafted vines in Syrah (Table 5). Similar results were found in Thompson Seedless and Red Globe varieties cultivated under hyper arid conditions [20]. St. George is a very vigorous rootstock and presents a long vegetative cycle and delays scion maturity [56–58]. Due to this, St. George is

not suitable for varieties with irregular set, such as Carménère, Pinot Noir, Chardonnay or Merlot [56]. Concerning N, this essential macronutrient was inversely correlated to bunches number per vines (r=-0.85), yield (r=-0.85), Ravaz index (r=-0.81) and fruitfulness percentage (r=-0.70). Guilpart et al. [59] reported that during critical period of vine growing (400- and 700-degree days) water and N stress drove bud fertility and berry number and determined 65–70 % of vine yield in next season.

Syrah ungrafted grapevines exhibited lower P and Ca petiole content than most of grafted vines and highest Mg petiole content, whereas St. George induced high P petiole content, while Harmony induced high Ca petiole content in Syrah grapevines (Table 5). Vines grafted onto St. George in high P soil availability induced less shoot dry weight than grafted onto Freedom, which was greatly inhibited by low P availability in soils [60]. St. George seemed to use P inefficiently for vine growth under these soil conditions. Recently, it has been reported that genetic background of vine rootstock may affect petiolar nutrient concentration in scions [8,61]. Rootstocks that presents *V*. *riparia* genetic background confer low P and Mg petiolar concentrations [8]. These findings are similar to our results since SO4 rootstocks leant to induce low P and Mg petiolar content compared to rest of rootstocks tested in Syrah grapevines (Table 5). Vines growing under low P soil availability showed symptoms of Mg deficiency that were relieved by application of P fertilizer [62]. This lack of P-uptake capacity could also affect uptake of Mg by rootstocks that presents *V*. *riparia* genetic background and its subsequent translocation to shoots [8]. St. George and Harmony rootstocks induced higher K petiole content than rest of rootstocks in Syrah grapevines (Table 5). Rootstock differed significantly in petiole K content at flowering and rootstocks that presents *V*. *berlandieri* genetic background contained lower levels of petiole K [63], which was in agreement in most of cases to our results (Table 5). High levels of K uptake may increase pH, leading to wine instability and altering taste, acidity and color in wines [47]. Based upon these findings, it is important to select rootstocks that confer low potassium absorption, even more in vines growing under hyper-arid climatic conditions probably by using *Vitis berlandieri* rootstocks. Season factor also affected most of the nutrient petiole content in Syrah grapevines (Table 5). The accumulation of K, Mg and especially Ca is higher in berries that have higher transpiration rates, even at veraison [45,64]. In addition, Ca content increased from anthesis to ripeness in the whole berry [65]. Confirming these findings, our results showed that in 2006-07 season Syrah grapevines presented higher content of several nutrients in petiole, including Ca, and highest SON Max thus nutrient petiole content could be determined by temperature accumulation between budburst and flowering.

Salt Creek rootstocks induced high Zn and Cu, and low Mn petiole content than most of grafted vines in Syrah, although 140 Ruggeri induced higher Mn petiole content than most of rootstocks, in Syrah (Table 5). High Cu concentration in vineyard soils may provoke increase in Cu toxicity symptoms in young vines [66]. Cu concentrations may affect Mn, Zn, and Fe concentrations in vine plant tissues but effects provoked by Cu toxicity on micronutrients concentrations (Mn and Zn) depend on rootstock genotype [67]. Season also affected the content of most of micronutrients in petioles of Syrah grapevines (Table 5). Commonly, concentrations of ions increased across berry development and ripening, but Zn and Mn is accumulated before veraison [45,68]. Salt Creek could be a rootstock that should not be used in Syrah due to its high Cu uptake, which could limit uptake of other micronutrients of importance to vines.

5. Conclusions

~~Independently,~~ rootstocks and seasons affected ~~yield components,~~ vigor and petiole nutrient content in Syrah grapevines grown under hyper-arid conditions. St. George rootstock resulted in lower yield than 1103 Paulsen in Syrah grapevines. ~~Number of bunches, bunch weight, budburst and fruitfulness were not affected by rootstocks.~~ Salt Creek induced higher plant growth vigor than ungrafted vines, which was correlated to P petiole content. However, Salt Creek and St. George rootstocks induced unbalanced vines with

an excess of vigor according to their Ravaz index values. St. George and Harmony rootstocks induced a high petiole N concentration than ungrafted vines in Syrah, whereas Salt Creek rootstocks induced high Zn and Cu petiole content than most of grafted vines in Syrah. In summary, *Vitis berlandieri* rootstocks induced intermediate and low levels of K concentration in petioles of Syrah grapevines. The use of St. George, Salt Creek and Harmony rootstocks in Syrah grapevines growing under hyper-arid conditions should be limited with the aim to avoid irregular set and umbalances of some nutrients. According to our results, rootstocks that presents *Vitis berlandieri* genetic background should be used in Syrah grapevines grown under hyper-arid conditions. These results are relevant for wine industry and may contribute to viticultural management of vines cultivated under hyper-arid climatic conditions. In this way, future research should be carried out considering the effect of rootstocks on oenological variables and thus have more information about its effects on the Syrah variety cultivated under hyper-arid conditions.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Interaction effect of Rootstock and Season for Mg (%) petiole concentration. Table S2: Pearson's correlation obtained from Principal Component Analysis of Rootstock. Table S3: Pearson's correlation obtained from Principal Component Analysis of Season.

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Data Availability Statement: The data presented in this study are available on request from the corresponding and the first author.

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