Scientia Horticulturae 174 (2014) 155–163

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Using differential thermal analysis to analyze cold hardiness in the roots of grape varieties

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ARTICLE INFO

Article history:
Received 23 November 2013
Received in revised form 7 May 2014
Accepted 8 May 2014
Available online 11 June 2014

Keywords:
Grape, Cold hardiness
LT–I
Root
DTA

ABSTRACT

In this study, a system for differential thermal analysis (DTA) was applied for low temperature exothermic (LTE) analysis of the roots of several grape varieties. We determined lethal temperature–injury (LT–I) values of phloem and xylem using a three-point method. We performed a comprehensive evaluation on LT_{20} to LT_{80} of different grape varieties using subordinate function value analysis. The result indicate that Beta had the strongest cold hardiness of all the rootstocks examined, followed by SO4, Dogridge, 3309C, 5BB, 101-14M and 140Ru, while 1103P and 110R had the weakest cold hardiness. Of the cultivars tested, Frontenac had the strongest cold hardiness, followed by Golden Queen, Chambourcin and Beck memento, with Summer Black, Merlot noir, Vidal and Riesling exhibiting moderate cold hardiness and Syrah, Carmenere, Dorn felder and Tannat the least cold hardy. We examined the root cross-sectional anatomy and determined the relative water content in 12 varieties with different cold hardiness gradients and performed correlation analysis of the subordinate function values. We found that in grape root tissue, low water content and free water content and high bound water and bound water/free water content resulted in large subordinate function values for LT_5, which indicates good cold hardiness. Grape varieties with thick root exodermis layers, thin phloem and a high proportion of xylem had better cold resistance than the other varieties.

1. Introduction

Freezing injury in grapes is a key factor restricting grape production. Freezing injury reduces grape yield significantly, and causes an enormous loss of manpower and material resources due to the requirement for re-plantation in severe cases (Clore et al., 1974; Zabadal et al., 2007). The roots of grapes are the organs with the weakest cold hardiness during the dormant period. Root injury is a major factor that leads to the death of the whole grape plant (Ahmedullah, 1985). The ability to resist cold temperatures is derived from genetic variation and natural selection and represents a long-term adaption to low-temperatures encountered in cold environments (Zabadal et al., 2007). Cold hardiness varies remarkably with species, variety and organ. Previous studies have shown that canes and buds in most grape varieties can survive at −20 °C (Clore et al., 1974; Gu, 1999; Mills et al., 2006), but cold hardiness in roots is much lower than that in aboveground portions (Guo et al., 1989; Ahmedullah and Kawakami, 1986; Okamoto et al., 2000).

Low temperature exothermic (LTE) analysis, which can be used to compare the cold hardiness of different plant varieties, has been undergoing improvement since the 1970s. LTE analysis is a technique used to detect, record and analyze low-temperature exotherms, which generate ice crystals under low-temperature conditions in the tissues, using a PC-aided differential thermal analysis (DTA) system and further to assess the cold hardiness of plant tissues (Guy, 2003; Repoa et al., 2008).

Initially, the half-lethal temperature (LT_{50}), LT_{10} or LT_{90} was applied to analyze initial low-temperature exothermic data (Andrews et al., 1984; Jones et al., 1999; Hartley et al., 2001; Mills et al., 2006; Ferguson et al., 2011; Proebsting et al., 1980), but these values failed to fully reflect the cold hardiness of different varieties. Using LT_{50} and slope Qlt (lethal temperature coefficient) to assess the cold hardiness of buds (Gu, 1999) provided a new concept for analysis of roots' cold hardiness.

Abbreviations: DTA, differential thermal analysis; LTE, low temperature exothermic; LT–I, lethal temperature–injury.

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http://dx.doi.org/10.1016/j.scienta.2014.05.002
0304-4238/

2. Materials and methods

2.1. Experimental materials

All grape roots at 10–15 cm soil depth examined in this study were sampled from the vineyard at the Horticultural Experiment Station of Shandong Agricultural University, Tai'an, China, early December, 2012. Tai'an county (116°20′–117°59′ E latitude, 35°38′–36°28′ N longitude, 697 mm annual rainfall, 13 °C annual temperature, −16 °C minimum temperature in winter) is situated in the central of Shandong. It has a continental monsoon climate featuring four distinct seasons. The rootstocks included 110R, 1103P, 140Ru, 101-14m, 5BB, 3309C, Dogridge, SO4 and Beta. *Vitis vinifera* cultivars (a Eurasian species) included Merlot noir, Syrah, Riesling, Tannat, Dorn Felder, Carmenere and Beck Memento; the interspecific hybrids (interspecific crossing) included Vidal (Jurancon Blanc × Saint Emilion), Chambourcin (Seyve Villard12-417 × Seibel 7053), Frontenac (Landot 4511 × Riparia 89), Summer Black (Kyoho × Thompson Seedless) and Golden Queen (Black Alicante × Ferdinand de Lesseps).

2.2. Experimental instruments

The DTA system consisted of a data acquisition system and a program-controlled cooling climate chamber. The program-controlled cooling climate chamber was cooled by a Model EHS-100 program-controlled freezer manufactured by Suzhou Zhihe Environmental Experimental Equipments Co., Ltd (KiangSu, China). The chamber size was 500 mm × 500 mm × 400 mm, the operating temperature ranged from −40 to +150 °C and the temperature fluctuation was ±0.5 °C. The data acquisition system employed in this experiment, which was developed by the Mechanical and Electronic Engineering School of Shandong Agricultural University (Hou et al., 2012), included 30 Peltier thermoelectric modules (TEMs, CP1.4-127-045L Melor), each of which was enclosed in a small, square box by PVC plates (40 mm × 40 mm × 15 mm). The function of this system was to sense and convert the freezing exotherms into voltage output while recording the temperature change during the exothermic process using a resistance temperature sensor (pt100, accurate up to 0.1%). The data were amplified using a signal amplification system and saved onto a secure digital memory card (SD card) in Excel format and synchronously displayed on a 3.4-in. TFT true color screen for real-time observation of the experimental process.

A model WAY-2S Abbe refractometer (manufactured by Shanghai Yice Instruments Equipments Co., Ltd., China) was used to measure refractive index in the range of 1.30 to 1.70 ± 0.0002, and the sucrose mass fraction was measured in the range of 0–95%.

2.3. Experimental methods

2.3.1. LTE determination method

Roots of different varieties were cut into sections (at a thickness 0.3–0.5 cm and a length of 2.5 cm) using a pruner and sealed with wax at both cut-ends. Each root section was placed into a single thermoelectric module (five to 16 replicates). The roots were pressed with a plastic foam plate (40 mm × 40 mm × 10 mm) to maintain adequate contact between the roots and the TEMs. The temperature was then reduced using the following scheme: room temperature to 0 °C for 1 h, 0 °C for 1 h, 0 °C to −32 °C for 8 h and −32 °C for 1 h (Andrews et al., 1984; Mills et al., 2006; Quamme, 1973; Wample et al., 1990).

2.3.2. Determination of bound water content and root anatomy

2.3.2.1. Determination of bound and free water contents. Roots with similar thicknesses (0.3–0.5 cm) were selected from different varieties, cut into 0.02–0.05 cm-long sections with pruners and placed into three randomly numbered 50 mL tared conical flasks. Next, 5 mL of 60% sucrose solution was added to approximately 0.8–1.0 g of root tissue. The sucrose solution in each flask was then accurately weighed. Each flask was sealed with film and shaken on a shaker for 12 h. The concentration of sucrose solution in each flask was determined using an Abbe refractometer. The concentration of the original sucrose solution was determined simultaneously. The fresh weights of the remaining roots were measured, and the roots were then incubated in an oven at 105 °C for 15 min to kill the tissues. The roots were then dried to constant weight at 80 °C. The water content in each tissues was calculated. The free and bond water contents were calculated using the following equations:

$$\text{Free water content}\,(\%) = G \times (D_1 - D_2)/W \times 100$$

where G represents the quantity of the sucrose solution (g), $D1$ represents the original sucrose solution (%), $D2$ represents the sucrose concentration (%) after root immersion and W represents the fresh weight (g).

$$\text{Bound water content}\,(\%) = \text{tissue water content}\,(\%) - \text{free water content}(\%)$$

2.3.2.2. Determination of root cross sectional area and proportions. Roots with similar thicknesses (0.3–0.5 cm) were selected from different grape varieties and cut transversely with a single-edge blade. The roots were cut as thinly as possible. Eight to 10 slices were placed onto each slide and pressed with another slide to help flatten the slices. The images were captured with an Olympus camera. The thickness of the (exodermis + cortex + endodermis), (periderm + pericycle + phloem) and (xylem + stele) were measured using PC-aided image processing software. The exodermis, cortex and endodermis, which are located outside of the periderm, are referred to as exodermis below; the periderm, pericycle and phloem are referred to as phloem below; the xylem and pith, which are located inside of the phloem, are referred to as xylem below. The proportions of exodermis to radius, phloem to radius and xylem to radius were calculated.

2.3.3. Evaluation of cold tolerance by the electrolyte leakage method

A semi-lethal low temperature (LT_{50}) was determined using 0.5 g roots of different grape varieties, according to methods described by Deng et al. (2011) with minor revision. The temperature treatments were 4, −3, −6 and −9 °C with 48 h treated time, respectively.

2.4. Data analysis

LT–I regression analysis was performed according to the bud cold-hardiness analysis method (Gu, 1999). Variance analysis was performed using the LSD method for LT_{P0}, LT_{P50}, LT_{P100}, LT_{X50} and LT_{X100} in Table 1. In Table 2, except for LT_{P100} obtained by LTE spectrum analysis (Table 1), the degrees of injury of all varieties at different temperatures were estimated from LT–I. Variance analysis was also performed using the LSD method for tissue water, free water, bound water contents in Table 3a and proportion of exodermis to radius, phloem to radius and xylem to radius in Table 4. Differential analysis was performed on the slope (lethal temperature coefficient, Qlt) using the method as found on the website (http://www.ats.ucla.edu/stat/stata/ado/analysis/). The cold hardiness of the roots was comprehensively assessed using the subordinate function value method.