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Changes on grape volatile composition through elicitation with methyl jasmonate, chitosan, and a yeast extract in Tempranillo (*Vitis vinifera* L.) grapevines

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ABSTRACT

Elicitors play a key role against pathogen infestation, becoming as an alternative to chemical pesticides by the improving secondary metabolites synthesis. Their effect on grape volatile compounds has been little investigated. This field trial aimed to study the foliar application of methyl jasmonate (MeJ), chitosan (CHT), and a yeast extract (YE) on grape volatile compounds by HS-SPME-GC–MS. The results showed that, in general, foliar elicitor application decreased the synthesis of grape volatile compounds. Depending of the treatment, MeJ and CHT showed the lowest total amounts of terpenoids, C_{13} norisoprenoids, benzenoids, and esters, together with the highest levels of C6 compounds. YE treatment applied to the grapevines barely affected the synthesis of volatile compounds in grapes. The importance of this work is to add information about the effects of elicitors on grape volatile composition in Tempranillo grapevines.

1. Introduction

Several hundreds of volatile compounds belonging to different chemical groups, such as terpenoids, C_{13} norisoprenoids, esters, benzenoid compounds, among others, are part of the grape aroma, being mainly responsible for the called varietal aroma in wines (Moreno-Arribas and Polo, 2009; Zalacain et al., 2007). These compounds are part of the secondary metabolites of plants and their synthesis depends on several factors, such as grape variety, viticultural practices, climate conditions, soil characteristics, and degree of maturation (Cabrita et al., 2007; Robinson et al., 2014; Garde-Cerdán et al., 2015; Hernández-Orte et al., 2015). Some terpenoids and C_{13} norisoprenoids are the most odoriferous compounds, due to their very low sensory thresholds. Thus, linalool, α-terpineol, nerol, geraniol, citronellol, β-damascenone, and β-ionone strongly contribute to floral fragrances (Ribéreau-Gayon et al., 2006). Hexanal, n-hexanol, (Z)-3-hexen-1-ol, and (E)-2-hexen-1-ol, which belonging to C6 compounds, are formed by enzymatic degradation of grape lipids after grape crushing (Jackson, 2008; Moreno-Arribas and Polo, 2009). These volatile compounds at high levels can provide undesirable herbaceous flavors to the wines (Pedroza et al., 2010; Cai et al., 2014a).

Elicitors are a specific class of purified molecules originating from microorganisms or plants which are able to trigger an innate immune response in plant cultures (Boller and Felix, 2009; Delaunois et al., 2014). One of the most used elicitors in plants is methyl jasmonate (Ruiz-García and Gómez-Plaza, 2013). This is a volatile organic compound derived from jasmonic acid that modulates chlorophyll degradation and anthocyanin biosynthesis (Ruiz-García and Gómez-Plaza, 2013). This elicitor has been mainly implicated as mediator in the plant responses triggered by wounding and insect feeding and is involved in pathogen resistance (Gozzo, 2003; Ruiz-García and Gómez-Plaza, 2013). Another molecule with elicitor capacity is chitosan. This is a polycationic β-1,4-linked-D-glucosamine polymer that form a semi permeable film around plant tissues, which allows to avoid the infection of pathogen microorganisms, triggering the accumulation of polyphenol-related enzymes, allowing the synthesis of secondary metabolites (El Ghaouth et al., 1994; Romanazzi et al., 2002; Aziz et al., 2006; Trotel-Aziz et al., 2006; Ruiz-García and Gómez-Plaza, 2013; Portu et al., 2016). Yeast extracts are considered as part of the biotic elicitors and their application in plant tissues allows to trigger plant defense mechanisms, leading to an accumulation of secondary metabolites, such as phenols, sesquiterpenoids, and other compounds, which has been described in several plant cultures (Ferrari, 2010; Abraham et al., 2011; Rahimi et al., 2014; Cai et al., 2014b; Loc et al., 2014).

Elicitors have been applied in the vineyard in order to trigger defense mechanisms to avoid pathogen infection (Delaunois et al., 2014). Along with this, elicitation in grapevines has allowed to improve the quality of grape berries, leading to the accumulation of phenolic

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compounds, which are responsible for the color and its stability during aging of the produced wines (Ruiz-García and Gómez-Plaza, 2013; Portu et al., 2016). The effects of the application of different elicitors, other than methyl jasmonate, such as chitosan and yeast extracts to the vineyard on grape organoleptic composition have been little studied. It has been reported that methyl jasmonate and yeast extracts applied to the vineyard increased grape and wine anthocyanin content when compared to the control (Portu et al., 2016). These authors showed also that the application of a yeast elicitor enhanced grape stilbene content, while the chitosan treatment did not have a substantial impact on the synthesis of phenolic compounds. Respect to amino acid composition, chitosan and yeast extract applications to vines decreased the must concentration of several amino acids, affecting its total content (Gutiérrez-Gamboa et al., 2017). However, methyl jasmonate treatment applied to these grapevines had a slight effect on grape amino acid content, increasing only the concentration of methionine and phenylalanine (Gutiérrez-Gamboa et al., 2017). These authors suggested that the resistance induction through chitosan and yeast extracts treatments may results in physiological costs to grapevines associated with a decrease in grape amino acid concentration or to a compartmentalization of these compounds in reserve tissues. In relation to the effects of elicitation on volatile compounds in grapes, the use of benzothiadiazole and methyl jasmonate in Monastrell grapevines increased the levels of volatile compounds in grapes, mainly terpenes and norisoprenoids in benzothiadiazole treated grapes (Gómez-Plaza et al., 2012). Moreover, in *Vitis vinifera* L. cv. Gropello Gentile, the application of benzothiadiazole increased total acetals and esters, while chitosan raised the levels of total acetals and alcohols compared with the use of conventional fungicides, such as penconazole and methyldinocap (Vitalini et al., 2014). Thus, to our knowledge, this is one of the first approaches that study the effects of different elicitors on grape volatile compounds in *Vitis vinifera* cv. Tempranillo grapevines.

Due to the aforementioned, the aim of this work was to study the effect of elicitation through methyl jasmonate, chitosan and a yeast extract applied foliarly to Tempranillo vines on grape volatile composition.

2. Materials and methods

2.1. Grapevine treatments

The field study was performed in a commercial vineyard located in Alfaro (La Rioja, Spain), during the 2014 growing season. Three elicitors were applied: methyl jasmonate (MeJ), chitosan (CHT), and a yeast extract (YE), as well as a control treatment with a manual pump sprayer. Two applications were made to the grapevines; the first was applied at veraison, and the second one week after the first application. The MeJ solution (Sigma-Aldrich, Madrid, Spain) was prepared according to Garde-Cerdán et al. (2016) at a concentration of 10 mM; 200 mL per plant were applied. The CHT solution (Sigma-Aldrich) was prepared according to Vitalini et al. (2014) at a concentration of 0.03% (w v^{-1}), with 572 µL of acetic acid L^{-1}. CHT solution was sprayed over leaves applying a total amount of 400 mL per plant. YE is a formulation of 100% natural, inactivated wine yeast (*Saccharomyces cerevisiae*) derivatives. The YE solution was prepared at a concentration of 1.69 g L^{-1}; 200 mL were sprayed per plant. In all cases, Tween 80 (Sigma-Aldrich) was used as the wetting agent (0.1% v v^{-1}). Control plants were sprayed only with Tween 80 solution (Garde-Cerdán et al., 2015). Treatments were applied to the grapevines during the morning on a day in full sun, without wind or precipitation. A completely randomized experimental design was set up consisting of three replicates of 10 grapevines per treatment.

The grapes were harvested at their optimum technological maturity. Subsequently, they were mechanically destemmed and crushed in the experimental winery. The oenological parameters were determined in the obtained musts. Aliquots of each sample were frozen in order to analyze their volatile composition.

2.2. Oenological parameters and yeast assimilable nitrogen (YAN)

In the musts were analyzed the probable alcohol, pH, titratable acidity, malic acid, and potassium, according to OIV methods (2003). Tartaric acid was performed according to Rebelein method (Lipka and Tanner, 1974). Yeast assimilable nitrogen (YAN) was determined according to the method described by Aerny (1996). Since treatments were performed in triplicate, the results of these parameters are the average of three analyzes (n = 3).

2.3. Analysis of grape volatile compounds by HS-SPME-GC–MS

Grape volatile compounds were analyzed by the method exposed by Garde-Cerdán et al. (2015). 2 g of NaCl were added to 12 mL of must sample. Then, the samples were conditioned during 15 min at 60 °C with stirring. Later, the extraction was performed at this temperature for 105 min with stirring. After concluding the extraction process, the SPME fiber (DVB/CAR/PDMS, 50/30 mm) (Supelco, Bellenfonte, PA, USA) containing the volatile compounds of the samples was manually introduced into the GC injection port at 250 °C (equipped with a glass liner, 0.75 mm I.D. (Supelco) and thermogreen™ LB-2 septum (Supelco)) and kept during 15 min for desorption. The desorbed compounds were separated in an Agilent 7890 A gas chromatograph system (GC) coupled to a quadrupole Agilent 5975C electron ionization mass spectrometric detector (Agilent Technologies, Palo Alto, CA, USA) equipped with a SPB™-20 fused silica capillary column (30 m x 0.25 mm I.D. × 0.25 µm film thickness) (Supelco). Helium was used as carrier gas at a flow rate of 1.2 mL min^{-1}. The injections were performed in splitless mode (1 min). The volatile compounds were separated using a temperature program with an initial oven temperature of 40 °C for 5 min, a temperature gradient of 2 °C min^{-1} to a final temperature of 220 °C, and a final time of 20 min. The ionization was performed at 70 eV and the detector temperature was 250 °C. The acquisitions were performed in Full Scan (35–300 *m/z*). Identification was carried out using the NIST library and by comparison with the mass spectrum and retention index of chromatographic standards (Sigma-Aldrich), and data found in the bibliography. Volatile compounds were expressed as average relative area, which was calculated as the area of each volatile compound divided by the total area (sum of the area of all the studied volatile compounds) × 1000, as it has been exposed by Mauriello et al. (2009) and Garde-Cerdán et al. (2018). The four treatments were carried out in triplicate at vineyard, so the results of grape volatile compounds correspond to the average of three different analysis (n = 3).

2.4. Statistical analysis

The statistical analysis in relation to oenological parameters, YAN, and grape volatile composition was performed using variance analysis (one-way ANOVA) by Statgraphics Centurion XVI.I (Warrento, Virginia, United States). Differences between samples were compared using the Duncan test at 95% probability level. Principal component analysis (PCA) was performed with volatile compounds content in the different samples using InfoStat Professional 2012 version (InfoStat, www.infostat.com.ar).

3. Results and discussion

3.1. Must oenological parameters

Table 1 shows the must oenological parameters of the different samples. No significant differences were observed among CHT and YE treatments in relation to the control. But, in case of MeJ application, must samples presented higher content of tartaric acid than the musts from vines treated with CHT and YE. However, MeJ applied to the