Scientia Horticulturae 125 (2010) 648–653

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Similarities between natural and chemically induced bud-endodormancy release in grapevine *Vitis vinifera* L.

Ricardo Vergara, Francisco J. Pérez*

Facultad de Ciencias, Lab Bioquímica Vegetal, Universidad de Chile, Casilla 653, Santiago, Chile

ARTICLE INFO

Article history:
Received 30 November 2009
Received in revised form 10 May 2010
Accepted 19 May 2010

Keywords:
Dormancy release
Grapevine buds
Hydrogen cyanamide

ABSTRACT

Bud-break in grapevines (*Vitis vinifera* L) can be poor and uneven in locations that have warm winters with insufficient chilling. Growers in these regions use hydrogen cyanamide (HC), a dormancy-breaking compound, to increase and synchronise bud-break. Previous studies demonstrated that HC upregulates the expression of select genes encoding enzymes belonging to the ascorbate glutathione cycle (AGC) (glutathione reductase, VvGR) to the oxidative pentose phosphate pathway (oPPP) (glucose-6-phosphate dehydrogenase, VvG6PD) and a key enzyme for dormancy release (1,3-β-glucanase, VvβGLU). Here, the expression of these genes was studied in grapevine buds throughout the natural progression of endodormancy (ED) and under the effects of the dormancy-breaking compounds, HC and sodium azide. Although gene expression increased with the progression of ED until mid-May, in early-June they turned off transiently and reappeared in mid-July before bud-break. The upregulation of these genes under both natural and artificial conditions suggests that dormancy-breaking compounds activate the same metabolic pathways that are naturally activated during the bud-ED release process. However, in contrast with the effects of dormancy-breaking compounds, artificial chilling did not upregulate the above genes.

1. Introduction

Bud dormancy in woody perennials is a physiological stage that enables plants to survive long periods of adverse conditions and is characterised by growth cessation, arrest of cell division, and reduced metabolic and respiratory activity (Faust et al., 1997; Arora et al., 2003). Different degrees of dormancy found in plant meristems have been defined as paradormancy (PD), endodormancy (ED) and ecodormancy (ECD) (Lang, 1987). During bud-ED, growth cessation is due to an endogenous signal, whereas the cessation during PD is due to apical dominance and during ECD to environmental conditions (Lang, 1987). In some grape varieties, decreasing photoperiod triggers the transition of buds into ED (Fennell and Hoover, 1991; Salzman et al., 1996; Wake and Fennell, 2000; Kühn et al., 2009). Moreover, the expression of PHYA and PHYB in grapevine buds suggests a role of this embryonic organ in the perception of photoperiod (Pérez et al., 2009a). On the other hand, exposure to low temperature commonly referred to as the chilling requirement, must be fulfilled by grapevines and other deciduous fruit trees for the release of buds from ED and for their homogeneous sprouting in spring (Saure, 1985; Dokoozlian et al., 1995). Warm winters limit the productivity of the table grape in many regions (Erez, 1995), and prolonged ED, has been identified in these regions as a major obstacle for commercial production (Shulman et al., 1983; Saure, 1985; George et al., 1986; Dokoozlian et al., 1995). Applications of hydrogen cyanamide (HC) in late winter are widely used by producers in these regions to homogenise and advance bud-break to ensure commercially viable yields (Henzell et al., 1991; Erez, 1995). Understanding how bud-ED release is regulated and the role that dormancy-breaking compounds and chilling play in the process may assist in developing new dormancy-breaking strategies that do not rely on chilling, and this understanding will facilitate the searching for traits that could be used as informative markers for low chilling requirements in breeding programs.

Several recent studies in grapes have focused on bud-ED release, and HC has been used as a tool because it provides a controlled, synchronised and relatively rapid induction of dormancy release (Pang et al., 2007; Halaly et al., 2008; Pérez et al., 2009b; Ophir et al., 2009). Although the mode of action of HC is unknown, it has been demonstrated that catalase expression and activity are inhibited and H_2O_2 levels increased in HC-treated buds, (Shulman et al., 1983; Nir and Lavee, 1993; Or et al., 2002; Pérez and Lira, 2005). Applications of HC to grape buds, also upregulate hypoxic (PDC, ADH, SuSy) and oxidative stress-responsive genes (TrxH, GST, APX, GR) (Halaly et al., 2008; Keilin et al., 2007; Pérez et al., 2008a). Moreover, comparative studies among different dormancy-releasing stimuli led to the hypothesis that as a consequence of mitochondrial perturbation, transient respiratory and oxidative stresses are involved in

* Corresponding author. Tel.: +56 2 9787249.
E-mail address: frperez@uchile.cl (F.J. Pérez).

0304-4238/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2010.05.020

the mechanism of dormancy release in grapevines (Keilin et al., 2007; Halaly et al., 2008; Pérez et al., 2009b; Ophir et al., 2009). In this paper, we studied, under controlled conditions, the effect of HC on the bud-break response of buds from different dormancy statuses. The expression of selected genes related to the dormancy release process was analysed throughout the dormancy cycle and compared with the effect of dormancy-breaking compounds and chilling on buds from the same dormancy status.

2. Material and methods

2.1. Plant material

Plant material was collected from 8 years-old (*Vitis vinifera* L cv. Thompson Seedless) vineyards growing at the experimental station of the Chilean National Institute of Agriculture Research (INIA) located in Santiago (33°34′S). Canes were randomly collected every 3 weeks starting in April and ending in late winter (mid-August). Canes were cut off at both ends leaving the central section with 10–12 buds for further experiments.

2.2. Effect of HC application date on the bud-break response of grapevines

On each collection date two groups of 30 single-node cuttings (10–12 cm long) was prepared. One group was treated with 5% (w/v) Dormex (a commercial formulation containing 49% hydrogen cyanamide (HC), SKW, Trotsberg, Germany) and the other group was treated with water as control. Immediately after treatments, the cuttings in the plastic tray were transferred to a growth chamber set at 23 ± 2 °C under a 16-h photoperiod at 350 μmol m^{-2} s^{-1} (forced conditions). Bud-break was assessed every 5 days for a period of 60 days and breaking of bud dormancy was indicated by the presence of visible green tissue appearing at the tip of the bud. A parameter developed to estimate the mean time required to reach 50% bud-break under forced conditions (BR_{50}) was used to determine the depth of bud-ED (Pérez et al., 2007). Differences in BR_{50} values between HC-treated and control buds (ΔBR_{50}) was used to measure the intensity of the HC effect on the bud-break response.

2.3. Chilling treatments in grapevine buds

Canes were randomly collected on 9 June during the phase of ED release in accordance with dormancy status analysis carried out previously (Pérez et al., 2007) and confirmed in this work. Canes were cut off, leaving 10–12 buds from the basal and central sections. Buds were wrapped in moist paper, placed in sealed plastic bags and moved to cold storage (4 °C) for 400 h without light exposure.

2.4. Primer design

Gene-specific primers were designed using Primer3 (Rozen and Skaletsky, 2000) based on the EST database from the University of California (http://cfg.ucdavis.edu) and corroborated by the Vitis genomic database GENOSCOPE (http://www.genoscope.cns.fr/vitis). For VvGR, VvG6PD-cyt and VvβGLU22, specific primers are described in (Pérez et al., 2009b), and primers for VvβGLU78 were designed based on the sequence in the NCBI database (http://www.ncbi.nlm.nih.gov) and corroborated by the Vitis genomic database (Table 1).

2.5. RNA isolation and cDNA synthesis

Total RNA was isolated from endodormant grapevine buds (0.5–0.7 g fr. wt.) using a modification of the Chang et al. (1993) method described in Noriega et al. (2007). DNA was removed by treatment with RNase-free DNase (1 U/μg) (Invitrogen, CA, USA) at 37 °C for 30 min. First-strand cDNA was synthesised from 5 μg of purified RNA with 1 μL oligo$(dT)_{12–18}$ (0.5 μg × μL^{-1}) as primer, 1 μL dNTP mix (10 mM) and the Superscript ™ III RNase H-RT kit (Invitrogen, CA, USA).

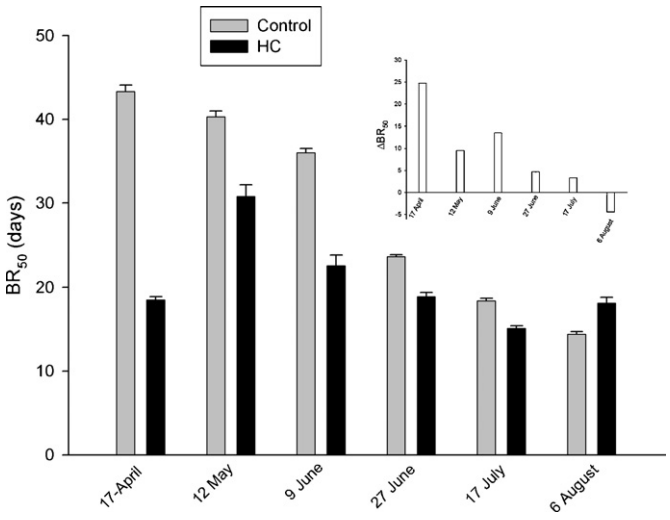

Fig. 1. Effect of HC application on the bud-break response of grapevine buds of different dormancy statuses. Grapevine buds were harvested on different dates throughout the fall winter season and sprayed with 5% (w/v) Dormex or water before placing them in the growth chamber at 23 ± 2 °C under a 16 h photoperiod. Bud-break was assessed every 5 days for a period of 60 days, and BR_{50}, a parameter that estimates the time required to reach 50% bud-break under forced conditions, was determined (see Section 2). Differences in BR_{50} between HC-treated and control buds (ΔBR_{50}) on the different harvest dates are shown as an insert.

2.6. PCR and sequencing

Template cDNA (1 μL) was placed in a final volume of 12.5 μL in PCR tubes containing 0.25 μL of each primer (10 μM), 0.2 μL Platinum Taq DNA Polymerase (Invitrogen, CA, USA) (5 U × μL^{-1}), 0.25 μL of dNTP Mix (10 mM), 1.25 μL of 10× PCR buffer (Tris-HCl 200 mM pH 8.4 and KCl 500 mM), 0.3 μL $MgCl_2$ (50 mM) and 9.1 μL DEPC water. PCR was performed in a thermocycler (MJ Research PTC-150, Watertown, MA, USA) with, 35 cycles of 30 s at 94 °C for denaturation, 30 s at 55 °C for annealing and 45 s at 72 °C for extension. A final extension was performed at 72 °C for 10 min. PCR products were separated in 1.5% (w/v) agarose on 1× TAE buffer (50× TAE: Tris, 0.038 M, EDTA1 mM, glacial acetic acid 1.1% (v/v)) at 70 V and visualised by ethidium bromide.

For sequence analysis, PCR-amplified DNA and cDNA were purified using Montage PCR Filter Units (Millipore, Amicon USA). Sequencing reactions were performed with 2 μL of PCR- amplified product (20 ng μL^{-1}), 2 μL DYENAMIC ET Terminator cycle kit (Amersham Bioscience) and 5 pmol of primer in 10 μL reaction volume. Sequencing fragments were separated and analysed with a 4-capillary sequencer ABI PRISM 3100-Avant genetic analyser (Applied Biosystems).

3. Results

3.1. Effect of HC application date on forced bud-break response in grapevines

The response of grapevine to HC varies according to the dormancy status of the buds (Erez, 1987; Wood, 1993; Or et al., 1999). Fig. 1 shows the effect of HC on the bud-break response of grapevine buds from different dormancy statuses. Our results confirmed the

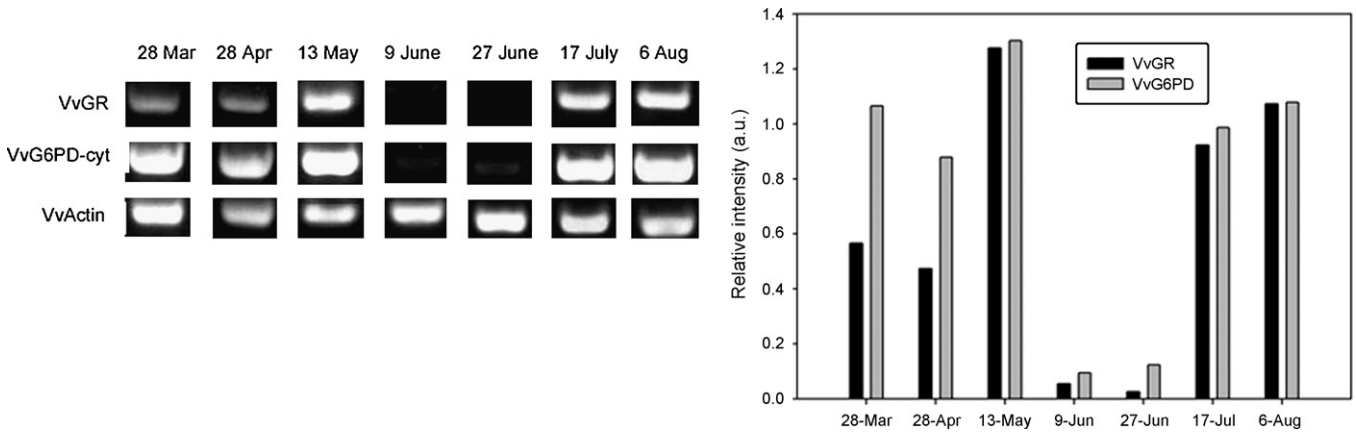

Fig. 2. Expression of VvGR and VvG6PD-cyt transcripts in grapevine buds cv. Thompson Seedless harvested on different dates during the fall winter season in Santiago, Chile (33°34′S). Expression analysis of transcripts was carried out by semi-quantitative RT-PCR on total RNA extracted from buds harvested on the dates indicated in the figure. The VvActin gene was used as internal control.

well-known fact that HC is more effective in advancing the bud-break response in buds that are deeply dormant. The depth of bud dormancy was determined through BR_{50}, a parameter that defines the time required to reach 50% bud-break under forced conditions (Pérez et al., 2007). Differences in BR_{50} values between HC-treated and non-treated buds (ΔBR_{50}) reflect the strength of the HC effect on the bud-break response (Fig. 1 insert). The strongest HC effect took place during mid-April (ΔBR_{50} = 25 days) (Fig. 1 insert). Later in the season, when buds were still deeply endodormant (12 May), the HC effect diminished and ΔBR_{50} dropped to 9.5 days, though it increases to 13.5 days on 9 June and remained relatively constant afterwards. During early August HC negatively affected the bud-break response (Fig. 1 insert).

3.2. Gene expression of VvGR and VvG6PD-cyt in grapevine buds throughout the dormancy cycle

The expression of VvGR and VvG6PD-cyt, encoding two major enzymes from the AGC and oPPP, was studied throughout the dormancy cycle in grapevine buds (Fig. 2). Under field conditions, VvGR and VvG6PD-cyt gene expression increased with the progress of the season between March and mid-May. Later in June, during the fall winter transition, both genes turned off transiently and reappeared in mid-July (Fig. 2).

3.3. Gene expression of VvβGLU78 and VvβGLU22 in grapevine buds throughout the dormancy cycle

Plant 1,3-β-glucanases (βGLU) are involved in pathogen defence and in diverse physiological and developmental processes like the release of buds from dormancy (Rinne et al., 2001) and the promotion of seed germination (Leubner-Metzger, 2003, 2005). In birch (Betula pubescens), βGLU removes the 1,3-D-glucan around plasmodesmata during the release of dormancy, a process that is stimulated by chilling (Rinne et al., 2001). To test whether these features also apply to grape buds, specific primers designed for VvβGLU were used in RT-PCR analysis, and the obtained amplicons were sequenced and designated VvβGLU78 and VvβGLU22. Blastx analysis of both amplicons against the NCBI database is shown in (Fig. 3). The expression of VvβGLU78 and VvβGLU22 increased with the progress of the season, reaching a maximum in mid-May (Fig. 4A). Later in June, both genes turned off transiently, only to reappear in mid-July and in early August, respectively. On the other hand, applications of dormancy-breaking compounds (HC and sodium azide) induced VvβGLU22 (Pérez et al., 2009b) and VvβGLU78 expression in endodormant buds (Fig. 4B).

3.4. Chilling effect on bud dormancy release

The effects of artificial chilling on the expression of VvGR, VvG6PD-cyt, VvβGLU22 and VvβGLU78 were studied in buds harvested on 9 June. None of the genes analysed was induced after chilling treatment (Fig. 5), whereas in buds from the same dormancy status HC and sodium azide induced their expression (Pérez et al., 2009b, Fig. 4B).

4. Discussion

4.1. Evolution of BR_{50} and ΔBR_{50} parameters throughout the bud-ED period

Previous studies in grapevines cv. Thompson Seedless showed that bud-ED is triggered by short day (SD)-photoperiod, and that in plants grown in Santiago of Chile, buds enter into ED in mid-January (Kühn et al., 2009). The changes in BR_{50} throughout ED and the strength of HC on the bud-break response (ΔBR_{50}) in buds from different dormancy statuses suggest that bud-ED could be divided in two phases. The first would be dormancy development that start in mid-January (Kühn et al., 2009) and ends in mid-April (deepest dormancy). The second would be dormancy release extending from mid-May to mid-July. The reduced effect of HC on buds harvested in mid-May is difficult to explain, as at that time buds were still deeply dormant. However, we conjectured that metabolic and developmental changes occurring within the bud at that time reduce the effect of HC on the bud-break response. The reduced values of BR_{50} and ΔBR_{50} during late transition stages (mid-July) indicate the end of ED and the toxicity of HC reflected by the negative ΔBR_{50} value detected during early August indicate that the population of buds is no longer endodormant. Although the mechanism by which HC exerts its dormancy-breaking effect is yet unknown, several pieces of evidence indicate that respiratory and oxidative stresses are produced after HC applications to grapevine buds (Keilin et al., 2007; Halaly et al., 2008; Pérez et al., 2009b). Because respiratory rate and metabolism increase significantly at the end of the ED period (Pérez et al., 2007; Trejo-Martínez et al., 2009), the ability of HC to induce oxidative and respiratory stress could be strongest once ED has finished, allowing it to reach toxic levels.

4.2. Expression of VvG6PD, VvGR, VvβGLU78 and VvβGLU22 genes throughout the bud-ED period

Different stimuli that induce bud-ED release in grapevines upregulate genes encoding enzymes belonging to the AGC, like

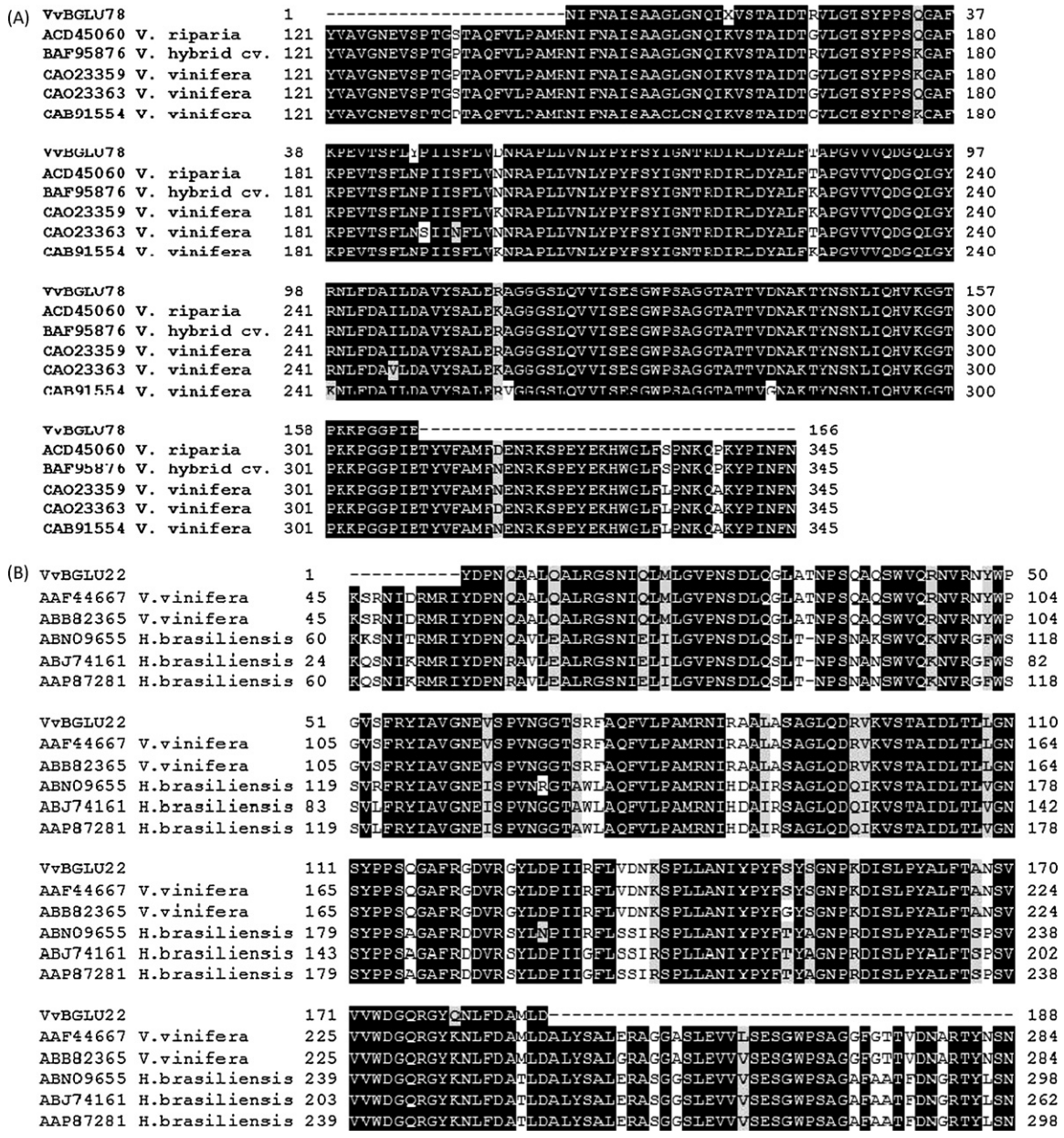

Fig. 3. Deduced amino acid sequence analysis of PCR-amplified fragments VvβGLU78 and VvβGLU22 (Blastx to NCBI database). (A) Analysis of VvβGLU78 showed 96% identity against *V. riparia* 1,3-β-glucanase, 96% against *V. hybrid* cultivar, 96% against *V. vinifera* unidentified protein, 94% against *V. vinifera* unidentified protein and 93% against *V. vinifera* 1,3-β-glucanase. (B) Analysis of VvβGLU22 showed 99% identity against *V. vinifera* 1,3-β-glucanase, 98% against *V. vinifera* 1,3-β-glucanase, 78% against *H. brasiliensis* 1,3-β-glucanase, 78% against *H. brasiliensis* 1,3-β-glucanase and 78% against *H. brasiliensis* 1,3-β-glucanase.

ascorbate peroxidase (VvAPX) and glutathione reductase (VvGR). To the oPPP like glucose-6-phosphate dehydrogenase (VvG6PD-cyt) and key enzymes for dormancy release like 1,3-β-glucanase VvβGLU (Pacey-Miller et al., 2003; Halaly et al., 2008; Ophir et al., 2009; Pérez et al., 2009b). The AGC is a metabolic pathway that detoxifies hydrogen peroxide (H_2O_2) and involves the metabolites, ascorbate, glutathione and NADPH and the enzymes linking these metabolites (Noctor and Foyer, 1998). In non-photosynthetic tissue, the oPPP is a major source of NADPH which maintains redox potential and plays important roles in regulating cell growth (Tian et al., 1998). VvβGLU78 is reportedly upregulated in grapevine leaves after Botrytis cinerea infection, while in berries it is upregulated after mechanical wounding and berry development (Kraeva et al., 2000). Interestingly, in other species, 1,3-β-glucanase has been implicated in the release of buds from dormancy (Rinne et al., 2001).

In grapevine buds, the expression and repression of VvG6PD-cyt, VvGR, VvβGLU78 and VvβGLU22 throughout the ED period could be due to developmental or environmental factors. However, because there are not great differences in temperature and

Table 1
Primer used in RT-PCR.

Genes	Code	Forward	Reverse
VvβGLU78	GSVIVT00025341001	5′-TGCCACCGCCAGGGCAAG-3′	5′-CACGTAAGTCTCGATAGGCC-3′

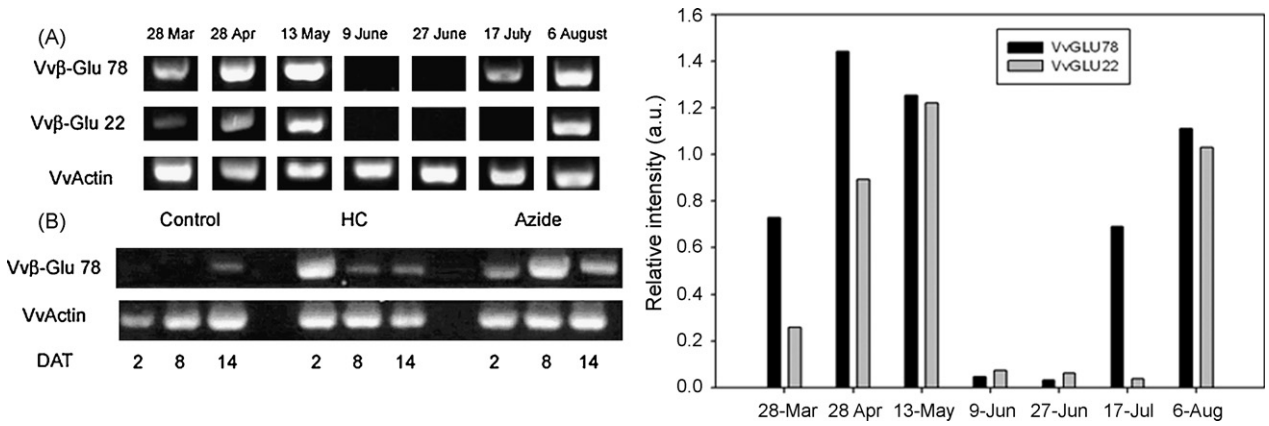

Fig. 4. Expression of VvβGLU78 and VvβGLU22 transcripts in buds of grapevine cv. Thompson Seedless harvested on different dates during the fall winter season in Santiago, Chile (33°34′S) (A) Expression of VvβGLU78 in buds harvested on 9 June and treated with 5% (w/v) Dormex or 2% (w/v) sodium azide before placing the buds in the growth chamber under forced conditions for 2, 8 or 14 days. (B) Transcript analysis was carried out by semi-quantitative RT-PCR on total RNA extracted from buds harvested on the dates indicate in the figure.

or day-length between mid-May and early-June, it is reasonable to suppose that developmental factors drive the observed changes in gene expression. Moreover, the concurrence between the induction of bud-break and the expression of these genes by dormancy-breaking compounds suggest that they are involved in the bud-break response and that their expression is controlled by developmental factors.

4.3. Chilling effects on the release of buds from ED

Grapevine buds need to be exposed to low temperatures during winter for some period of time to achieve release from ED, the so-called chilling requirement (Weaver et al., 1974; Saure, 1985; Dokoozlian et al., 1995). However, the chilling requirement markedly varies with fruit tree species, growing area and the year among other factors (Westwood, 1978), and it has been reported to range from 50 to 400 h for grapevines (Dokoozlian, 1999). In grapevine buds, artificial chilling treatments applied during the phase of dormancy release (early-June) did not affect the expression of VvG6PD, VvGR, VvβGLU78, VvβGLU22 (Fig. 5). However, in buds of the same dormancy status, application of dormancy-breaking compounds induced the expression of all these genes (Pérez et al., 2009b and Fig. 5B). Artificial chilling treatments applied to grapevine buds (400 h at 4 °C) during the phase of dormancy release (9 June) corresponded to the chilling hours (CH) accumulated between early-June and mid-July in Santiago, Chile (Pérez et al., 2008a,b). Additionally, this duration of chilling is adequate to satisfy the chilling requirement of grapevine *V. vinifera*

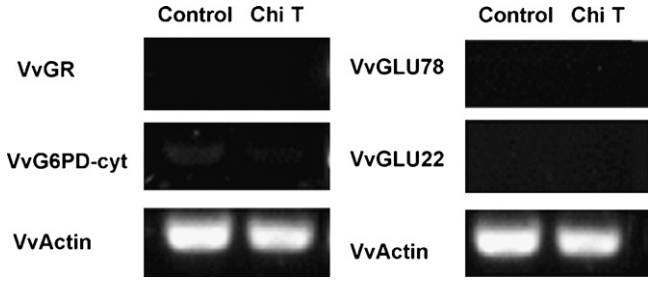

Fig. 5. Expression of VvGR and VvG6PD-cyt in grapevine buds harvested on 9 June and subsequently exposed to chilling (4 °C) for 400 h (Chi *T*) or maintained at ambient laboratory temperature for the same period of time (control). Transcript analysis was carried out by semi-quantitative RT-PCR on total RNA extracted from buds harvested according to defined conditions.

(Dokoozlian et al., 1995). However, some errors in the estimation of chilling requirements could alter the results, as the biological significance of CH calculated according to Weinberger (1950) has been questioned because it considers only temperatures between 0 and 7 °C and not the full range of temperatures (Allan, 2004). Better models have therefore been developed that are more accurate, and the best model so far is the dynamic model that quantifies chilling as chilling portions (CP) (Fishman et al., 1987; Erez et al., 1988). According to the dynamic model, approximately 26 CP are accumulated in Santiago, Chile between early-June and mid-July, while approximately 14 CP accumulate after continuous exposure to 400 h at 4 °C (Pérez et al., 2008a,b). In consequence, artificial chilling treatment only fulfilled about half of the chilling requirements of *V. vinifera* L, so it seems reasonable that an extension of the chilling period is necessary to stimulate the expression of genes related with the release of buds from ED. However, our results, like those reported in buds of *V. riparia* exposed to 500–2000 CH (Mathiason et al., 2009), indicate the down regulation of two genes encoding 1,3-β-glucanase isoforms, and genes encoding VvG6PD-cyt and VvGR, whose expression seems necessary for releasing buds from dormancy. Moreover, a careful examination of the genes differentially expressed after chilling treatments (Mathiason et al., 2009) and after treatments with dormancy-breaking compounds (Ophir et al., 2009) showed a limited correspondence between them. Thus while treatments with dormancy-breaking compounds upregulated genes related with energy and carbohydrate metabolism, which are necessary for growth resumption, chilling treatments, downregulated or had no effect on them. In addition to its dormancy release effect, chilling induces cold hardiness or freeze-tolerance in grapevine buds (Hubácková, 1996; Mills et al., 2006), so the differential genes expression observed after chilling treatments could be related either to the dormancy release or to the cold acclimation or to both processes. Therefore, following the deepness of bud-ED throughout the dormancy cycle in plants exposed and non-exposed to chilling could be a better approach to study the role of chilling in the dormancy release and in the cold acclimation processes and its interrelation with the bud-break response.

Acknowledgement

The financial support of FONDECYT project 1080013 is gratefully acknowledged.

References

Allan, P., 2004. Winter chilling in areas with mild winters. Its measurement and supplementation. Acta Hortic. 662, 47–52.

Arora, R., Rowland, L.J., Tanino, K., 2003. Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38, 911–921.

Chang, S., Puryear, J., Cairney, J.A., 1993. Simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Rep. 11, 113–116.

Dokoozlian, N.K., Williams, L.E., Neja, R.A., 1995. Chilling exposure and hydrogen cyanamide interact in breaking dormancy of grape buds. HortScience 30, 1244–1247.

Dokoozlian, N.K., 1999. Chilling temperature and duration interact on the budbreak of Perlette grapevines cuttings. HortScience 34, 1–3.

Erez, A., 1987. Chemical control of bud-break. HortScience 22, 1240–1243.

Erez, A., Fishman, S., Gat, Z., Couvillon, G.A., 1988. Evaluation of winter climate for breaking bud rest using the dynamic model. Acta Hortic. 232, 76–89.

Erez, A., 1995. Means to compensate for insufficient chilling to improve bloom and leafing. Acta Hortic. 395, 81–95.

Faust, M., Erez, A., Rowland, L.J., Wang, S.Y., Norman, H.A., 1997. Bud dormancy in perennial fruit trees; physiological basis for dormancy induction maintenance and release. HortScience 32, 623–629.

Fennell, A., Hoover, E., 1991. Photoperiod influences growth, bud-dormancy and cold acclimation in *V. labruscana* and *V riparia*. J. Am. Soc. Hortic. Sci. 116, 270–273.

Fishman, S., Erez, A., Couvillon, G.A., 1987. The temperature dependence of dormancy breaking in plants: two step model involving cooperative transition. J. Theor. Biol. 124, 473–483.

George, A.P., Nissen, R.J., Baker, J.A., 1986. Low chill peach and nectarine cultivars Qld. Agric. J. 112, 27–33.

Henzell, R.F., Briscoe, M.R., Gravett, I., 1991. Improving kiwifruit vine productivity with plant growth regulators. Acta Hortic. 297, 345–350.

Halaly, T., Pang, X., Batikoff, T., Crane, O., Keren, A., Venkateswari, J., Ogrodovitch, A., Sadka, A., Lavee, S., Or, E., 2008. Similar mechanisms might be triggered by alternative external stimuli that induce dormancy release in grape buds. Planta 228, 79–88.

Hubácková, M., 1996. Dependence of Grapevine bud cold hardiness on fluctuations in winter temperatures. Am. J. Enol. Viticult. 47, 100–102.

Keilin, T., Pang, X., Venkateswari, J., Halaly, T., Crane, O., Keren, A., Ogrodovitch, A., Ophir, R., Volpin, H., Galbraith, D., Or, E., 2007. Digital expression profiling of grape EST collection leads to new insight into molecular events during grapebud dormancy release. Plant Sci. 173, 446–457.

Kraeva, E., Renault, A., Terrier, N., Tesniere, C., Romieu C., Sauvage, F.X., Bierne, J., Deloire, A., 2000. Beta 1,3-Glucanase mRNA synthesis in grapevine leaves after *Botrytis* inoculation, and in berries after wounding or during ripening. Proceedings of the Vth International Symposium on Grapevine Physiology. In: B.A. Bravdo (Ed.), Acta Hort, 526, 429–435.

Kühn, N., Ormeño-Nuñez, J., Jaque-Zamora, G., Pérez, F.J., 2009. Photoperiod modifies the diurnal expression profile of VvPHYA and VvPHYB transcripts in field-grown grapevine leaves. J. Plant Physiol. 166, 1172–1180.

Lang, G.A., 1987. Dormancy: a new universal terminology. HortScience 22, 817–820.

Leubner-Metzger, G., 2003. Function and regulation of β-1,3-glucanase during seed germination, dormancy release and after ripening. Seed Sci. 13, 17–34.

Leubner-Metzger, G., 2005. β-1,3-Glucanase gene expression in low hydrated seeds a mechanism for dormancy release during tobacco after ripening. Plant J. 4, 133–145.

Mathiason, K., Dong, H., Grimplet, J., Venkateswari, J., Galbraith, D.W., Or, E., 2009. Transcript profiling in *Vitis riparia* during chilling requirement fulfillment reveals coordination of gene expression patterns with optimized bud break. Funct. Integr. Genom. 9 (1), 81–96.

Mills, L.J., Ferguson, J.C., Keller, M., 2006. Cold-hardiness evaluation of grapevine buds and cane tissues. Am. J. Enol. Viticult. 57, 194–200.

Nir, G., Lavee, S., 1993. Metabolic changes during cyanamide induced dormancy release in grapevines. Acta Hortic. 329, 271–274.

Noctor, G., Foyer, C.H., 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Mol. Biol. 49, 249–279.

Noriega, X., Burgos, B., Pérez, F., 2007. Short-day photoperiod triggers and low temperatures increase expression of peroxidase RNA transcripts and basic peroxidase isoenzymes activity in grape buds. Phytochemistry 68, 1376–1383.

Ophir, R., Pang, X., Halaly, T., Venkateswari, J., Lavee, S., Galbraith, D., Or, E., 2009. Gene expression profiling of grape bud response to two alternative dormancy release stimuli expose possible links between impaired mitochondrial activity, hypoxia, ethylene-ABA interplay and cell enlargement. Plant Mol. Biol. 71, 403–423.

Or, E., Nir, G., Vilozny, I., 1999. Timing of hydrogen cyanamide application to grapevine buds. Vitis 38, 1–6.

Or, E., Vilozny, I., Fennell, A., Eyal, Y., Ogrodovitch, A., 2002. Dormancy in grape buds: isolation and characterization of catalase cDNA and analysis of its expression following chemical induction of bud dormancy release. Plant Sci. 162, 121–130.

Pacey-Miller, T., Sott, K., Ablett, E., Tingey, S., Ching, A., Henry, R., 2003. Genes associated with the end of dormancy in grapes. Funct. Integr. Genom. 3, 144–152.

Pang, X., Halaly, T., Crane, O., Kellin, T., Keren, A., Ogrodovitch, A., Galbraith, D., Or, E., 2007. Involvement of calcium signalling in dormancy release of grape buds. J. Exp. Bot. 58, 3249–3262.

Pérez, F.J., Lira, W., 2005. Possible role of catalase in post-dormancy bud-break in grapevines. J. Plant Physiol. 162, 301–308.

Pérez, F.J., Rubio, S., Ormeño-Nuñez, J., 2007. Is erratic bud-break in grapevines grown in warm winters areas related to disturbances in mitochondrial respiratory capacity and oxidative metabolism? Funct. Plant Biol. 34, 624–632.

Pérez, F.J., Vergara, R., Rubio, S., 2008a. H_2O_2 is involved in the dormancy-breaking effect of hydrogen cyanamide in grapevine buds. Plant Growth Regul. 55, 149–155.

Pérez, F.J., Ormeño, J., Reynaert, B., Rubio, S., 2008b. Use of the dynamic model for the assessment of winter chilling in a temperate and a subtropical climatic zone of Chile. Chilean J. Agric. Res. 68, 198–206.

Pérez, F.J., Kühn, N., Ormeño-Nuñez, J., Rubio, S., 2009a. The expression of VvPHYA and VvPHYB transcripts is differently regulated by photoperiod in leaves and buds of grapevines. Plant Signal. Behav. 4 (7), 1–3.

Pérez, F.J., Vergara, R., Or, E., 2009b. On the mechanism of dormancy release in grapevine buds: a comparative study between hydrogen cyanamide and sodium azide. Plant Growth Regul. 59, 145–152.

Rozen, S., Skaletsky, H., 2000. Primer3 on the www for general users and for biologist programmers. Methods Mol. Biol. (Clifton, NJ) 132, 365–386.

Rinne, L.H., Kaikuranta, P.M., van der Schoot, C., 2001. The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. Plant J. 26, 249–264.

Salzman, R.A., Bressan, R.A., Hasegawa, P.M., Ashworth, E.N., Bordelon, B.P., 1996. Programmed accumulation of LEA-like proteins during dessication and cold acclimation of overwinter grape buds. Plant Cell Environ. 19, 713–720.

Saure, M.C., 1985. Dormancy release in deciduous fruit trees. Hortic. Rev. 7, 239–299.

Shulman, Y., Nir, G., Lavee, S., 1983. The effect of cyanamide on the release from dormancy of grapevine buds. Sci. Hortic. 19, 97–104.

Tian, W.N., Braunstein, L.D., Pang, J., Stuhlmeier, K.M., Xi, Q.C., Tian, X., Stanton, R.C., 1998. Importance of glucose-6-phosphate-dehydrogenase activity for cell growth. JBC 273, 10609–10617.

Trejo-Martínez, M.A., Orozco, J.A., Almaguer-Vargas, G., Carvajal-Millán, E., Gardea, A.A., 2009. Metabolic activity of low chilling grapevine buds forced to break. Termochim. Acta 481, 28–31.

Wake, C.M.F., Fennell, A., 2000. Morphological, physiological and dormancy response of three Vitis genotypes to short photoperiod. Physiol. Plant. 109, 203–210.

Weaver, R.J., Manivel, L., Jensen, F.L., 1974. The effect of growth regulators, temperatures and drying on *Vitis vinifera* buds. Vitis 13, 23–29.

Weinberger, J.H., 1950. Chilling requirements of peach varieties. Proc. Am. Soc. Hortic. Sci. 56, 122–128.

Westwood, M.N., 1978. Temperature zone Pomology. W.H. Freeman and Co., San Francisco, pp. 299–332.

Wood, B.W., 1993. Hydrogen cyanamide advances Pecan budbreak and harvesting. J. Am. Soc. Hortic. Sci. 118, 690–693.