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Short communication

Exploring variability and the putative influence of rootstocks in fruit from 'Muscat of Alexandria' clones selected at La Marina Alta (Spain), growing under rain-fed conditions

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Summary

Background of the study – **An increment in drought and negative changes of grapevine quality are being associated to climate change. Selecting clones and rootstock-scion combinations can help to cope with these matters.** *Objectives* – **To explore the variability for bunch and berry characteristics, as well as the putative influence of rootstock in these traits in three 'Muscat of Alexandria' clones selected at La Marina Alta (Alicante, Spain).** *Methods* – **Intravarietal variability was studied by ampelographic and eno-carpological descriptors and chemical analysis in plants of 'Muscat of Alexandria' CM11, CM14, and CM16 clones grafted onto 110R, 1103P, and 41B rootstocks and cultured under rain-fed conditions.** *Results* – **Variability among clones and influence of rootstock in parameters affecting wine quality are found, all of them showing good acidity in rain-fed growing conditions. Differences for ampelographic and eno-carpological descriptors in bunches and berries are also noted, highlighting the elongated berries and late ripening of the clone CM14.** *Conclusions* – **The intra-variability found for visual and/or quality traits as well as the acquired knowledge of the rootstock influence in these parameters is of interest for this globally distributed grape variety, used for winemaking and as a table grape; especially in the current scenario of climate change.**

Keywords
grapes, *Vitis vinifera*, ampelography, quality

Significance of this study

What is already known on this subject?

- In vegetatively propagated crops, studying variability is especially useful for selecting resilient materials and diversify. It is also useful to assess the rootstock's influence on fruit quality, as it can affect it.

What are the new findings?

- Variability in traits like berry shape, acidity, soluble solids content, and polyphenols has been observed in 'Muscat of Alexandria' clones (CM11, CM14, CM16) grafted onto 1103P, 110R, and 41B rootstocks and grown under rain-fed conditions.

What is the expected impact on horticulture?

- 'Muscat of Alexandria' is economically important worldwide as a table grape, raisin, and wine grape. These results aid in selecting the best rootstock-scion combination based on the intended use. The study was conducted under growing conditions relevant to climate change.

Introduction

'Muscat of Alexandria' is one of the most representative varieties among the grapevine 'Muscat' family, characterized by a pronounced floral aroma and a typical 'Muscat' flavour which is greatly appreciated (Mateo and Jiménez, 2000). This variety resulted from a natural crossing of 'Muscat à Petit Grains blanc' and 'Axina de Tres Bias' (also named 'Heptakilo') (Cipriani et al., 2010), and it was involved in the pedigree of a large number of varieties (Peiró et al., 2018). Grapes from 'Muscat of Alexandria' are consumed fresh, dried or used for elaborating wines, as the traditional Italian Moscato, the fortified Spanish *Moscatel* or the French *vins doux naturels*. Interest of wine production with this variety has globally increased in past years. In Spain, it is key at several Protected Designation of Origin (PDO) like 'Vinos de Alicante' (subzone Marina Alta), 'Valencia' (subzone Moscatel de Valencia), 'Málaga', 'Jeréz', or 'Condado de Huelva', and it is authorized in most Spanish wine producing areas. As in many crop areas, grapevine is cultivated as a grafted plant, frequently onto these three rootstocks 110R, 140Ru, and 1103P (Peiró et al., 2020).

Nowadays, there is great concern about the consequences of climate change and anthropogenic global warming on grapevine cultivation areas. The resulting erratic precipitation patterns and increased temperatures are expected make drought events more frequent. In terms of quality, the decoupling between technological maturity parameters and phenolic maturity (possibly leading to an excessive alcoholic content or the production of non-equilibrated wines) will be aggravated under a global warming scenario (Marín et al., 2021; Mira de Orduña, 2010; Mosedale et al., 2016). For instance, the longer and the warmer the ripening period, the lower will be the acidity at the end of the season. The choice of more adequate rootstock-scion combination has been proposed as a measure of adaptation (Romero et al., 2018; Van Leeuwen et al., 2019; Peiró et al., 2020). The recovery

of minor varieties as well as the exploration of intravarietal variability has been also suggested as a strategy to face this issue (Gisbert et al., 2022). Climate changes will not affect all cultivation areas to the same extent, but it may lead to the establishment of vine cultivation in novel areas and/or a substitution of varieties by ones which are better adapted to new conditions. In this context, the characterization of the existent variability as well as the study of rootstock-scion combinations (mainly in drought conditions), is necessary. For a given cultivar in a specific region each rootstock should be evaluated due to the complex interactions among rootstocks, soil, and climatic conditions (Wang et al., 2019).

In this work, the variability of 'Muscat of Alexandria' clones and the putative influence of rootstocks on bunch and berry characteristics has been performed in a three-year evaluation of nine rootstock-scion combinations, managed under rain-fed conditions.

Material and methods

Plant materials

Plants of 'Muscat of Alexandria' clones 11 (CM11), 14 (CM14) and 16 (CM16) analysed in our study were grafted onto 110R, 1103P and 41B rootstocks in a globed system at a spacing of 2.5 × 1.8 m in a field of Teulada (X 246801,33; Y 4287901,81) in 2009. Vine rows have an East–West orientation. Short pruning and flaking (pruning in green) were done in spring. No banding or blunting system or hormonal treatment has been used to facilitate curdling. The vineyards were managed, without irrigation, using standard practices according to the production standards of the PDO 'Vinos de Alicante' appellation. Fruits were collected on 5th September in 2017, 2018 and 2019. A completely randomized design was used as the statistical model, arranged in a factorial system (3 clones × 3 rootstocks) with three replications per year.

Clones analysed in the present work had been, previously selected in the period 1980–1990 at 'La Marina Alta'; CM11 and CM14 by members of the Local Agriculture Ministry, and CM16, by J.X. Soler, who obtained vegetative progenies of these three clones. According to the climatic classification for grape-growing regions proposed by Tonietto and Carbonneau (2004), the experimental conditions were warm, with temperate nights, and moderately dry (averaged values for the period of this study: annual rain fall 521 ± 38.12 mm; Temperature 17.5 ± 0.02°C; T max 36.5°C ± 0.37; Humidity 67 ± 0.12%).

Ampelographic descriptors

Fourteen OIV ampelographic descriptors related to bunch and grape characteristics (202, 203, 204, 206, 208, 209, 220, 221, 223, 238, 242, 243, 502 and 503) were measured during three consecutive years in plants grafted onto the 41B rootstock. Measures were taken each year in three representative bunches of each clone (Table 1). Ten seeds were used for taking their weight, five berries were used for analysing berry traits, and at last three measures per bunch were made for noting the rest of the descriptors. The following ratios were calculated: length/width for bunches and berries and seed weight/ berry weight.

Eno-carpological attributes and chemicals

Yield in grapes (%) and in scrape (%) were determined. Eno-carpological descriptors 505, 506, and 508 corresponding to sugar, total acidity (TA), and pH of must were measured following the OIV (2018) indications: 1) pH values were obtained by potentiometry using an MP 220 digital pH-meter (Mettler Toledo, Barcelona, Spain); 2) Soluble Solids Content (SSC, expressed in °Brix) in the must were measured with a N-8 R hand-held refractometer (Atago Co. Ltd., Tokyo, Japan); 3) The total acidity of must was determined by titration with sodium hydroxide up to the pH value = 8.2, and expressed as g of tartaric acid L^{-1}. Reducing sugars (glucose + fructose) were determined by the enzymatic method, and expressed in g L^{-1}. The intensity of colour was calculated as the

Table 1. Mean value and standard error of fourteen OIV descriptors related to bunch and berry traits of three 'Muscat of Alexandria' clones (CM11, CM14, and CM16) from la Marina Alta (Alicante, Spain) evaluated in three consecutive years.

Trait	Clone	CM11	CM14	CM16
202: Bunch: length (peduncle excluded)-	NS	16.79 ± 1.78 [a]	17.11 ± 0.49 [a]	20.32 ± 0.84 [a]
203: Bunch: width	NS	13.41 ± 0.46 [a]	11.97±0.98 [a]	12.48 ± 0.21 [a]
Bunch: ratio length/width	*	1.25 ± 0.12 [a]	1.44±0.08 [ab]	1.63 ± 0.09 [b]
204: Bunch: density	NS	3.67 ± 0.67 [a]	3.00 ±0.00 [a]	3.00 ± 0.00 [a]
206: Bunch: length of peduncle of primary bunch	NS	3.94 ±0.54 [a]	3.61 ± 0.40 [a]	3.88 ± 0.46 [a]
208: Bunch: shape 208	NS	1.83 ± 0.17 [a]	2.00 ± 0.00 [a]	1.33 ± 0.33 [a]
209: Bunch: number of wings of the primary bunch	NS	2.33 ± 0.33 [a]	1.67 ± 0.33 [a]	2.33 ± 0.67 [a]
220: Berry: length	*	24.55 ± 0.21 [a]	26.59 ± 0.10 [b]	24.87 ± 0.3 [a]
221: Berry: width	*	20.76 ± 0.19 [b]	18.80 ± 0.10 [a]	21.52 ± 0.19 [c]
Berry: ratio length/width	*	1.18 ± 0.02 [a]	1.41 ± 0.01 [b]	1.16 ± 0.01 [a]
223: Berry: shape	*	3.00 ± 0.00 [a]	4.00 ± 0.00 [b]	3.00 ± 0.00 [a]
238: Berry: length of pedicel	NS	6.07 ± 0.41 [a]	6.02 ± 0.20 [a]	6.42 ± 0.33 [a]
242: Berry: length of seeds	NS	6.53 ± 0.13 [a]	6.82 ± 0.13 [a]	6.79 ± 0.06 [a]
243: Berry: weight of seeds	NS	0.45 ± 0.02 [a]	0.41 ± 0.01 [a]	0.45 ± 0.02 [a]
502: Bunch: single bunch weight	*	408.1 ± 38.5 [ab]	320.8 ± 19.5 [a]	419.5 ± 17.5 [b]
503: Berry: single berry weight	*	6.22 ± 0.21 [a]	5.65 ± 0.15 [a]	7.05 ± 0 .21 [b]
Ratio seed weight/berry weight	NS	0.07 ± 0.01 [a]	0.07 ± 0.02 [a]	0.06 ± 0.01 [a]
Ratio seed weight/berry weight (%)	NS	7.22 ± 0.61 [a]	7.34 ± 0.34 [a]	6.40 ± 0.39 [a]

NS and * indicate clone effect with p value > 0.01 and < 0.01, respectively. Different letters in the same column shows significant differences (P-value < 0.01) between values.

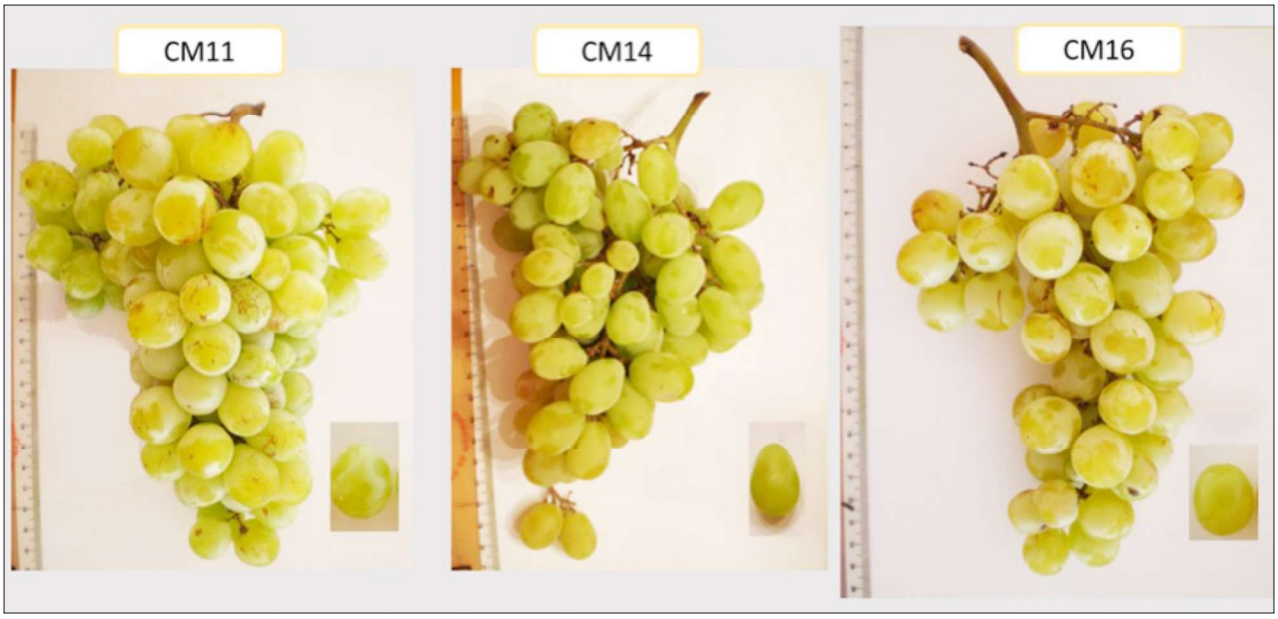


Figure 1. Bunches of the 'Muscat of Alexandria' clones CM11, CM14, and CM16 from La Marina Alta (Alicante, Spain). Detail of a single berry of each clone.

sum of absorbances at 420, 520 and 620 nm, using a Jenway 6305 UV-VIS spectrophotometer (Jenway, Essex, U.K.). The Total Phenolic Index (TPI) was determined by measuring the absorbance at 280 nm wavelength (Ribereau-Gayon et al., 2006) using a Jenway 6305 UV-VIS spectrophotometer (Jenway, Essex, U.K.). The index of phenolic maturity was set by the Glories method (Saint Cricq et al., 1998), which is based on the determination of seed tannins (%), extractability of anthocyanins (mg L^{-1}), and total anthocyanins (mg L^{-1}). Total phosphorus (mg P_2O_5 L^{-1}) was analyzed according to the molybdovanadate method (OIV-MA-AS321-04) using a Jenway 6305 UV-VIS spectrophotometer (Jenway, Essex, U.K.). Potassium (mg L^{-1}) was analyzed by flame photometry (OIV-MA-AS322-02B method) using a Jenway PFP7 flame photometer, and calcium (mg L^{-1}) was analyzed by atomic absorption spectrophotometry (OIV-MA-AS322-04 method) using a Thermo Elemental (SOLAAR AA Spectrometers, Cambridge, U.K.) spectrometer.

Statistical analysis

Data was analysed using the software Statgraphics® Centurion XVII. Data was tested using a two-way ANOVA to determine the effects of clone, rootstock, and year. Differences among groups were tested with the Least Significant Differences (LSD) post-hoc test. Results were considered statistically significant at $P < 0.01$.

Results and discussion

In this work, bunch and berry characteristics of three 'Muscat of Alexandria' clones (CM11, CM14, and CM16) se-

Table 2. Bunch weight, grape percentage, total acidity, pH, soluble solids content (SSC), reducing sugars and colour intensity in grapevines from three 'Muscat of Alexandria' clones (CM11, CM14, and CM16) grafted onto the rootstocks (110R, 1103P and 41B) during three consecutive years (2017, 2018, and 2019). Total acidity, pH and SSC correspond to the OIV descriptors 506, 508 and 505, respectively.

Muscat of Alexandria clone	Bunch weight (g)	Grape percentage (%)	Total acidity	pH	SSC (°Brix)	Reducing sugars	Colour intensity
	*	NS	*	NS	*	*	NS
CM11	361 ± 11 [b]	96.5 ± 0.2 [a]	5.55 ± 0.11 [a]	3.33 ± 0.01 [a]	20.4 ± 0.2 [b]	17.1 ± 4 [b]	0.38 ± 0.03 [a]
CM14	295 ± 12 [a]	96.5 ± 0.2 [a]	6.58 ± 0.11 [b]	3.36 ± 0.01 [a]	19.4 ± 0.2 [a]	15.2 ± 4 [a]	0.39 ± 0.03 [a]
CM16	379 ± 11 [b]	95.9 ± 0.2 [a]	5.57 ± 0.11 [a]	3.34 ± 0.01 [a]	20.4 ± 0.2 [b]	17.2 ± 4 [b]	0.40 ± 0.03 [a]
Rootstock	*	NS	*	*	NS	NS	NS
110R	364 ± 11 [b]	96.4 ± 0.2 [a]	5.57 ± 0.11 [a]	3.33 ± 0.01 [a]	20.1 ± 0.2 [a]	16.7 ± 4 [a]	0.40 ± 0.03 [a]
1103P	309 ± 12 [a]	96.2 ± 0.2 [a]	6.18 ± 0.11 [b]	3.41 ± 0.01 [b]	20.1 ± 0.2 [a]	16.7 ± 4 [a]	0.39 ± 0.03 [a]
41B	362 ± 11 [b]	96.2 ± 0.2 [a]	5.72 ± 0.11 [a]	3.32 ± 0.01 [a]	19.9 ± 0.2 [a]	16.1 ± 4 [a]	0.41 ± 0.03 [a]
Year	NS	NS	*	*	*	*	NS
2017	352 ± 12 [a]	96.6 ± 0.2 [a]	6.02 ± 0.12 [a]	3.30 ± 0.01 [a]	21.9 ± 0.2 [b]	18.1 ± 4 [b]	0.41 ± 0.03 [a]
2018	335 ± 12 [a]	96.4 ± 0.2 [a]	6.57 ± 0.12 [b]	3.43 ± 0.01 [c]	18.9 ± 0.2 [a]	15.6± 4 [a]	0.42 ± 0.03 [a]
2019	348 ± 10 [a]	95.8 ± 0.2 [a]	6.48 ± 0.09 [b]	3.35 ± 0.01 [b]	18.4 ± 0.2 [a]	15.7 ± 3 [a]	0.39 ± 0.03 [a]

NS and * indicate effect with p value > 0.01 and < 0.01, respectively. Different letters in the same column shows significant differences (P-value < 0.01) between values.

Table 3. Total phenolic index (TPI), Anthocyanins, Tannins, Phosphorus, Potassium and Calcium analysed in three 'Muscat of Alexandria' clones (CM11, CM14, and CM16) grafted onto the rootstock (110R, 1103P, and 41B). Data is the average of measures obtained in years 2017, 2018, and 2019.

Muscat of Alexandria clone	TPI	Anthocyanins	Tannins	Phosphorus	Potassium	Calcium
	NS	NS	NS	NS	NS	NS
CM11	16.2 ± 0.5 [a]	1.47 ± 0.17 [a]	11.2 ± 0.4 [a]	54.6 ± 2.7 [a]	917 ± 26 [a]	46.6 ± 3.6 [a]
CM14	17.7 ± 0.5 [a]	1.22 ± 0.17 [a]	12.1 ± 0.4 [a]	58.4 ± 2.7 [a]	927 ± 27 [a]	51.5 ± 3.6 [a]
CM16	16.5 ± 0.5 [a]	1.28 ± 0.17 [a]	11.2 ± 0.4 [a]	53.5 ± 2.7 [a]	881 ± 26 [a]	51.9 ± 3.5 [a]
Rootstock	NS	NS	NS	NS	NS	*
110R	16.4 ± 0.5 [a]	1.15 ± 0.17 [a]	11.1 ± 0.4 [a]	56.0 ± 2.7 [a]	880 ± 26 [a]	56.3 ± 3.6 [b]
1103P	16.9 ± 0.5 [a]	1.18 ± 0.17 [a]	11.1 ± 0.4 [a]	53.2 ± 2.7 [a]	942 ± 27 [a]	55.5 ± 3.7 [b]
41B	17.3 ± 0.5 [a]	1.94 ± 0.17 [b]	12.5 ± 0.4 [b]	57.3 ± 2.7 [a]	905 ± 26 [a]	38.3 ± 3.5 [a]
Year	*	*	*	*	*	*
2017	18.8 ± 0.6 [b]	1.93 ± 0.23 [b]	13.1 ± 0.5 [b]	71.6 ± 2.9 [b]	1,048 ± 28 [c]	60.4 ± 3.9 [b]
2018	16.2 ± 0.6 [a]	1.21 ± 0.23 [a]	11.0 ± 0.4 [a]	49.0 ± 2.9 [a]	879 ± 28 [b]	44.2 ± 3.8 [a]
2019	15.6 ± 0.4 [a]	0.91 ± 0.18 [a]	10.5 ± 0.4 [a]	46.0 ± 2.3 [a]	798 ± 22 [a]	45.4 ± 3.1 [a]

NS and * indicate effect with p value > 0.01 and < 0.01, respectively. Different letters in the same column shows significant differences (P-value < 0.01) between values.

lected and growing in La Marina Alta (Alicante, Spain) under rain-fed conditions, were compared over three years to study the intravarietal variability, which is especially interesting in vegetatively propagated crops, like grapevine.

In 'Muscat of Alexandria', differences for berry traits (berry length, berry width, single bunch weigh, berry weight, brix, and total acids) were reported in biotypes from France, Greece, Italy, and Spain (De Lorenzis et al., 2017). In Spanish clones of 'Muscat of Alexandria', genetic variability was also found by Peiró et al. (2018) and Esteras et al. (2019). In these last works, the clones CM11 and CM16, both from 'La Marina Alta', grouped in different clusters which is consistent with the differences found in our characterization. The clone CM16 had more elongated bunches (higher ratio for length/ width of bunch) as well as wider and heavier berries than the CM11 clone. However, the clone CM14, which was not previously evaluated, had more elongated and narrower berries than CM11 and CM16 (Table 1; Figure 1). The ratio length/width of berries in CM14 was also higher than those calculated for the biotypes compared in De Lorenzis et al. (2017). The clone CM14, also showed in comparison to CM11 and CM16, lighter bunches with berries that contain higher TA, and lower SSC contents (Tables 1 and 2). Differences in acidity are of interest for wine elaboration, but acidity is also involved in the sensory quality of table grapes (Muñoz-Robredo et al., 2011). Between CM11 and CM16 no differences were found for the eno-carpological attributes (total acid, pH, and sugar content of must), showing both good acidity at 20° Brix (Table 2). No differences were found among the three clones for the other evaluated traits as the percentage of grapes per bunch (i.e., equal quantity of scrape), pH, colour intensity, total phenolic index, anthocyanins, tannins, phosphorus, potassium, or calcium (Tables 2 and 3). The lower brix measured in the berries from the clone CM14 shows a tendency of this clone for retarding maturation that is of interest in the context of climate change that has anticipated ripening and decoupled phenolic and alcoholic maturation (Marín et al., 2021; Mira de Orduña, 2010; Mosedale et al., 2016). However, an in deep characterization will be performed to test tartaric and malic content as it matures.

The putative effect of the rootstock on berry traits, was evaluated as it is widely accepted that rootstocks can affect berry composition, despite the inconsistence of the results that has been reported due to: influence of soil, environmental conditions, management practices or rootstock-scion interactions (Wang et al., 2019). In this work, the clones of 'Muscat of Alexandria' were grafted onto the rootstocks 110R and 1103P, mainly used in the European wine producing countries (Peiró et al., 2020), and onto the rootstock 41B, also used in the Mediterranean area for its adequacy for chalky soils (Hidalgo, 1999). Despite the rootstock 110R and 1103P being more proximate genetically (both resulted from *V. berlandieri* × *V. rupestris* crosses) than 41B (which include *V. vinifera* in its pedigree) lighter bunches were found, in plants grafted onto 1103P, in addition to berries with higher TA and pH (around 0.1). Similar effect on berries was also reported by Pou et al. (2022) for Tempranillo 1103P-grafted plants when comparing to plants grafted onto 110R, 41B or 161-49 rootstocks. Vigorous rootstocks, like 1103P, has also been linked with low-quality grape parameters such as a higher pH, higher potassium, or lower polyphenol content (Cortell et al., 2008; Romero et al., 2018). Although in our case the increase in pH is moderated, it could be due to a higher uptake of potassium, which partly neutralize organic acids (Poni et al., 2018). In addition, a higher content in anthocyanins and tannins as well as lower Ca^{2+} was found in grapes from plants grafted onto the rootstock 41B. Differences in anthocyanins between two grape vines were attributed by Nelson et al. (2016) to the variations in the canopy size, which can mediate the sunlight exposure. This could also be applied, in our situation where plants grafted onto the rootstock 41B had a reduced canopy in comparison to those grafted on 110R or 1103P rootstocks. Nedelkovski et al. (2017) also indicated that the rootstock 41B could increase total anthocyanins concentrations in the berries. Anthocyanins are important parameters of vine growth and wine quality, because in addition to affect colour, they have antioxidative capacity, influence in wine astringency, in protection against a variety of pathogens, etc. (Garrido and Borges, 2011; Schaefer et al., 2004). Although all the compared plants are in the same field (under rain-fed conditions) some differences in the water uptake among rootstock-scion combinations could be having influence in berry composition.

Among years, differences for some must components have been registered in 2017 compared to 2018 and 2019 (Tables 2 and 3). These differences could be associated to lower rain fall in 2017 and/or the age of the grafted plants more variable when younger.

Conclusions

Overall, the intravarietal variability found for visual attributes and berry composition and maturation among the three 'Muscat of Alexandria' clones selected in La Marina Alta, as well as the acquired knowledge about the influence of rootstocks, is of use for selecting the appropriate rootstock-clone combination depending on the purpose of its application. This variety, which is consumed as a table grape or dried (as raisin), and is used for making different kinds of wines, has global economic importance, and, therefore, the use of its variability, could have great economic impact.

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