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## Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature



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#### ABSTRACT

The intra-varietal genetic diversity of grapevine (*Vitis vinifera* L.) may be exploited to maintain grape quality under future warm conditions, which may alter grape berry development and composition. The present study assesses the effects of elevated temperature on the development of berry, grape composition and anthocyanins:sugars ratio of thirteen clones of *V. vinifera*. cv. Tempranillo that differed in length of the ripening period (time from veraison to berry total soluble solids, mainly sugars, of ca. 22 °Brix). Two temperature regimes (24 °C/14 °C or 28 °C/18 °C, day/night) were imposed to grapevine fruit-bearing cuttings from fruit set to maturity under greenhouse-controlled conditions. Elevated temperature hastened berry development, with a greater influence before the onset of ripening, and reduced anthocyanin concentration, colour intensity and titratable acidity. The clones significantly differed in the number of days that elapsed between fruit set and maturity. At the same concentration of total soluble solids, the anthocyanin concentration was lower at 28 °C/18 °C than 24 °C/14 °C, indicating a decoupling effect of elevated temperature during berry ripening. Thermal decoupling was explained by changes in the relative rate of response of anthocyanin and sugar build-up, rather than delayed onset of anthocyanin accumulation. Clones differed in the degree of thermal decoupling, but it was directly associated with differences neither in the length of their ripening period nor in plant vigour.

#### 1. Introduction

Climatic conditions have changed over the past decades, and simulations with different scenarios of greenhouse gas emissions show that the observed tendencies will continue in the near future [1]. According to the Intergovernmental Panel on Climate Change (IPCC), the increase of global mean surface temperature by the end of the 21 st century (2081–2100), relative to the current reference period (1986–2005), is likely to be from 0.3 °C to 4.8 °C depending on the mitigation scenario [1]. In addition, it is likely that heat waves will occur with higher frequency and longer duration as a consequence of the increase in temperature variability [1,2].

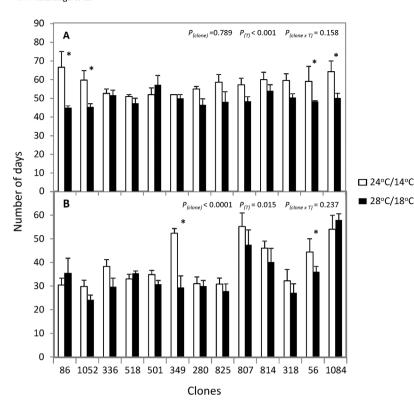
Among human activities, agriculture —in particular viticulture—is highly dependent upon climatic conditions during the growing season [3]. The grape berry is one of the fruits whose composition is highly sensitive to the environment [4], temperature being an important

environmental factor during berry development and ripening [5]. Many studies have linked increases in temperature to accelerated phenology, with the potential to greatly affect grape attributes for the production of red table wines. For example, increased background temperature has been reported to advance budburst, flowering and to hasten berry development [6-8]. Simulations using a model for the developmental stages of Riesling and Gewurztraminer predict an earlier onset of veraison (up to 23 days before), by the end of the present century compared with its timing in 1976–2008, resulting in an important increase in mean temperatures (more than 7 °C) during the ripening period [9]. Such changes will likely impact on grape and wine quality. One of the clearest relationships between temperature and fruit quality concerns grape berry acidity, as high temperatures reduce the concentration of organic acids, especially malic acid [10-12], desynchronizing sugar and organic acid metabolisms [13,14]. In addition, high temperatures during ripening decrease anthocyanin concentration in grapes [15-17],

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**Fig. 1.** Elapsed days from fruit set to mid-veraison (A) and from mid-veraison to maturity (B) of fruit-bearing cuttings of thirteen clones of Tempranillo grown at 24 °C/14 °C and 28 °C/18 °C (day/night) from fruit set to maturity. Bars are means  $\pm$  SE, n = 4–6. Probability values (*P*) for main effects of clone  $P_{(clone)}$ ; temperature,  $P_{(T)}$ ; and their interaction  $P_{(cloneT)}$ . Asterisks indicate significant differences between the two temperature regimes within each clone.

due to the inhibition of anthocyanin biosynthesis, chemical or enzymatic degradation and/or the imbalance in the expression and function of specific transmembrane transporters [16,18,19]. Elevated temperature can also uncouple berry traits, leading to an unbalanced wine. In previous studies, seed ripening was advanced in relation to other berry tissues, and this asynchrony may have direct oenological implications affecting the resultant phenolic composition and sensory attributes of wines [15,20]. In addition, a consistent thermal decoupling of anthocyanins and sugars was observed in berries of cv. Cabernet Sauvignon and Shiraz regardless of the irrigation regime and source: sink ratio, with consequences for the colour-alcohol balance of wine [21].

In order to avoid quality alterations caused by high temperatures during fruit ripening, phenology should be delayed [3]. With this aim in mind, besides changes in vineyard location (higher latitudes and altitudes) or modifications of training systems (higher trunks, late pruning, minimal pruning of reduced leaf area to fruit weight ratios) [3,22–24], plant material is a major tool for adapting vineyards to warm temperatures. Thus, ripeness can be delayed by the use of late-ripening varieties. However, if the objective is to maintain wine typicity, one alternative is to explore in depth the existing intra-varietal genetic variability. Because much of grapevine plants are reproduced by vegetative propagation, spontaneous mutations can accumulate over time [25]. When these natural events have significant phenotypical effects, the new plant can bear interesting traits, thus leading to somatic variants within a variety that can be exploited for clonal selection and propagation [26-28]. During the last two centuries, clonal selections were performed to improve vineyard health and production traits (yield, precocity, flavour and colour among others) [29]. The existing clone collections worldwide can be explored to detect any phenotypic variation that could be powerful means of adaptation to climate change, looking for either late-ripening clones or clones with a high ability to maintain some required characteristics under warm conditions.

The objective of this study was to evaluate the response of thirteen clones of Tempranillo to elevated temperature, focusing on the phenology of grape development, berry composition and the thermal decoupling of sugar and anthocyanin accumulations. The study tries to

explore the possibility of using the intra-varietal variation of the Tempranillo cultivar to maintain high quality standards in berries under future warmer conditions.

#### 2. Material and methods

#### 2.1. Plant material and growth conditions

Dormant cuttings of thirteen clones of grapevine (V. vinifera L.) cv. Tempranillo were obtained from the germplasm bank of the Institute of Sciences of Vine and Wine (Rioja Government, Spain) located in "La Grajera" (La Rioja, Spain). The studied clones were: 86, 1052, 336, 518, 501, 349, 280, 825, 807, 814, 318, 56, and 1084. They had been previously characterized in the clone bank for three years (2009-2011) focusing on phenological development (dates of budburst, flowering, veraison and maturity), grape production and must composition at harvest time, and had showed differences in the length of the ripening period (number of days between veraison and berry with total soluble solids (TSS, mainly sugars) of ca. 22 °Brix) (unpublished data). Cuttings 400-500 mm-long were selected to maximize the chances of them bearing fruit, as described in Mullins and Rajasekaran (1981) [30], with slight modifications. Briefly, rooting was induced using indole butyric acid (400 mg  $L^{-1}$ ) in a heat-bed (27 °C) kept in a cold room (5 °C). Once cuttings had developed roots, they were transplanted to 6.5 L pots containing 2:1 peat:perlite (v/v) and transferred to growth chamber – greenhouses (GCGs). Initial growth temperature conditions until fruit set were 25 °C/15 °C (day/night). Plants grew with natural light supplemented with a system of high-pressure sodium lamps (HQT-TS 400W/D Osram, Augsburg, Germany), which was triggered when photosynthetically active radiation (PAR) dropped below a photon flux density of 1000 µmol m<sup>-2</sup> s<sup>-1</sup> and used to maintain a photoperiod of 14 h (more details about GCGs in Morales et al. [31]). Plants were irrigated with the nutritive solution described by Ollat et al. [32]. Under these conditions, the bud-break took place after 1 week. Only one leaf was allowed to grow in the developing shoot, manually removing the rest of the leaves. The tip of the shoot was manually excised above the inflorescence. A new shoot (lateral) was then allowed to develop and

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