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Thermal history parameters drive changes in physiology and cold hardiness of young grapevine plants during winter

Francisco Gonzalez Antivilo[a,b,*], Rosalía Cristina Paz[c], Mariela Echeverria[d], Markus Keller[e], Jorge Tognetti[f,g], Roberto Borgo[h], Fidel Roig Juñent[b]

[a] INTA EEA-San Juan, Calle 11 y Vidart (5427), Villa Aberastain, San Juan, Argentina
[b] Laboratorio de Dendrocronología e Historia Ambiental, IANIGLA, CCT-CONICET-Mendoza, Av. Ruiz Leal s/n, Parque Gral. San Martín, CC 330, PO Box 5500 Mendoza, Argentina
[c] CIGEOBIO (FCEFyN, UNSJ/CONICET), Av. Ignacio de la Roza 590 (Oeste), J5402DCS, Rivadavia, San Juan, Argentina
[d] Conicet-Universidad Nacional de Buenos Aires, Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA), Buenos Aires, Argentina
[e] Department of Horticulture, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA 99350, USA
[f] Laboratorio de Fisiología Vegetal - Facultad de Ciencias Agrarias – Universidad Nacional de Mar del Plata, Buenos Aires, Argentina
[g] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina
[h] Cátedra de Fisiología Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500, Luján de Cuyo, Mendoza, Argentina

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ABSTRACT

Vitis vinifera is mainly cultivated in temperate areas, where seasons are well defined and winter conditions might be severe. To survive under these conditions during the dormant season, grapevines sense environmental parameters to trigger different protective mechanisms that lead to cold hardiness (CH). Crop yield and sustainability will be determined according to the level of CH reached in each organ. Moreover, different cultivars of *V. vinifera* exhibit different behavior throughout the dormant season, attaining a different status of CH. However, there is scarce information concerning how the same cultivar behaves under contrasting thermal environments. The aim of our research was to unveil how CH varies in trunks of the same cultivar under two contrasting environments and define which are the main thermal and biochemical parameters involved in this process. We submitted 2-year old plants of the same clone of cv. Malbec to two different thermal conditions: natural winter (control) and artificially warm winter (treatment). CH status, thermal and biochemical parameters in trunks were measured periodically over the dormant season, and this experiment was repeated for three years. Our results suggest that grapevine trunks subjected to a different environment reach dissimilar CH status, except at the end of winter. In addition, we determined that daily minimum temperature is the main thermal parameter that drives changes in CH. Also, we found that the total soluble sugars have the greatest relative weight in determining the CH compared with the other compounds evaluated. These results have practical implications in the establishment of vineyards for new growing regions. Moreover, with rising minimum temperature predicted by climate change scenarios, grapevines may be more vulnerable to cold events during the dormant season.

1. Introduction

Vitis vinifera is a perennial liana adapted to temperate climates, capable of surviving to relatively low temperatures during the winter. The acquisition of dormancy and cold hardiness (CH) is an active, dynamic and complex process with physiological-biochemical adaptations (Weiser, 1970; Shaulis, 1971; Chen and Li, 1977). Classically, the dormant period is divided into three stages: acclimation, characterized as a period of transition from the non-hardy to the fully hardy state; ii) mid-winter, characterized as a period of most severe cold and greatest CH; and iii) deacclimation, characterized as a period of transition from the fully hardy to the non-hardy state and active growth (Howell, 2000; Ferguson et al., 2014). Traditionally it is thought that during mid-winter CH reaches a threshold of maximum resistance, a factor that is considered constant and independent of weather, even if a warm event occurs (Proebsting et al., 1980; Zabadal et al., 2007; Beck et al., 2004). For that, CH had been described as a U-shaped curve with a maximum hardiness level MHL) thought to be as constant for each year

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* Corresponding autor at: INTA EEA-San Juan, Calle 11 y Vidart (5427), Villa Aberastain, San Juan, Argentina.
E-mail address: ing.gonzalezantivilo@gmail.com (F. Gonzalez Antivilo).

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(Proebsting et al., 1980). CH is usually measured as the lethal temperature required to kill 50% of tissues (LT_{50}).

Surviving cold temperatures may involve different plant strategies, namely freezing tolerance and/or freezing avoidance, as defined by Levitt (1980). The avoidance of ice formation in plant tissues is linked to cryoprotective compounds that have the function of lowering the freezing point of the cytoplasm (supercooling). Some compounds that were reported to have cryoprotectant properties include simple soluble sugars and free amino acids (Pierquet and Stushnoff, 1980; Guy, 1990; Fennell, 2004). Moreover, tissue dehydration has also been cited as another mechanism to prevent ice formation. The other mechanism is freezing tolerance, which is the capacity to tolerate both ice in the apoplast and the high concentration of solutes in cells (Levitt, 1980).

Grapevine exploits both of these mechanisms. In buds the CH is based predominantly on supercooling but in woody tissues the two mechanisms occur simultaneously (Burke et al., 1976; Andrews et al., 1984; Badulescu and Ernst, 2006). The efficacy of these mechanisms depends on temperature, species, and cultivar in question, meaning that there is a genetic potential of CH (Keller, 2010). It has been reported that *V. vinifera* is not particularly cold hardy, suffering more freeze damage during winter compared with American grapevines species (Londo and Kovaleski, 2017).

Macroclimatic changes may affect local winter conditions. Mendoza Province (MZA), Argentina, between 32° and 36 °South latitude, is the most important grapevine (*V. vinifera*) production region in South America (almost 160,000 ha). Its macroclimate is dry and temperate with a high continentality due to the proximity of the Andes mountain range. This results in a large thermal amplitude over the day/night cycle and between different seasons (Gonzalez Antivilo et al., 2017). Moreover, during winter and spring a strong, dry and warm föhn wind called Zonda is common (Norte and Simonelli, 2016) and may be followed by freezing events that cause injury in fruit trees and reduce plant yields (Caretta et al., 2004).

MZA is a desert region with less than 200 mm/year of precipitation. Therefore, the crops are irrigated with water from melting snow in the Andes, which leads to the crops being concentrated in four small productive oases with different agroclimatic characteristics, partially defined by their geographic location as North, East, Central and South oases (Suppl Fig1A; González et al., 2009; DACC, 2013). More than 20 grapevine cultivars are grown in MZA. The most emblematic cultivar is Malbec which has experienced a strong increase in the last 15 years, more than doubling in the production area to reach 40,000 ha, and distributed in all oases (Instituto Nacional de Vitivicultura, INV, 2016).

According to the IPCC (Stocker et al., 2013) projections, a temperature increase between 2 °C and 4 °C for the next 100 years is expected worldwide. In MZA there has already been an increase in the average minimum winter temperature over the last 50 years (Deis et al., 2015). Other predictions also indicate that climate contingencies will be more extreme, including cold and heat wave events, and a longer frost-free period (Aruani, 2010). Plants will live in a riskier environment if it is fluctuating (Londo and Kovaleski, 2017). In the last decade, hard winters with very low absolute temperatures and late frosts were registered, affecting several production areas in MZA (DACC, 2013). For example, severe freezing events were recorded in large parts of the province, both during the dormant ($<$ -15 °C) and growing ($<$ -4 °C) seasons during 2015 and 2016. By coincidence, the INV indicated grape harvest losses up to 30% for these seasons, compared with the previous harvests. Therefore, as cold injury affects both yield and vineyard sustainability, it is necessary to enhance local information to assist producers and government agencies in zoning existing cultivated areas by variety and in better matching varieties to specific zones at the time of planting a new vineyard.

The objective of this study was to determine if the CH status of *V. vinifera* can be affected by the thermal history during the dormant season. Our strategy consisted in subjecting plants to two contrasting thermal environmental and to evaluate the change in CH in order to establish the relationship between the process of acclimation-deacclimation and different thermal parameters. With this, we tried to establish which parameters explain this relationship better. Moreover, we wanted to unveil periods throughout the dormant season during which the thermal history can influence the maintenance of CH. This information could be linked to agroecological characteristics of each production oases of MZA within the framework of climate change predictions. Finally, we measured the seasonal changes of different physiochemical parameters involved in cold acclimation in order to determine which one may be the most influential in this process.

2. Materials and methods

2.1. Field experiments

2.1.1. Locations and plant material

Three independent experiments were conducted during the winter season (June to September) of the years 2012, 2013, and 2016 (hereinafter referred to as Y-1, Y-2, and Y-3, respectively). During the first two years, assays were carried out in Luján de Cuyo (33° 35′ 24″ S; 68° 30′ 00″ W; 925 m asl), whereas in the third year, it was conducted in Godoy Cruz (32°55′6.69″S; 68°50′32.82″O; 787 m asl), both located in the northern agricultural oases of MZA, Argentina.

For each experiment, 200 2-year-old, own-rooted Malbec certificated clone Perdriel plants were used. Plants were grown in 7-liter pots filled with a mixture of soil:sand:perlite (2:1:1 by vol). During the dormant season (autumn to winter), plants were watered every 15 days. During the growing season (end of winter and spring), at the start of bud burst, watering frequency was increased to twice a week. In order to maintain the canopy in healthy conditions standard pest control strategies were applied until the natural leaf fall. During the months prior to the application of thermal treatments (March to May), plants were grown outdoors.

2.1.2. Thermal treatments, monitoring and ecological characterization

At the beginning of the winter season, plants were randomly divided into 2 groups of 100 plants each. Each group was assigned to a different thermal treatment: natural winter (W_N) and artificially warm winter (W_W). The W_N was considered as control, and consisted of maintaining plants under natural winter field conditions, whereas the W_W treatment consisted of increasing the temperature by using a greenhouse and adding an external source of heat. During Y-1 and Y-2, a 1000 W electric fan heater installed 1 m above soil level was placed within a 2 × 3 m greenhouse coated with 200-micron crystal polyethylene UV protection. In Y-3, a natural gas heater of 3000 cal/hour was placed 10 cm above the ground in a 3 × 4 m greenhouse with the same coating. Heating was performed throughout every night (approximately from 8:00 PM to 7:00 AM of the next day). There were differences in the way heat was applied: i) in Y-1, the electric heater was programmed to be turned on 30 min and turned off 30 min each hour; ii) in Y-2, the heater remained on for 2 h and off for 1 h per cycle; iii) the natural gas heater remained on all night long.

During Y-1 and Y-2, the temperature was monitored using iButton sensors (Thermochron DS1922L-F5 temperature loggers, Maxim Integrated, San Jose, CA, USA, with a measurement range of -40 to + 125 °C, and accuracy ± 0.5 °C); whereas during Y-3, an Arduino mega 2560 logger integrated with DS18B20 sensors developed by IANIGLA-CONICET was used after contrasting and checking with iButton. In all years, two sensors per treatment were installed.

In order to characterize and compare the ecological environments generated by treatments over grapevine physiology during the dormant season (from April 1 until August 31) of Y-1, Y-2 and Y-3, two ecological indices were calculated according to Deis et al (2015): i) ΣTmin, corresponding to the summation of daily minimum temperature (Tmin) during the dormant season and ii) n°D $<$ -3, corresponding to the total number of days that reached temperatures equal to or lower than