Research Article

Study on the expression of dehydrin genes and activities of antioxidative enzymes in floral buds of two sand pear (*Pyrus pyrifolia* Nakai) cultivars requiring different chilling hours for bud break

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Abstract: Seasonal expression pattern of antioxidative genes (dehydrins), activities of antioxidant enzymes and H_2O_2 contents, and their relations to dormancy phases in floral buds of sand pear (*Pyrus pyrifolia* Nakai 'Cuiguan' and 'Wonhwang') were documented in the present study. The results showed that the expression patterns of *PpDHN1*, *PpDHN2*, and *PpDHN3* in the two cultivars were similar, but the expression peak time and quantity were different. In the floral buds of Cuiguan, expression of all three dehydrin genes (*PpDHN1*, *PpDHN2*, and *PpDHN3*) was detected during endodormancy, reached the highest levels in ecodormancy, and then declined during the bud break stage. In the floral buds of Wonhwang, the expression of all the dehydrin genes (*PpDHN1*, *PpDHN2*, and *PpDHN3*) were at their highest in endodormancy and declined in ecodormancy. High activities of superoxide dismutase (SOD) were detected in endodormancy and decreased during ecodormancy, then increased again during bud break stage. Peroxidase (POD) quantities were different between the two cultivars, but followed similar activity patterns: POD activities were low during endodormancy, reached their lowest point during ecodormancy, and then increased again at the bud break stage. Catalase (CAT) activities were increased during endodormancy and reached their peak level in ecodormancy before decreasing in the bud break stage in both cultivars on different sampling dates. Ascorbate peroxidase (APX) activities were decreased in endodormancy, increased in ecodormancy, and at their peak in the bud break stage, with different amounts of ascorbate in both cultivars. The highest level of H_2O_2 content was detected during endodormancy release in floral buds of Cuiguan, while it was decreased during endodormancy in Wonhwang; their content decreased in bud break stage. During dormancy, the activities of SOD, POD, and APX decreased while CAT activity increased with the expression of dehydrin genes and H_2O_2 content. Therefore, the changes in gene expression, enzyme activities, and H_2O_2 content may be involved in the antioxidant defense mechanisms in floral buds of pear cultivars during dormancy.

Key words: Antioxidant enzymes, dehydrin genes, dormancy, flower buds, H_2O_2, *Pyrus pyrifolia*

1. Introduction

Pear (*Pyrus* spp.) belongs to the family Rosaceae and is one of the most economically important temperate fruit trees. China is the world's leading country in pear production and pear ranks third among Chinese fruit industries after orange and apple (Teng, 2011). Like other deciduous trees, pears have a key feature of transition from growth to dormancy during their annual growth cycles. Pear trees must pass through dormancy for their normal growth and development.

Dormancy may be defined as the time between bud set in fall and bud burst in spring. Dormancy is a complex phase of plant development that allows plants to survive under unfavorable environmental conditions in winter (Faust et al., 1997). It can be divided into five phases of variable duration and intensity: paradormancy, endodormancy, ecodormancy, and the two transitional phases from para- to endodormancy and endo- to ecodormancy (Lang et al., 1987). Dormancy is marked by the accumulation of chilling hours, leading to dormancy release, and differs among tree species (Lang et al., 1987; Arora et al., 2003). Therefore, clarifying dormancy mechanisms is important for controlling plant growth and development. Considerable improvement has been made in identifying dormancy-associated genes in woody and herbaceous perennial plants, including Japanese pear (Saito et al.,

2013), peach (Jimenez et al., 2010), apple (Wisniewski et al., 2011), Japanese apricot (Yamane et al., 2008), and poplar (Ruttink et al., 2007), for the purpose of investigating dormancy. Most of these studies suggested that bud dormancy involves many biochemical pathways related to photoperiod, temperature, circadian clocks, water, energy, reactive oxygen species (ROS), and hormones. Many genes involved in dormancy transition have been identified and they are providing useful references for studying perennial plant dormancy. However, the whole picture of the molecular and biochemical mechanisms that control the processes of dormancy in woody perennial plants remains elusive (Dennis, 1994; Chao, 2002). Transcriptome analysis of pears has been used to examine global gene expression during dormancy (Liu et al., 2012; Bai et al., 2013) and has revealed dramatic expression changes of some genes, including dehydrin genes, which might play a very important role during dormancy (Liu et al., 2012).

Dehydrins are one of the most extensively studied putative dehydration protective molecules, group 2 of the late embryogenesis abundant protein family. The expression of dehydrin genes is associated with freezing tolerance (Kosová et al., 2007) and plays an important role in providing protection during dormancy (Yakovlev et al., 2008). Yakovlev et al. (2008) detected reduced transcript levels in most of the 15 dehydrin genes as Norway spruce transitioned from closed to bud burst.

During dormancy, abiotic stresses direct a series of morphological, physiological, biochemical, and molecular changes that negatively affect plant growth and development (Wang et al., 2001). Antioxidant enzymes are activated to cope with the harmful effect of oxidative stress under low temperatures, which is correlated with high stress tolerance (Kang and Saltveit, 2002; Huang et al., 2005), because nearly all abiotic stresses produce ROS during dormancy (Mittler et al., 2004). In pear floral buds, ROS metabolism changes greatly during the dormancy stage (Shao and Ma, 2004). Protection against free oxygen radicals during dormancy is achieved through the action of antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), and ascorbate peroxidase (APX; EC 1.11.1.11).

The plant antioxidation system is composed of a series of complex mechanisms. Many antioxidants, including some special proteins, are involved, which usually accumulate in response to environmental stresses during dormancy (Sun and Lin, 2010). Antioxidants are the first line of defense against free radical damage. Both antioxidant enzymes and dehydrins have been broadly studied for their roles in environmental stress tolerance and they are natural defensive elements in living organisms that scavenge excessive ROS (Chen, 2011). Lately, antioxidative and anti-ROS activities have been thought to be an important

function of dehydrins and antioxidant enzymes (Sun and Lin, 2010; Chen, 2011). It has been suggested that dehydrins and antioxidant enzymes probably play a role in antioxidative defense response directly by their ROS scavenging activity (Mittler, 2002; Hara et al., 2004).

The present study was conducted to obtain data for the assessment of chilling requirements and to check the dormancy status of floral buds in two pear cultivars. Different seasonal expression patterns of dehydrin genes (*PpDHN1*, *PpDHN2*, and *PpDHN3*) and changes in activities of antioxidant enzymes (SOD, POD, CAT, and APX) and H_2O_2 content during dormancy under natural growing conditions were assessed in the floral buds of two pear cultivars requiring different chilling hours for their bud break. The aim of our study was to examine the relationships among dehydrin genes, antioxidant enzymes, and H_2O_2 content in the antioxidant defense mechanism in floral buds of two pear cultivars during dormancy.

2. Materials and methods

2.1. Plant materials
To illustrate the expression patterns of three dehydrin genes (*PpDHN1*, *PpDHN2*, and *PpDHN3*), activities of four antioxidant enzymes (SOD, POD, CAT, and APX), and H_2O_2 content during dormancy, floral buds of Cuiguan and Wonhwang were grafted on *P. calleryana* Decne. Rootstocks were collected at a commercial pear orchard located in Fuyang, Zhejiang Province, China. One-year-old shoots were collected from October 2011 to mid-March 2012, at approximately 7- or 15-day intervals. Floral buds were scraped from the shoots, put immediately in liquid N_2, and then stored at –80 °C.

2.2. Monitoring of temperature
Ambient air temperature in the field was recorded hourly during the experiment with an automatic temperature-humidity recorder (ZDR-20, Hangzhou Zeda Instruments Co., Ltd., China). The data are shown in Figure 1.

2.3. Assessment of chilling requirements
Hourly temperature data were used to calculate the chilling requirements using three chilling models: chill hours (CH), hours below 7.2 °C (Weinberger, 1950); chill units, Utah model (CU) (Richardson et al., 1974); and chill portions (CP), dynamic model (Luedeling and Brown, 2011).

2.4. Determination of dormancy status
Bud break percentage was examined following the method of Liu et al. (2012). In the present study, dormancy depth was described as the number of days until the first bud break (or time for the first bud to break) under control conditions (Hauagge and Cummins, 1991). Bud dormancy strength was grouped into shallow (<10 days to first bud break), medium (10 to 21 days to first bud break), and deep (>21 days to first bud break) dormancy.
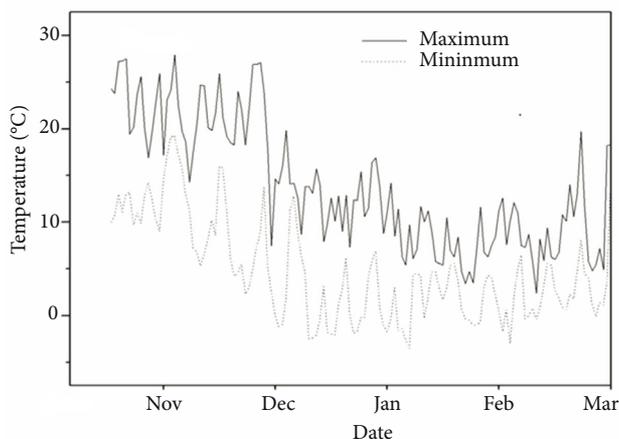
Figure 1. Daily maximum and minimum temperatures of the experimental orchard during dormancy.

2.5. RNA extraction and quantitative PCR analysis

On the basis of a transcriptome library constructed from dormant Suli pear, three dehydrin genes were retrieved: *PpDHN1*, *PpDHN2*, and *PpDHN3*, with accession numbers of DQ660905, JQ649464, and JQ649456, respectively. Total RNA was isolated from the floral buds by the CTAB method of Zhang et al. (2005), with a slight modification. Quality and quantity of RNA was examined by gel electrophoresis and spectrophotometer test. Total RNA concentration was measured after DNase-I digestion. First-strand cDNA was synthesized from 4 µg of DNA-free RNA using a Revert Aid First-Strand cDNA Synthesis Kit (Fermentas, USA) and 3 µL of the diluted cDNA was used for quantitative PCR (Q-PCR) analysis. Q-PCR reactions were performed for the measurement of the expression of selected genes with specific forward and reverse primers that were designed using Primer 3.0 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (Table 1). Total volume for the Q-PCR reaction was 15 µL, containing 7.5 µL of SYBR Premix Ex Taq TM (Takara, Japan), 3 µL of cDNA, 0.5 µL of each primer (10 µM), and 3.5 µL of RNase-free water. Three replications of each sample were performed. The reaction was performed on a LightCycler 1.5 (Roche, Germany), with a preliminary step of 30 s at 95

°C followed by 40 cycles that included 95 °C for 5 s and 60 °C for 20 s. Verification for the specificity of the amplifications was performed by obtaining a single melting curve peak and a single band in agarose gel electrophoresis. *PpActin* (JN684184) was used as a housekeeping gene.

2.6. Antioxidant enzyme assays

For enzyme extraction, 0.5 g of floral buds was ground in 5 mL of extraction buffer (50 mM potassium phosphate, 1 mM EDTA, 1% PVP, 1 mM DTT, and 1 mM PMSF with a pH of 7.8) under ice-cold conditions. The extractions were centrifuged at 15,000 × *g* for 30 min at 4 °C and the supernatant was used for the determination of the following enzyme activities.

SOD activity was measured spectrophotometrically at 560 nm by recording the rate of *p*-nitro blue tetrazolium chloride reduction using an Eppendorf BioSpectrometer according to the method of Giannopolities and Rise (1977).

POD, CAT, and APX activities were examined following the method of Zhang and Kirkham (1996) by observing the changes in absorbance at 470, 240, and 290 nm, respectively, using an Eppendorf BioSpectrometer.

2.7. Hydrogen peroxide determination

The H_2O_2 content was measured using the method of Bernt and Bergmeyer (1974) with slight modifications. A sample of powdered floral buds (0.5 g) was homogenized in 1 mL of 100 mM sodium phosphate buffer (pH 6.8), and the extractions were centrifuged at 16,000 × *g* for 20 min at 4 °C. Then 0.17 mL of supernatant was added to 0.83 mL of peroxidase reagent containing 83 mM sodium phosphate (pH 7.0) and 0.005% (W/V) *o*-dianisidine; 40 µg peroxidase/mL of 1 N perchloric acid was added to stop the reaction. The absorbance was read at 436 nm. The H_2O_2 concentration was calculated by using a standard curve with known concentrations.

2.8. Statistical analysis

Data were managed with the Data Processing System (DPS, v. 7.05, Zhejiang University, China). Two-way analysis of variance (ANOVA) was carried out, followed by the least significant difference (LSD) test at P ≤ 0.05 for separation of means.

Table 1. List of primer sequences used for Q-PCR.

No.	Gene name	Product length, nt	Primer sequences (forward/reverse)
1.	*PpDHN1*	99	GTCTGGTCTGACCTCGGTTG/GGGAACGGCAGACAAAATTA
2.	*PpDHN2*	105	CCCTGTGATGGGTGGTTTAT/AAGGTCCTCCCAAACCAAAG
3.	*PpDHN3*	396	GCACCGAACACTACGCAATA/CCGTTACTCGAACGAGCACT
4.	*PyActin*		CAGATCATGTTTGAGACCTTCAATGT/CATCACCAGAGTCCAGCACAAT

3. Results

3.1. Chilling requirements of two pear cultivars

Our results revealed that the chilling requirements, using the 7.2 °C, Utah, and dynamics models, respectively (Table 2), were 296 CH, 304 CU, and 20 CP for Cuiguan and 1065 CH, 992 CU, and 50 CP for Wonhwang. This suggests that Cuiguan has lower chilling requirements than Wonhwang for its bud break.

3.2. Dormancy status in floral buds of pear cultivars

Fewer than 50% of Cuiguan buds had broken on shoots sampled before 22 December, while more than 50% of buds had broken after 22 December (Figure 2). Therefore, floral buds were determined to be in the endodormancy phase before 22 December. Floral buds were estimated to be in the endodormancy release phase between 15 and 22 December and in the ecodormancy phase after that, because of more than 50% bud break. However, in Wonhwang, fewer than 50% of buds broke on shoots sampled before 13 January; more than 50% had broken on shoots sampled on 30 January (Figure 2). Therefore, floral buds were estimated to be in the endodormancy phase before 13 January, and the ecodormancy phase was considered to last from 30 January to the end of the sampling dates. Furthermore, medium dormancy was observed in Cuiguan from 30 October to 30 November, while shallow dormancy was observed from 15 December to the end of the endodormancy release (Table 3). In Wonhwang, deep dormancy was observed on 30 November and 15 December; medium dormancy was observed on the majority of sampling dates shown in Table 3.

3.3. Seasonal expression pattern of dehydrin genes in floral buds during the dormancy

To elucidate the expression of dehydrin genes during the dormancy period, transcript levels of dehydrin genes
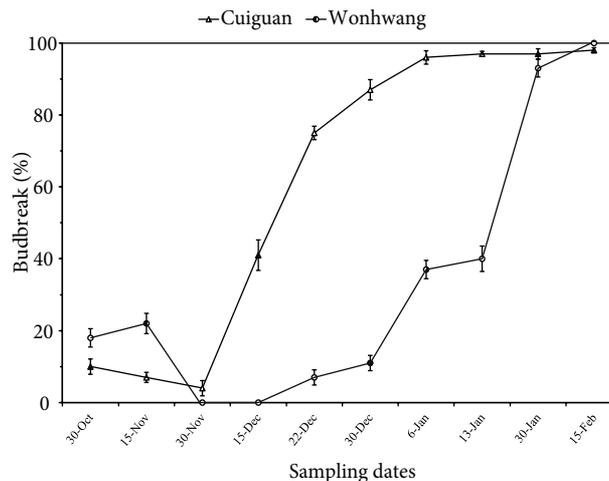

Figure 2. Floral bud break percentage of Cuiguan and Wonhwang after 21 days of forced conditions. Dormant shoots of field-grown pear trees were collected on 30 October, 15 November, 30 November, 15 December, 22 December, 30 December, 6 January, 13 January, 30 January, and 15 February. The shoots were kept in water-containing vials in a phytotron at day/night 25 ± 1/18 ± 1 °C, with a 12-h photoperiod of white light (320 µmol m^{-2} s^{-1}) and 75% humidity. The bud break percentage in 12 shoots per sampling time was measured after 21 days.

were determined in floral buds of two pear cultivars from November to March (Figures 3A–3C). *PpDHN1*, *PpDHN2*, and *PpDHN3* expression was markedly upregulated in floral buds of both cultivars in endodormancy. The highest peaks in the expression of *PpDHN1*, *PpDHN2*, and *PpDHN3* were detected during ecodormancy in Cuiguan and during endodormancy in Wonhwang, with different amplitudes of transcription (Figures 3A–3C). After reaching a peak, their expressions decreased

Table 2. Chilling requirements for pear cultivars according to 7.2 °C, Utah, and dynamics models.

Cultivars	7.2 °C model/CH	Utah model/CU	Dynamics model/CP
Cuiguan	296	304	20
Wonhwang	1065	992	50

Table 3. Seasonal dormancy depth in the floral buds of two pear cultivars using whole branches.

		Days to floral bud break of pear cultivars by sampling dates									
Cultivar	Location	30.10	15.11	30.11	15.12	22.12	30.12	6.1	13.1	30.1	15.2
Cuiguan	Fuyang	+	+	+	++	++	++	++	++	++	++
Wonhwang	Fuyang	+	+	–	–	+	+	+	+	++	++

First bud break was observed within 10 days (++), observed within 21 days (+), or not observed within 21 days (–).
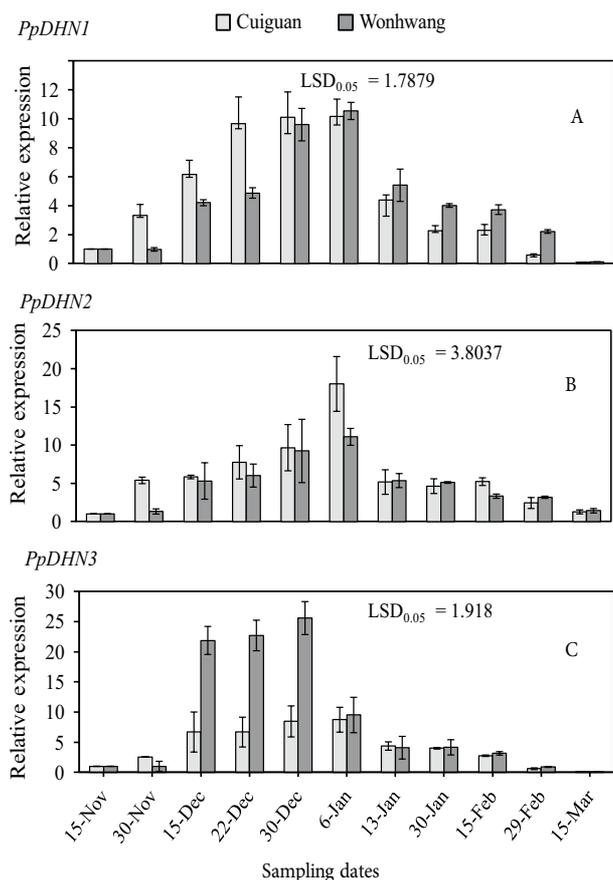
Figure 3. Transcript levels of dehydrin genes *PpDHN1*, *PpDHN2*, and *PpDHN3* in floral buds collected from two pear cultivars during the dormancy period. A) *PpDHN1* expression; B) *PpDHN2* expression; C) *PpDHN3* expression. The transcription level for each gene was calculated by data obtained from Q-PCR and normalized against an actin gene primer. Data represent means ± standard deviations (n = 3 in all the sampling dates). Different bars indicate statistical significant differences based on the LSD test at P ≤ 0.05 between time points with three replications.

gradually in both cultivars (Figure 3A). Compared to Wonhwang, in Cuiguan the intensity of *PpDHN1* and *PpDHN2* was high during upregulation and low during downregulation (Figures 3A and 3B). *PpDHN3* expression disappeared during the bud break stage in both cultivars. *PpDHN3* expression in Wonhwang was much higher than in Cuiguan during the whole dormancy period (Figure 3C). Our results demonstrated that the expression level of *PpDHN3* varied greatly in floral buds between the two cultivars during dormancy.

3.4. Changes in antioxidant enzymes in floral buds of pear cultivars during dormancy

SOD, POD, CAT, and APX activities were studied in floral buds of two pear cultivars during dormancy (Figures 4A–4D).

SOD, POD, CAT, and APX are key antioxidant enzymes. In the present study, SOD and POD activities in Cuiguan and Wonhwang were somewhat similar during the whole dormancy period, though with different amounts. SOD and POD activities were decreased during endodormancy and changed very slightly in ecodormancy, while they increased at the bud break stage in Cuiguan. In Wonhwang, SOD and POD activities were decreased in endodormancy and increased during ecodormancy until bud break (Figures 4A and 4B). SOD activity was higher in Wonhwang than in Cuiguan during the whole dormancy period, while in Cuiguan POD activity was higher than in Wonhwang in ecodormancy and lower during endodormancy and bud break (Figures 4A and 4B).

In the present experiment, CAT activity was increased in endo- and ecodormancy and decreased during the bud break stage in both cultivars (Figure 4C). However, the activity levels of CAT in Cuiguan were highest during endodormancy, while in Wonhwang the highest peak was observed during ecodormancy (Figure 4C).

APX activity was similar in both Cuiguan and Wonhwang. The lowest APX activity point was observed in endodormancy and ecodormancy in Cuiguan and Wonhwang, respectively (Figure 4D). Furthermore, after reaching its lowest level, APX activity increased and reached its highest point during the bud break stage, though with different amounts in the two cultivars (Figure 4D).

3.5. Changes in H_2O_2 content in floral buds of pear cultivars during dormancy

In Cuiguan, the H_2O_2 content was low in endodormancy and increased rapidly, reaching its highest point during the endodormancy release stage; it decreased again in ecodormancy and reached its lowest level in the bud break stage (Figure 5). However, in Wonhwang, the H_2O_2 content was increased during endodormancy and reached its peak in endodormancy release, then decreased at bud break stage (Figure 5). H_2O_2 content was higher in Wonhwang from endodormancy to bud break.

4. Discussion

Dormancy in the floral buds of perennial fruit plants plays an imperative role for survival under harsh environmental conditions. During the dormancy period, different kinds of physiological, molecular, and biochemical changes occur in buds (Viti et al., 2010; Gai et al., 2013). The expression of 15 dehydrin genes appeared to be downregulated close to bud burst in Norway spruce (Yakovlev et al., 2008). The association of dehydrin genes during the dormancy period has received some attention in perennial plants, particularly in temperate fruit trees. However, there is still little information regarding their role during the dormancy period in perennials. In the present study, the
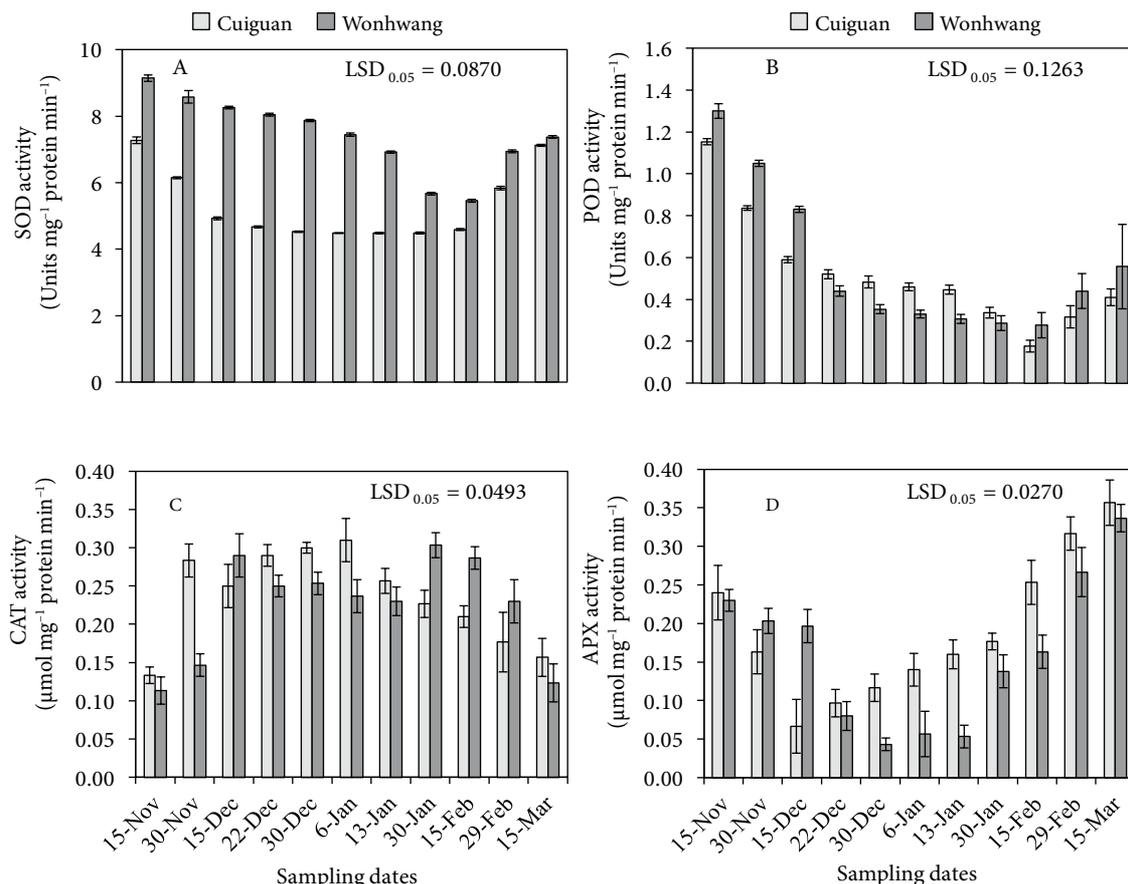
Figure 4. Seasonal responses of SOD, POD, CAT, and APX antioxidant enzymes activities in the floral buds of two pear cultivars during dormancy on different sampling dates. A) Superoxide dismutase (SOD) activities; B) peroxidase (POD) activities; C) catalase (CAT) activities, and D) ascorbate peroxidase (APX) activities. Activities are presented as means ± standard errors of three replicates based on LSD test at P ≤ 0.05.
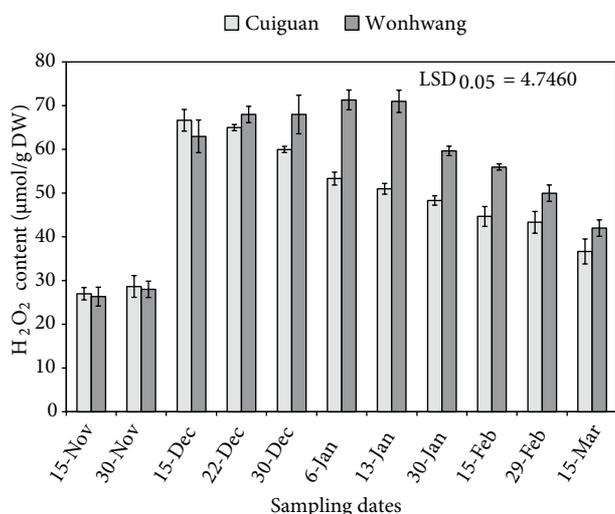

Figure 5. Changes in H_2O_2 content in the floral buds of two pear cultivars during dormancy on different sampling dates. Changes in content are presented as means ± standard errors of three replicates based on the LSD test at P ≤ 0.05.

expressions of three dehydrin genes (*PpDHN1*, *PpDHN2*, and *PpDHN3*) were analyzed in the floral buds of two pear cultivars requiring different chilling requirements for their bud break.

Significantly higher expressions of *PpDHN1*, *PpDHN2*, and *PpDHN3* were detected during ecodormancy in Cuiguan and during endodormancy in Wonhwang; thenceforth, the expression levels of these genes rapidly decreased (Figures 3A–3C). Liu et al. (2012) recently reported that pear dehydrin was highly expressed during the transition from endo- to ecodormancy. The expression of all three genes remained for a longer time in Wonhwang (high chilling) than in Cuiguan (low chilling), with different expression levels (Figures 3A–3C). Recent findings of Welling et al. (2004) and Yamane et al. (2006) showed that dehydrin transcription levels were found in birch and Japanese apricot during endodormancy or in response to low temperature. The dormant floral buds of two Japanese apricot cultivars accumulated *Pmdhn1* in different amounts (Yamane et al., 2006). *PpDHN3* was more strongly

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expressed in dormant floral buds of Wonhwang than in those of Cuiguan (Figure 3). In addition, the amount of *PpDHN3* increased in Wonhwang during endodormancy in the end of December, whereas in Cuiguan, its expression increased up to bud break (Figure 3). Recent studies have reported that the transcript levels of *Pmdhn1* began earlier in Japanese apricot cultivar Ellching (low chilling cultivar) than in Nanko (high chilling cultivar), but the dormant buds of Nanko accumulated more *Pmdhn1* than Ellching (Yamane et al., 2006). *PpDHN1*, *PpDHN2*, and *PpDHN3* levels were downregulated at the end of ecodormancy and during the bud break stage in the floral buds of both cultivars (Figures 3A–3C). Similar results were obtained by Welling et al. (2004) and Yamane et al. (2006); the expression of dehydrin genes seems to be downregulated in bud tissue during the breaking of dormancy. Based on our results and previous findings, it seems that accumulation of dehydrin is a common feature of cold acclimation and coincides with the beginning of dormancy, and the levels of dehydrin transcription seem to stay very high during the dormancy. We assume that dehydrin genes play an important protective role during dormancy, but the majority of molecular mechanisms related to dormancy transitions in pear cultivars remain largely unknown. To best of the present authors' knowledge, this is the first study on dehydrin gene expression during dormancy in two different pear cultivars.

Environmental stress, such as low temperature, has profound negative impacts on deciduous fruit trees during dormancy. Antioxidative systems prevent or ease oxidative stress, which is an important factor in relation to a chilling injury resulting from ROS under low temperature in plants (Chiang et al., 2006). Plants have a set of enzymatic scavengers, including SOD, POD, CAT, and APX, to keep ROS under control (Garbero et al., 2011).

SOD may be the key step in protecting against chilling temperature and ROS production, thus playing a vital role in the plant antioxidant system. The role of SOD during environmental adversity has received much attention, since ROS have been found to be produced during many stress conditions in plants (Gong et al., 2001). SOD and POD activities were high in endodormancy, while their activities declined and reached the lowest point during ecodormancy in Cuiguan and endodormancy in Wonhwang (Figures 4A and 4B). Wang et al. (1991a, 1992b) and Abassi et al. (1998) also showed that SOD and POD activities were very low in apple buds during dormancy and increased during bud break, which confirmed that the dormant tissues have very low levels of O_2^- free radicals. After cold temperatures and the bud break stage, reactive O_2^- is overproduced; therefore, SOD and POD activities increase to protect the cells from damage. Our results confirm the findings of Wang et al. (1991b) and Shao and

Ma (2004); they stated that SOD and POD activities rise during the breaking of dormancy. The increasing activities of SOD and POD at the bud break stage (Figures 4A and 4B) confirm Wang et al.'s (1991a) suggestion that the SOD and POD enzymes are involved in breaking bud dormancy by detoxifying the tissue of free radicals. In previous findings, Wang et al. (2009) reported that the POD activity in alfalfa roots decreased when subjected to chilling stress, but two cultivars showed the same POD activity. Various components of antioxidative defense systems involved in ROS scavenging have been manipulated; overexpression or downregulation of SOD and POD provides new knowledge of the role of the antioxidant systems.

CAT is a protective enzyme, like SOD. It traps H_2O_2 and converts it to water and oxygen. Our results showed that CAT activity was low in the transition and bud break stage of dormancy and high during endodormancy and ecodormancy in floral buds of both cultivars (Figure 4C). Shao and Ma (2004) stated that CAT activity in pear floral buds was high during the dormant stage, which strongly supports our result. Gao et al. (2002a) stated earlier that CAT activity is decreased during dormancy release, which is very similar to our findings. The significant increase in CAT activity during dormancy observed here suggests a more efficient scavenging of H_2O_2, which may result in better protection against peroxidation. These results suggest that CAT activity is directly correlated with dormancy in floral buds of both cultivars.

APX plays a vital role in the control of intracellular ROS levels and also reduces H_2O_2 to water using ascorbate as the electron donor, resulting in the formation of dehydroascorbate. During dormancy, APX activity changes seasonally in fruit stress (Yuan et al., 2003). APX enzymes also act in tandem with SOD to scavenge H_2O_2 generated through SOD action, such as superoxide anion radical (O_2^-) dismutated to H_2O_2. In the present study, APX activity was decreased during endodormancy and reached its lowest level in the transition from endo- to ecodormancy and then increased again, reaching its highest level in the bud break stage (Figure 4D). The present results about APX activities in pear floral buds are consistent with the previous findings of Shao and Ma (2004), who reported that the APX activity decreased during the dormancy phase and then increased during bud break. This means that ROS metabolism has a very strong link with the natural dormancy of pear floral buds during dormancy.

Due to the long evolution process in plants, they have developed intracellular enzymatic and nonenzymatic defense systems to reduce ROS damage. In this experiment, H_2O_2 content was increased during endodormancy release and decreased in ecodormancy and bud break (Figure 5). This result is consistent with the previous finding of Gao

et al. (2002b), who stated that the changes in H_2O_2 content are very closely related to dormancy in peach. They further stated that in dormant phases, H_2O_2 content was increased, but by the end of dormancy, the content was decreased. The present results about H_2O_2 are also very similar to the previous findings of Shao and Ma (2004), who observed that during dormant stage in pear floral buds, the H_2O_2 content increased during dormancy and decreased in bud break.

Low temperature induces ROS production that can damage chloroplasts, mitochondria, membrane lipids, and proteins (Prasad, 1996). Plant cells are equipped with antioxidant enzymatic machinery (SOD, POD, CAT, and APX) that protects plants against environmental stresses, but enzymatic activities are rather complex and cannot protect the plant completely from outside invaders such as ROS. However, plants employ multiple mechanisms to strengthen their protective system, such as the accumulation of soluble sugars, proline, hormones, and antioxidative genes (Koster and Lynch, 1992; Tao et al., 1998). Dehydrins belong to the family of antioxidant genes, which protect the plant against the damaging effects of certain environmental stresses (Close, 1996; Sun and Lin, 2010). To find out whether dehydrin genes are involved in antioxidative mechanisms and thus reinforce the antioxidant enzyme activity of plants, three dehydrin genes and four antioxidant enzymes were analyzed in the present study. With a decrease in temperature during dormancy, the amount of ROS increased, which can be seen in the increase of H_2O_2 content in the present study (Figure 5). Xu et al. (2014) reported that H_2O_2 content was high after cold temperature treatment in loquat fruitlets. SOD is the main enzyme responsible for scavenging ROS and for converting it into oxygen and H_2O_2 (Cruz-de-Carvalho, 2008), but in our results, increases in H_2O_2 content were accompanied by decreases in SOD activity.

The results of the present study show that there might be some other factors involved in ROS scavenging. On the other hand, dehydrin gene expression increased with the increase of H_2O_2 content from endodormancy to ecodormancy in our study. It can thus be hypothesized that dehydrin gene expression could be one of the factors involved in protecting plant membranes against ROS.

In summary, despite their contrasting chilling requirements and levels of dehydrin gene expression and antioxidative enzyme levels, similar trends in the expression of the three dehydrin genes, antioxidative enzymes activities, and H_2O_2 content were observed in both cultivars during dormancy stages. Q-PCR analysis detected the expression of three dehydrin genes (*PpDHN1*, *PpDHN2*, and *PpDHN3*) during endodormancy in the floral buds of both cultivars, but the transcription peak occurred during ecodormancy in Cuiguan and during endodormancy in Wonhwang. The expression level of *PpDHN3* was more abundant in Wonhwang than Cuiguan, but the expression patterns were similar for the three genes in both cultivars. Data indicated that the expression of the three dehydrin genes during the endodormancy in floral buds of both cultivars was associated with the same trends but in different amounts. However, CAT activity and H_2O_2 content were higher during dormancy and SOD, POD, and APX activities were lower during dormancy and higher during the bud break stage. This additive action may protect and stabilize the cells to make the plant hardier during dormancy.

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