Scientia Horticulturae 240 (2018) 378–386

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Methyl jasmonate treatment to increase grape and wine phenolic content in Tempranillo and Graciano varieties during two growing seasons

Javier Portu, Rosa López, Pilar Santamaría, Teresa Garde-Cerdán*

Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja, CSIC, Universidad de La Rioja). Carretera de Burgos Km. 6, 26071 Logroño, Spain

ARTICLE INFO

Keywords:
Elicitation
Methyl jasmonate
Phenolic
Anthocyanins
Flavonols
Stilbenes
Viticulture

ABSTRACT

Phenolic compounds include a heterogeneous group of secondary metabolites that play diverse biological functions. Moreover, these compounds play a key role in grape and wine organoleptic and health promoting properties. Therefore, these compounds have been the subject of recent studies aimed at increasing their concentration in both grape and wine. The exogenous application of elicitors, like methyl jasmonate, stands out among these practices. We aimed to contribute to this growing area of research by carrying out this practice with two different grape varieties, Tempranillo and Graciano, during two growing seasons, providing therefore relevant information of the effect of this practice on the grape and wine phenolic composition. Despite the huge influence of the growing season and grape variety, a significant influence of MeJ treatment was found in grape phenolic composition, especially in anthocyanins, flavonols, and stilbenes. Moreover, certain wine chromatic parameters were also significantly improved by MeJ treatment. In conclusion, MeJ foliar application led to obtain grapes with a higher concentration of phenolic compounds.

1. Introduction

Phenolic compounds comprise a heterogeneous group of compounds that are formed through the phenylpropanoid pathway, starting with the amino acid phenylalanine. These secondary metabolites are divided according to their structure in non-flavonoids (i.e. phenolic acids and stilbenes) and flavonoids (i.e. anthocyanins, flavonols, and flavanols). Together with the volatile compounds, the phenolic compounds are the major responsible for grape and wine quality, taking part in color, mouthfeel properties and wine aging potential. Moreover, phenolic compounds have drawn the attention during the last decade of many studies, given their role in the beneficial health properties related to the moderate consumption of wine. In this respect, it is noteworthy their antioxidant properties, as well as biological activities like anticarcinogenic or cardioprotection (Xia et al., 2010), which could depend on the gut microbiota composition (Espín et al., 2017).

In view of the foregoing reasons, various studies have evaluated different tools to increase grape and wine phenolic content. However, grape phenolic composition depends on many factors that include the grape variety (Mazza et al., 1999), climate factors (Carbonell-Bejerano et al., 2014), biotic factors (Romero-Pérez et al., 2001), as well as viticultural practices such as early leaf removal (Diago et al., 2012), cluster thinning (Avizcuri-Inac et al., 2013) or the establishment of vegetal ground cover crops (Bouzas-Cid et al., 2016).

Among the viticultural practices aimed at improving grape phenolic composition, the application of elicitors has drawn the attention of different studies in recent years (Ruiz-García and Gómez-Plaza, 2013). Previous works have demonstrated that exogenous application of substances known as elicitors may induce plant defense mechanisms. Thus, plants could react to elicitor application by inducing the phenylpropanoid pathway and accumulating phenolic compounds (Dixon et al., 2002). In this respect, *in vitro* and *in vivo* studies have shown that the elicitor methyl jasmonate could improve grape and wine phenolic content in grape varieties like Tempranillo or Monastrell (Portu et al., 2016; Ruiz-García et al., 2012).

In addition, an improvement in grape phenolic composition has a special relevance in the current context of the climate change, which is known to accelerate grapevine phenology (Trought et al., 2015) and, in consequence, the challenge in warm areas is nowadays to get grapes with an optimal phenolic ripeness but not too high sugar levels.

Therefore, the aim of this study was to evaluate the foliar application of methyl jasmonate, as a promising tool to improve grape and wine phenolic composition, by studying the detailed grape and wine phenolic composition. We aim to contribute to this growing area of research by carrying out this practice with two different grape varieties, Tempranillo and Graciano, both originated in Rioja wine region, a region susceptible to climate change impact. Moreover, this work has also been conducted during two growing seasons, providing therefore

* Corresponding author.
E-mail address: teresa.garde@icvv.es (T. Garde-Cerdán).

https://doi.org/10.1016/j.scienta.2018.06.019
Received 2 March 2018; Received in revised form 6 June 2018; Accepted 8 June 2018

relevant information of this strategy.

2. Materials and methods

2.1. Vineyard site and experimental layout

This study was conducted during two growing seasons (2015 and 2016) with two different *Vitis vinifera* grape varieties: Tempranillo and Graciano.

Tempranillo commercial vineyard was located in Alfaro (Rioja Baja, Spain) at an altitude of 335 m above sea level (m.a.s.l.). The exact locations was 42° 10′ 2″ north latitude; 1° 49′ 53″ west longitude. Vines were planted in 1999 in north–south rows 2.80 m apart, with 1.20 m within-row spacing, resulting in a plant density of 3000 plants ha^{-1}, and grafted onto rootstock 1103-Paulsen.

As for Graciano grape variety, in 2015 the experimental site was located in Alfaro at an altitude of 345 m.a.s.l. The exact location was 42° 9′ 36″ north latitude; 1° 50′ 6″ west longitude. Vines were planted in 1997 in east-west rows 3.00 m apart, with a 1.28 m within-row spacing, resulting in a plant density of 2600 plants ha^{-1}. In 2016, Graciano trial was moved to a nearby vineyard, also located in Alfaro at an altitude of 465 m.a.s.l. The exact location was 42° 7′ 36″ north latitude; 1° 52′ 52″ west longitude. Vines were planted in 2002 in northwest-southeast rows 2.90 m apart with a 1.20 m within-row spacing, resulting in a plant density of 2900 plants ha^{-1}.

All vineyards were trained to a VSP (vertical shoot positioned) trellis system and managed according to the standard viticultural practices for the cultivars and region. Climatic conditions were recorded by a local weather station belonging to the Agroclimatic Information Service of La Rioja (SIAR). The growing season in 2015 was drier and slightly warmer than 2016. In this respect, annual rainfall in 2015 was 301 mm and average annual temperature was 14.1 °C. In 2016, annual rainfall was 386 mm while average annual temperature was 13.9 °C. Climatic conditions during vegetative growth period (i.e. from April to the end of September) followed a similar pattern: accumulated rainfall and average temperature during this period were, respectively, 128 mm and 19.5 °C in 2015; 145 mm and 19.1 °C in 2016.

The experimental design was set up as a completely randomized block design with three replicates of ten vines. The methyl jasmonate (MeJ) solution was prepared according to Portu et al. (2015b) at a concentration of 10 mM; 200 mL per plant were applied using Tween 80 as the wetting agent (0.1% v/v). Control plants were sprayed with Tween 80 aqueous solution. The treatments were carried out twice, at veraison and one week later.

2.2. Harvest and must parameters

Grapes were harvested when they reached an average ºBrix between 22.5 and 24. Harvest dates for Tempranillo were 17th of September in 2015 and 9th of September in 2016. Harvest dates for Graciano were 10th of September in 2015 and 6th of October in 2016. From each replicate, about 150 berries were separated and frozen at −20 °C in order to determine grape monomeric phenolic composition. Another set of 400 berries per replicate was separated and crushed in order to determine must parameters.

ºBrix was determined by refractometry. pH, total acidity, and potassium were analyzed in musts according to the International Organization of Vine and Wine (2013), while the tartaric acid was determined following the Rebelein method (Lipka and Tanner, 1974). An automatic analyser (Miura One, TDI, Barcelona, Spain) was used to determine malic acid.

Since treatments were performed in triplicate, the results of these parameters are the average of the analyses of three samples (n = 3).

2.3. Vinification and wine parameters

Grapes from each field replicate were destemmed and crushed and vinified in the experimental winery of the Instituto de Ciencias de la Vid y del Vino (ICVV, Logroño, Spain). Vinifications were performed at room temperature and potassium metabisulfite was added to the samples to give a final total SO_2 concentration of 50 mg L^{-1} and then the musts were inoculated with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand, St Simon, France) (20 g hL^{-1}). Caps were punched down daily and fermentation activity was followed by determining must temperature and ºBrix decrease.

Once the alcoholic fermentation was finished, wines were pressed and inoculated with the commercial *Oenococcus oeni* strain Uvaferm α (Lallemand) (1 g hL^{-1}) in order to perform the malolactic fermentation (MLF) under controlled conditions at 20 °C. The evolution of the MLF was followed by analyzing malic acid content. Once the MLF was finished, aliquots of each wine were frozen and stored at −20 °C until the analyses of monomeric phenolic compounds were carried out. Wines were then characterized by measuring the alcoholic degree, pH, total acidity, hue and color intensity (CI) according to the International Organization of Vine and Wine (2013). Tartaric acid was determined by Rebelein method (Lipka and Tanner, 1974). Miura One (TDI) was used to determine Folin-Ciocalteu index and the concentration of malic and lactic acids. Total phenolics were determined as total polyphenol index (TPI) by spectrophotometric absorbance at 280 nm after previous dilution of samples (Ribéreau-Gayon and Stonestreet, 1965). Ionised anthocyanins were determined according to Glories (1978) and polymerization index was calculated according to Ruiz (1999). The total antioxidant activity in wines was determined according to the DPPH method following the methodology described by Nixdorf and Hermosín-Gutiérrez (2010). Spectrophotometric analyses were carried out with the following spectrophotomers: Helios Omega (Thermo Fisher Scientific, Waltham, USA) for IC, hue and TPI; DR 5000 (Hach, Dusseldorf, Germany) for ionized anthocyanins and polymerization index; Cary 60 (Agilent, Palo Alto, USA) for the total antioxidant activity.

Since field treatments were performed in triplicate and one vinification was performed from each replicate, the results of wine parameters correspond to the average of the analyses of three samples (n = 3).

2.4. Determination of grape and wine low molecular weight phenolic compounds

2.4.1. Sample preparation

Phenolic compounds were extracted from grape berries according to the method described by Portu et al. (2016). Moreover, in order to isolate grape and wine non-flavonoid compounds, a purification step by solid phase extraction (SPE) was performed using PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent) placed in a Visiprep™ Vacuum Manifold extraction system (Sigma-Aldrich, San Luis, USA) (Portu et al., 2015a). The anthocyanin-free fraction was used to analyze flavonols, flavanols, hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes.

2.4.2. Analysis of phenolic compounds by HPLC-DAD

Phenolic compounds but stilbenes were analyzed using an Agilent 1260 Infinity chromatograph, equipped with a diode array detector (DAD). The chromatographic procedure was as described by Portu et al. (2016) using a Lichrospher® 100 RP-18 reversed-phase column (250 × 4.0 mm; 5 µm packing; Agilent) with pre-column Lichrospher® 100 RP-18 (4 × 4 mm; 5 µm packing; Agilent), both thermostated at 40 °C. For the analysis of anthocyanins, 10 µL of the grape extract or wine were injected into the system. For the analysis of non-anthocyanin phenolic compounds fractions, the injection volume was 20 µL. Flow rate was set at 0.630 mL min^{-1}. For anthocyanin analysis, a gradient solvent system consisting of acetonitrile–water–formic acid (3:88.5:8.5, v/v/v) (eluent A) and acetonitrile–water–formic acid (50:41.5:8.5, v/v/