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Carbon isotope discrimination ($\delta^{13}C$) as an indicator of vine water status and water use efficiency (WUE): Looking for the most representative sample and sampling time

Amani Bchir[a,1], José M. Escalona[b,*,1], Alexander Gallé[c], Esther Hernández-Montes[b], Ignacio Tortosa[b], Mohamed Braham[a], Hipolito Medrano[b,*]

[a] Olive Tree Institute, Unit of Susah, Tunisia
[b] Grup de Recerca en Biologia de les Plantes en Condicions Mediterrànies, Departament de Biologia (UIB-IMEDEA), Universitat de les Illes Balears, Carretera de Valldemossa Km 7.5, 07122 Palma de Mallorca, Balears, Spain
[c] Bayer, Crop Science SA_NV, Technologiepark 38, 9052, Gand Zwijnaarde, Belgium

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ABSTRACT

Dry mass Carbon isotope composition ($\delta^{13}C$) is a representative parameter of water use efficiency (WUE) in plants, as previously demonstrated by different authors for different species under different environmental conditions. Nevertheless, the relationship between $\delta^{13}C$ and WUE in grapevines shows important variations among different experiments thus limiting its interest as indicator of water status or WUE. Our hypothesis was that such representativeness could be improved choosing an adequate sample and sampling time. Thus, the main objective of the present work was to identify the most representative sample and sampling time for a better assessment of grapevine water status and WUE by measuring: (i) the variability of WUE and $\delta^{13}C$ in leaves and berries along growing season; (ii) the effect of water availability on WUE parameters and on $\delta^{13}C$; (iii) the relationships between leaf and plant WUE and the $\delta^{13}C$. Experiments were performed in the field at the Univertsity of Balearic Islands (Mallorca, Spain). The first experiment was carried out during the 2012 and 2013 seasons using cv. Tempranillo plants growing in 30-l pots under three levels of soil water content: (i) Control (100% of Field Capacity); (ii) Moderate water deficit (50% of Field Capacity) and (iii) Severe water deficit (25% of Field Capacity). The second experiment was performed under field-grown grapevines in the 2013 season, in cvs. Tempranillo and Grenache under two treatments: (i) Control (irrigation, 50% ETo) and (ii) No irrigation. In both experiments the correlation between the measured $\delta^{13}C$, in both young leaves (located in the middle zone of stem) and mature leaves (located in the basal zone of the stem) and berries in different growth stages and water use efficiency measured at the leaf level defined as intrinsic WUE (A_N/g_s) were studied, as well as at the whole plant level, defined as WUE_{plant} (biomass increment/water consumed) and at the crop level, WUEc (yield/water applied). The best correlations were attained between $\delta^{13}C$ and soil water status or intrinsic WUE (A_N/g_s). Berries at ripening showed to be the most appropriate organ to estimate both, the plant water status and the leaf and plant water use efficiency in grapevines.

1. Introduction

The climatic conditions within the Mediterranean region are characterised by a typical summer drought, which corresponds with the vegetative and reproductive growth of grapevines. This constrain is promoting an increased use of irrigation, raising important concerns on the sustainability of the crop (Medrano et al., 2015). With an increase of temperature and water scarcity in arid areas as predicted in the near future according to global climate models (IPCC, 2013), water deficits may become still a more limiting factor in wine production and quality (Chaves et al., 2007). Global warming is also affecting grapevine development, as shown by changes in phenology and earlier harvests throughout the world (Jones and Davis, 2000; Webb et al., 2007). The current and future predicted changes in climatic conditions, reinforce the need to improve water use efficiency (WUE) in order to provide an environmentally friendly and sustainable viticulture. In this way, deficit

* Corresponding authors. Fax: +34 971173184.
E-mail address: jose.escalona@uib.es (J.M. Escalona).
[1] These authors contributed equally to this study.

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irrigation has been desmostrated as a tool to optimize the source to sink balance avoiding excessive vigor and improving WUE and grape quality (Flexas et al., 2010; Tomás et al., 2012; Medrano et al., 2015).

The concept of WUE always reflects a balance between gains (carbon acquisition or crop yield) and costs (water consumed by transpiration and water applied). This balance can be measured at different levels from instantaneous fluxes of CO_2 and water vapor at the leaf, plant and crop levels although in a wider context this concept is also applied to whole agricultural systems (Pou et al., 2011). At the leaf level, the rate of net CO_2 assimilation (A_N) and transpiration (E) and stomatal conductance for water vapour (g_s) allow determining the "instantaneous water use efficiency (A_N/E, WUE_{inst})", and the "intrinsic water use efficiency (A_N/g_s, WUE_i)" (Fischer and Turner, 1978). At this level, WUE can be easily measured but always at short time intervals (instantaneous gas exchange measurements).

Carbon and other stable isotopes are found in a certain concentration in the atmosphere and plant physiological processes discriminate in some way their abundance in plant tissues, thus, the isotope composition results are of great interest for multiple purposes as recently reviewed for grapevines (Santesteban et al., 2015). The carbon isotope ratio of plants dry matter ($\delta^{13}C$) is determined during photosynthesis by the differential diffusion of the two carbon isotopes ^{13}C and ^{12}C between the atmosphere (C_a) and the chloroplast (C_c), but mainly because of the important discrimination against $^{13}CO_2$ in the reaction center of Ribulose Bisfosphate carboxylase/oxydase (Rubisco). However, this Rubisco discrimination becomes less intense when CO_2 is scarce in the chloroplast, for instance due to stomatal closure. The differential amount of carbon isotopes in the plants dry matter results in an integrative estimation of the ratio between photosynthesis rate and stomatal aperture (WUE) over a determined growth period, (Farquhar and Richards, 1984). Thus, less negative $\delta^{13}C$ corresponds to higher WUE. Analysis of carbon isotopes composition in different plant tissues allows determining the average leaf WUE. The discrimination against ^{13}C is therefore a widely used indicator of WUE and water stress in different plants becoming also an interesting parameter for different purposes in relation with water use in grapevines (Gómez-Alonso and García-Romero, 2010; Santesteban et al., 2015).

Soil water content variations induced by different irrigation regimes are clearly reflected in the $\delta^{13}C$ of different tissues of grapevines, as reported by Chaves et al. (2007), De Souza et al. (2005), Romero et al. (2010), Pou et al. (2008) among others. Different plant tissues can be taken as samples for ^{13}C detection, leaves and berries being the most common ones.

For leaf samples, the correspondence can be quite high (Tomás et al., 2012), but variable depending on the environmental conditions (Medrano et al., 2015). However, when leaves and berries are sampled, berries usually show better correspondence than leaf with respect to the variations in soil water availability (estimated as leaf Ψ_{PD} or Ψ_{stem}), as clearly shown by Chaves et al. (2007). However, the correspondence resulted similar for leaves and berries in De Souza et al. (2005), and recent publications show the interest of berries, or pulp as indicators of the water status of the vine (Santesteban et al., 2012; Gómez-Alonso and García-Romero, 2010). Berry $\delta^{13}C$ has recently been proposed also as a good long-term indicator of average water status and water use efficiency for field growing grapevine (Santesteban et al., 2015), as well as to map vineyard's water availability (Herrero-Langreo et al., 2013; Costantini et al., 2010).

The lower correspondence showed by leaves is related to the fact that carbon stored in the leaves is mainly takenup before the typical summer water stress, meanwhile fruit sugar accumulation occurs later in the growing season (Chaves et al., 2007; De Souza et al., 2005; Santesteban et al., 2015). Thus, the average stomatal closure for the whole leaf-life span seems to be determinant for the value of $\delta^{13}C$ as indicator of leaf water status or leaf WUE. Most of the leaf WUE responses to plant water status are studied by instantaneous measurements (A_N/E or A_N/g_s) in potted plants without fruit and, in consequence, the leaf $\delta^{13}C$ results of particular interest to qualify this response in a wider time-scale, thus, a more detailed analysis of these correspondences would be of particular research interest.

Moreover, the $\delta^{13}C$ as surrogate character for WUE is of increasing interest to quantify the genotype variations in WUE. In fact, there are different works demonstrating the variability of the $\delta^{13}C$ and the WUE among different grapevine genotypes showing commonly good relationship among both parameters (Gaudillere et al., 2002; Tomás et al., 2012, 2014; Chaves et al., 2010; Gómez-Alonso and García-Romero, 2010; Bota et al., 2016).

From previous studies, it can be assumed that WUE parameters and $\delta^{13}C$ are affected by: (a) genotype, (b) soil water availability (c) environmental conditions (d) sampling type and sampling time. All those sources of variation complicate the reliability of $\delta^{13}C$ as indicator of plant WUE. In this sense, the main objective of the present work was to identify the most representative sample and sampling time for a better assessment of grapevine WUE based on $\delta^{13}C$ measurements. To achieve that, it is required to determine: (i) the variability of WUE and $\delta^{13}C$ in leaves and berries between different phenological stages; (ii) the effect of water availability on WUE parameters and $\delta^{13}C$; (iii) The relationships between leaf and plant WUE with respect to $\delta^{13}C$.

2. Material and methods

2.1. Plant material and treatments

2.1.1. Experiment 1(Pot experiment)

The experiment was performed in two consecutive years with Tempranillo cultivar grafted on 110-Richter. The first year experiment was carried out in the summer of 2012 (from June 1st to August 24th). The second year experiment was performed in the summer of 2013 (from June 11th to August 15th). The experiment was located at the Campus of the Balearic Islands (Mallorca, Spain). Plants were grown in 15 L pots filled with organic substrate and perlite mixture (3:1). Plants were irrigated daily from May until the start of the experiment, and supplemented with organic-mineral fertilizer containing (%): N, 5; P_2O_5, 8; K_2O 15; MgO, 2; organic C, 17.4, humic acid, 5; SO_3, 15; Fe, 1; Zn 2×10^{-3}; Mn 1×10^{-2}. A thick layer of perlite was added to the surface of each pot to decrease water loss through evaporation.

Three levels of soil water content were established as treatments, defined by the field capacity (FC): (i) control (100% of FC); (ii) moderate water stress (50% of FC) and (iii) severe water stress (25% of FC) with 5 replicates for each treatment. Once the levels of water stress per treatment were reached, soil moisture content of each pot was maintained constant until the end of the experiment by replenishing daily the amount of water used. This was monitored by weight losses using mini-lysimeter (Bench scale, TQ 30P, 30 × 40 cm, 30 kg/2 g, Baxtran). The daily weight of pots was obtained for each treatment (100% FC, 50% FC or 25% FC), before and after irrigation. Water consumption (WC) was calculated as:

$WC(day_n)$ = pot weight after irrigation ($day_{(n-1)}$) – pot weight before irrigation (day_n).

The water amount used to maintain the 100% of FC treatment (soil moisture between 35% and 45%) were about 171 L/plant in 2012 and 156 L/plant in 2013 (Table 6). Plants subjected to 50% of FC treatment consumed 108 L and 115 L in 2012 and 2013, respectively, and plants irrigated at 25% ETo needed a 38 and 52 L per plant in 2012 and 2013 respectively. At the begining of the experiment,