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Phenolic composition of Tempranillo grapes following foliar applications of phenylalanine and urea: A two-year study

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ABSTRACT

Foliar fertilization with nitrogen compounds has been reported to have a substantial impact on grape composition. Overall, this practice has been associated with an increase of grape nitrogen compounds. However, much less is known about its real impact on phenolic composition, especially through more than one year. In the current work, grape phenolic composition was studied after carrying out foliar treatments with two nitrogen sources (phenylalanine and urea) at 0.9 kg N/ha over two consecutive vintages. Phenylalanine treatments increased the concentration of a few anthocyanin compounds in comparison with control, although hydroxycinnamic acids were decreased in the first year of the study. In contrast, urea application increased some flavanol compounds in the second year, while no effect was observed in the first one. Overall, it was not observed a significant decrease of phenolic compounds which, in contrast, has been traditionally associated with high nitrogen supply by soil fertilization. In conclusion, the foliar application of phenylalanine and urea at 0.9 kg N/ha did not exert a big impact on grape phenolic composition.

1. Introduction

Foliar application of nitrogen (N) compounds to grapevine has emerged as an interesting tool for increasing must yeast assimilable nitrogen (YAN), grape amino acid concentration (Garde-Cerdán et al., 2014; Lacroux et al., 2008; Neilsen et al., 2013), and grape volatile composition (Garde-Cerdán et al., 2015). Previous studies have been mainly focused on the application of urea (Ur), which is rapidly taken up by the leaf cuticle due to its small molecular size and high water solubility. However, a few studies have also investigated the effectiveness of the application of other nitrogen sources, such as commercial fertilizers or the amino acid phenylalanine (Phe) (Garde-Cerdán et al., 2014). Recently, Sánchez-Gómez et al. (2016) also proved the potential usefulness of foliar application of grapevine shoots extract, vineyard waste by-product, in order to increase grape amino acid content, which resulted as well in higher concentration of wine volatile compounds after the alcoholic fermentation.

Recent evidence suggests that veraison is the optimal moment for Ur foliar application (Lasa et al., 2012; Verdenal et al., 2015). At veraison, the application of N compounds could prevent the competition between vegetative (shoots) and reproductive (grapes) sinks, a competition that has been often associated with high N supply to soil at earlier stages of the annual vine cycle. This competition could lead to excessive vine growth which in turn could result in bunch shading, increasing disease incidence, imbalanced grape ripening, and consequently, decreasing grape and wine quality. Not only foliar N application may prevent these problems when compared to earlier soil N applications, but it has also been reported to be more effective at increasing grape amino acids concentration when compared to soil fertilization at the same timing (Hannam et al., 2016).

It is known that fermentation kinetics and synthesis of secondary metabolites during fermentation, especially higher alcohols and esters, are strongly affected by must amino acid composition (Cramer et al., 2002; Garde-Cerdán and Ancín-Azpilicueta, 2008; Taillandier et al., 2007). In addition, the aromatic amino acids Phe and tyrosine (Tyr) are as well precursors in the biosynthesis of phenolic compounds (Vogt, 2010). As a matter of fact, some studies have described that the addition of precursors of phenylpropanoid pathway (i.e. precursor feeding) induces the accumulation of phenolic compounds in lentil and wheat sprouts (Seo et al., 2015; Świeca et al., 2014; Świeca, 2015). Moreover, previous studies have reported that foliar application of Ur increases the concentration of Phe (Garde-Cerdán et al., 2014; Hannam et al., 2016; Verdenal et al.,

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2015) or Tyr (Garde-Cerdán et al., 2014; Verdenal et al., 2015) in grape must. Similarly, Phe application to grapevine leaves has been reported to increase grape Tyr concentration, although it seems that the most noteworthy outcome could be a marked increase in the concentration of Phe itself (Garde-Cerdán et al., 2014). Manela et al. (2015) observed that increasing internal concentrations of Phe and Tyr in *Vitis vinifera* cell suspensions caused a significant increase in the accumulation of phenolic compounds, suggesting therefore that Phe and Tyr availability could be a limiting factor in the synthesis of phenolic compounds. However, Chassy et al. (2014) proposed that Phe may not be a simply direct precursor of phenolic compounds, but it could be accumulated in grape berries as an intermediate substrate pool (i.e. phenolamides) and being further metabolized under demand from different metabolic pathways. At this point, it should be remembered that Phe is not only precursor in phenolic compounds biosynthesis, but also in the synthesis of other secondary metabolites like the volatile compounds 2-phenylethanol and 2-phenylethanal (Garde-Cerdán et al., 2015). Taking into account all these studies, it seems that there could be a direct effect of foliar application of Phe and Ur on grape phenolic compounds, as it has been recently observed (Portu et al., 2015a).

As above mentioned, results from previous researches suggest that foliar application of Phe and Ur may have a potential effect on phenolic compounds biosynthesis by grapevine. However, the research under field conditions is scarce, highlighting the need of further studying the influence of field applications of N compounds on grape phenolic composition, especially over different vintages. Therefore, the current work aims to complete previous studies performed by our research group (Portu et al., 2015a,b). In those studies, Phe and Ur were applied each at two different doses (0.9 kg N/ha and 1.5 kg N/ha) to a Tempranillo vineyard located in Rioja Alta (subregion in Rioja characterized by a significant influence of Atlantic climate; i.e. highest rainfall and lowest temperatures). Moreover, they were focused on a single year and the effect over consecutive vintages remained uncertainty. Therefore, in order to extend our knowledge on this matter, we aimed to continue our studies by carrying out the treatments with the lowest dose (0.9 kg N/ha) in a Tempranillo vineyard located in Rioja Baja; evaluating the detailed grape phenolic composition over two consecutive vintages. The lowest dose from the preliminary studies was selected since the previous results suggested that it was the dose that exerted a major impact on the phenolic composition (Portu et al., 2015a,b).

2. Materials and methods

2.1. Plant material and experimental design

The experiment was conducted over two consecutive years (2014 and 2015) in a commercial vineyard located in Alfaro, Rioja Baja (warmest and driest area of La Rioja, Spain). The altitude of the location was 335 m. Tempranillo (*Vitis vinifera* L.) vines were grafted onto 1103-Paulsen rootstock, planted in 1999 and trained to a VSP (vertical shoot positioned) trellis system. Planting density was 2976 plants/ha with vine spacing between rows and within the row of 2.80 × 1.20 m, respectively. The soil was classified as Typic Haplocalcids according to the American Soil Taxonomy (Soil Survey Staff, 2014). The commercial vineyard was fertilized in both years during winter, by applying 250 kg/ha of a NPK fertilizer (15-5-10 + 2% Mg) which resulted in 33.5 kg N/ha. Apart from the nitrogen foliar treatments, no fertilizer was applied during the growing season. Weather conditions were recorded by a meteorological station belonging to the Agroclimatic Information Service of La Rioja (SIAR) installed at about 5 km from the experimental field. Accumulated rainfall from the beginning of April to the harvest date was 174 mm in 2014 and 127 mm in 2015, while the average temperatures for the same period were 18.7 and 19.8 °C, respectively.

The trial involved the foliar application of a control and two nitrogen compounds: Phe and Ur solutions were prepared using Tween 80 as wetting agent (0.1% v/v). Control plants were sprayed with water solution of Tween 80 alone. The treatments were applied to grapevine twice, at veraison and one week later. The treatments were applied in triplicate and were arranged in a complete randomized block design with ten vines per replicate. The application was carried out using a knapsack sprayer. For each treatment, 6 L of the solution previously described were prepared. 2 L were therefore applied to each replicate, half on one side of the canopy and half on the other. Since each replicate consisted of ten vines, 200 mL/plant were sprayed over leaves, so the total amount applied in each treatment was 0.9 kg N/ha.

2.2. Grape sampling and analysis of vegetative and yield components, must oenological parameters and yeast assimilable nitrogen

Grapes were harvested on 8th September (2014) and 16th September (2015). At harvest, the total yield for each replicate was determined. Then, a random set of 150 berries per replicate was collected and frozen at −20 °C until the analyses of grape monomeric phenolic compounds were carried out. Another set of 200 berries was separated, counted, and weighed to obtain the average berry weight. Grape berries were then crushed and oenological parameters were determined in the musts. °Brix was determined by refractometry. pH, total acidity, malic acid, and potassium were analyzed in musts according to ECC official methods (1990), while the tartaric acid was determined following the Rebelein method (Lipka and Tanner, 1974). Yeast assimilable nitrogen (YAN) was determined according to the method described by Aerny (1996).

In winter, shoots per plant were counted and then pruned. Shoots of each replicate were bundled together and weighed using a hanging scale. Ravaz index was calculated dividing the yield by the pruning weighed.

Since field treatments were performed in triplicate, the results for oenological parameters and YAN are the average of the analyses of three samples (n = 3).

2.3. Determination of grape low molecular weight phenolic compounds

2.3.1. Extraction of grape phenolic compounds

Grape phenolic compounds were extracted according to Portu et al. (2015a). Briefly, 50 g of each frozen grape sample were immersed into 50 mL of a mixture of methanol/water/formic acid (50:48.5:1.5, v/v/v) and then homogenized by Ultra-Turrax T-18 (IKA, Staufen, Germany) at high speed (18,000 rpm) for 1 min, obtaining a smooth paste. Afterwards, samples were macerated in an ultrasonic bath (JP Selecta, Barcelona, Spain) for 10 min and were centrifuged at 5000 rpm at 10 °C for 10 min. The supernatant was separated and the pellet was again redissolved and extracted with 50 mL of the solvent mixture. It was performed up to three extractions. The supernatants thus obtained were combined and the volume was annotated. The grape extracts were transferred to vials and stored at −20 °C until the analyses were carried out.

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

Isolation of non-anthocyanin compounds was carried out based on Castillo-Muñoz et al. (2007). PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA) containing a mixture of reverse-phase and cation-exchanger materials were used. Cartridges were placed in the extraction system (VisiprepTM Vac-