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Phenylalanine and urea foliar application: Effect on grape and must microbiota

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ABSTRACT

The main aim of this study was to describe the impact of foliar phenylalanine and urea application on grape and must microbial populations. The tool used to perform the ecological study was DGGE conducted with several infusions in non-enriched and enriched liquid media, as well as direct DNA extractions of grapes and musts. A total of 75 microbial species were found in the study. The alpha diversity indices of grape after both foliar nitrogen treatments did not show significant changes in comparison to the control samples, but were modified in some indices in must samples. The phenylalanine must sample was similar to the control, while foliar urea application caused significant changes in microbial diversity and population structure in comparison to the control must. Further research would be necessary to properly predict the impact on winemaking of the effects observed in this study for grape and must microbiota, especially regarding the foliar application of urea.

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1. Introduction

Nitrogen is quite an important molecule in oenology. Playing a key role during winemaking, this macronutrient is involved in vine growth and is absolutely vital during the fermentation process. Consequently, nitrogen could be considered essential for elaborating high quality wine. From all must nitrogen compounds, ammonium ions and free amino acids can be readily assimilated by yeasts. In this regard, the usable nitrogen fraction corresponds to the combination of ammonium ions and amino acids except proline, and it is referred to as yeast assimilable nitrogen (YAN). A concentration in excess of 140 mg N/L of YAN is usually established as the threshold concentration for the completion of fermentation (Bely et al., 1990).

As a result, an inadequate concentration of nitrogen could contribute to sluggish fermentation. When its supplementation in the vineyard is scarce and there is a lack of nitrogen content, alcoholic fermentation (AF) cannot finish properly because yeasts do not get the necessary nutritional requirements. In contrast, excessive nitrogen availability may result in the synthesis of undesirable intermediary metabolites such as ethyl-carbamate or biogenic amines that could be detrimental to the organoleptic and health properties of the wine.

Recently, some studies have been conducted to optimize traditional nitrogen supplementation of the soil by promoting the foliar application of different nitrogen sources such as phenylalanine and urea (Portu et al., 2015a). In this respect, foliar feeding may offer some advantages with respect to traditional soil fertilization (Haytova, 2013). One of the most important advantages is the fact that foliar sprays can be applied throughout the growing season and nutrient uptake is reported to be faster compared to soil fertilization. Therefore, treatments carried out at veraison may prevent the competition between vegetative (shoots) and reproductive (grapes) sinks, a competition that has often been associated with high nitrogen supply to soil at earlier stages of the annual vine growth cycle.

Phenylalanine is a very interesting amino acid in oenology not only as a nitrogen molecule, but also a precursor in phenolic compound synthesis by the phenylpropanoid biosynthetic pathway. Thus, it is involved in the aromatic profile of wines because some yeasts can metabolize it and synthesize into intermediate aromatic compounds (Santamaría et al., 2015). Furthermore, urea is a small molecule which is highly soluble in water and easily absorbed by leaf cells. Recent studies have determined that the foliar application of both nitrogen sources, especially urea, promoted the synthesis of amino acids in musts (Garde-Cerdán et al., 2014). Furthermore, both compounds have been reported to enhance the concentration of grape phenolic compounds (Portu et al., 2015a, 2015b). Studies of foliar application of phenylalanine and urea targeted to the phenolic composition of wine have also demonstrated that anthocyanin and flavonol concentration increased in wines made from treated grapevines, which resulted in an improvement of wine colour characteristics (Portu et al., 2015a).

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The nitrogen content of musts is directly linked to the nutritional requirement of several microorganisms (Bell and Henschke, 2005). Many authors have described the importance of this compound in fermentation kinetics, focusing on the *S. cerevisiae* yeast (Bach et al., 2011; Bell and Henschke, 2005). In contrast, research into the importance of nitrogen supplementation for lactic acid bacteria (LAB) has not been extensively reviewed (Remize et al., 2006; Remize et al., 2005). Apart from studies focusing on such spoilage microorganisms as acetic acid bacteria (AAB), or even on nitrogen-fixing or environmental bacteria (EB), no references are found.

Knowledge about the impact of viticulture practices on the microbial communities of grapes and musts is scarce (Martins et al., 2012). In fact, little research has been performed in that sense. Cederlund et al. (2014) observed a change in the abundance of soil bacterial species in long-term nitrogen fertilizer regimes and Zhou et al. (2016) described how this type of fertilization reduced the soil fungal biodiversity. In addition, Zheng et al. (2016) determined an improvement in soil microbial activity after nitrogen and phosphorous fertilization.

The lack of knowledge regarding grape and must microbiota after foliar feeding, along with the significance of describing possible effects of foliar phenylalanine and urea applications on the microbial populations, defined the main objectives for this research. For these reasons, this study tries to achieve its goal by using a wide range of different methods to describe the microbial diversity of yeasts, moulds and bacteria, in grapes and musts after applying phenylalanine and urea to grapevine leaves.

2. Materials and methods

2.1. Grapevine treatments and sampling

The study was conducted in the experimental *Vitis vinifera* L. cv. Tempranillo vineyard in La Grajera-ICVV (Northern region of La Rioja, Spain) as Portu et al. (2015a) described. The vineyard was managed under a conventional soil tillage management system. Two nitrogen sources were individually applied: 250 mg N/plant of phenylalanine (Phe) and 250 mg N/plant of urea (Ur). Aqueous solutions were prepared with the corresponding concentration of both nitrogen sources using Tween 80 as wetting agent (0.1% v/v). Control plants (C) were sprayed with a water solution of Tween 80 alone. The foliar treatments were applied to grapevines at the moment of veraison and one week later. For each application, 200 mL/plant was sprayed over the leaves. Treatments were applied in triplicate and arranged in a completely randomized block design with 3 vines per replicate (Fig. 1).

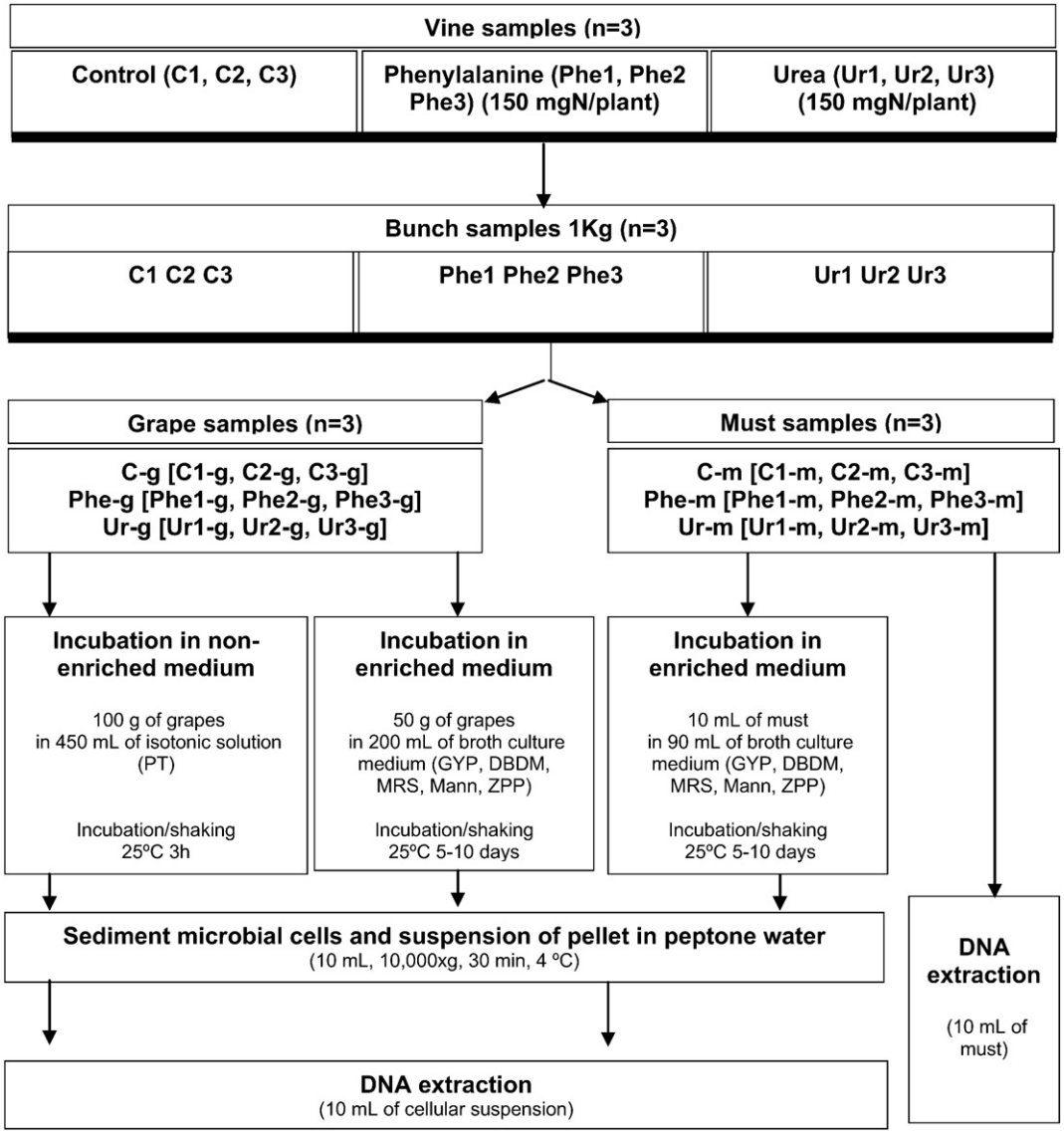

Fig. 1. Flow sheet of the experiment.

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