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Biochemistry

Kaolin exogenous application boosts antioxidant capacity and phenolic content in berries and leaves of grapevine under summer stress

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ABSTRACT

Heat waves, high light intensities and water deficit are becoming important threats in many important viticultural areas worldwide, so the implementation of efficient and cost-effective mitigation strategies is crucial for the production of premium wines while maintaining productivity. In this context, the foliar application of kaolin, a chemically inert mineral with excellent reflective properties, is being developed and experimented as a strategy to reduce the impact of heat and drought in Douro vineyards (Northern Portugal), already revealing promising results. In the present study we investigated if an improved antioxidant capacity is part of the beneficial effects of kaolin, by studying changes in the enzymatic and nonenzymatic antioxidant system in leaves and berries (cv Touriga Nacional). Results showed that mature grape berries contained higher amounts of total phenols (40%), flavonoids (24%), anthocyanins (32%) and vitamin C (12%) than fruits from control vines, and important changes were also measured in leaves. In parallel, kaolin application improved the antioxidant capacity in berries, which was correlated with the observed increased content in secondary metabolites. Kaolin application also regulated secondary metabolism at the transcriptional level through the increase in the transcript abundance of genes encoding phenylalanine ammonia lyase and chalcone synthase.

1. Introduction

Douro Demarcated Region (DDR), recognized in 2001 by UNESCO as World Heritage, is one of the iconic wine regions in the world. This widespread area of vineyards, with a crucial socioeconomic and cultural relevance, has peculiar soil and climatic characteristics providing a suitable "terroir" for premium wine production (Likar et al., 2015). Excess of temperature, high irradiance and water scarcity are well-known environmental stress factors that severely limit grapevine productivity in Douro, which are becoming particularly frequent in the context of ongoing climate change (Fraga et al., 2014).

High irradiance, including UVB radiation, is absorbed by cellular components such as proteins and nucleic acids, resulting in biomass reduction, impaired photosynthesis, reduced protein synthesis, damage to DNA and to other chloroplast functions (Schultz, 2000). Also, leads to oxidative stress (Molassiotis et al., 2006) when excess of reactive oxygen species (ROS) are produced from the disruption of metabolic activities and through the activation of membrane localized NADPH oxidase (Majer and Hideg, 2012). It is well known that oxygen radicals are remarkably reactive and cytotoxic in all organisms, since they can react with unsaturated fatty acids and thus induce the peroxidation of essential membrane lipids or intracellular organelles (Zimmermann and Zentgraf, 2005). Peroxidation leads to cell leakage, fast dehydration and finally cell death. Damage

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to intracellular membranes may influence mitochondrial respiration and induce the degradation of pigments and a loss of the CO_2 fixation ability as well as photoinhibition (Zimmermann and Zentgraf, 2005).

The negative impacts of extreme heat, water scarcity and high irradiance in vineyards prompted the search for short mitigation strategies through the application of exogenous compounds that could maintain or even improve plant productivity under such environmental stresses. Although the literature reports encouraging results in other crops, these strategies are so far less explored in grapevine. Indeed, exogenous mannitol application to salt-stressed wheat, a plant unable to synthesize this polyol, significantly increased its salt tolerance, mainly by stimulating the activity of antioxidant enzymes (Seckin et al., 2009). Also, in maize, an exogenous application of glycinebetaine limited the adverse effects of water stress by modulating water relations (Nawaz and Ashraf, 2007), and its application in tomato (Park et al., 2006), a fleshy fruit, enhanced tolerance to chilling by protecting membranes and macromolecules directly and by inducing antioxidant enzymatic mechanisms. Abscisic acid (ABA) is an important phytohormone responsible for activating drought resistance, and has been successfully experimented as an exogenous protective compound against abiotic stress in maize (Hose et al., 2000), tomato (Aroca et al., 2008), spring wheat and poplar (Du et al., 2013).

From a set of promising compounds, we experimented the exogenous application of kaolin, which is a chemically inert mineral with excellent reflective properties that has yielded promising results. Kaolin reduced leaf surface temperatures, and improved fruit maturation and quality in apple (Wand et al., 2006), and reduced leaf surface temperature and increased CO_2 assimilation rates in olive (Nikoleta-Kleio et al., 2012). In tomato, kaolin application reduced the number of sunburned fruits, exhibited protective properties against insect attack (Cantore et al., 2009) and influenced the physiological response to salinity (Boari et al., 2014). Also, in Merlot grape (*Vitis vinifera* L.), kaolin application enhanced the total amount of berry anthocyanins (Song et al., 2012) and we recently showed that in Touriga Nacional it induced a protective effect on photosystem II structure and function (Dinis et al., 2016). The protective properties of kaolin make imperative to investigate in more detail the protective properties of kaolin in grapevine against heat waves, high irradiance and water scarcity, and if an improved antioxidant capacity is part of its beneficial effects also due to its potential useful role in water management.

In the present study the effect of kaolin exogenous application was investigated on the enzymatic and nonenzymatic antioxidant capacity of leaves and berries from grapevine (cv Touriga Nacional) through approaches that included ROS-scavenging assessment by non-enzymatic methods, such as $ABTS^{\bullet+}$ and β-carotene and hydroxyl radical-scavenging activity assays, the quantification of key secondary compounds and antioxidants, and molecular biology approaches to study the effect of kaolin on the expression of two important genes of the secondary metabolism, *VvPAL1* and *VvCHS1*, which encode phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), respectively. The observed lower ROS levels, increased hydroxyl radical scavenging and enhanced production of antioxidants compounds, including phenolics, flavonoids and anthocyanins, all key metabolites in berry, are likely important mechanisms underlying the mitigation effects of kaolin on adverse abiotic climatic stresses in grapevines.

2. Material and methods

2.1. Chemicals

All chemicals and reagents were of analytical grade and were obtained from commercial sources (Sigma–Aldrich, Merck, and Pronalab). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Plant material and samples preparation

Samples were obtained from Touriga Nacional (*Vitis vinifera* L.) cultivar grafted on 110 R located in a commercial vineyard "Quinta do Vallado" in the Douro Demarcated Region (Denomination of Origin Douro/Porto) located at Peso da Régua (41°09′44.5″N 07°45′58.2″W), in northern Portugal. Touriga Nacional is considered the finest Portuguese red grape variety, and in Douro wine region is particularly important in the production of Port. The climate is typically Mediterranean-like, with a warm-temperate climate and dry and hot summers (Kottek et al., 2006), with higher precipitation during the winter months and very low during the summer. The soil, essentially of schist origin with a loam-dominated texture, is classified as dystric-surribi aric anthrosols (Agroconsultores and Coba, 1991). Vines were managed without irrigation and grown using standard cultural practices as applied by commercial farmers. Monthly maximum temperature (T_{max}) and precipitation values (April to October) are shown in Fig. 1. Temperature values were higher in July and September, while precipitation values were lower in June and August.

Three vineyard rows, with twenty plants each, were sprayed soon after veraison (17th July 2014) with 5% (w/v) Kaolin (Surround WP; Engelhard Corp., Iselin, NJ), according to previous work done by our team (Dinis et al., 2016). A second application in the same day was done to ensure Kaolin adhesion uniformity. Other three vineyard lines, with twenty plants each, were maintained as control, i.e. without Kaolin application. All rows are located side-by-side (ensuring the same edaphoclimatic conditions) on a steep hill with an N-S orientation. The vines, with 7 years, were trained to unilateral cordon and the spurs were pruned to two nodes each with 10–12 nodes per vine.

Leaves (six fully expanded leaves of the first third of the shoot per treatment) were sampled in three different dates: 23th July (one

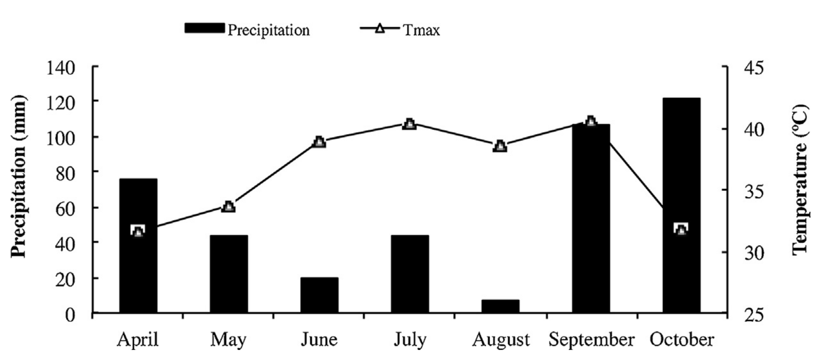

Fig. 1. Monthly values of maximum temperature (T_{max}) and precipitation at the experimental site during 2014.