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Effects on grape amino acid concentration through foliar application of three different elicitors

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ABSTRACT

Elicitors play an important role in the defense against pathogens as an alternative to chemical pesticides by increasing secondary metabolites. Their effect on grape amino acid has been little investigated. Thus, the aim of this research was to study the influence of methyl jasmonate (MeJ), chitosan (CHT), and a yeast extract (YE) on grape amino acid composition, through foliar applications to grapevines. The must amino acid concentration was analyzed by HPLC. The results showed that CHT and YE treatments decreased the must concentration of several amino acids, affecting total amino acid content (from 2364 to 1961, and 1818 mg/L, respectively). However, MeJ treatment had a slight effect on grape amino acid content, increasing the concentration of Met (from 8.95 to 12.13 mg/L) and Phe (from 7.96 to 9.29 mg/L). It seems to be that, the resistance induction through CHT and YE treatments results in physiological costs to grapevines associated with a decrease on grape amino acid concentration. Consequently, MeJ applications, as a viticultural practice, could be a better tool than CHT and YE treatments, because did not affect grape amino acid concentration.

1. Introduction

The nitrogen composition of the grapevines is the primary determinant of must composition prior to fermentation (Bell & Henschke, 2005). Thus, grape and consequently, must nitrogen composition play an important role on final wine quality. Yeast growth, fermentation kinetics and flavor metabolism are all greatly affected by the nitrogen status of the must (Bell & Henschke, 2005). A deficient nitrogen concentration in the must may cause stuck or sluggish fermentations, which is a persistent problem in wine production (Bisson & Butzke, 2000). As it has been extensively studied, many factors can influence the nitrogen composition such as cultural practices, soil management, nitrogen form, timing or rate of applications, among others (Huang & Ough, 1989; Lasa et al., 2012; Pérez-Álvarez, Garde-Cerdán, García-Escudero, & Martínez-Vidaurre, 2017; Stines et al., 2000).

Elicitors are a specific class of purified molecules originating from microorganisms or plants which are able to stimulate an innate immune response in plant (Boller & Felix, 2009; Delaunois et al., 2014). Elicitors act as signal compounds at low concentrations, providing information to the plants to trigger different defense mechanisms, distinguishing elicitors from toxins, which may act only at higher concentrations and/or affect the plant detrimentally, without active the plant metabolism (Boller, 1995). The role of elicitors in inducing resistance in plants against pathogen infection, related to the elicitor signal transduction mechanisms, which activate plant primary immune response, among others, was reviewed by Thakur and Sohal (2013).

In this way, in the wine industry, elicitors are used as an alternative strategy to chemical pesticides in order to induce defense mechanisms against important grapevine pathogens such as *Botrytis cinerea*, *Plasmopara viticola* and *Uncinula necator* (Aziz et al., 2003; Campbell & Latorre, 2004; Jacometti, Wratten, & Walter, 2010). In their report, Oliva, Garde-Cerdán, Martínez-Gil, Salinas, and Barba (2011) showed that the application of pesticides to the grapevines led to a decrease on the grape concentration of several amino acids. Likewise, regarding elicitors, only a few studies have been carried out in order to study its effect on grape or wine quality and most of them are focused in the study on phenolic and volatile composition of grapes and wine (Gil-Muñoz, Fernández-Fernández, Crespo-Villegas, & Garde-Cerdán, 2017; Gómez-Plaza, Mestre-Ortuño, Ruiz-García, Fernández-Fernández, & López-Roca, 2012; Portu, López, Baroja, Santamaría, & Garde-Cerdán, 2016; Ruiz-García et al., 2012). However, to our knowledge, there is only one report that studies the effect of the foliar application of an elicitor on the grape amino acid composition, so Garde-Cerdán, Portu, López, and Santamaría (2016) showed in their study, that the grapes treated with methyl jasmonate led to an increase of eight amino acids content, especially on phenylalanine must concentration. The effects above mentioned are probably due to that the application of jasmonates activate the phenylpropanoid pathway,

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leading to the accumulation of phenolic compounds (Belhadj et al., 2008) and possibly of others compounds.

On the other hand, it is of great interest to study the effect of other elicitors on grape amino acid concentration. Thus, chitosan is a polycationic β-1,4-linked-D-glucosamine polymer that form a semi permeable film around plant tissues, inhibiting to a several pathogenic fungi, and also induce host-defense responses (El Ghaouth et al., 1994). Chitosan has been studied for disease control improving development and protects plants against *B. cinerea* (Romanazzi, Nigro, Ippolito, Di Venere, & Salerno, 2002), inhibiting the development *in vitro* studies of *B. cinerea* (Trotel-Aziz, Couderchet, Vernet, & Aziz, 2006) and inducing grapevine defense reaction and resistance to *B. cinerea* and *P. viticola* (Aziz et al., 2006). *In vitro* studies have shown that diverse chitosan oligomers of different molecular weight and degree of acetylation, triggered an accumulation of phytoalexins, *trans* and *cis*-resveratrol and their derivatives ε-viniferin and piceid in grapevine leaves (Aziz et al., 2006). In addition, it has been exhibited that the treatment of grapevine leaves by chitosan led to marked induction of lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL) and chitinase activities, three markers of plant defense responses (Trotel-Aziz et al., 2006). Therefore, its application to the grapevines could have a significant effect on grape amino acid concentration.

On another note, yeast extracts are biotic elicitors which have been used in plant tissue culture due to their ability to stimulate the defense mechanisms, leading an increase of secondary metabolite production (Abraham, Bhatt, Keng, Indrayanto, & Sulaiman, 2011). These compounds are regarded as triggers of various modes of plant defense (Ferrari, 2010). It has been showed *in vitro* studies that yeast extract induces the accumulation of defensive metabolism such as the accumulation of sesquiterpenoids (Rahimi et al., 2014), and the accumulation of phenolic acids in cell suspension culture of *Malus* × *domestica* Borkh (Cai, Kastell, & Smetanska, 2014), the increase in solasodine content in cell cultures of *Solanum hainanense* Hance plants (Loc, Anh, Khuyen, & An, 2014). Therefore, its use in grapevines may have a significant effect on grape quality. In this sense, Portu et al. (2016) studied the effect of foliar applications of methyl jasmonate, chitosan and a yeast extract on grape and wine phenolic content showing that, methyl jasmonate and yeast extract increased grape and wine anthocyanin content compared with control samples. In addition, chitosan treatment did not have a substantial impact on phenolic compounds. It is important to note that, to our knowledge, the effect of chitosan and yeast extract by foliar application on grape amino acid concentration has not yet been carried out.

Due to above mentioned, the aim of this study was to evaluate the effect of different elicitors foliar applications to Tempranillo vines on grape amino acid concentration. In this regard, three different elicitors were studied: methyl jasmonate, chitosan, and a yeast extract.

2. Materials and methods

2.1. Study site, grapevine treatments and harvest

The field study was conducted on a commercial vineyard located in Alfaro (warmest and driest area of La Rioja, Spain), during the 2014 growing season. The altitude of the plot location was 335 m.a.s.l. Red Tempranillo grapevines (*Vitis vinifera* L), clone RJ 43 were used in this trial. Information about plant material, vineyard orientation, planting distance, viticultural management, weather conditions, and soil classification of the study site is stated in Portu et al. (2016). The vineyard was managed according to the standard viticultural practices for the variety and region.

The field trial involved the application of three elicitors: methyl jasmonate (MeJ), chitosan (CHT), and yeast extract (YE), as well as a control treatment. The MeJ solution (Sigma-Aldrich, Madrid, Spain) was prepared according to Portu, Santamaría, López-Alfaro, López, and Garde-Cerdán (2015). The CHT solution (Sigma-Aldrich) was prepared according to Vitalini et al. (2014). YE (LalVigne MATURE®, Lallemand) is a formulation of 100% natural, inactivated wine yeast (*Saccharomyces cerevisiae*) derivatives. Information about application dose, and timing is stated in Portu et al. (2016). A completely randomized experimental design was set up consisting of three replicates of ten grapevines per treatment.

Grapes were harvested at their optimum technological maturity, and then were destemmed and crushed. The oenological parameters were determined in the musts obtained. Aliquots of each sample were frozen in order to analyze their free amino acids content.

2.2. Oenological parameters and yeast assimilable nitrogen (YAN)

Musts were physico-chemically characterized by determining probable alcohol, pH, titratable acidity, malic acid, and potassium according to the ECC (1990) and tartaric acid according to Rebelein method (Lipka & 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 amino acids by HPLC

Different methodologies for grape amino acids determination have been outlined. Derivatization is habitually required, using various reagents, including *o*-phthaldialdehyde (OPA), for primary amino acids, and 9-fluorenylmethylchloroformate (FMOC) for secondary amino acids. The reaction with these two reagents is fast, sensitive and the products are visible in both diode array detector (DAD) and fluorescence detector (FLD) (Callejón, Troncoso, & Morales, 2010). The must amino acids analysis was performed by the method described by Garde-Cerdán et al. (2014). Free amino acids were analyzed by reversal-phase HPLC using an Agilent 1100 Series (Palo Alto, USA), equipped with an ALS automatic liquid sampler, a (FLD) and a DAD. Each sample was centrifuged at 4000 rpm for 10 min at 20 °C and then, 5 mL of the sample was mixed with 100 μL of norvaline, internal standard for quantify all amino acids except proline, and 100 μL of sarcosine, internal standard for quantify proline. This mixture was filtered through 0.45 μm OlimPeak pore filter (Teknokroma, Barcelona, Spain) and submitted to an automatic precolumn derivatization with OPA (Agilent) and with FMOC (Agilent). The injected amount from the derivatized sample was 10 μL at 40 °C. All separations were performed on a Hypersil ODS (250 × 4.0 mm, I.D. 5 μm) column (Agilent).

Two eluents, previously filtered through 0.45 μm Millipore pore filter, 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. For the quantification, different amino acid solutions were prepared in HCl at 0.1 N in the range of the amino acid concentrations usually found in Tempranillo musts. The calibration curve for each amino acid was made with 7 points. The concentration range was from 0.57 mg/L for Orn to 927.54 mg/L for Pro (R^2 > 0.993). The accuracy of the analytical method was from 83% (GABA) to 123% (Asp); the repeatability was from 0.21% (Tyr) to 6.03% (Pro); and the sensitivity was from 0.01 L/mg (Pro) to 0.29 L/mg (His).

The treatments were carried out in triplicate, so the results for free amino acids correspond to average of three analyses (n = 3).

2.4. Statistical analysis

A statistical analysis on oenological parameters, yeast assimilable nitrogen (YAN) and amino acids composition was performed using variance analysis (one-way ANOVA), by Statgraphics Centurion XVI.I