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Changes on grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea

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ABSTRACT

Grapevines may require the input of nitrogen to grow and to guarantee an appropriate grape composition. Recently there has been a growing interest in foliar fertilization, which entails a fast and efficient assimilation of the products. The aim of this work was to study the influence of foliar applications of phenylalanine and urea, at two different doses, on grape anthocyanins, flavonols, flavan-3-ols, phenolic acids, and stilbenes. All treatments were applied at veraison and one week later at doses of 0.9 and 1.5 kg N/ha. The results showed that the synthesis of phenolic compounds was favoured by foliar applications of phenylalanine and urea. The application of the lowest dose of urea was the most effective treatment, increasing the content of several anthocyanins and flavonols. Moreover, none of the foliar treatments worsened the grape phenolic composition. In conclusion, foliar application of phenylalanine and especially urea, could be an interesting management tool for improving grape quality and their health-promoting properties.

1. Introduction

Nitrogen is an essential macronutrient for the correct growth and development of grapevine (Bell & Henschke, 2005). Moreover, nitrogen composition of the must plays a key role in the development of alcoholic fermentation, affecting the fermentation kinetics (Bell & Henschke, 2005) and the formation of secondary metabolites, especially higher alcohols and esters (Garde-Cerdán & Ancín-Azpilicueta, 2008). As a consequence, nitrogen deficiencies are the leading cause of stuck or sluggish fermentations (Bisson, 1999) and sulphurous off-flavor formation (Giudici & Kunkee, 1994).

Thus, nitrogen fertilization is carried out in order to guarantee vine growth and a correct grape and must composition. Nitrogen fertilization has traditionally been performed by adding fertilizer to the soil, to be absorbed by plant roots. Nonetheless, the current pollution problems arising from excessive use of soil fertilizers have led to the rise of new fertilization techniques, that allow more precise and effective applications. One of these techniques is foliar fertilization, because of the quick and efficient assimilation of applied products by the plant (Lasa et al., 2012), that reduces costs and contributes to sustainable eco-friendly agriculture.

In recent years, some studies about foliar treatments to vineyards that modified grape and wine composition have been reported. In that respect, some works have investigated the use of biostimulants, such as oak extracts, as products able to stimulate the synthesis of aroma compounds (Martínez-Gil, Garde-Cerdán, Zalacain, Pardo-García, & Salinas, 2012; Pardo-García, Serrano de la Hoz, Zalacain, Alonso, & Salinas, 2014) and phenolic compounds (Pardo-García et al., 2014) by grapevine. Moreover, different works have considered the foliar application of hormones or growth regulators in order to trigger the secondary metabolism of grapevine. In regard of this, an increase in anthocyanin synthesis has been observed after foliar application of abscisic acid (ABA) to Cabernet Sauvignon (Balint & Reynolds, 2013), or Malbec grapevines (Berli, Fanzone, Piccoli, & Bottini, 2011).

Regarding foliar application of nitrogen compounds, urea has been shown to change the wine aroma profile (Lacroux et al., 2008; Ancín-Azpilicueta, Nieto-Rojo, & Gómez-Cordón, 2013) and to increase both berry yeast assimilable nitrogen (YAN) and some amino acids in grapes (Lasa et al., 2012; Neilsen, Neilsen, Hannam, Millard, & Midwood, 2013). A recent approach has been established with the aim of studying the effect of the foliar application of different nitrogen compounds on the grape amino acid content (Garde-Cerdán et al., 2014). In the latter study, foliar appli-

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cations of phenylalanine and urea were able to enhance the synthesis of most of the amino acids by the plant, where both treatments gave a similar effect.

On another note, grapes, and therefore wine, are one of the most important sources of phenolic compounds when compared to other fruits and vegetables. Phenolic compounds are secondary metabolites of the grape which have a great importance in plant metabolism. They are usually synthesized by grapevines under different stress conditions, like UV radiation, drought, or predator and pathogen attacks. Moreover, phenolic compounds contribute significantly to the organoleptic characteristics of both grape and wine since they are responsible for the color, its stability and mouthfeel sensations such as astringency sensation or bitterness (Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014). These compounds also determine the oenological capacity of a wine for aging in oak barrels (Rubio-Bretón, Lorenzo, Salinas, Martínez, & Garde-Cerdán, 2012). Last but not least, the health benefits of phenolic compounds have been widely studied in recent years, including cardioprotective, anti-inflammatory, anti-carcinogenic, and antimicrobial activities, mainly attributed to their antioxidant and radical scavenging properties, and their metal chelation ability (Del Rio et al., 2013). The majority of phenolic compounds are found in grape skin and seeds. Grape phenolic composition is affected by many factors such as environmental conditions, cultural practices as well as cultivar genetics (Kennedy, Matthews, & Waterhouse, 2002; Downey, Dokoozlian, & Krstic, 2006; Lijavetzky et al., 2006). Therefore, grape phenolic composition is subjected to a great variation.

The implication of soil nitrogen fertilization on grape quality components was reviewed by Bell and Henschke (2005). These authors found contradictory results between different studies and stated that there is not a clear relationship between phenolic compounds and soil nitrogen fertilization since the effect could be dependable on genetic, environmental or cultural factors. Previous studies about the impact of foliar applications of different nitrogen compounds to grapevine on grape phenolic composition have not been found. Urea, due to its intrinsic characteristics such as small molecular size and high water solubility, is rapidly taken up by the leaf cuticule. Phenylalanine is not an abundant amino acid in grapes but yet it is the precursor of various phenolic compounds, and of 2-phenylethanol, a positive compound for wine aroma with a rose odor descriptor. Recently, it has been reported that phenylalanine incorporation into grape berries influences berry phenolic metabolism (Chassy, Douglas, Laurie, & Waterhouse, 2012). In the latter study, it was demonstrated that labeled phenylalanine was absorbed by grape berries and metabolized into labeled flavonoids. Due to the lack of information in relation to the impact of nitrogen foliar applications on grape phenolic content, the objective of this work was to study the effects of foliar applications of phenylalanine and urea on grape phenolic composition.

2. Materials and methods

2.1. Reagents and standards

All solvents (methanol, acetonitrile, and formic acid) were of HPLC quality. All chemicals were analytical grade (>99%) unless otherwise stated, and were purchased from Panreac (Barcelona, Spain). Water was of Milli-Q quality (Millipore, Bedford, USA). Nitrogen compounds (phenylalanine and urea) and Tween 80 were purchased from Sigma-Aldrich (Madrid, Spain). The following commercial standards were purchased from Sigma-Aldrich: (−)-epicatechin, (+)-catechin, (−)-epicatechin-3-gallate, rutin, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, kaempferol, myricetin, piceatannol, *trans*-resveratrol, *trans*-piceid, gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and caftaric acid. Malvidin-3-*O*-glucoside and procyanidin B2 were purchased from Extrasynthèse (Genay, France), whereas procyanidin B1 was from Phytolab (Vestenbergsgreuth, Germany).

2.2. Samples and grapevine treatments

Nitrogen foliar treatments were applied to Tempranillo (*Vitis vinifera* L.) grapevines located in the experimental vineyard of La Grajera (northern region of La Rioja, Spain) in 2013. Apart from control, four nitrogen treatments were carried out using two nitrogen sources at two different doses each: 150 and 250 mg N/plant of phenylalanine (Phe1 and Phe2), and 150 and 250 mg N/plant of urea (Ur1 and Ur2). To carry out the treatments, aqueous solutions were prepared with the corresponding concentration of phenylalanine (Phe), and urea (Ur), using Tween 80 as a wetting agent (0.1% v/v). Control plants were sprayed with water solutions of Tween 80 alone. The treatments were applied to the grapevine twice, at veraison and one week later. For each application, 200 mL/plant were sprayed over leaves, so the total amount applied in each treatment was 0.9 kg N/ha for Phe1 and Ur1 and 1.5 kg N/ha for Phe2 and Ur2, assuming 3000 plants/ha. Experiments were performed in triplicate ($n = 3$) and were arranged in a complete randomized block design with 3 vines for each experiment replication.

Grapes were harvested when they reached an average probable alcohol content of 14%, common in wines from our region. From each treatment, about 150 berries were separated and frozen at −20 °C in order to determine their monomeric phenolic composition. Grapes were destemmed and crushed and oenological parameters were determined in the musts.

2.3. Oenological parameters and yeast assimilable nitrogen analysis

Musts were physicochemically characterized by determining °Brix by refractometry, probable alcoholic strength, pH, total acidity, malic acid and potassium content according to ECC methods (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 analyses ($n = 3$).

2.4. Analysis of grape phenolic compounds

2.4.1. Grape sample extraction

About 50 g of each frozen grape sample were immersed into 50 ml of aqueous methanol solution (50% v/v) at pH 2 adjusted with formic acid (>96%). Immediately after, grapes were homogenized using a Ultra-Turrax T-18 (IKA, Staufen, Germany) at high speed (18,000 rpm) for 1 min, obtaining a smooth paste in which there were not visible pieces of seeds or skin. Samples were then maintained in an ultrasonic bath (JP Selecta, Barcelona, Spain) for 10 min and were centrifuged at 5000 rpm at 10 °C for 10 mins. A second extraction of the resulting pellets was completed using the same volume of the solvent mixture (50 ml). The supernatants were combined and the volume was annotated. Each sample was maintained at −20 °C until the analyses were carried out.

2.4.2. Sample preparation for the analysis of non-anthocyanin phenolic compounds

Anthocyanins may cause interferences in the chromatographic separation and identification of other phenolic compounds. Therefore, an extraction on PCX SPE cartridges (500 mg, 6 ml; Bond Elut Plexa, Agilent, Palo Alto, CA, USA) containing a mixture of reverse-