Plant Mol Biol (2012) 79:171–178
DOI 10.1007/s11103-012-9904-3

Hypoxia and hydrogen cyanamide induce bud-break and up-regulate hypoxic responsive genes (HRG) and *VvFT* in grapevine-buds

Ricardo Vergara · Sebastián Rubio · Francisco J. Pérez

Received: 16 January 2012 / Accepted: 10 March 2012 / Published online: 31 March 2012

Abstract It has been reported that dormancy-breaking compound hydrogen cyanamide (HC) stimulates the fermentative pathway and inhibits respiration in grapevine-buds, suggesting in this way, that a respiratory stress must be involved in the release of buds from dormancy. Here, we tested low-oxygen effect (hypoxia) on the bud-break response of endodormant grapevine buds, and HC and hypoxia effects on the expression of hypoxic responsive genes (HRG) *PYRUVATE DECARBOXYLASE* (*VvPDC*), *ALCOHOL DEHYDROGENASE* (*VvADH2*), *SUCROSE SYN THASE* (*VvSUSY*), *non-symbiotic HEMOGLOBIN* (*VvnsHb*), and on *FLOWERING LOCUS T* (*VvFT*), a transcription factor related to dormancy release in *Vitis*. Hypoxia as HC, induce transiently the expression of HRG and *VvFT* and hasten the sprouting of endodormant grapevine-buds. During the first 24 h after treatment, HRG and *VvFT* were strongly induced by hypoxia, subsequently, their expressions fell, and 14 days post-treatment increased again above control levels. These results indicate that in the short-term, a respiratory stress, caused either by oxygen deprivation or by inhibitors of respiration, induces transiently the expression of HRG and *VvFT*, and in the long-term, along with the advancement of bud-break, the expression of these genes move forward in treated buds, suggesting that these second induction that occurs just before bud-break is developmentally regulated.

Keywords *Vitis vinifera* · Hypoxia · Bud-dormancy · Dormancy-breaking compounds

R. Vergara · S. Rubio · F. J. Pérez (✉)
Laboratorio de Bioquímica Vegetal, Facultad de Ciencias, Universidad de Chile, Casilla, 653 Santiago, Chile
e-mail: frperez@uchile.cl

Introduction

As most buds of temperate fruit trees, grapevine-buds require chilling exposure during the winter in order to exit from endodormancy (ED) and to sprout homogeneously in spring (Saure 1985; Dokoozlian et al. 1995). Dependence on natural climate to induce bud-break has limitations, especially in warm-winter regions where high winter temperatures may lead to uneven bud-break resulting in different fruit size, different time of harvest, and eventually in difficulties in market planning (Saure 1985; Pérez et al. 2007). To overcome low and uneven bud-break, hydrogen cyanamide (HC) is widely used by growers (Shulman et al. 1983; Erez 1987; Henzell et al. 1991). Although the extensive commercial use of HC worldwide, the mechanism that underlies its dormancy-breaking effect has still not been fully revealed. For a long time, it has been hypothesized that the inhibition of catalase and the subsequent increase in H_2O_2 levels were the main metabolic changes produced by HC that explained its dormancy breaking effect in grapevine-buds (Shulman et al. 1983; Nir and Lavee 1993; Or et al. 2002; Pérez and Lira 2005). However, recent genomic studies indicate that a plethora of genes is up or down-regulated in grapevine-buds after its applications (Halaly et al. 2008; Keilin et al. 2007; Pérez et al. 2008; Ophir et al. 2009), and possible links between impaired mitochondrial activity, hypoxia, and ethylene signaling pathway with the dormancy release mechanism have been proposed (Ophir et al. 2009; Pérez et al. 2009). In plants, low O_2 and chemical inhibition of mitochondria (Mit) respiration reconfigure central carbon metabolism to overcome limited ATP production and regeneration of NAD^+ by enhancing glycolysis and fermentative pathway respectively (Fukao and Bailey-Serres 2004; Bailey-Seeres and Chang 2005). Genomic and proteomic studies in

Arabidopsis confirmed this metabolic shift demonstrating that genes involved in glycolysis and fermentative pathway are among those most strongly induced by hypoxia (Chang et al. 2000; Klok et al. 2002; Liu et al. 2005). It has also been reported that gene expression of non-symbiotic hemoglobins (nsHb) (Nie and Hill 1997), is enhanced under hypoxia or by chemical inhibition of Mit respiration.

Dormancy breaking-compound HC stimulates the fermentative pathway in grapevine-buds (Ophir et al. 2009), the increase of this pathway under low oxygen concentration, is a conserved metabolic response in most organisms including plants. Here, we reported that hypoxia hastens the sprouting of endodormant grapevine-bud, and that both HC and hypoxia, induce the expression of HRG (*VvADH*, *VvPDC*, *VvSUSY*, *VvnsHbs*) and of *VvFT*, an homologue of Arabidopsis *FLOWERING LOCUS T* that in *Vitis* has been related with dormancy (Pérez et al. 2011), providing thus further evidences supporting the hypothesis that a respiratory stress is necessary to initiate the bud-break response in grapevines.

Materials and methods

Plant material

Eight years old *Vitis vinifera* L cv. Thompson Seedless grown at the experimental station of the Chilean National Institute of Agriculture Research (INIA) located in Santiago (33°34′S) were used as plant material. Canes for bud-break experiments were collected on the 19 April of 2010 and 2011 once ED was fully reached, and on the 8 June of 2010 and 2011 at the onset of the ED release stage, according to previous assessments of bud-dormancy status (Pérez et al. 2007; Vergara and Pérez 2010). Canes were cut-off at both ends leaving the central section with 10–12 buds for further experiments.

Assessment of bud-break in grape cuttings exposed to hypoxia

Canes collected on the 19 April and on the 8 June were used to prepare three groups of 30 single-bud cuttings. Group one was treated 24 h under hypoxia (8 % O_2), group two was treated 48 h under hypoxia (5.2 % O_2) and group three was sprayed with water and served as control. To obtain low oxygen concentrations, cuttings were placed in a glass chamber filled with water in the bottom and N_2 flushed continuously at a rate of 100 mL min^{-1}. The oxygen concentration in the bulk solution of the measuring chamber, was recorded polarographically using a Clark type electrode and after 24 and 48 h of bubbling the O_2 concentration was 105 nmol mL^{-1} (8.5 %) and 65 nmol mL^{-1} (5.2 %). After the treatment, cuttings were placed under forcing conditions in a growth chamber set at 23 ± 2 °C, 14 h light (150 μmol m^{-2} s^{-1}) for the periods indicated in the legends. Bud-break percentage and BR_{50}, a parameter developed to estimate the mean time required to reach 50 % bud-break under forced conditions, were used to assess the effect of the different treatments on dormancy release (Pérez et al. 2007). Each value of BR_{50} and its corresponding standard error was calculated by means of probit analysis a statistical test used for transforming discrete variables into continues (Cox and Oaks 1984).

RNA purification and cDNA synthesis

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

Primer design

A survey of grape-bud EST collection representing a wide range of bud development stages (Keilin et al. 2007) indicated that two alcohol dehydrogenase isogenes (*VvADHs*) corresponding to locus GSVIVT00014300001 (*VvADH1*) and **GSVIVT00034834001** (*VvADH2*), three pyruvate decarboxylase genes (*VvPDC*) corresponding to locus **GSVIVT00001355001**; GSVIVT00035905001 and GSVIVT00035907001 and one Sucrose synthase gene (*VvSUSY*) corresponding to locus **GSVIVT00016378001** in the *Vitis* genomic database GENOSCOPE (http://www.genoscope.cns.fr/vitis) are expressed in grapevine buds. Bold sequences indicate specific primers designed against *VvADH2, VvPDC* and *VvSUSY*. Specific primers against *VvnsHb1* (GSVIVT00036442001) *VvnsHb2* (GSVIVT00036443001) and *VvFT* (GSVIVT00012870001) were designed using sequences from GENOSCOPE (Table 1).

qPCR analysis

Quantitative real-time PCR was carried-out in an Eco Real-Time PCR system (illumina, Inc. SD, USA) using the intercalation dye SYBRGreen I as a fluorescent reporter and Platinum Taq DNA Polymerase (Invitrogen, CA, USA). Primers suitable for amplification of 80-150 bp products for each gene under study were designed using the PRIMER3 software (Rozen and Skaletsky 2000) (see Table 1). Amplification of cDNA was carried-out under the

Table 1 List of the primers used in this study

Gene	Forward primer	Reverse primer
VvADH2	5′CTGTGGTTCATGTTGGTTGC 3′	5′ACCGAAAATGGCGACTGAT 3′
VvPDC	5′TTGCTTCATTGAGGTGATCG 3′	5′CTTTCAGCAAGAGGCAGTCC 3′
VvSUSy	5′AACCCAGAGAGGAGACATGG 3′	5′GCGATGGGCAGTTAGAGTTT 3′
VvnsHb1	5′ATGAAGAGTGCTTGGGCAGA3′	5′AGACTTTTCTCGAGCCGTTCT3′
VvnsHb2	5′TGAGATTGCACCATCAGCTC3′	5′ACAGCCATAGCATGGGACTT3′
VvFT	5’ACATTGGAGGGGATGACTTG3’	5’AAGTTTGCCCCAGTAGTTGC3′

following conditions: 2 min denaturation 94 °C; 40 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s. Three repetitions were performed for each sample (technical replicates). Melting curves for each PCR were determined by measuring the decrease in fluorescence with increasing temperature (from 55 to 95 °C). The folds that transcript level was induced or repressed, was calculated by the ΔΔCq method (Livak and Schmittgen 2001) using *VvACTIN* and *VvUBIQUITIN* as reference genes. Results obtained with both reference genes were closely related. The efficiency for reference and studied genes were determined by standard curves and was 95 %.

Promoter analysis

Sequence of 1,000 or 1,500 bp upstream from the transcription start site of the selected genes were downloaded from the *Vitis vinifera* genomic database GENOSCOPE (http://www.genoscope.cns.fr) and from phytozome (http://www.phytozome). Identification of *cis*-acting regulatory elements (CARE) within the promoter dataset was carried-out using the web-based analysis tool PlantCare (Rombauts et al. 1999).

Results

Hypoxia advances the sprouting of endodormant grapevine buds

The exposure of grape cuttings to hypoxia treatments (8 % O_2; 24 h) and (5 % O_2; 48 h) hastened the sprouting of endodormant grapevine-buds harvested on the 19 April, and on the 8 June (Fig. 1). No major differences were detected between both hypoxia treatments; nevertheless, the developmental stage of the buds affected significantly the response to hypoxia treatments. Thus, in buds harvested on the 8 June, the time required to reach 50 % bud-break (BR_{50}) was reduced in approximately 30 % with respect to control buds, while in buds harvested on the 19 April, BR_{50} was reduced only in 12 %.

Anaerobic *cis*-acting regulatory element (ARE) was found in promoters of *Vitis* HRG and *VvFT*

As expected, *cis*-acting regulatory elements essential for the anaerobic induction (ARE) was found in the promoter of all *Vitis* HRG analyzed (Fig. 2a) However, surprisingly it was also found in the promoter of *VvFT*, moreover ARE is present in the promoter of all FT genes of other species whose genome or FT gene has been sequenced (Fig. 2b).

Short and long-term effects of hypoxia and HC on the expression of HRG in endodormant grapevine-buds

The effect of hypoxia on the expression of HRG was analyzed in grapevine buds shortly after treatment by qRT-PCR (Fig. 3a). The short-term effect of HC on the expression of HRG in grapevine buds was taken from Ophir et al (2009) (Fig. 3b). A comparison between both results indicates that hypoxia and HC induce transiently the expression of HRG in grapevine buds. However, while all HRG respond similarly to HC treatment reaching its highest level of expression 24 h post-treatment. Three different expression patterns can be distinguished in buds exposed to hypoxia. *VvADH2* reached its maximum level of expression immediately after the hypoxia treatment, and subsequently decreased continuously with time. *VvPDC* and both *VvnsHbs*, reached its maximum level of expression 6 h post-treatment, afterward the expression level fell drastically until reaching control levels. Finally, *VvSUSY* reached its maximum level of expression 24 h post-treatment, to decrease later continuously. In contrast, both HC and hypoxia treatments showed a similar long lasting effect on the expression of HRG, reaching the highest level of expression, with the exception of *VvSUSY*, 14 days post-treatment (Fig. 4a, b).

Effect of hypoxia and HC on the expression of *VvFT* in endodormant grapevine buds

In order to analyze whether *VvFT*, the *Vitis* homologue of Arabidopsis *FLOWERING LOCUS T* (*FT*), is related to bud-dormancy release in grapevines, we studied the short

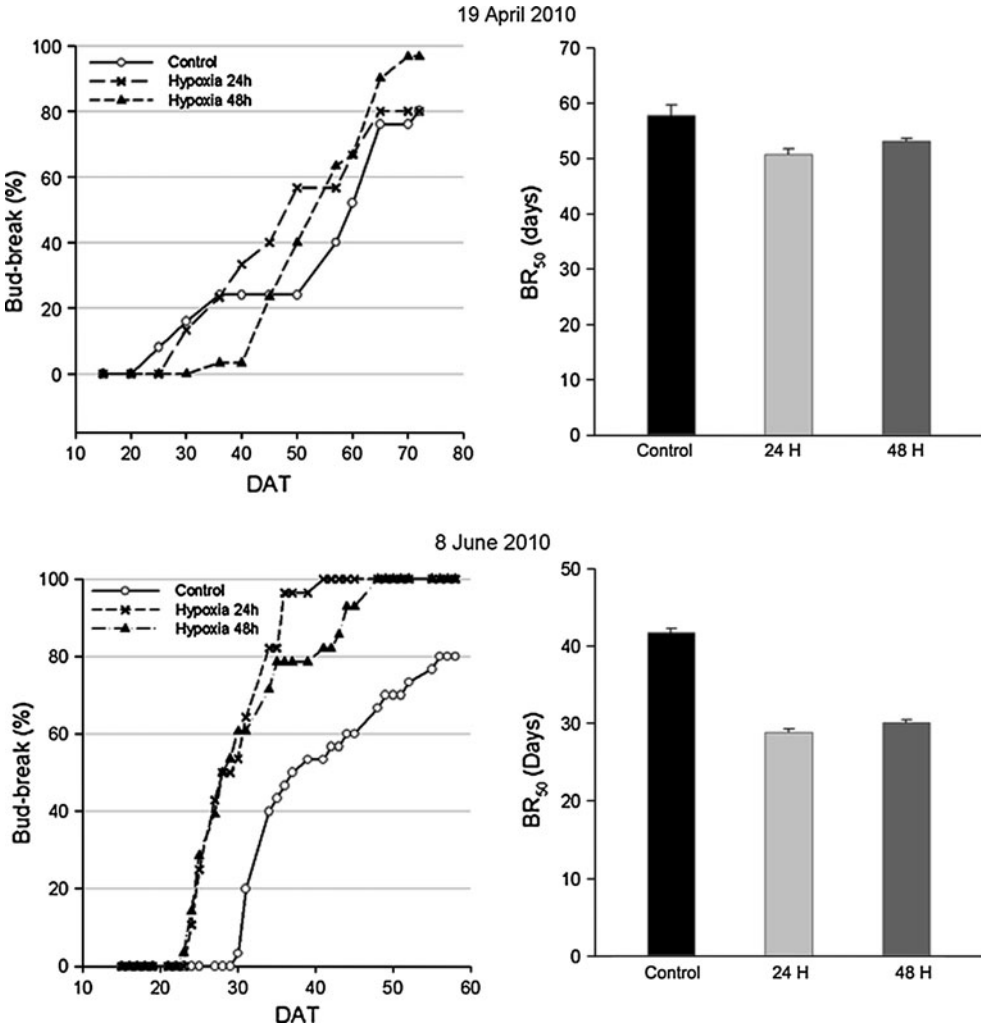

Fig. 1 Hypoxia advances bud-break response in grapevines. Buds were harvested on the 19 April and on the 8 June when they were at the endodormant stage according to previous assessment of grape dormancy status (Vergara and Pérez 2010). Results are expressed as percentage of bud-break and as BR_{50} a parameter that indicates the time required to reach 50 % of bud-break. Each value of BR_{50} and its corresponding standard error was calculated by mean of the probit analysis a statistical test used for transforming discrete variables into continues (Cox and Oaks 1984)

and long-term effects of hypoxia and the long-term effect of HC on its expression in endodormant grapevine buds. Hypoxia (8 % O_2; 24 h), in the short-term, induced transiently the expression of *VvFT*, maximum level (1,200-fold above the control) was reached 6 h post-treatment, afterward the expression of *VvFT* decreased drastically (Fig. 5a). Two days post- treatment, *VvFT* fell below the levels observed in control buds, a situation that continued until day 14. Thereafter, *VvFT* levels increased again above control levels (Fig. 5b). In HC treated buds, 2 days post-treatment, *VvFT* level was still above control buds, but later 8 days post-treatment, fell below to increase again at day 14 (Fig. 5c).

Discussion

Hypoxia advances bud sprouting in grapevines

The low-oxygen concentration (hypoxia) positively affected the breaking of buds in grapevines, advancing its response. The effect of hypoxia was bigger in buds that were in a more advanced developmental stage. These results provide new evidences indicating that a respiratory stress caused either by inhibitors of respiration or by restrictions in oxygen availability, activates a dormancy release program within the grape bud.

HRG and dormancy release in grapevine buds

Plant cells, as most eukaryotic cells, respond to low oxygen concentration altering their energetic metabolisms by increasing carbohydrate consumption to overcome ATP limitations through glycolysis and to regenerate NAD^+ through ethanolic fermentation. This common response of eukaryotic cells to oxygen deficiency is known as the Pasteur Effect (Bailey-Seeres and Voesenek 2008; Mustroph et al. 2010). Microarray studies carried-out in different plant species confirmed that genes involved in carbohydrate metabolism, glycolysis and fermentation, are up-regulated under anaerobic conditions (Gonzali et al. 2005; Lasanthi-Kudahettige Magneschi et al. 2007). In

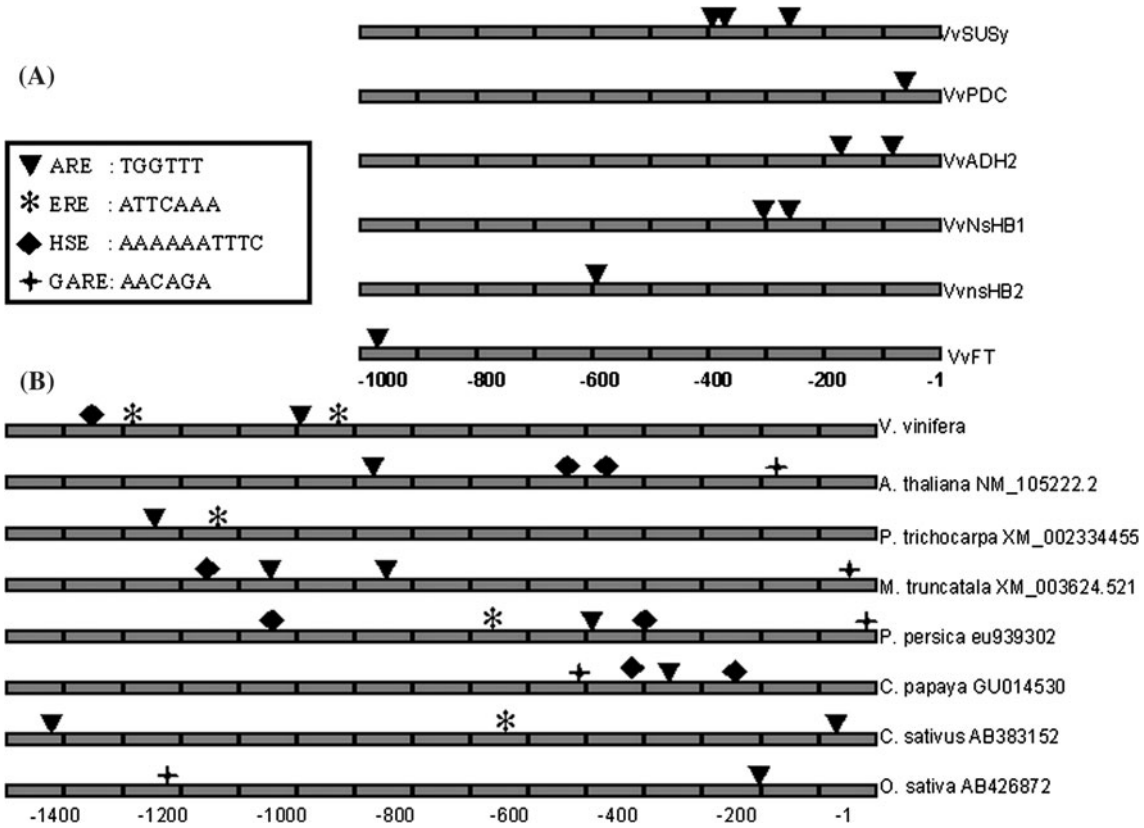

Fig. 2 **a** Position of ARE (anaerobiosis responsive elements) putative *cis*-regulators within the 1 kb promoter region of HRG and *VvFT*. **b** Position of *ARE* (anaerobiosis responsive elements), *ERE* (ethylene responsive elements), *HSE* (heat stress element), *GARE* (giberellic acid responsive element) putative *cis*-regulators within the 1.5 kb promoter region of *FT* from different species

roots of *Arabidopsis*, a set of genes that respond rapidly to a moderate decrease in oxygen levels was identified and regarded as hypoxia responsive genes (HRG) (van Dongen et al. 2009). This set includes genes related to carbohydrate metabolism (*SUSY*), to glycolysis and fermentation (*PDC* and *ADH*) and non-symbiotic hemoglobins (*nsHb*). In *Vitis*, all HRG shows the presence of ARE in their promoter, and hypoxia and HC induce transiently the expression of HRG in the buds. The fact that both stimuli up-regulated HRG suggests that the sensing and signaling that leads to modification in their expression, may be triggered by a change in cellular homeostasis rather than a direct sensing of the oxygen concentration. It is still unclear if an oxygen sensor exists in plants (Geigenberger 2003; Bailey-Seeres and Chang 2005). Among HRG, the two fermentative genes *VvPDC* and *VvADH2* increased their expression 14 days post-treatment, suggesting that they play a crucial role before bud-break. In pollen, PDC and ADH accumulate to high levels irrespective of oxygen availability, and under situations of high energy demand and high rate of sugar metabolism, respiration and fermentation took place concurrently (Tadege and Kuhlemeier 1997; Gass et al. 2005). The breaking of buds produces a high energy demand, and therefore, the increased expression of fermentative genes just before the occurrence of bud-break, suggests that like in pollen, respiration and fermentation can occur simultaneously during the breaking of buds.

VvFT and bud-dormancy release

The expression of *VvFT* in *Vitis* has been related with the transition of buds into ED, a process triggered by decreasing photoperiod (Kühn et al. 2009). *VvFT* is down-regulated by SD-photoperiod in leaves and buds of grapevines, and remains repressed within the bud during the stage of ED (Kühn et al. 2009; Pérez et al. 2011). Moreover, it has been shown that the expression of *VvFT* is induced previous to the sprouting of buds, and that is up-regulated by HC (Pérez et al. 2011), suggesting that its transcriptional activation is related with the resumption of bud growth after the recess period. This behavior of *VvFT* is similar to that described in poplar *pFT2* (Hsu et al. 2011). Confirming the presence of ARE in *VvFT* promoter, hypoxia in the short-term, induces transiently *VvFT*

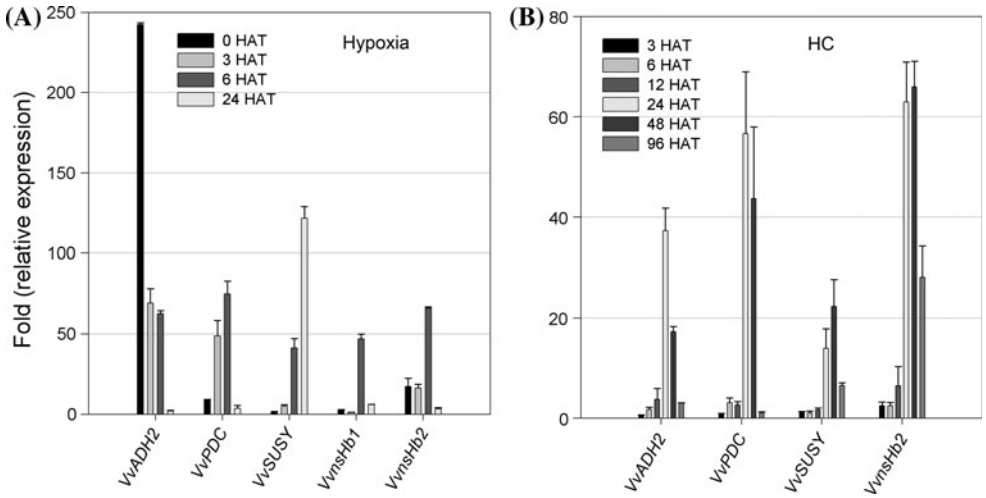

Fig. 3 Short-term effects of hypoxia and HC, hours after treatment (*HAT*), on the expression of hypoxic responsive genes (*HRG*) *VvADH2, VvPDC, VvnsHb1, VvnsHb2* and *VvSUSY* in grapevine buds. **a** Effect of hypoxia was performed on buds collected on the 19 April and gene expression analysis was carried-out by qRT-PCR, data for hypoxia were normalized against *VvACTIN*. **b** Data for HC effect on HRG was taken from Ophir et al. 2009. Values correspond to the average of three technical replicates and *bars* represent standard deviation

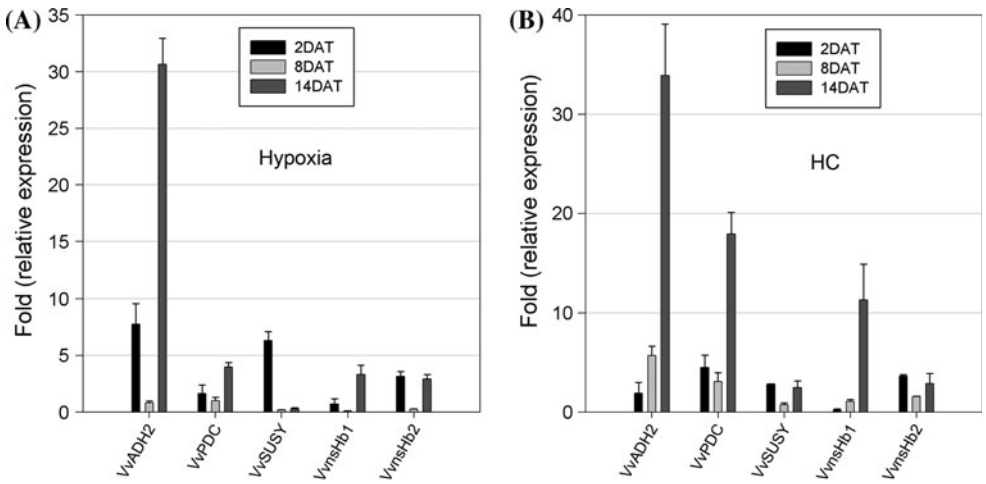

Fig. 4 Long-term effects of hypoxia and HC, days after treatment (*DAT*), on the expression of hypoxic responsive genes (*HRG*) *VvADH2*, *VvPDC*, *VvnsHb1*, *VvnsHb2* and *VvSUSY* in grapevine buds. **a** Effect of hypoxia and **b** effect of HC was performed in buds collected on the 8 June and gene expression analysis was carried-out by qRT-PCR 2, 8 and 14 days after treatment and data were normalized against *VvACTIN* in both cases. Values correspond to the average of three technical replicates and *bars* represent standard deviation

expression reaching a maximum 6 h post-treatment and 24 h post-treatment level-off to control buds. However, 14 days after treatment, *VvFT* increased again above control levels, suggesting that this last up-regulation could be developmentally controlled. Although, the short-term effect of HC on the expression of *VvFT* was not analyzed, the long-term effect also showed an increase in its expression 14 days post-treatment. Moreover, the expression of HRG also increased 14 days post-treatment, suggesting that both HC and hypoxia, along with hastening the sprouting of buds, moves forward the expression of these genes whose activation could be part of a genetic program in charge of the release of buds from ED. The transient induction of HRG and *VvFT* shortly after the hypoxia and the HC treatment could be related with the signal that activates the genetic program leading to dormancy release. Interestingly, it has been reported recently that *FT* is strongly induced by chilling in dormant buds of populus (Rinne et al. 2011) and chilling promotes bud-ED release in this specie (Rinne et al. 2001). However, in *Vitis* we found that although hypoxia and HC up-regulate the expression of *VvFT* and advance the bud-break response,

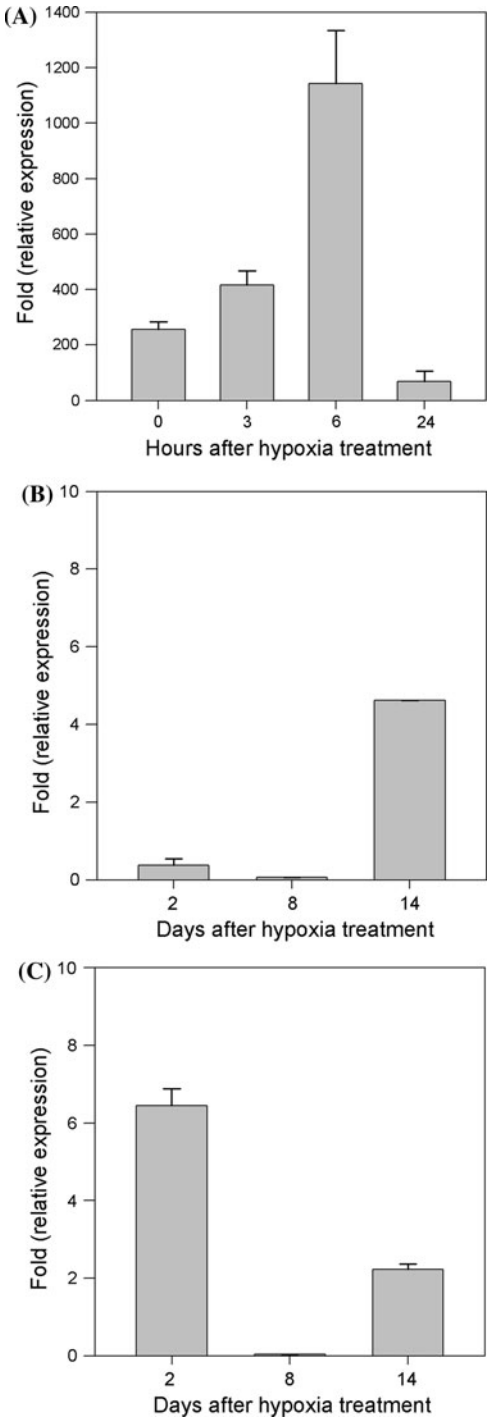

Fig. 5 Short and long-term effects of hypoxia and long term-effect of HC on the expression of *VvFT*. **a** Short-term effect of hypoxia on *VvFT* gene expression, **b** long-term effect of hypoxia on *VvFT* gene expression and **c** long term effect of HC on *VvFT* gene expression. Gene expression analysis was performed by qRT-PCR in buds harvested on 19 April for the short-term effect and on 8 June for the long-term effect, data were normalized against VvUBIQUITIN. Values correspond to the average of three technical replicates and *bars* represent standard deviation

chilling did not up-regulate *VvFT* and did not advance the bud-break response (results not shown). This discrepancy could be explained by the fact that in poplar there are two *FT* paralogs, *pFT1* and *pFT2* that differ functionally. Thus while *pFT1* is induced by chilling, is expressed during the winter and is related to reproductive growth, *pFT2* is induced by LD and warm temperatures and is related to vegetative growth (Hsu et al. 2011). Although, the induction of FT and the exit of buds from dormancy have been related in populus and in *Vitis*, further studies will be necessary to reveal the role of *FT* in the exit of buds from dormancy.

Acknowledgments Financial support of Fondecyt Project 1110056 is gratefully acknowledged.

References

Bailey-Seeres J, Chang R (2005) Sensing and signaling in response to oxygen deprivation in plants and other organisms. Ann Bot 96:507–518

Bailey-Seeres J, Voesenek LA (2008) Flooding stress: acclimations and genetic diversity. Ann Rev Plant Biol 59:313–339

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

Chang WWP, Huang L, Shen M, Webster C, Burlingame AL, Roberts JKM (2000) Pattern of protein synthesis and tolerance to anoxia in root tips of maize seedlings acclimated to a low oxygen environment and identification of protein by mass spectrometry. Plant Physiol 122:295–317

Cox DR, Oaks D (1984) Analysis of survival data. Chapman & Hall, London

Dokoozlian NK, Williams LE, Neja RA (1995) Chilling exposure and hydrogen cyanamide interact in breaking dormancy of grape buds. Hortic Sci 30:1244–1247

Erez A (1987) Chemical control of bud-break. Hortic Sci 22: 1240–1243

Fukao T, Bailey-Serres J (2004) Plant responses to hypoxia is a survival balancing act. Trends Plant Sci 9:449–456

Gass N, Glagotskaia T, Mellema S, Stuurman J, Barone M, Mandel T, Roessner-Tunali U, Kuhlemeier C (2005) Pyruvate decarboxylase provides growing pollen tubes with a competitive advantage in petunia. Plant Cell 17:2355–2368

Geigenberger P (2003) Response of plant metabolism to too little oxygen. Curr Opin Plant Biol 6:247–256

Gonzali S, Loreti E, Novi G, Poggi G, Alpi A, Peralta P (2005) The use of microarrays to study the anaerobic response in arabidopsis. Ann Bot 96:661–668

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

Henzell RF, Briscoe MR, Gravett I (1991) Improving kiwifruit vine productivity with plant growth regulators. Acta Hortic 297: 345–350

Hsu C-Y, Adams JP, Klim H, No K, Ma C, Strauss SH, Drnevich J, Vandervelde L, Ellis JD, Rice MD, Wickett N, Gunter LE,

Tuskan GA, Brunner AM, Page GP, Barakat A, Carlson JE, dePamphilis CW, Luthe DS, Yuceer C (2011) *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. PNAS 108(26):10756–10761

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 grape-bud dormancy release. Plant Sci 173:446–457

Klok EJ, Wilson IW, Wilson D, Chapman SC, Ewing RM, Somerville SC (2002) Expression profile analysis of the low oxygen response in Arabidopsis roots cultures. Plant Cell 14:2481–2494

Kühn N, Ormeño J, Jaque-Zamora G, Pérez FJ (2009) Photoperiod modifies the diurnal expression profile of *VvPHYA* and *VvPHYB* transcript in field-grown grapevines. J Plant Physiol 166: 1172–1180

Lasanthi-Kudahettige Magneschi L, Loretti, Gonzali S, Licausi F, Novi G (2007) Transcript profiling of the anoxic rice coleoptiles. Plant Physiol 144:218–231

Liu F, Vantoai T, Moy L, Bock G, Linford LD, Quackenbush J (2005) Global transcription profile reveals novel insights into hypoxic response in *Arabidopsis*. Plant Physiol 137:387–396

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real time quantitative PCR and the $\Delta\Delta$CT method. Methods 25:402–408

Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Masihi A, Fukao T, Bailey-Serres J (2010) Cross-Kingdom comparison of transcriptomic adjustments to low oxygen stress highlights conserved and plant specific response. Plant Physiol 152:1484–1500

Nie X, Hill RD (1997) Mitochondria respiration and hemoglobin gene expression in barley aleurone tissue. Plant Physiol 114:835–840

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

Noriega X, Burgos B, Pérez FJ (2007) Short-day photoperiod triggers and low temperature increase expression of peroxidase RNA transcripts and basic peroxidase isoenzyme activity in grape buds. Phytochem 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, Vilozny I, Fennell A, 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

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

Pérez FJ, Rubio S, Ormeño-Nuñez J (2007) Is erratic bud-break in grapevines grown in warm winter areas related to disturbance in mitochondrial respiratory capacity and oxidative metabolism? Function Plant Biol 34:624–632

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

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

Pérez FJ, Kühn N, Vergara R (2011) Expression analysis of phytochromes A, B and floral integrator genes during the entry and exit of grapevine-buds from endodormancy. J Plant Physiol 168:1659–1666

Rinne PLH, Kaikuranta PM, van der Schoot (2001) The shoot apical meristem restores its symplasmic organization during chilling induced release from dormancy. Plant J 26:249–264

Rinne PLH, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjärvi, van der Schoot (2011) Chilling of dormant buds hyperinduces FLOWERING LOCUS T and recruit GA- inducible 1,3-β-glucanase to reopen signal conduits and release dormancy in populus. Plant Cell 3:130–146

Rombauts S, Dehais P, van Montagu M, Rouze P (1999) PlantCare, a plant *cis*-acting regulatory elements database. Nucleic Acid Res 27:295–296

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

Saure MC (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. Scientia Hortic 19:97–104

Tadege M, Kuhlemeier C (1997) Aerobic fermentation during tobacco pollen development. Plant Mol Biol 35:345–354

van Dongen JT, Froehlich A, Ramírez-Aguilar SJ, Schauer N, Fernie AR, Erban A, Kopka J, Clark J, Langer A, Geingenberger P (2009) Transcript and metabolic profiling of the adaptative response to mild decrease in oxygen concentration in the roots of *Arabidopsis* seeds following low oxygen treatment. Ann Bot 103:269–280

Vergara R, Pérez FJ (2010) Similarities between natural and chemically induced bud- endodormancy release in grapevine *Vitis vinifera* L. Scientia Hortic 125:648–653