Possible role of catalase in post-dormancy bud break in grapevines

Francisco J. Pérez*, Waldo Lira

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

KEYWORDS
Catalase;
Grapevines;
Bud;
Dormancy;
Hydrogen peroxide;
Hydrogen cyanamide

Summary

Changes in the activity of catalase (Cat) and in the levels of H_2O_2 were followed throughout dormancy in buds of grapevines (*Vitis vinifera* L.). In grapevines grown in the Elqui valley in Chile, a region with warm-winters, the activity of Cat increased during the recess period of buds, reaching a maximum and thereafter decreased to less than one third of its maximal activity. Three isoforms of Cat were detected in extracts of buds by native PAGE analysis, and the extracted activity was inhibited competitively by hydrogen cyanamide (HC), a potent bud-break agent. Furthermore, HC applications to field-grown grapevines in addition to the expected effect on advancing bud break, reduced the Cat activity during bud dormancy. Similar reductions were observed during dormancy in buds of grapevines grown in the Central valley in Chile, a region with temperate winters, suggesting that HC and winter chilling inhibits the activity of the main H_2O_2 degrading enzyme in grape buds. A transient rise in H_2O_2 levels preceded the release of buds from endodormancy, moreover, the peak of H_2O_2 and the onset of bud break occurred earlier in HC treated than in control grapevines, suggesting the participation of H_2O_2 as a signal molecule in the release of endodormancy in grape buds. The relationship between Cat inhibition, rise in H_2O_2 levels and initiation of bud break are discussed.

Introduction

In woody plants, seasonal cycles of growth and dormancy occur in over-wintering buds. At the end of the growing season, perennial plants cease development and assume a dormant and freezing tolerant state protecting buds against unfavourable winter conditions (Saure, 1985). Lang (1987) classified various states of dormant buds as para-dormant, endodormant, and ecodormant. During

Abbreviations: Cat, catalase; HC, hydrogen cyanamide; PAGE, polyacrylamide gel electrophoresis; ROS, radical oxygen species

*Corresponding author.
E-mail address: frperez@uchile.cl (F.J. Pérez).

paradormancy, growth is regulated by plant growth regulators (PGR) originating outside the bud and is related to apical dominance. During endodormancy, growth is repressed by endogenous factors within the buds and chilling requirements need to be satisfied before growth is reassumed. During ecodormancy, bud break is limited by adverse environmental factors, and generally it takes place during the late winter and spring. Dormancy induction and release is genetically and environmentally controlled, and photoperiod and low temperatures play a major role in these process. However, little is known about the events that occur in the bud during dormancy and on the mechanisms that control bud induction and release.

In warm-winter regions, prolonged endodormancy is considered a major obstacle to economic production of temperate fruits (Shulman et al., 1983; Erez, 1987). In these regions, the need for artificial means to compensate for natural chilling becomes a dominant factor for maintaining economic production (Erez, 1987, 1995).

In grapevines, as in other perennial woody plants, induction and release mechanisms of bud dormancy are unknown, however, decreases in Cat activity during bud dormancy have been observed (Nir et al., 1986). Furthermore, it has been reported that low temperatures and HC, an effective dormancy-breaking agent, inhibits Cat activity and reduces levels of Cat mRNA transcripts shortly after its application (Nir et al., 1986; Or et al., 2001). Inhibition of Cat activity led to oxidative stress in several systems due to increased H_2O_2 content (Godon et al., 1998; Prasad, 1996), however, little is known about their function during stress conditions. Recent findings indicate that heat shock leads to a reduction in Cat level and a significant increase in H_2O_2 (Dat et al., 1998). Salicylic acid (SA), a signal for the activation of plant defence has similar effects (Foyer et al., 1997). More recently, it has been suggested that the fall in Cat activity is a phenomenon occurring in many plant species under oxidative stress and is related to the accumulation of SA in oxidatively stressed plants (Shim et al., 2003).

Here we studied the interplay of H_2O_2 and catalase activity in relation to bud dormancy and release. The experiments were performed in the Elqui and central valleys in Chile, two regions with warm and temperate winters, respectively.

Material and methods

Plant material. The experiments were conducted from 2002 to 2003 in commercial vineyards in the Elqui (27°18′S, IV region) and central valley (33°29′S, RM) in Chile. The $4 \times 4\,m^2$ planted vines of *Vitis vinifera* L. cv. Thompson seedless were 8 years old, drip irrigated, and conducted in a horizontal system. All plants were subjected to the cultural practices commonly used in these regions.

Hydrogen cyanamide (HC) applications. Dormancy breaking treatments were applied after winter pruning (25 June), corresponding to the date commonly used for H_2CN_2 application in the Elqui valley. The experiments were designed as randomised blocks with four replications. Vines were pruned to 8–9 buds per cane on average; four vines in each replicate were sprayed with H_2CN_2 and the other four were sprayed with water as a control. Dormex (SKW, Trostberg, Germany), a commercial formulation containing 49% H_2CN_2, was applied at a concentration of 5% (v/v). The vines were sprayed at full volume until runoff with a hand-operated sprayer. In each vine, four canes oriented N, S, E, W were marked with ribbons of different colours and left for monitoring bud break. Buds from the rest of the canes were used for laboratory analysis and removed weekly from the application date until the onset of bud break. Bud break was monitored weekly for the first 12 weeks after H_2CN_2 applications, and bud-break percentages were calculated. Bud break was defined as the stage when green tissue was visible.

Bud break under forced conditions. Since ecodormancy is a limiting factor for natural bud break in the Central valley in Chile, we used bud-break response of single bud cuttings under forced conditions to differentiate between ecodormant and endodormant buds (Koussa et al., 1994). Endodormancy was monitored by changes in bud-break percentages and timing on single node cuttings. Canes were collected at 3-week intervals from May to middle September. In each collection date, four homogeneous canes per vine oriented N, S, E, W containing approximately 10 buds each, were selected for forcing bud break. Three groups of 10 single node cuttings for each cane position were prepared on each collection date and forced for 21 days at 25 °C under 12 h light: 12 h dark photoperiods. After incubation, bud break was used to express the dormancy depth of vines. For laboratory analysis, 5 buds per vine were removed on each collection date.

Catalase isoforms in grape buds. Buds were ground to a fine powder in liquid N_2, extracted in 500 μL buffer (0.5 M Tris-HCl, pH 7.5, 5 mM DTT, 1 mM $MgCl_2$, 10 μM PMSF, 2% insoluble PVP and 12.5% glycerol) and the homogenate centrifuged in a bench-centrifuge for 15 min at 13,000*g*. The

precipitate was resuspended in the same buffer plus the addition of 2% Triton-X 100 final concentration. After 15 min of incubation at 30 °C, the homogenate was centrifuged again. Samples of the homogenate were separated on 10% native PAGE run at 100 V for 6 h at 4 °C in running buffer Tris-glycine pH 8.3 without Urea and SDS. After electrophoresis, the gel was soaked in distilled water for 15 min. Subsequently, the gel was incubated in 0.03% H_2O_2 for 5 min and carefully washed to remove the residual H_2O_2. Catalase activity was detected by soaking the gel in a solution containing 1% (w/v) ferric chloride and 1% (w/v) potassium ferricyanide. The gel was stained uniformly blue except at positions exhibiting Cat activity. When maximum contrast was achieved the reaction was stopped by rinsing the gel with H_2O (Fath et al., 2002).

Catalase assays. Catalase activity was determined following O_2 evolution using a Clark-type oxygen electrode (Hansatech, UK). Measurements were carried out at 30 °C in 1 ml 0.1 M phosphate buffer pH 7.0 and 1 mM H_2O_2. Inhibition studies of Cat by H_2CN_2 and SA were performed varying the H_2O_2 concentration in the range of 5–25 mM with H_2CN_2 concentrations of 0, 2, 10 and 20 mM and SA concentrations of 0, 10 and 15 mM. The data were analysed by Lineweaver–Burk plots.

H_2O_2 measurements. H_2O_2 concentration was measured by chemiluminescence (CL) in a ferricyanide-catalyzed oxidation of luminol (5-amino-2,3-dihydro-1,4-phtalazinedione) (Warm and Laties, 1982). Buds (0.1 g fr. wt) were ground in liquid N_2, half of the powder was homogenised with a 5-fold volume of 5% TCA and the other half was used for enzymatic and proteins determinations. The homogenate was centrifuged in a bench-centrifuge at 13,000*g* for 5 min. To eliminate phenols and low molecular weight compounds that interfere with CL measurements, extracts were shaken with 0.01 g insoluble PVP for 30 min and centrifuged again for 10 min. The extracts were diluted 1:10 in 0.2 M NH_4OH (pH 9.5) before CL measurements. Sample solutions (30 μL) consisting in 5 μL of diluted extract, 20 μL of NH_4OH buffer, and 5 μL of distilled water, and control solutions, in which distilled water was replaced by 50 units of catalase (bovine liver, Sigma, USA), were incubated for 15 min in a water bath at 30 °C. After incubation, the amount of H_2O_2 was tested by adding 30 μL of sample or control solution into 0.9 ml of 0.2 M NH_4OH (pH 9.5) plus 50 μL 0.5 mM luminol. The CL reaction was initiated by adding 50 μL of 0.5 mM $K_3Fe(CN)_6$ into the mixture. The emitted photons were counted over 15 s. with a luminometer HY-LITE® 2 (Merck, Germany). The difference between sample and control measurements was considered as H_2O_2-specific CL, and CL data were transformed to H_2O_2 concentration by means of a calibration curve. To assess the recovery of H_2O_2, the liquid nitrogen powder corresponding to one bud was divided in two halves, to one half 0.1 μmol of H_2O_2 was added as an internal standard, and to the other an equivalent volume of distilled water was added. Both halves were processed in parallel and the differences in H_2O_2 concentration indicated that 88% of the added H_2O_2 was recovered, signalling that the method is appropriate for H_2O_2 determinations in buds of grapevine.

Protein determinations. Protein concentrations were determined using Bradford method (Bradford, 1976).

Results

Hydrogen cyanamide (HC) induction of bud break

After winter pruning, applications of HC to grapevines grown in the Elqui valley induced 50% bud break after 8 weeks from application date, while buds on control vines (without HC application) remained dormant (Fig. 1). Maximum rates of bud break were higher in HC-treated than in control buds and were reached 40 days earlier. Advance and uniformity in bud break represents economic benefits for table grape producers from warm winter regions.

Inhibition of Cat activity in extracts of grape buds by HC and SA

HC and SA within the milimolar range inhibited the activity of Cat in extracts of bud grapevines (Fig. 2A). The nature of the inhibition by both compounds was determined by Lineweaver–Burk plots. Results show that HC-inhibited competitively the activity of Cat (Fig. 2B), while SA inhibited it in a non-competitive manner (Fig. 2C).

Catalase isoforms identified by native PAGE in extracts of grape bud

Extracted Cat activity from grape buds is insoluble, and additions of 2% final concentration Triton-X 100 were necessary to solubilise it. Soluble homogenate containing the enzyme activity was separated by native PAGE and assayed further in the gel using bovine Cat as positive control. Three Cat isoforms were revealed in the gel as white spots (Fig. 3).

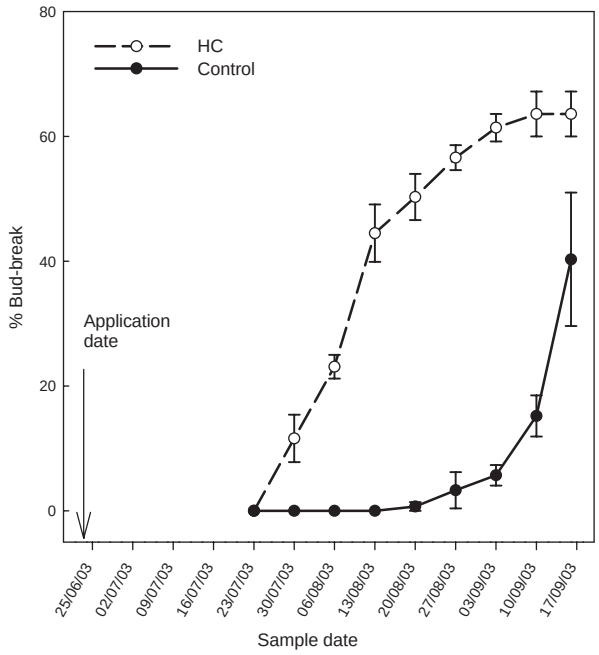

Figure 1. Influence of the H_2CN_2 application (2.5%) on bud break in Thompson seedless grapevines grown in the Elqui valley in Chile. The application was carried out 2 weeks after winter pruning. Mean values are of four replications, and each replication consisted of four vines per treatment. Bars represent standard deviation.

Evolution of Cat activity in HC treated and control buds during dormancy

In buds of grapevines grown in the Elqui valley, Cat activity increased markedly with the progress of dormancy (Fig. 4). Similar increases have been reported in cv. Perlette grapevines grown in the Jordan valley in Israel (Nir et al., 1986), a region in which the weather conditions resemble those of the Elqui valley. As a direct consequence of Cat inhibition by HC, buds of HC-treated grapevine showed reduced levels of Cat activity throughout dormancy (Fig. 4). It is likely that in the Elqui valley, advances in bud break induced by HC applications are due to a shortening in bud endodormancy, since under the climatic conditions of the Elqui valley, ecodormancy should not impair or delay bud break. Moreover, a shortening in bud endodormancy could be related to Cat inhibition through H_2O_2 accumulation in bud tissues. An excess of H_2O_2 in bud tissues could generate an oxidative stress or act as a chemical signal that triggers the expression of genes related to endodormancy release. It is well known that H_2O_2 can function as a signalling molecule in plants (Foyer et al., 1997; Neill et al., 1999; Bolwell, 1999; Durner and Klessig, 1999; Dat et al., 2000). Therefore, changes in levels of H_2O_2 during dormancy were studied in buds of HC-treated and control grapevines.

Evolution of H_2O_2 levels in HC treated and control buds during dormancy

Two transient increases in H_2O_2 levels were detected in buds of HC-treated grapevines. The first one, occurred 3 weeks from the application date when buds were fully dormant and Cat activity was low (Fig. 5). The second H_2O_2 peak occurred after 8 weeks from the application date, when most buds were open and Cat was at high levels of activity (Fig. 5). On the other hand, in buds of control grapevines, a single transient increase in H_2O_2 was detected and it occurred 6 weeks after the application date when buds were fully dormant and Cat reached its maximum activity (Fig. 5).

Since chilling is an environmental factor that inhibits Cat activity in grape buds (Nir et al., 1986), it was interesting to analyse during bud dormancy the interplay between Cat activity and H_2O_2 levels in grapevines grown in the central valley in Chile. In this region, HC applications are not required to advance bud break, since chilling requirements are satisfied during winter and a high and uniform bud break is obtained during spring. However, natural bud break occurs several weeks after the end of endodormancy since low temperatures at the beginning of spring impairs bud break, and thus, buds remain ecodormants until temperature rises. Since endodormancy and ecodormancy are not distinguishable by eye, bud-break response of single-bud cuttings under forcing conditions was used as an indicator to differentiate between both dormant states (Shulman et al., 1983). Fig. 6 shows the interplay between Cat activity and H_2O_2 levels during bud dormancy in grapevines grown in the central valley. In contrast to buds of grapevine grown in the Elqui valley, Cat remained at low levels of activity during most of the endodormancy period and H_2O_2 peaked before endodormancy ended. Afterward, at the end of endodormancy, the activity of Cat increased drastically and a second rise in H_2O_2 was observed. These results, resemble those obtained in buds of HC-treated grapevines grown in the Elqui valley, suggesting that winter chilling and HC inhibits the activity of Cat during bud endodormancy and, as a result, an early H_2O_2 peak is produced.

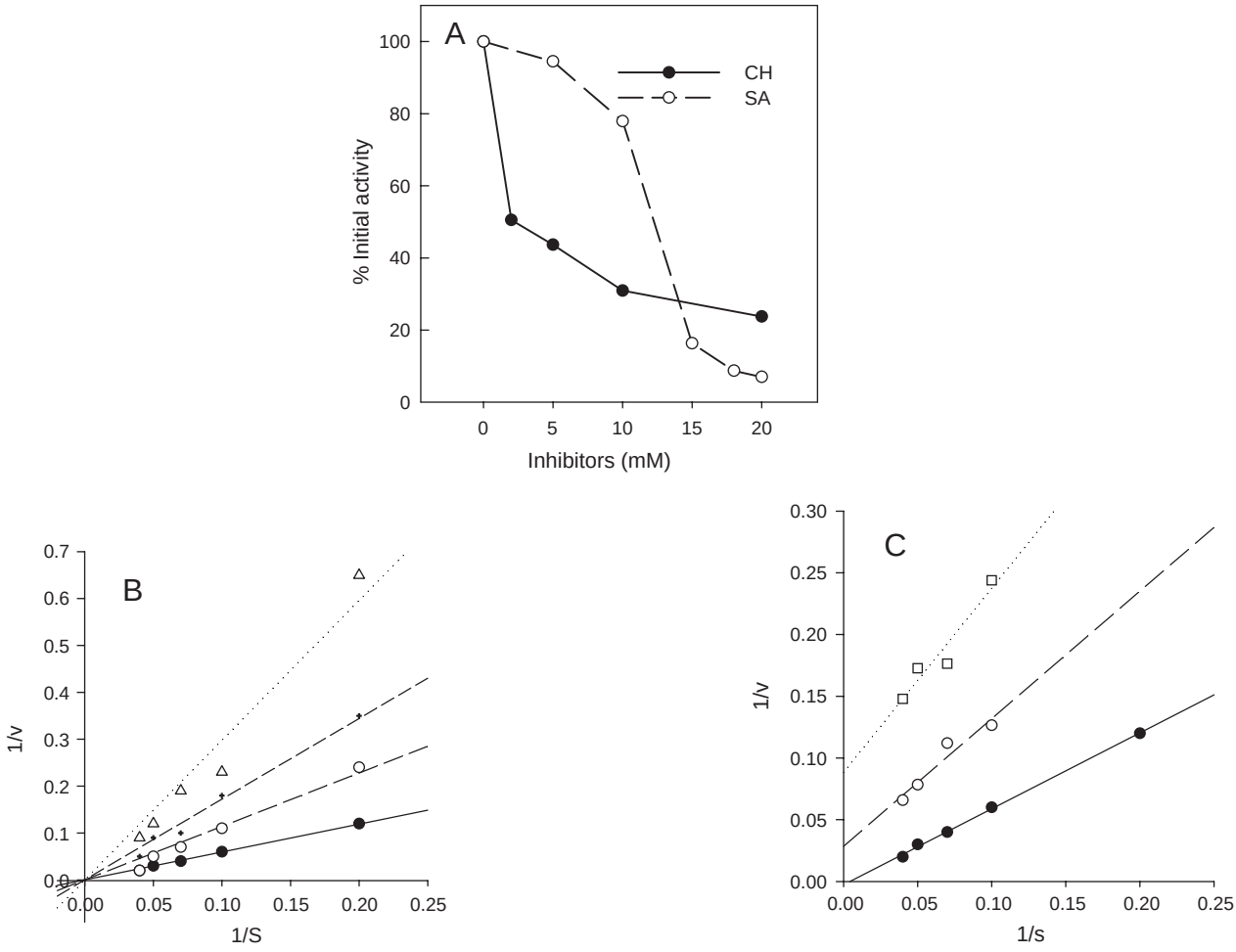

Figure 2. Inhibition of catalase activity in extracts of bud grapevines by H_2CN_2 and salicylic acid (SA). Measurements were carried out at 30 °C in buffer 0.1 M phosphate pH 6.8 and 1 mM H_2O_2. Initial catalase activity was 18 μmol O_2 min^{-1} mg^{-1} prot (A). Lineweaver–Burk plots of H_2CN_2 inhibition (B) and SA inhibition (C).

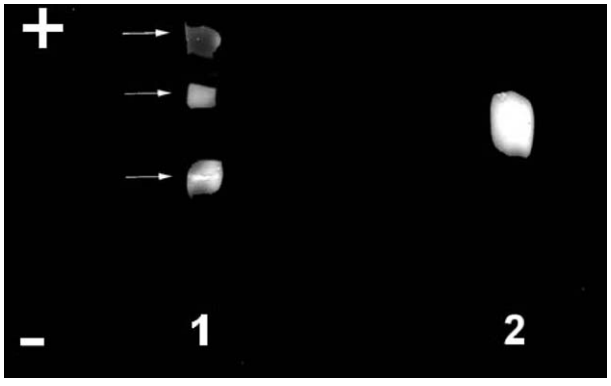

Figure 3. Activity gel assay of catalase. Bud of grapevines were extracted and catalase solubilised in the homogenate. Sample of the homogenate were separated by native PAGE and the activity of catalase measured in the gel. Clear areas of the gel indicate the presence of enzyme activity. Lane 1, catalase isoforms from extracts of bud grapevine. Lane 2, bovine catalase used as positive control.

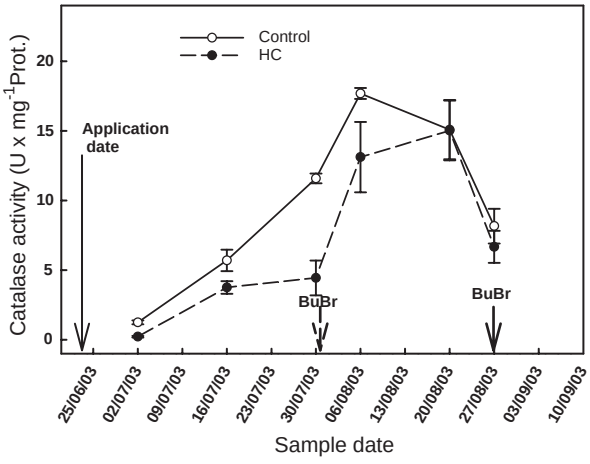

Figure 4. Evolution of catalase activity in buds of H_2CN_2 treated (-●-) and control (-○-) grapevines during dormancy. Vines were from a commercial vineyard located in the Elqui valley in Chile. Dates of bud-break initiation and date of H_2CN_2 application are indicated by arrows. Values are averages of four independent repetitions and bars represent standard deviation.

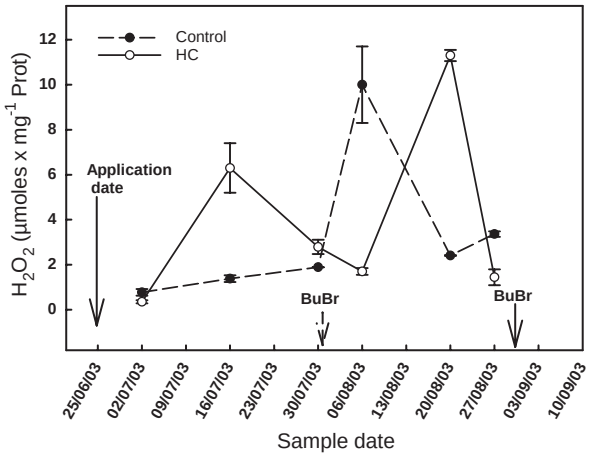

Figure 5. Evolution of H_2O_2 levels in buds of H_2CN_2 treated (-○-) and control (- -●- -) grapevines during dormancy. Dates of bud-break initiation and date of H_2CN_2 application are indicated by arrows. Values are averages of four independent repetitions and bars represent standard deviation.

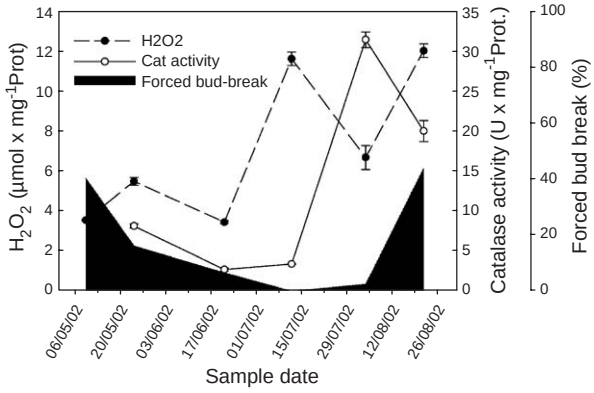

Figure 6. Evolution of H_2O_2 levels (- -●- -) and catalase activity (-○-) in buds of grapevine grown in the Central valley in Chile. Endodormancy is expressed as percentage of bud break under forcing conditions. Bud break was measured after 21 days of bud incubation at 25 °C under a photoperiod of 12 h light.

Discussion

Catalase is a tetrameric enzyme containing an iron heme prosthetic group in each of its subunits and is present in almost all aerobic organisms. In animals, there is only one Cat isoform encoded by a single gene. In contrast, Cat in plants is present as multiple isoforms encoded by a small gene family (Scandalios et al., 1997). It exists in three biochemical distinct isoforms in monocots, such as maize, and in dicots, such as tobacco, arabidopsis and pumpkin. In grape, the isolation of a c-DNA clone encoding a Cat has been recently reported (Or et al., 2001). Comparison of the deduced amino acid sequence with the sequence database revealed that the putative protein encoded by the c-DNA clone was most closely related to Cat-1 from tobacco. Furthermore, Southern blot analysis of genomic DNA from *V. vinifera* cv. Perlette suggested the existence of a small family of genes that code for Cat in the grape genome (Or et al., 2001). At the protein level, three Cat isoforms were found in extracts of bud grape, which agrees with results obtained in other plant species (Scandalios et al., 1997). Moreover, the in vitro activity of Cat was inhibited competitively by HC, suggesting a probable binding of HC to the iron-heme prosthetic group. In vivo, Cat activity was also inhibited by HC, and thus after commercial HC applications to the vines, the activity of Cat remained reduced during the endodormancy period of buds. The same reduction in Cat activity was observed in buds of grapevines grown in the central valley, indicating that HC and winter chilling inhibits its activity during endodormancy. The question emerging from these results is whether the inhibition of Cat during bud dormancy is related to the release of buds from their endodormancy stage. The early peak of H_2O_2 observed in buds of HC-treated grapevines could be the result of the inhibition of Cat by HC and, furthermore, it could act as a chemical signal inducing the expression of genes related to endodormancy release. Several H_2O_2 induced genes have been identified in *Arabidopsis* suspension cultures (Desikan et al., 2000). Moreover, genes encoding signalling proteins, such as transcription factors, protein kinases, and protein phosphatases, are up-regulated by H_2O_2 (Neill et al., 2002). On the other hand, as cytosolic calcium elevation is a common early response to H_2O_2 in plant cells (Price et al., 1994), it is likely that activation of calcium-dependent protein kinases and phosphatases occurs as an early step in the H_2O_2 signal transduction system. Recently, a SNF-like protein kinase GDBRPK was identified in buds of grapevine, which is up-regulated upon chemical induction by HC. The authors (Or et al., 2000) hypothesised that GDBRPK may be involved in the perception of a stress signal induced by HC. Whether the stress signal is mediated by ROS and specifically by H_2O_2 is yet unknown, but it is strongly suggested by the fact that an early H_2O_2 peak, which was not observed in untreated buds, was induced by HC applications. Intracellular accumulation of H_2O_2 is associated with conditions that increase its production or that interfere with its degradation. The two main sources of H_2O_2 generation via electron transport in green tissues, photosynthesis and respiration,

are lacking or function at very low levels in bud tissues, hence it seems likely that accumulation of H_2O_2 depends mainly on mechanisms that interfere with H_2O_2 degradation. Thus, the regulation of Cat, which is the major antioxidative enzyme that degrade H_2O_2 into water and oxygen, should be relevant in the accumulation of H_2O_2 in bud tissues. Although the peak of H_2O_2 in Figs. 5 and 6 is held by a single point, in both cases it represents the average of four independent replicates, and moreover, since H_2O_2 recovery from bud extracts was not affected by the Cat activity present in the extract, the rises in H_2O_2 observed during dormancy are well sustained.

However, the fact that in buds of control grapevines grown in the Elqui valley the H_2O_2 peak coincides with a maximum in Cat activity, introduces some doubts about the role of Cat by itself in regulating H_2O_2 accumulation during dormancy. Furthermore, the presence of peroxidase activities with the ability to oxidise NADH generating H_2O_2 in grape buds (results not shown), could be an alternative way to regulate the levels of H_2O_2.

From these results, we conclude that a transient increase in H_2O_2 levels precedes the release of endodormancy in buds of grapevine. The H_2O_2 peak could act as a signal triggering the expression of genes related to endodormancy release. Moreover, the early occurrence of a H_2O_2 peak in HC-treated buds could be due to the inhibition of Cat and could be the cause of endodormancy shortening and of earlier bud-break response. However, further studies are necessary to establish the direct participation of H_2O_2 as a signal molecule that triggers gene expression related to endodormancy release and the role of Cat in regulating its levels.

Acknowledgements

The financial support of FONDECYT project 10200096 is gratefully acknowledged.

References

Bolwell GP. Role of active oxygen species and NO in plant defence. Curr Opin Plant Biol 1999;2:287–94.

Bradford MM. The quantitation of micrograms quantities of proteins utilising the principles of protein-dye binding. Anal Biochem 1976;72:248–54.

Dat JF, Lop H, Foyer CH, Scott IM. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol 1998;116:1351–7.

Dat JF, Vandenbeele S, Vranova E, Van Motagu M, Inze D, Van Breusegem F. Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 2000;57:779–95.

Desikan R, Neill SJ, Hancock JT. Hydrogen peroxide induced gene expression in *Arabidopsis thaliana*. Free Rad Biol Med 2000;28:773–8.

Durner J, Klessig DF. Nitric oxide as a signal in plants. Curr Opin Plant Biol 1999;2:369–74.

Erez A. Chemical control of bud break. Hortic Sci 1987;22:1240–3.

Erez A. Means to compensate for insufficient chilling to improve bloom and leafing. Acta Horticult 1995;395: 81–95.

Fath A, Bethke P, Beligni V, Jones R. Active oxygen and cell death in cereal aleurone cells. J. Exp. Botany 2002;53:1273–82.

Foyer CH, López-Delgado H, Dat J, Scott IM. Hydrogen peroxide and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol Plantarum 1997;100:241–54.

Godon C, Lagniel G, Lee J, Buhler J, Kieffer S, Perrot M, Boacherie H, Toledo MB, Labarre J. The H_2O_2 stimulon in sacharomyces cervisiae. J Biol Chem 1998;273:22480–9.

Koussa T, Broquedis M, Bouard J. Changes of absisic acid during the development of grapevine latent buds particularly in the phase of dormancy break. Vitis 1994;33:63–7.

Lang GA. Dormancy a new universal terminology. Hortic Sci 1987;22:817–20.

Neill SJ, Desikan R, Clarke A, Hancock JT. H_2O_2 signalling in plant cells. In: Clavert C, Smallwood M, Bowles D editors. Plant response to environmental stress. UK: BIOS Scientific Publishers; 1999. p. 59–63.

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. Hydrogen peroxide and nitric oxide as signalling molecules in plants. J. Exp Botany 2002;53:1237–47.

Nir G, Shulman Y, Fanberstein L, Lavee S. Changes in the activity of catalase in relation to the dormancy of grapevine (*Vitis vinifera* L.) buds. Plant Physiol 1986;81:1140–2.

Or E, Vilozny I, Eyal Y, Ogrodovitch A. The transduction of the signal for grape bud dormancy breaking induced by hydrogen cyanamide may involve the SNF-like protein kinase GDBRPK. Plant Mol Biol 2000;43:483–94.

Or E, Vilozny I, Eyal Y, Ogrodovitch A. Dormancy in grape buds: isolation and characterization of catalase cDNA and analysis of its expression following chemical induction of bud dormancy release. Plant Sci 2001;162:121–30.

Prasad TA. Mechanism of chilling induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids and proteases activities. Plant J 1996;10: 1017–26.

Price AH, Taylor A, Ripley SJ, Griffiths A, Trewavas AJ, Knight RM. Oxidative signals in tobacco increase cytosolic calcium. Plant Cell 1994;6:1301–10.

Saure M. Dormancy release in deciduous fruit trees. Hort Rev 1985;7:817–20.

Scandalios JG, Guan L, Polidoros A. Catalase in plant gene structure, properties, regulation and expression. In: Scandalios J editor. Oxidative stress and molecular biology of antioxidant defences. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1997. p. 343–406.

Shim I, Momose Y, Yamamoto A, Kim D, Usui K. Inhibition of catalase activity by oxidative stress and its relationships to salicylic acid accumulation in plants. Plant Growth Regulat 2003;39:285–92.

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

Warm E, Laties GG. Quantification of hydrogen peroxide in plant extracts by the chemiluminiscense reaction with luminol. Phytochemistry 1982;21:827–31.