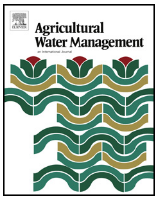
Agricultural Water Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat



Influence of irrigation strategy and mycorrhizal inoculation on fruit quality in different clones of Tempranillo grown under elevated temperatures

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ARTICLE INFO

Article history:
Received 8 May 2017
Received in revised form
21 November 2017
Accepted 2 December 2017
Available online xxx

Keywords:
Anthocyanins
Arbuscular mycorrhizal fungi (AMF)
Climate change
Clonal variability
DPPH assay
Deficit irrigation

ABSTRACT

The projected climate scenario for South Mediterranean Europe predicts lower precipitation and higher temperatures that will negatively affect viticulture in the region. The application of moderate deficit irrigation at crucial moments of berry ripening has been found to improve berry quality. Furthermore, grapevine association with arbuscular mycorrhizal fungi (AMF) may improve grapevine's ability to cope with abiotic stresses. Therefore, the aims of this research were: (1) to characterize the response of three clones of *Vitis vinifera* L. cv. Tempranillo to the combination of different water deficit programs and AMF inoculation under elevated temperatures, and (2) to determine whether AMF inoculation can improve berry antioxidant properties under these conditions. The study was carried out on three fruit-bearing cuttings clones of cv. Tempranillo (CL-260, CL-1089 and CL-843) inoculated (+M) or not (−M) with AMF and subjected to two temperature regimes (24/14 °C and 28/18 °C (day/night)) combined with three irrigation regimes during berry ripening. Irrigation treatments were: (i) water deficit from fruit set to veraison (early deficit, ED); (ii) water deficit from veraison to maturity (late deficit, LD); and (iii) full irrigation (FI). Although each Tempranillo clone seemed to have different abilities to respond to elevated temperatures and water supply, in general, at 24/14 °C the LD treatment performed better than ED. Differences among clones were attenuated at 28/18 °C. In addition, potential benefits of the LD treatment were improved by AMF inoculation. Thus, in all clones the loss of anthocyanins at 28/18 °C detected in −M plants after applying LD did not occur in the +M plants. Moreover, AMF inoculation increased must antioxidant capacity in CL-843 under these environmental conditions. Our results suggest that the implementation of measures to promote the association of grapevines with appropriate AMF for each variety could contribute to optimize effects of irrigation strategy on berry properties under future warming conditions.

1. Introduction

The grapevine is one of the most widely cultivated crops, with a total global surface area of 7.5 million ha under vines. Most harvested grapes are processed into wine, leading to a global production of 274 million hectoliters in 2015, with Spain being the third largest producer in the world (14% of the total world wine production) (OIV, 2016a). However, the future climate scenario for South Mediterranean Europe is not favorable for agriculture in general and for viticulture in particular (Chaves et al., 2010; Lionello et al., 2014) due to the predicted decreased precipitation, increased air and soil temperatures and extreme climate events (IPCC, 2014). Grapevine development has already suffered from significant impact from global climate change (Teixeira et al., 2013). Thus, a growing body of evidence indicates that as the climate warms, grapevine phenology progresses at a faster rate, grapes ripen earlier (Webb et al., 2012), berry sugar content (and subsequent alcohol in the wine) tends to increase (Petrie and Sadras, 2008) and phenolic ripeness is not always achieved (Mori et al., 2007; Sadras and Moran, 2012). In addition, the tendency toward a decreased acidity of must (Sweetman et al., 2014) has potential effects on wine aging capacity.

In the Mediterranean region, the climate may be quite dry during the grapevine growing season and vines may require additional irrigation to counteract water deficit stress (Chaves et al., 2007, 2010). Currently, irrigation of vineyards is below 10% of the total

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https://doi.org/10.1016/j.agwat.2017.12.004
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Please cite this article in press as: Torres, N., et al., Influence of irrigation strategy and mycorrhizal inoculation on fruit quality in different clones of Tempranillo grown under elevated temperatures. Agric. Water Manage. (2017), https://doi.org/10.1016/j.agwat.2017.12.004

area in Europe, but the tendency towards irrigation is increasing in order to mitigate the negative impact of climate change (Costa et al., 2016). Severe water deficit exacerbates the accelerated accumulation of sugars in grapes caused by warm temperatures (Bonada et al., 2015), which results in an imbalance between the levels of sugars and the phenolic ripening in berries (Sadras and Moran, 2012). In contrast, several studies (Santesteban et al., 2011; Zarrouk et al., 2012; Niculcea et al., 2014) have demonstrated that, under moderate water restriction, berries from red wine varieties (such as Tempranillo) had increased levels of sugars and anthocyanins. For several years, moderate deficit irrigation has been applied in order to improve cluster microclimate, increase water use efficiency, control the vegetative development of grapevines, reduce berry size and induce the accumulation of sugars and polyphenols in fruits (Wample and Smithyman, 2002). Different deficit irrigation programs maintain plants at some degree of water deficit for a prescribed part of the season (Basile et al., 2011; Intrigliolo et al., 2012). Nevertheless, high temperatures can constitute a relevant constraint to the implementation and success of the deficit irrigation (Shellie, 2011) and the timing of water deficit might need to be revised to account for the deleterious effects of elevated temperatures in water-stressed plants (Edwards et al., 2011). Thus, it has been reported that alterations in berry primary metabolism (such as sugars, organic acids and amino acids) due to warm temperatures was higher than in secondary metabolism (i.e., anthocyanins and flavonoids), which was mainly affected by timing of water deficit throughout ripening (Torres et al., 2017).

Soil microorganisms can help crops to cope with abiotic stresses (Grover et al., 2011). Amongst these microorganisms, arbuscular mycorrhizal fungi (AMF) have received increasing attention due to their numerous benefits for their host plants. The symbiotic association of plants with AMF is a common phenomenon observed in nearly 80% of plant species, including grapevines (Balestrini et al., 2010). For this reason, considerable progress has been made in the last decade towards the use of these symbiotic fungi to improve grapevine growth and yield. Mycorrhizal symbiosis has been associated with improved growth, increased tolerance against drought and/or enhanced mineral uptake from soils (Trouvelot et al., 2015). Moreover, mycorrhizal plants can accumulate higher levels of phenolic compounds in their tissues than non-mycorrhizal plants and this phenomenon is more evident when plants undergo water deficit rather than optimal irrigation (Baslam and Goicoechea, 2012). In grapevines, mycorrhizal colonization enhances water use efficiency under drought (Valentine et al., 2006) and induces the accumulation of phenolics in leaves (Eftekhari et al., 2012; Torres et al., 2015) and berries (Torres et al., 2016) under optimal irrigation, with these latter results being highly dependent on intravarietal differences of grapevines and air temperatures throughout grapevine cultivation. The phenolic compounds detected in grapes have generated remarkable interest because they have antioxidant properties that are beneficial for human health (Georgiev et al., 2014).

Currently, deficit irrigation in viticulture can be managed in order to increase the concentrations of phenolics in berries with attain the final objective of enhancing must quality and its nutraceutical properties. However, to our knowledge, no studies have assessed the contribution of AMF for improving or maintaining the benefits that different deficit irrigation programs can exert on berry quality in a future scenario of climate change. In our previous studies, we showed that the effects of warm temperatures on berry composition of Tempranillo depended on the deficit irrigation system applied and on the clone chosen (Torres et al., 2017). Moreover, under warm temperatures, the benefits of AMF inoculation on berry properties were also modulated by type of clone (Torres et al., 2016). Taking into account all these precedents, the aims of the current research were (1) to characterize the response of three clones of *Vitis vinifera* L. cv. Tempranillo to the combination of different water deficit programs (pre- and post-veraison deficit irrigation) and AMF inoculation under elevated day/night temperatures; and (2) to determine whether AMF inoculation can improve berry antioxidant properties under different climatic scenarios. Previous research (Antolín et al., 2010; Morales et al., 2016) has demonstrated that fruit-bearing cuttings are a meaningful and useful model system to study the response of berry ripening to environmental factors. Thus, potted vines were used to ensure that all clones experienced the same conditions and to control mycorrhizal inoculation and to have comparable non-inoculated plants.

2. Material and methods

2.1. Biological material and growth conditions

Dormant 400–500 mm long *Vitis vinifera* (L.) cuttings from different clones of Tempranillo were collected during the winter of 2014 from an experimental vineyard of the Institute of Sciences of Vine and Wine (Logroño, Spain) (Denomination of Origin Rioja, North of Spain). Three clones (CL-260, CL-1089 and CL-843) were selected in the field on the basis on their different agronomic traits (Table S1) and on the basis of our previous finding, which showed that phenolic content and antioxidant activity were stimulated by the combination of elevated temperature and AMF inoculation (Torres et al., 2015, 2016). Cuttings of each clone were selected for fruit-bearing according to the steps originally outlined by Mullins (1966) with some modifications as described in Ollat et al. (1998) and Antolín et al. (2010). Briefly, rooting was made in a heat-bed (27 °C) kept in a cool room (4 °C). One month later, the cuttings were planted in 6.5-L plastic pots containing a mixture of vermiculite–sand–light peat (2.5:2.5:1, v:v:v). Properties of the peat (Floragard, Vilassar de Mar, Barcelona, Spain) were pH 5.2–6.0, nitrogen 70–150 mg L^{-1}, P_2O_5 80–180 mg L^{-1}, and K_2O 140–220 mg L^{-1}. The peat was previously sterilised at 100 °C for 1 h on 3 consecutive days. At transplanting, half of the plants were inoculated with the mycorrhizal inoculum GLOMYGEL Vid, Olivo, Frutales (Mycovitro S.L., Pinos Puente, Spain) (+M plants). The concentrated commercial inoculum was derived from an *in-vitro* culture of AMF *Rhizophagus intraradices* (Schenck & Smith) Walker & Schüßler comb. nov. (Krüger et al., 2012) that contained 2000 mycorrhizal propagules (inert pieces of roots colonized by AMF, spores and vegetative mycelium) per mL inoculum. The selection of *in vitro*-produced inoculum of *R. intraradices* was based on two expected benefits: easy application of the product and low colonization of grape roots by contaminant fungi (Vimard et al., 1999). In order to facilitate its application, the concentrated commercial inoculum was diluted with distilled water to a mycorrhizal inoculum of 250 propagules mL^{-1}. Each +M plant received 8 mL diluted mycorrhizal inoculum close to the roots, thus making 2000 propagules in total. In order to compensate for a possible partial disinfection of mycorrhizal spores during the production of high quantities of the commercial inoculum, a filtrate was added to plants that did not receive any inoculum (−M plants) with the objective of restoring the helper microorganisms accompanying spores and hyphae of AMF and which play an important role in the uptake of soil resources as well as on the infectivity and efficiency of AMF isolates (Agnolucci et al., 2015). The filtrate was obtained by passing diluted mycorrhizal inoculum through a layer of 15–20-mm filter paper with particle retention of 2.5 mm (Whatman 42; GE Healthcare, Little Chalfont, UK), and each −M plant received 8 mL filtrate close to the roots.

After transplanting, the fruit-bearing cuttings were transferred to greenhouses, which were adapted to simulate climate change conditions (see details in Morales et al., 2014) until berry matu-

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