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Foliar application of methyl jasmonate to Graciano and Tempranillo vines: effects on grape amino acid content during two consecutive vintages

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ABSTRACT

Aim: The study of elicitors in grapevines has focused on inducing resistance to diseases and improving phenolic and volatile composition of grapes and wines. Due to the importance of nitrogen compounds in grape quality, the aim of this work was to study the effect of methyl jasmonate (MeJ) applied to two grapevine varieties on grape amino acid content during two consecutive seasons.

Methods and results: Amino acids were analyzed by HPLC-DAD. The results showed that MeJ applications to Tempranillo grapevines decreased the concentration of certain amino acids during both seasons, but did not affect total amino acid content. Moreover, MeJ applications to Graciano grapevines increased the concentration of several amino acids during the second season.

Conclusions: The effects of MeJ on grape amino acid concentration were conditioned by variety and vintage.

Significance and impact of the study: The importance of this work is to provide more information with respect to the effects of elicitors on grape quality for good viticultural management.

KEYWORDS

elicitors, methyl jasmonate, grapevines, amino acids, vintage effect

INTRODUCTION

Elicitors are a specific class of molecules originating from different organisms, which are able to trigger plant defense responses contributing to plant resistance against pathogen attacks. Fungicide treatments represent more than half of pesticides applied in viticulture and their use is not without risks to human health (Delaunois *et al.*, 2014). Due to this, several studies have focused on finding more sustainable alternatives for grapevine production (Jacometti *et al.*, 2010). Currently, elicitors are used as an alternative strategy to chemical fungicides, with the aim to induce defense mechanisms against different grapevine pathogens (Romanazzi *et al.*, 2002; Romanazzi *et al.*, 2013; Delaunois *et al.*, 2014). Likewise, it has been shown that the application of elicitors to grapevines increases the concentration of phenolic compounds (Ruiz-García *et al.*, 2012; Portu *et al.*, 2016; Gil-Muñoz *et al.*, 2017). Chemical pesticides have a detrimental effect on grape nitrogen composition (Oliva *et al.*, 2011). However, to our knowledge, there are only two reports describing the effects of foliar elicitor treatments on grape amino acid content. Garde-Cerdán *et al.* (2016) observed that methyl jasmonate (MeJ) applications increased the concentration of certain amino acids, especially phenylalanine, in Tempranillo grapes. Gutiérrez-Gamboa *et al.* (2017) reported that MeJ applied to grapevines increased Met and Phe content, while other elicitors such as chitosan and yeast extract decreased the concentration of several amino acids in grapes. However, despite the effects exposed in the aforementioned reports, it is important to study how different factors, such as variety and season, can affect grape amino acid concentration through the application of MeJ to the grapevines.

Nitrogen composition of grapevines plays an important role in wine quality. This affects the development of alcoholic fermentation, the growth of the microbiological population of must and wine, the synthesis of volatile compounds, especially of higher alcohols and ethyl esters that contribute to the pleasant aroma of wine, and the production of undesirable carcinogenic compounds in wines, such as ethyl carbamate and biogenic amines (Bell and Henschke, 2005). Stuck and sluggish fermentations are major oenological problems resulting in increased vinification time and spoilage of wine (Bisson and Butzke, 2000), which in turn lead to logistical problems into the wine cellar and economic losses for the winery. Thus, nitrogen composition of must fundamentally depends on the grape variety and viticultural practices (Rodriguez-Lovelle and Gaudillère, 2002). However, the effect of elicitor application to grapevines on grape amino acid concentration is not yet fully understood.

Graciano is a red variety originally from the La Rioja and Navarra regions of Spain, which is usually used to improve blends containing Tempranillo, Garnacha and Mazuelo, giving them more aroma and color (Cirami *et al.*, 2000; Núñez *et al.*, 2004). However, to our knowledge, there are no studies characterizing the amino acid composition of this variety. For this, the aim of this research was to study the effect of foliar MeJ applications to Graciano and Tempranillo vines on grape amino acid content during two consecutive seasons.

MATERIALS AND METHODS

1. Study site

The field study was conducted in a commercial vineyard located in Alfaro, Rioja Baja (warmest and driest area of La Rioja, Spain), during the 2015 and 2016 growing seasons. The altitude of the plot was 335 meters above sea level. Tempranillo (*Vitis vinifera* L.) vines were grafted onto 1103 Paulsen rootstock, planted in 1999 and trained to a vertical shoot positioned (VSP) trellis system. Planting density was 2,976 plants/ha, with vine spacing between rows and within rows of 2.80 × 1.20 m, respectively. Graciano (*Vitis vinifera* L.) vines were grafted onto 1103 Paulsen rootstock, planted in 1997 and trained to a VSP trellis system. Planting density was 2,600 plants/ha, with vine spacing between rows and within rows of 2.60 m × 1.10 m, respectively.

2. Grapevine treatments and harvest

The field trials involved the application of MeJ, as well as a control treatment. MeJ solution was prepared according to Garde-Cerdán *et al.* (2016) at a concentration of 10 mM. Tween 80 was used as wetting agent (0.1 % v/v). Control plants were sprayed with a Tween 80 solution. Two hundred milliliters were applied per plant in control and MeJ treatments. The applications were carried out twice, at veraison and one week later. A completely randomized experimental design was set up considering three replicates (ten plants per replicate). Grapes were harvested, destemmed and crushed. The oenological parameters were determined in musts. Aliquots of each sample were frozen in order to determine their free amino acid content.

3. Oenological parameters and yeast assimilable nitrogen (YAN)

Musts were physicochemically characterized by determining probable alcohol, pH, total acidity, malic acid, and potassium according to the OIV (2003) and tartaric acid according to the Rebelein method (Lipka and Tanner, 1974). YAN was determined according to the method described by Aerny (1996). Since treatments were performed in triplicate, the results of these parameters are expressed as the average of three analyses (n = 3).

4. Analysis of amino acids by HPLC

The analysis of amino acids in must was performed by the method described by Garde-Cerdán *et al.* (2014). Free amino acids were analyzed by reverse-phase HPLC using an Agilent 1100 Series (Palo Alto, USA). Each sample was centrifuged at 4,000 rpm for 10 minutes at 20 °C, then 5 mL of the sample was mixed with 100 µL of norvaline, the internal standard to quantify all amino acids except proline, and 100 µL of sarcosine, the internal standard to quantify proline. This mixture was filtered through 0.45 µm OlimPeak filter (Teknokroma, Barcelona, Spain) and submitted to an automatic precolumn derivatization with *o*-phthaldialdehyde (OPA reagent, Agilent) and 9-fluorenylmethylchloroformate (FMOC reagent, Agilent). The injected amount from the derivatized sample was 10 µL. All separations were performed on a Hypersil ODS (250 × 4.0 mm, I.D. 5 µm) column (Agilent) at 40 °C.

Two eluents were used as mobile phases : eluent A : 75 mM sodium acetate, 0.018 % triethylamine (pH 6.9) + 0.3% tetrahydrofuran ; eluent B : water, methanol and acetonitrile (10:45:45, v/v/v). Identification of compounds was performed by comparison of their retention times with their pure reference standards. The pure reference compounds and internal standards were obtained from Sigma-Aldrich (Madrid, Spain). The treatments were carried out in triplicate, so the results for free amino acids correspond to the average of three analyses (n = 3).

5. Statistical analysis

The statistical analysis of oenological parameters, YAN and amino acid concentration was performed using variance analysis (one-way ANOVA), by Statgraphics Centurion XVI.I. The multivariate factor analysis was performed with all amino acids using Statgraphics Centurion XVI.I. Differences between samples were compared using the Duncan test at 95 % probability level. A principal component analysis (PCA) was performed with the percentage of all amino acids with respect to their total concentration (InfoStat, www.infostat.com.ar).

RESULTS AND DISCUSSION

1. Oenological parameters and YAN

The oenological parameters for Graciano and Tempranillo grapes, during the 2015 and 2016 vintages, are summarized in Table 1. Slight differences were found after MeJ application. In 2015, there were no significant differences between the treatments in any of the oenological parameters for both varieties. In 2016, MeJ application had a differentiated effect depending on the variety. In Graciano grapes, MeJ treatment increased total acidity and tartaric acid with respect to control samples. Meanwhile, in Tempranillo grapes, MeJ application decreased malic acid and potassium content with respect to control. These differences in relation to season have been evidenced by Ruiz-García *et al.* (2012). On the other hand, statistical differences were found in must oenological parameters comparing both seasons for each variety. In Graciano grapes, pH, malic acid and potassium in 2015 showed higher level than in 2016. However, in this last season, the concentration of tartaric acid was higher than in 2015. In Tempranillo grapes, the total acidity in 2015 was higher than in 2016, while in this last season, pH was higher than in 2015. However, the differences in must oenological parameters after MeJ application were slight.

Previous studies showed similar results in must oenological parameters after elicitor applications to grapevines. Ruiz-García *et al.* (2012) reported that in the first season, the grapes from MeJ treatment showed higher total acidity and tartaric acid content. In the following season, those differences were not appreciated. Romanazzi *et al.* (2013) and Garde-Cerdán *et al.* (2016) showed that none of the studied oenological

TABLE 1. Oenological parameters and yeast assimilable nitrogen (YAN) in Graciano and Tempranillo musts from untreated (Ctr) and treated grapevines with methyl jasmonate (MeJ) in 2015 and 2016 vintages.

	Graciano			
	2015		**2016**	
	Ctr	**MeJ**	**Ctr**	**MeJ**
Probable alcohol (% v/v)	13.85 ± 0.64 a,A	13.71 ± 0.52 a,A	14.29 ± 0.49 a,A	13.79 ± 0.63 a,A
pH	3.31 ± 0.04 a,B	3.37 ± 0.04 a,B	3.19 ± 0.02 a,A	3.15 ± 0.03 a,A
Total acidity (g/L)*	7.23 ± 0.23 a,A	7.06 ± 0.37 a,A	7.10 ± 0.05 a,A	7.72 ± 0.02 b,B
Tartaric acid (g/L)	6.98 ± 0.22 a,A	7.87 ± 0.57 a,A	9.94 ± 0.12 a,B	10.55 ± 0.25 b,B
Malic acid (g/L)	1.80 ± 0.16 a,B	1.79 ± 0.12 a,B	1.05 ± 0.15 a,A	0.92 ± 0.13 a,A
Potassium (mg/L)	1536.67 ± 39.63 a,B	1545.00 ± 37.32 a,B	1345.67 ± 57.19 a,A	1254.00 ± 92.15 a,A
YAN (mg N/L)	198.67 ± 18.15 a,B	204.00 ± 40.73 a,A	148.00 ± 7.00 a,A	141.00 ± 13.45 a,A
	Tempranillo			
	2015		**2016**	
	Ctr	**MeJ**	**Ctr**	**MeJ**
Probable alcohol (% v/v)	12.34 ± 1.22 a,A	13.19 ± 1.06 a,A	13.94 ± 0.25 a,A	14.01 ± 0.38 a,A
pH	3.46 ± 0.05 a,A	3.43 ± 0.06 a,A	3.83 ± 0.06 a,B	3.78 ± 0.03 a,B
Total acidity (g/L)*	4.63 ± 0.11 a,B	4.78 ± 0.18 a,B	3.90 ± 0.13 a,A	3.96 ± 0.06 a,A
Tartaric acid (g/L)	6.88 ± 0.18 a,A	6.86 ± 0.05 a,A	6.63 ± 0.07 a,A	6.72 ± 0.12 a,A
Malic acid (g/L)	1.33 ± 0.25 a,A	1.29 ± 0.17 a,A	1.76 ± 0.06 b,B	1.52 ± 0.05 a,A
Potassium (mg/L)	1401.00 ± 152.29 a,A	1398.67 ± 85.41 a,A	1664.93 ± 16.47 b,B	1537.70 ± 49.69 a,A
YAN (mg N/L)	175.00 ± 10.10 a,A	164.27 ± 22.63 a,A	187.00 ± 6.24 b,A	149.67 ± 4.73 a,A

All parameters are given with their standard deviation (n = 3). For each season and variety, different lowercase letters in the same row indicate significant differences between treatments ($p \leq 0.05$); for each variety and treatment, different capital letters in the same row indicate significant differences between vintages (2015 vs 2016) ($p \leq 0.05$). *As g/L of tartaric acid.

parameters in must was significantly affected by elicitors applied to grapevines. Portu *et al.* (2016) reported that MeJ applications to grapevines exhibited slight differences in must oenological parameters with respect to the rest of the study treatments. It is possible to confirm that MeJ applications to grapevines slightly affect must oenological parameters.

The concentration of YAN in must ranged from 141 to 204 mg N/L. Stuck or sluggish fermentations can occur when YAN amount is lower than 140 mg N/L (Bisson and Butzke, 2000). MeJ application slightly affected must YAN concentration. In 2016, YAN concentration in must decreased after MeJ application to Tempranillo grapevines. Comparing must YAN concentration between seasons, YAN concentration in Graciano was higher in 2015 than in 2016.

2. Grape amino acid content

Figure 1 shows the amino acids (%) from untreated (Ctr) grapes in a) Graciano and b) Tempranillo, during the 2015 and 2016 seasons. To our knowledge, this is the first report that characterizes grape amino acid profile in Graciano. The most abundant amino acids found in untreated Graciano grapes were Arg, Pro, Gln, Gaba and Ala, representing around 78 and 82 % of total amino acids in 2015 and 2016, respectively. The most abundant amino acids found in untreated Tempranillo grapes in 2015 were Arg, Pro, Gln, Glu and Gaba, accounting for around 85 % of total amino acid content. Meanwhile, in 2016, the most abundant amino acids were Arg, Pro, Gaba, Gln and Ala, representing around 74 % of total amino acid content. These amino acids are the most abundant in several grape varieties, as has been evidenced by certain authors (Hernández-Orte *et al.*, 2002 ; Gutiérrez-Gamboa *et al.*, 2017). The least abundant amino acids found in Graciano

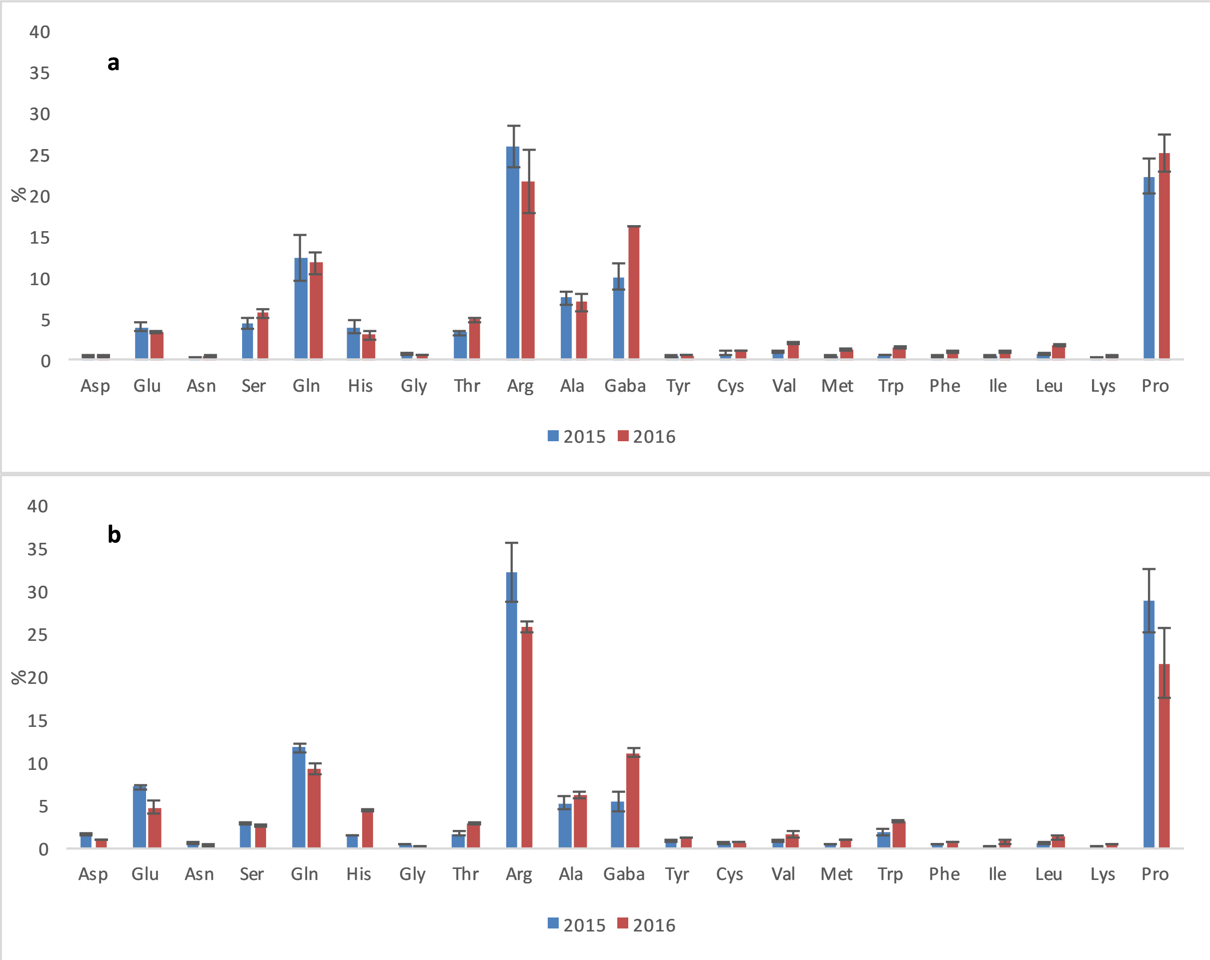

FIGURE 1. Amino acid content (%) in must from untreated (Ctr) grapevines : a) Graciano ; b) Tempranillo, during the 2015 and 2016 vintages. Aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), γ-aminobutyric acid (Gaba), tyrosine (Tyr), cysteine (Cys), valine (Val), methionine (Met), tryptophan (Trp), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys) and proline (Pro). All parameters are shown with the standard deviation (n = 3).

grapes in 2015 were Met, Phe, Ile, Asn and Lys, accounting for around 1.64 % of total amino acid content. Meanwhile, in 2016, the least abundant amino acids were Gly, Tyr, Asn, Lys and Asp, representing around 2.41 % of total amino acid content. With respect to Tempranillo, the least abundant amino acids in 2015 were Met, Cys, Gly, Ile and Lys, accounting for around 1.73 % of total amino acid content. Meanwhile, the least abundant amino acids in 2016 were Cys, Phe, Lys, Gly and Asn, accounting for around 2.12 % of total amino acid content. As reviewed by Bell and Henschke (2005), all amino acids were found in the range described for these compounds. In addition, among the amino acids, the best nitrogen sources for the yeasts are Glu, Gln, Asp, Asn, Thr, His, Ala, Tyr and Arg (Jiranek and Henschke, 1991 ; Bell and Henschke, 2005). In Graciano, the percentage of these amino acids ranged from 58 to 53 % in 2015 and 2016, respectively, while in Tempranillo, the percentage ranged from 62 to 55 %, respectively.

The effect of MeJ application to Graciano and Tempranillo grapevines on grape amino acid content, in 2015 and 2016, is shown in Table 2. MeJ applied to Tempranillo grapevines had lesser effect on the content of these compounds than in Graciano. In Graciano, the content of Pro (37 %) was increased by the MeJ applications with respect to control samples in 2015, while Ser (39 %), Gly (39 %), Thr (59 %), Gaba (27 %), Tyr (39 %), Cys (48 %), Met (38 %),

TABLE 2. Amino acid concentration (mg/L) in musts from treated (MeJ) and untreated (Ctr) Graciano and Tempranillo grapevines in 2015 and 2016 vintages.

	Graciano 2015		Graciano 2016		Tempranillo 2015		Tempranillo 2016	
	Ctr	MeJ	Ctr	MeJ	Ctr	MeJ	Ctr	MeJ
Asp	10.68 ± 1.38 a,B	11.92 ± 1.78 a,B	2.93 ± 0.59 a,A	4.65 ± 0.33 a,A	22.71 ± 0.69 b,B	19.43 ± 1.14 a,A	16.67 ± 0.91 a,A	20.76 ± 1.06 b,A
Glu	89.98 ± 13.26 a,B	92.93 ± 19.50 a,B	23.07 ± 0.57 a,A	32.78 ± 6.57 a,A	105.20 ± 4.02 b,A	93.38 ± 5.58 a,B	82.13 ± 14.57 a,A	74.72 ± 8.19 a,A
Asn	6.50 ± 1.24 a,B	7.07 ± 2.95 a,A	3.01 ± 0.53 a,A	3.24 ± 0.63 a,A	7.94 ± 1.59 a,B	6.81 ± 1.11 a,B	4.80 ± 0.49 a,A	4.45 ± 0.58 a,A
Ser	100.98 ± 15.65 a,B	108.60 ± 23.56 a,B	38.24 ± 3.12 a,A	53.21 ± 1.38 b,A	41.79 ± 1.92 a,A	40.02 ± 4.42 a,A	44.70 ± 2.05 a,A	45.69 ± 1.28 a,A
Gln	283.22 ± 63.24 a,B	326.62 ± 110.38 a,A	80.40 ± 8.91 a,A	96.10 ± 23.83 a,A	172.17 ± 7.71 a,A	155.63 15.57 a,B	158.69 ± 11.04 b,A	113.24 ± 8.20 a,A
His	89.97 ± 15.90 a,B	96.91 ± 34.55 a,A	20.50 ± 3.36 a,A	24.38 ± 1.19 a,A	22.17 ± 0.32 a,A	36.14 ± 4.93 b,A	75.56 ± 2.19 b,B	55.88 ± 5.71 a,B
Gly	17.75 ± 2.49 a,B	21.69 ± 5.57 a,B	4.09 ± 0.22 a,A	5.68 ± 0.19 b,A	5.54 ± 0.58 a,A	5.86 ± 0.71 a,A	4.89 ± 0.07 a,A	4.65 ± 0.72 a,A
Thr	73.97 ± 6.20 a,B	79.08 ± 18.72 a,A	32.98 ± 2.24 a,A	52.33 ± 6.04 b,A	25.07 ± 3.62 a,A	22.17 ± 1.01 a,A	48.25 ± 2.84 a,B	47.69 ± 1.55 a,B
Arg	586.98 ± 58.71 a,B	650.73 ± 135.21 a,B	147.51 ± 26.14 a,A	208.91 ± 44.76 a,A	408.04 ± 120.11 b,A	318.18 ± 54.30 a,A	443.91 ± 10.88 a,A	422.82 ± 22.51 a,B
Ala	170.71 ± 18.80 a,B	206.43 ± 42.05 a,B	47.48 ± 7.68 a,A	67.21 ± 14.28 a,A	77.39 ± 10.58 a,A	68.40 ± 6.73 a,A	106.41 ± 5.85 a,B	104.38 ± 6.44 a,B
Gaba	228.26 ± 36.81 a,B	235.72 ± 26.20 a,B	110.70 ± 0.31 a,A	141.04 ± 3.34 b,A	80.05 ± 15.40 a,A	64.41 ± 6.33 a,A	191.96 ± 7.57 a,B	183.50 ± 24.22 a,B
Tyr	9.03 ± 0.58 a,B	10.00 ± 3.94 a,B	3.41 ± 0.40 a,A	4.74 ± 0.35 b,A	11.87 ± 2.69 a,A	11.41 ± 1.33 a,A	20.49 ± 1.12 a,B	18.84 ± 0.81 a,B
Cys	17.42 ± 4.59 a,B	19.27 ± 6.61 a,A	7.51 ± 0.38 a,A	11.09 ± 1.44 b,A	7.05 ± 1.78 a,A	7.10 ± 1.40 a,A	10.96 ± 0.54 a,B	9.69 ± 0.80 a,B
Val	19.27 ± 2.91 a,B	24.30 ± 7.11 a,A	13.69 ± 1.56 a,A	19.38 ± 1.30 a,A	12.44 ± 0.76 a,A	12.23 ± 1.05 a,A	27.24 ± 5.16 a,B	21.55 ± 2.04 a,B
Met	8.95 ± 0.95 a,A	11.19 ± 4.39 a,A	7.59 ± 0.95 a,A	10.48 ± 0.60 b,A	7.15 ± 0.54 a,A	6.11 ± 0.85 a,A	15.83 ± 0.94 a,B	14.05 ± 0.93 a,B
Trp	11.92 ± 1.15 a,A	15.79 ± 6.19 a,A	10.35 ± 0.97 a,A	16.15 ± 0.45 b,A	27.54 ± 6.79 a,A	27.05 ± 1.31 a,A	53.38 ± 3.82 a,B	55.82 ± 3.84 a,B
Phe	8.77 ± 1.11 a,B	10.27 ± 3.43 a,A	6.01 ± 1.03 a,A	8.32 ± 0.37 a,A	7.55 ± 0.54 a,A	7.56 ± 0.99 a,A	9.94 ± 0.17 a,B	8.71 ± 1.26 a,A
Ile	7.98 ± 1.64 a,A	8.19 ± 3.76 a,A	7.28 ± 0.87 a,A	11.65 ± 1.48 b,A	3.22 ± 0.45 a,A	3.10 ± 0.04 a,A	12.02 ± 2.61 a,B	7.42 ± 1.57 a,B
Leu	17.58 ± 2.64 a,B	21.03 ± 7.73 a,A	11.90 ± 1.19 a,A	17.16 ± 1.79 b,A	8.42 ± 1.51 a,A	8.00 ± 0.47 a,A	21.36 ± 3.86 b,B	14.40 ± 1.87 a,B
Lys	5.08 ± 0.68 a,B	5.76 ± 2.68 a,B	2.99 ± 0.24 a,A	2.48 ± 0.40 a,A	2.69 ± 0.16 a,A	2.57 ± 0.20 a,A	6.00 ± 0.70 a,B	5.91 ± 0.11 a,B
Pro	505.20 ± 46.52 a,B	689.76 ± 46.76 b,B	170.49 ± 15.24 a,A	263.87 ± 17.63 b,A	426.48 ± 53.82 a,A	472.33 ± 87.92 a,B	372.26 ± 69.09 a,A	288.58 ± 48.32 a,A
Total aas	2270.22 ± 296.46 a,B	2653.24 ± 513.08 a,B	682.68 ± 19.23 a,A	983.06 ± 230.42 a,A	1479.59 ± 125.43 a,A	1398.78 ± 110.51 a,A	1729.03 ± 73.03 a,A	1527.96 ± 117.98 a,A
Total aas without Pro	1765.03 ± 249.94 a,B	1963.48 ± 466.32 a,B	512.19 ± 32.49 a,A	710.64 ± 216.92 a,A	1053.11 ± 165.38 a,A	926.45 ± 86.20 a,A	1356.78 ± 29.40 a,B	1239.38 ± 70.68 a,B

All parameters are given with their standard deviation (n = 3). For each season and variety, different lowercase letters in the same row indicate significant differences between treatments ($p \leq 0.05$); for each variety and treatment, different capital letters in the same row indicate significant differences between vintages (2015 vs 2016) ($p \leq 0.05$). Aas : amino acids.

TABLE 3. Percentage of variance attributable to treatment, season, variety, and their interaction [(treatment × season), (treatment × variety), (season × variety), and (treatment × season × variety)] for each amino acid.

	Treatment (%)	Season (%)	Variety (%)	Treatment x season (%)	Treatment x variety (%)	Season x variety (%)	Treatment x season x variety (%)	Residual (%)
Asp	0.44*	6.83***	83.20***	2.45***	0.31 NS	3.76***	1.68***	1.28
Glu	0.25 NS	47.24***	24.33***	0.13 NS	2.12 NS	15.23***	0.01 NS	10.7
Asn	0.00 NS	67.22***	7.40*	0.09 NS	2.54 NS	1.33 NS	0.50 NS	20.91
Ser	1.20 NS	23.79***	33.94***	0.45 NS	1.73 NS	30.55***	0.04 NS	8.31
Gln	0.12 NS	43.95***	7.68*	0.35 NS	3.77 NS	26.54***	0.00 NS	17.6
His	0.84 NS	7.70*	2.95 NS	2.24 NS	0.41 NS	71.87***	1.39 NS	12.61
Gly	1.05 NS	33.82***	32.98***	0.36 NS	1.07 NS	25.62***	0.11 NS	5
Thr	1.65 NS	1.36 NS	33.84***	1.03 NS	2.91*	50.63***	0.53 NS	8.07
Arg	0.38 NS	26.56***	0.09 NS	0.66 NS	4.70*	55.03***	0.27 NS	12.32
Ala	0.92 NS	17.01***	15.61***	0.10 NS	2.52*	56.12***	0.28 NS	7.45
Gaba	0.01 NS	0.69 NS	21.63***	0.18 NS	1.30 NS	69.91***	0.09 NS	6.19
Tyr	0.34 NS	3.43**	55.75***	0.32 NS	0.38 NS	34.75***	0.12 NS	4.92
Cys	1.24 NS	8.84*	28.06***	0.09 NS	3.82 NS	37.94***	0.58 NS	19.43
Val	1.42 NS	15.32**	1.64 NS	0.46 NS	10.42*	56.33***	1.80 NS	12.61
Met	0.52 NS	33.98***	4.35*	0.34 NS	8.03**	45.50***	0.25 NS	7.04
Trp	0.66 NS	12.87***	68.50***	0.35 NS	0.50 NS	15.08***	0.00 NS	2.05

Statistically significant at *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001, respectively. NS : not significant. Aas : amino acids.

Trp (56 %), Ile (60 %), Leu (44 %) and Pro (55 %) content was improved in 2016, without affecting the total amino acid content. In Tempranillo, Asp (14 %), Glu (11 %) and Arg (33 %) content was decreased by MeJ applications, while His (63 %) content increased with respect to control samples in 2015. MeJ application decreased Gln (29 %), His (26 %) and Leu (33 %) content, while it increased Asp (25 %) content with respect to control in 2016, without affecting the total amino acid content.

Currently, the study of elicitors carried out by different researchers has been focused mainly on inducing defense mechanisms against pathogens and improving grape and wine phenolic composition (Portu *et al.*, 2016 ; Gil-Muñoz *et al.*, 2017). However, Garde-Cerdán *et al.* (2016) reported that the concentration of certain amino acids was increased as a consequence of the use of MeJ. These results differ to those obtained in Tempranillo, but were similar to those observed in Graciano in the second season. There is probably a differentiated varietal response regarding the effect of MeJ on grape amino acid composition. MeJ has an important influence on phenylalanine ammonia-lyase activity (PAL). The exogenous application of this compound increases the concentration of phenolic compounds in grapes, as has been reported by different authors (Ruiz-Garcia *et al.*, 2012 ; Portu *et al.*, 2016) ; however, its effect on grape amino acid concentration is not entirely known. Nitrogen composition strongly affects the expression of resistance induced by elicitors (Dietrich *et al.*, 2004). Resistance induction through the use of chemical inducers often results in physiological costs to the plant decreasing the nitrogen concentration of plant tissues (Barbosa *et al.*, 2008). However, the physiological costs of resistance induced by elicitors can be observed only in Tempranillo (Table 2).

Table 3 shows the percentage of variance attributable to treatment, season, variety and their interaction on grape amino acid content. Season × variety was the most dominant factor for the concentration of His, Thr, Arg, Ala, Gaba, Cis, Val, Met, Phe, Leu, Lys, total amino acids and total amino acids without Pro. Season was the most dominant factor of variation for the concentration of Glu, Asn, Gln, Gly, Ile and Pro. Variety was the most dominant factor of variation for the concentration of Asp, Ser, Tyr and Trp. Gutiérrez-Gamboa *et al.* (2018) showed that variety had higher effect on must amino acid composition than the elicitation through MeJ and yeast extract treatments and their interaction (variety × treatment).

To classify the different treatments, PCA was performed using the individual amino acid concentration in untreated (Ctr) and MeJ-treated

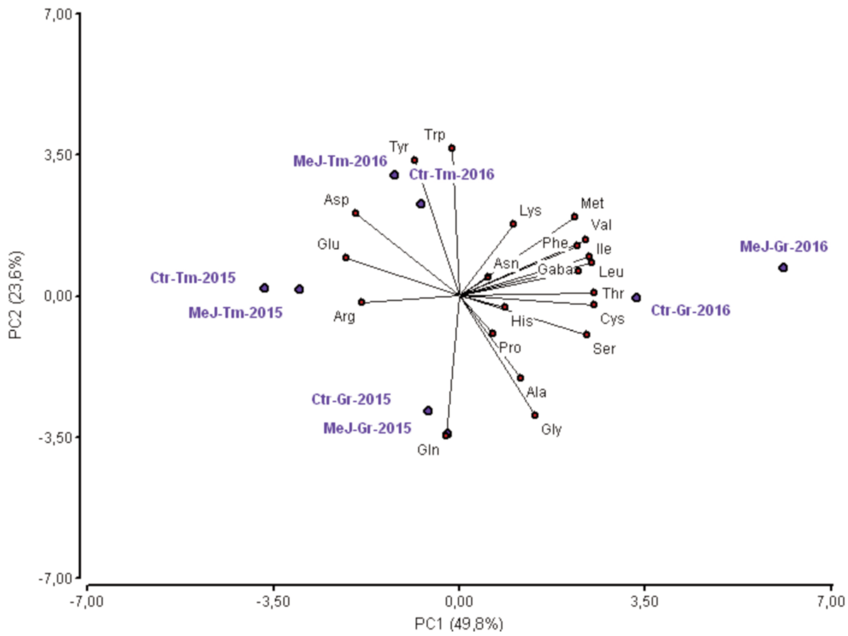

FIGURE 2. Principal components analysis (PCA) performed with all amino acids (mg/L) in Graciano (Gr) and Tempranillo (Tm) musts from untreated (Ctr) and treated grapevines with methyl jasmonate (MeJ) in 2015 and 2016 vintages.

Graciano (Gr) and Tempranillo (Tm) grapes, in 2015 and 2016 (Figure 2). PC1 explained 49.8 % of the variance and PC2 explained 23.6 % of the variance, representing 73.4 % of all variance. PC1 was strongly correlated with Glu, Ser, Thr, Gaba, Cys, Val, Met, Phe, Ile and Leu. PC2 was strongly correlated with Gln, Gly, Tyr and Trp. Ctr-Tm-2015 and MeJ-Tm-2015 samples were positively correlated with Glu and Arg, and negatively correlated with Thr, Cyr, Leu, Gaba and Ile. Ctr-Gr-2015 and MeJ-Gr-2015 samples were positively correlated with Gln, and negatively correlated with Tyr and Trp. Ctr-Tm-2016 and MeJ-Tm-2016 samples were positively correlated with Tyr and Trp, and negatively correlated with Gln. Ctr-Gr-2016 and MeJ-Gr-2016 samples were positively correlated with Thr, Cyr, Leu, Gaba and Ile, and negatively correlated with Glu and Arg.

CONCLUSIONS

The effect of MeJ application on grape amino acid concentration depended on the variety and season. Thus, this treatment decreased the concentration of certain individual amino acids in Tempranillo in both seasons. However, in Graciano, MeJ application increased the concentration of several amino acids in the second season. Season × variety was the main factor that determined the concentration of several amino acids, followed by season and variety factors

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