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Screening and Expression Analysis of *POD* Gene in POD-H_2O_2 Pathway on Bud Dormancy of Pear (*Pyrus pyrifolia*)

Xuwen Xiang [1,2], Keke Song [1,2], Yinyin Li [1,2], Chenyu Zhang [1,2], Ruiqi Zhou [1,2], Yu Feng [1,2], Jingnan You [1,2], Jingdong Wu [3], Yanhui Zhang [4], Cuicui Jiang [5,*] and Yongyu Li [1,2,*]

1 Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, China
2 Institute of Natural Products of Horticultural Plants, Fujian Agriculture and Forestry University, Fuzhou 350002, China
3 Economic Crop Station, Agricultural and Rural Bureau of Jianning County, Sanming 354500, China
4 Economic Crop Station, Agricultural and Rural Bureau of Yongtai County, Fuzhou 350700, China
5 Fruit Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350002, China
* Correspondence: cuiliao2046@163.com (C.J.); yongyu.li@fafu.edu.cn (Y.L.)

Abstract: Bud endodormancy represents a pivotal and intricate biological process influenced by both genetic and epigenetic factors, the exact mechanism of which remains elusive. Hydrogen peroxide (H_2O_2) functions as a signalling molecule in the regulation of dormancy, with peroxidase (POD) playing a crucial role in governing H_2O_2 levels. Our prior transcriptomic and metabolomic investigations into diverse pear dormancy phases posited that *POD* predominantly oversees pear bud dormancy. In this study, we utilised qRT-PCR to screen the most significantly expressed gene, Pyrus pyrifolia *POD4-like* (*PpPOD4-like*), from seven *POD* genes. Subsequently, H_2O_2 test kits, overexpression methods, and subcellular localisation techniques were employed to assess changes in H_2O_2 content, POD activity, *PpPOD4-like* expression, and its cellular positioning during pear bud dormancy. Subcellular localisation experiments revealed that PpPOD4-like is situated on the cell membranes. Notably, H_2O_2 content exhibited a rapid increase during endodormancy and decreased swiftly after ecodormancy. The fluctuation pattern of POD activity aligned with that of H_2O_2 content. Additionally, *PpPOD4-like* expression was markedly upregulated, displaying an overall upward trajectory. Our findings indicate that *PpPOD4-like* modulates H_2O_2 levels by regulating POD activity, thereby actively participating in the intricate regulation of pear dormancy processes.

Keywords: endodormancy; overexpression; H_2O_2; *POD* genes

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1. Introduction

Pears are cultivated from perennial fruit trees belonging to the Pyrus L. genus (*Rosaceae*), representing one of the most extensively planted fruit trees globally. In China, pears rank third in fruit production, following apples and citrus fruits [1]. According to the United Nations Food and Agriculture Organization (FAO) statistical database (http://www.fao.org), China led the world in pear planting, with a planting area of 913,400 hm² and a total output of 19,261,500,000 kg in 2022. The northern regions predominantly cultivate late-ripening pear varieties, typically maturing in mid-September, while the southern regions exploit the advantage of early ripening, often entering the market before August. However, the warming global climate and the pronounced greenhouse effect have led to frequent occurrences of "warm winter" in many southern regions of China, significantly affecting agriculture and forestry production, particularly deciduous fruit trees such as pears, which exhibit the biological characteristic of winter bud dormancy. Inadequate endodormancy during warm winters adversely impacts bud quality, resulting in reduced production in the subsequent growing season [2,3]. Therefore, investigating and comprehending the

regulatory mechanism of winter bud dormancy in pears holds not only considerable biological significance for species survival and reproduction, but also substantial economic importance in agricultural production.

Reactive oxygen species (ROS), encompassing superoxide anion radical ($O^{2-}\cdot$), hydroxyl radical (OH·), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), etc., constitute a class of oxygen-containing substances with active chemical properties and potent oxidation capacity in both plants and animals [4]. ROS, particularly H_2O_2, are recognised as crucial signalling molecules in plants, governing development and growth [5]. They activate second messengers, modulate gene transcription, and influence enzyme activity [4–6]. AS the precursor for most other ROS, $O_2^-\cdot$ could be catalysed into H_2O_2 by superoxide dismutases (SODs) and further reduced to the hydroxyl radical or water by peroxidases [4]. Normal cell aerobic metabolism and responses to oxidative stress promote ROS production in vitro [7]. Under stress conditions, the balance between ROS production and removal is disrupted, leading to the accumulation of ROS, including H_2O_2, in plants. This accumulation triggers peroxidation of the plasma membrane, loss of membrane permeability, and a cascade of physiological and biochemical reactions, ultimately causing metabolic disorders and injury [8]. H_2O_2, as the most studied ROS signalling molecule, plays a vital role in plant biology [9]. The winter dormancy of plant buds is a physiological response to short sunshine or low-temperature stress, and studies have identified the presence of ROS, particularly H_2O_2, in dormant buds of various plants, including pear [10], grape [11], apple [12]. H_2O_2 content in pear buds exhibits dynamic changes throughout the dormancy period, corresponding to the natural dormancy process [13,14]. Consequently, H_2O_2 emerges as a key signalling molecule in regulating the dormancy process in fruit trees.

Peroxidase (POD) is a multifunctional enzyme containing heme in plant, and the regulation of peroxidase by hydrogen peroxide is highly intricate [15]. POD serves as a secondary metabolite hydrogen donor, extensively participating in physiological and biochemical processes such as auxin degradation and the regulation of extracellular H_2O_2 levels [16,17]. Simultaneously, POD acts as an enzyme in response to stress. Its affinity for H_2O_2 is 1000 times that of catalase (CAT), playing a pivotal role in reactive oxygen species (ROS) clearance and serving as one of the key enzymes in the plant enzymatic defence system under stress conditions [18]. Studies have indicated that the transition of grape buds from internal dormancy to ecodormancy is closely related to POD, and changes in POD can be employed as a marker to study this transition [11]. Ambient temperature variations can regulate the synthesis and transport of POD in *Crocus sativus* bulbs [19]. According to research, the relief of seed dormancy in *Paeonia rockii* is closely related to changes in POD activity [20]. Low temperatures can increase the expression of the *POD* gene during the dormancy of grape buds [21]. Additionally, the overexpression of the cell wall-associated peroxidase gene (*TPX2*) in potatoes increases seed germination rates under high salt and osmotic stress [22]. Notably, 114 *POD* genes crucial in the growth process of pear fruit were identified in the *POD* gene family of pears [23]. Interestingly, H_2O_2 content accumulates during endodormancy and decreases during dormancy release in buds of many plants, suggesting that H_2O_2 is an important signalling molecule regulating the dormancy process [24,25]. The above results indicate that the POD-H_2O_2 pathway plays a crucial role in plant growth and development. In our previous study, we observed that H_2O_2 plays an important role in regulating the dormancy process of pear buds [10]. Additionally, through miRNAs [26] and the lncRNAs transcriptional database [19], we identified *POD* genes with a differential expression over five times, which may be of great importance in the dormancy process of pear buds. In this study, a peroxidase gene (*PpPOD4-like*) was cloned from pears, and the sequence structure, physical, and chemical properties of the gene were predicted to explore the function of the *PpPOD* gene in pear bud dormancy. Additionally, the change trend of the gene, POD activity, and H_2O_2 content during the dormancy of pear buds was analysed. The correlation of H_2O_2 content and POD activity with the *PpPOD4-like* gene was also investigated.

2. Materials and Methods

2.1. Plant Materials

Ten-year-old pear trees ("*Huanghua*", *Pyrus pyrifolia*), cultivated in an experimental orchard in Jianning Town, Fujian Province, China, were utilized for this study. Following the procedure outlined by Li et al. [10], one-year-old branches with healthy floral buds were collected on 4 December 2022. Agronomic measures, such as tree body pruning and pesticide spraying, were discontinued one month prior to sampling. After collection, the buds were carefully wrapped in moist cotton wool and transported to the laboratory. Subsequently, pruning was performed approximately 1 cm from the base, and the branches were placed in clean water and promptly transferred to cold storage for hydroponics (4 ± 1 °C) to maintain the endodormancy state. For each time point (0 d, 7 d, 14 d, 21 d, 28 d, 35 d, 42 d, 49 d), seventy-five branches were randomly selected, with forty-five branches dedicated to germination rate statistics and thirty branches earmarked for collecting flower buds for subsequent treatments.

To determine the stage of pear bud dormancy, the methods outlined in previous studies [10,27] were employed. Fifteen branches were randomly selected from each batch of samples and placed in an artificial climate chamber (maintained under conditions of day/night: 12/12 h, temperature of day/night: $25 \pm 1/20 \pm 1$ °C, relative humidity 75%). Branches were pruned approximately 1 cm from the base every 2 days, and the water was changed. After 21 days, the germination rate was measured with 3 replicates.

Additionally, for studying changes in H_2O_2 content, POD activity, and related gene expression in floral buds at different dormancy stages of pear, thirty branches from each batch of samples were used to collect flower buds. Full flower buds were randomly selected, and, after removing scales and villi, the flower bud primordia were extracted using tweezers and immediately utilized for the detection of H_2O_2 content and POD activity. The remaining flower buds were stored at −80 °C.

2.2. Statistics of Germination Rate and Detection of H_2O_2 Content and POD Activity in Different Dormancy States of Pear

The germination rate of pear flower buds was calculated according to Liu's method [28], and the determination of dormancy stages followed Lang's [29] and Yamane's [27] methods. When the overall germination rate was less than 50%, the flower bud was in the internal dormancy stage. If the germination rate was ≥50%, the flower bud was in the release date of internal dormancy. Additionally, H_2O_2 content and POD activity were determined using a hydrogen peroxide content detection kit (Beijing Soleibao Technology Co., Ltd., Beijing, China) and a peroxidase activity detection kit (Beijing Soleibao Technology Co., Ltd., Beijing, China), as per the kit's instructions. Statistical analysis was conducted using Excel 2010 and SPSS 22.0 software.

2.3. RNA Extraction

Total RNA from pear buds at different dormancy stages was extracted using the RNAprep Pure Plant Plus Kit (Tiangen Biotechnology, Beijing, China). The template cDNA was obtained using reverse transcription using TranScript One-Step gDNA removal and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) for subsequent PCR amplification.

2.4. Screening Determination of POD Gene in Bud Dormancy of Pear

The determination of *POD* genes involved in the dormancy process of pear commenced by extracting information from the transcriptome database showcasing differential expression at distinct resting stages, constructed within our laboratory. Subsequently, a search was conducted in the NCBI pear genome database [30], leading to the identification of specific genes, namely *PpPOD4-like* (Gene ID: 103945527), *PpPOD8* (Gene ID: 103964015), *PpPOD12* (Gene ID: 103944515), *PpPOD18* (Gene ID: 103947315), *PpPOD24* (Gene ID: 103953768), *PpPOD33* (Gene ID: 103960463), and *PpPOD47* (Gene ID: 103934752).

The genomic DNA (gDNA), coding sequence (CDS), and their respective protein sequences were downloaded for further analysis.

To identify the most significantly expressed genes, *PpActin* served as the internal reference gene, with specific primers detailed in Table 1. The qPCR reaction was executed using the TRANPerfectStart Green qPCR SuperMix kit (TransGen Biotech, Beijing, China) in accordance with the manufacturer's instructions. The assays were conducted on a Light Cycler 96 (Light Cycler® 96, Roche Company, Basel, Switzerland) to analyse the expression of *POD*-related genes at different dormancy stages in pear. The qPCR reaction comprised a total volume of 10 µL: 0.5 µL of cDNA template, 0.2 µL of F/R primers, 5 µL of SuperMix, and 4.1 µL of ddH_2O. Additionally, the relative expression levels of genes were calculated using the formula [31].

Table 1. List of primers used.

Primer Name	Primer Sequences (5′-3′)	Use
PpPOD4-like-F	ATGGCTTCCAGTAATACTTTTTCTTTGT	Clone
PpPOD4-like-R	CTAATTAGGCTTCCTGCAGTTCAATCT	Clone
NCPpPOD4-like-F	agtggtctctgtccagtcctATGGCTTCCAGTAATACTTTTTCTTTGT	Vector construction
NCPpPOD4-like-R	ggtctcagcagaccacaagtCTAATTAGGCTTCCTGCAGTTCAATCT	Vector construction
PpActin-F	CCATCCAGGCTGTTCTCTC	qRT-PCR
PpActin-R	GCAAGGTCCAGACGAAGG	qRT-PCR
PpPOD4-like-qF	CCCACTGCTACCCTCAAC	qRT-PCR
PpPOD4-like-qR	CTGCCGCTGTACGATTTA	qRT-PCR
PpPOD8-qF	TCACTCGGCTTCACTTCC	qRT-PCR
PpPOD8-qR	CGCTGTTGTGCTATCCCT	qRT-PCR
PpPOD12-qF	TCAAACCGCCTTTACAAT	qRT-PCR
PpPOD12-qR	GGACTTCCCAAACTCGTC	qRT-PCR
PpPOD18-qF	CGTGGTGTCATGTGCTGA	qRT-PCR
PpPOD18-qR	GTTTAGGTTTCGGGTTGG	qRT-PCR
PpPOD24-qF	TTGGTTTGGGAAGAAGAG	qRT-PCR
PpPOD24-qR	GCAGCATTGATGGTGGAT	qRT-PCR
PpPOD33-qF	GAGACTTGGTTGCCTTATC	qRT-PCR
PpPOD33-qR	ATCGCTTGTATTTATCGTG	qRT-PCR
PpPOD47-qF	GAGTTGGGAAGATTGGAT	qRT-PCR
PpPOD47-qR	ATGTTGATGGCTATGTCG	qRT-PCR

2.5. Gene Cloning

Primer design for PCR amplification of the complete open reading frames (ORFs) of *PpPOD4-like* was conducted using Premier 5.0 software, and the preceding cDNAs were diluted ten times for this purpose (Table 1). The PCR Thermal Cycler (Bio-Rad company, Hercules, CA, USA) was employed for the amplification process. The 10 µL PCR reaction system comprised 5 µL of 2 × TransTaq HiFi PCR SuperMix, 3.8 µL of ddH_2O, 0.4 µL of template cDNA, and 0.4 µL of F/R primers. The PCR conditions involved pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 58.4 °C for 30 s, extension at 72 °C for 50 s, repeated for 35 cycles, and a final extension at 72 °C for 10 min. Detection of PCR products was accomplished through 1% agar-gel electrophoresis. T1 clones were executed with the pEASY-T1 kit, and Trans1-T1 receptive cells underwent transformation. PCR was conducted using the *PpPOD4-like*-F/R primers for bacterial liquid detection.

2.6. Structural Characterization of PpPOD4-like

Predictions of pear basic physicochemical properties, secondary structure, transmembrane structure, signal peptide, domain, and conserved motifs of *PpPOD4-like* protein were carried out using ExPASy (https://web.expasy.org/protparam/, accessed on 13 August 2023), SOPMA (http://npsa-prabi.ibcp.fr/, accessed on 13 August 2023), TMHMM Server v2.0 (http://www.cbs.dtu.dk/services/TMHMM/, accessed on 13 August 2023), SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/, accessed on 13 August

2023), CDD (https://www.ncbi.nlm.nih.gov/cdd, accessed on 13 August 2023), and MEME (http://meme-suite.org/).

A Blastp search for *PpPOD4-like* protein was conducted in NCBI, and protein sequences displaying high homology with *PpPOD4-like* in different plants were downloaded. The screening criteria were Score > 100, with a value of E < 1 e^{-10}. The evolutionary tree was constructed using MEGA6.0 with default parameters. The GDR *Rosaceae* genome website (https://www.rosaceae.org/) facilitated the download of the pear *PpPOD4-like* initiation codon upstream 2000 bp sequences, which were subsequently submitted to the online Plantcare website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 13 August 2023) for the prediction of promoter cis-elements.

2.7. Subcellular Localization and Determination of H_2O_2 Content in Overexpression

Cello v2.5 (http://cello.life.nctu.edu.tw/, accessed on 13 August 2023) was employed for predicting the subcellular localization of pear *PpPOD4-like* protein. Specific primers with NC splices were designed to amplify full-length *PpPOD4-like* CDS (de-stop codon) sequences with NC splices. The purified PCR products with NC splices were linked enzymatically to the pNC-Cam1304-SubN (GFP) expression vector using the NC cloning method [32], yielding the recombinant expression vector p1304-GFP-*PpPOD4-like*. Following the kit's instructions, the recombinant plasmid was transformed into *E. coli* Trans1-T1, and plasmid extraction occurred after bacterial liquid PCR and sequencing identification. Agrobacterial-mediated injection was employed for transforming *Nicotiana benthamiana*, where the lower epidermis was removed. The subcellular localization of *PpPOD4-like* protein was observed using a laser scanning confocal microscope under the 488 nm laser channel. The H_2O_2 content in the transient *N. benthamiana* leaves, with the lower epidermis removed, was determined, and un-transfected *N. benthamiana* leaves were used as control (CK).

2.8. Analysis of PpPOD4-like Gene Expression during Pear Dormancy

The expression of *PpPOD4-like* in pear during various dormancy stages was examined using the Light Cycler 96 (Light Cycler® 96, Roche Company, Basel, Switzerland), employing the aforementioned method.

2.9. Correlation Analysis of H_2O_2 Content and POD Activity with PpPOD4-like

IBM SPSS Statistics 22 was utilised to conduct bivariate correlation analysis between H_2O_2 content, POD activity, and *PpPOD4-like* gene expression. According to Pearson's correlation coefficient, when the correlation coefficient $|r| \geq 0.8$, a high correlation between the two variables was established. For $0.5 \leq |r| < 0.8$, a moderate correlation existed. When $0.3 \leq |r| < 0.5$, a low correlation was observed. If $|r| < 0.3$, there was no discernible correlation between the two variables.

3. Results

3.1. Statistical Analysis of Germination Rate and Determination of Pear Dormancy Stage

The germination rate of pear floral buds subjected to dormancy treatment at 4 °C is depicted in Figure 1. During the initial 0–21 days, the germination rate remained below 50%, signifying the presence of the endodormancy state in floral buds. Subsequently, after 28 days, the germination rate surpassed 50%, indicative of the fulfilment of the requisite low-temperature accumulation for dormancy, transitioning the flower into the ecodormancy state. Therefore, based on the germination rate during the dormancy stages of pear floral buds, it can be inferred that the flower bud was in the internal dormancy stage at 0–21 days, transitioning from endodormancy to ecodormancy between 21–28 days, and conclusively in the ecodormancy stage at 28 days.

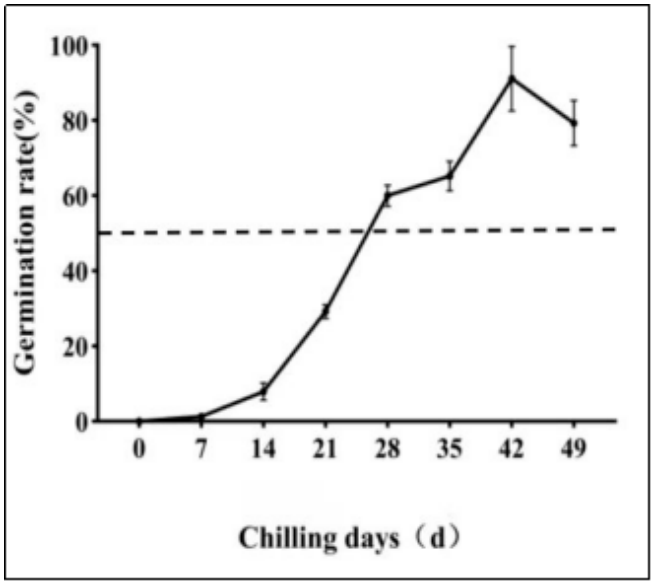


Figure 1. The change of bud germination rate of pear under different low temperature duration. The error bars represent the mean ± Standard error (SE).

3.2. Alterations in H_2O_2 Content and POD Activity across Distinct Dormancy Phases in Pear

Subjected to the low-temperature regimen at 4 °C, the H_2O_2 content exhibited a pattern of initial escalation followed by a decline. It demonstrated a rapid increase during the endodormancy phase, subsequently sharply decreasing upon entering ecodormancy. The zenith of H_2O_2 content coincided with the release of endodormancy (Figure 2a). The trajectory of POD activity mirrored that of H_2O_2 content, albeit with the peak manifesting later than that of H_2O_2. Throughout the dormancy stage spanning 0 to 21 days, H_2O_2 levels exhibited a continuous ascent, while POD activity remained low and constant (Figure 2b). By day 28, following the release of bud endodormancy, H_2O_2 content dwindled, and POD activity experienced a sudden surge followed by a subsequent decline.

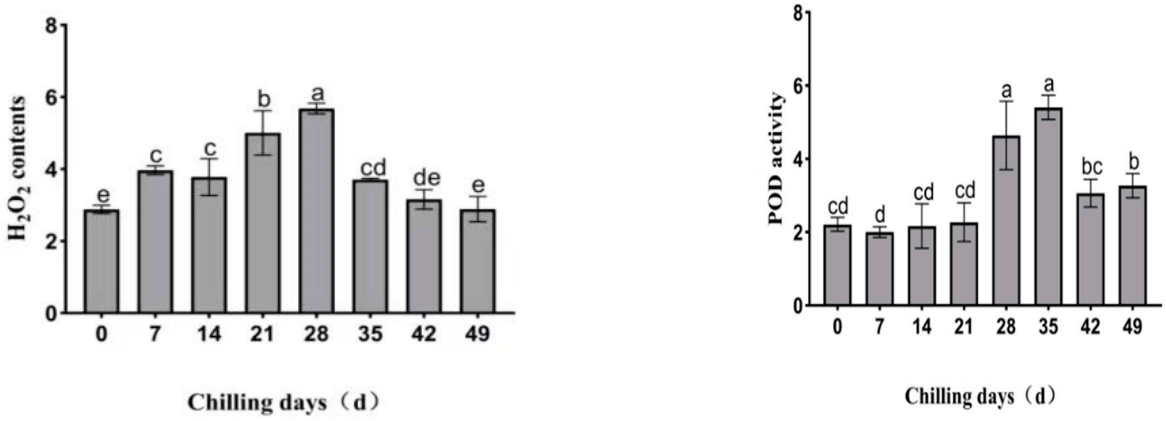


Figure 2. (**a**) H_2O_2 content (μmol/g) of floral buds in pear under cold treatment. (**b**) POD activity (U/mg) of floral buds in pear under cold treatment. The error bars represent the mean ± Standard error (SE); different letters indicate significant differences ($p < 0.05$, Tukey's test).

3.3. Screening Determination of PpPOD4-like from POD Genes

The findings revealed that during the endodormancy phase at 4 °C, the expression levels of *PpPOD4-like*, *PpPOD12*, *PpPOD24*, *PpPOD33*, and *PpPOD47* exhibited an initial increase followed by a subsequent decrease. This suggests that these genes might undergo

transient induction in response to low temperatures. As the transition from endodormancy to ecodormancy unfolded, the expression of *POD* genes (Figure 3), excluding *PpPOD18*, *PpPOD33,* and *PpPOD47*, displayed a notable upward trajectory. This observation indicates a pivotal role for these genes in regulating H_2O_2 levels during this stage. In the ecodormancy phase, these genes exhibited stable fluctuations. Notably, *PpPOD4-like* and *PpPOD12* demonstrated an oscillating upward pattern. Moreover, the expression of *PpPOD4-like* and *PpPOD12* witnessed upregulation upon sufficient cooling of the buds, eventually settling into a stable and elevated expression state. During this stage, *PpPOD4-like* and *PpPOD12* primarily participated in the elimination of excess H_2O_2. Remarkably, the expression trend of *PpPOD4-like* closely correlated with the natural dormancy process.

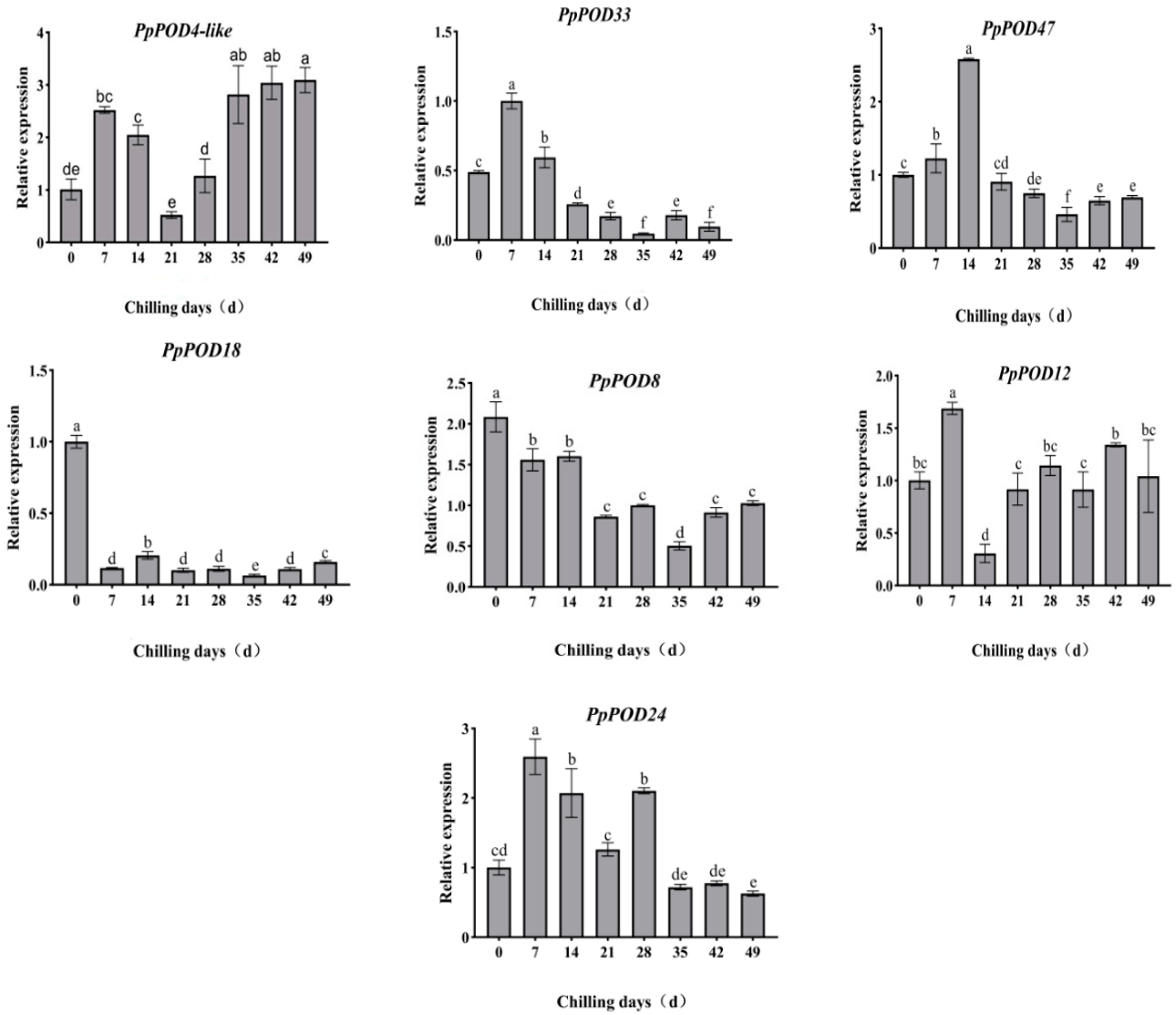


Figure 3. Expression of *POD* genes during the dormancy process of pear flower buds. The error bars represent the mean ± Standard error (SE), different letters indicate significant differences ($p < 0.05$, Duncan's multiple range test).

3.4. cDNA Cloning of PpPOD4-like Gene from Pear

A target strip of 984 bp in length was obtained through RT-PCR (Figure 4). Sequencing results revealed a high similarity of 99.59% between the cloned sequence and the CDS sequence of *Pyrus x bretschneideri* (GeneID: 103945527) in the Dangshan pear database. This indicates the robust reliability of the cloned *PpPOD4-like*. Given that the reference genome material is *Pyrus x bretschneideri*, a hybrid offspring of Dangshan pear and sand

pear, classified under the white pear strain [30], and considering that "*Huanghua*" pear in this study belongs to the sand pear strain *Pyrus pyrifolia*, it suggests a high conservation of the *POD* gene in pear.

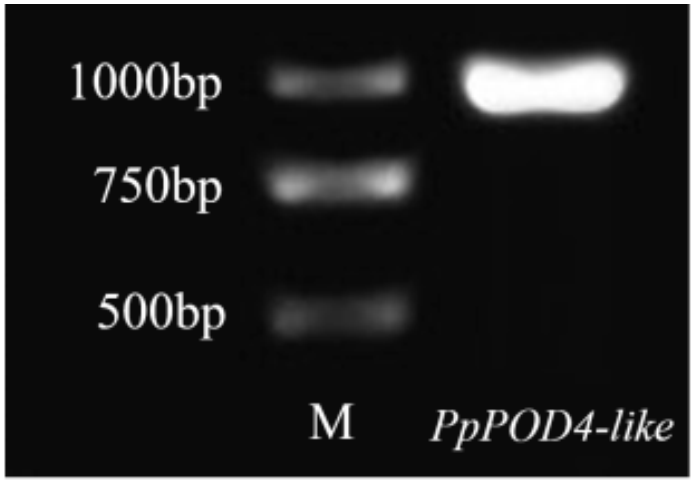


Figure 4. Electrophoresis detection result of the amplified complementary DNA sequence of *PpPOD4-like*. M: Trans2K DNA Marker.

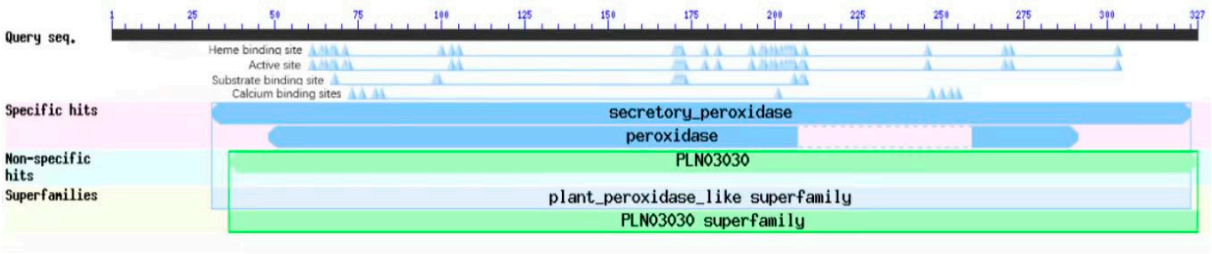
3.5. Bioinformatics Analysis of PpPOD4-like

3.5.1. Physicochemical Properties of PpPOD4-like Protein in Pear

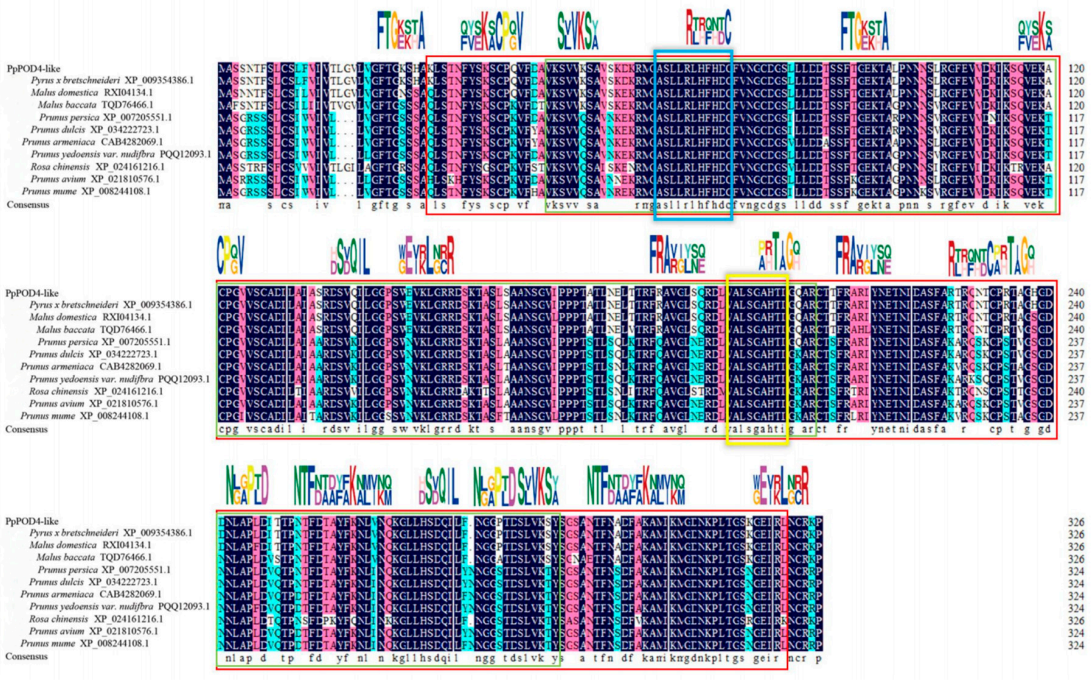
ExPASy predicted that the molecular weight of PpPOD4-like protein was 35,036.79, the fat coefficient was 80.55, the isoelectric point was 9.29, the hydrophilic coefficient was −0.181, and the instability index was 24.62, which was a stable basic hydrophilic protein. The prediction of secondary structure showed that random crimp (42.20%) dominated. TMHMM prediction results showed that the protein had a transmembrane domain, which was located at positions 7–26 of the amino acid sequence, belonging to the transmembrane protein. The SignalP 5.0 program was used to predict the signal peptide of PpPOD4-like protein. The results showed that PpPOD4-like protein had a signal peptide structure, and the signal peptide shear site was between the 30th and 31st amino acids, which belonged to the secreted protein.

3.5.2. Protein Domain and Motif Prediction Protein Sequence Alignment Analysis

Domain prediction results (Figure 5a) revealed three proteins matching the gene, including two specific and one non-specific binding sites. These encompassed heme binding, active site, substrate binding, and calcium binding sites. The conserved secretory peroxidase domain, belonging to the plant heme-dependent class III peroxidase superfamily, was identified between amino acids 31–325. The conserved peroxidase domain spanned amino acids 48–291, while the conserved cationic peroxidase (PLN03030) domain was located between amino acids 36–327. These findings confirmed the affiliation of *PpPOD4-like* to the peroxidase gene family. MEME prediction indicated an E value of 1.8 e^{-52}, unveiling the positions of 10 conserved motifs in the protein (Figure 5a). Protein sequence comparison demonstrated the highest sequence similarity (99%) between *PpPOD4-like* and white pear peroxidase4-like (XP_009354386.1). Furthermore, similarity with plant proteins such as apple, peach, cherry, and apricot exceeded 80%, all featuring secretory peroxidase and peroxidase domains (Figure 5b).



(**a**)

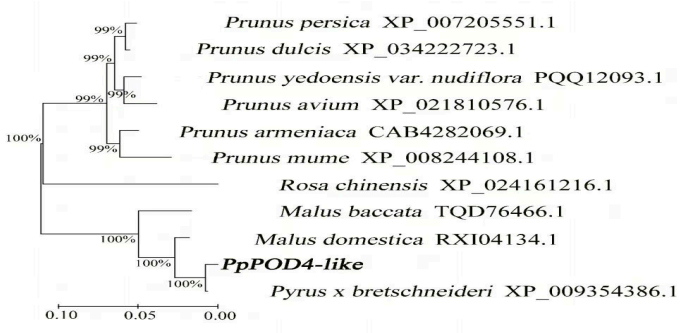


(**b**)

Figure 5. Conserved domains of *PpPOD4-like* (**a**) and Alignment of the amino acid sequence deduced by *PpPOD4-like* with other homologous protein (**b**).

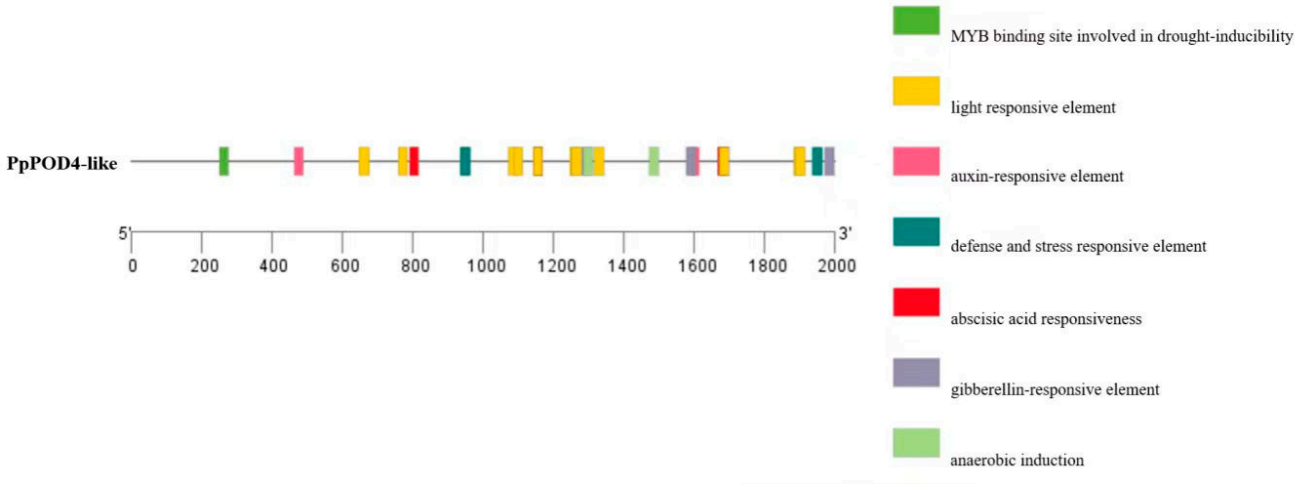
3.5.3. Evolutionary Tree and Promoter Cis-Acting Element Analysis

The results of phylogenetic tree analysis illustrated that *PpPOD4-like* exhibited the closest evolutionary relationship with the peroxidase4-like protein in white pear, followed by apple, mountain thorn, Chinese rose, plum, and others (Figure 6a). Examination of multiple sequences revealed the presence of the peroxidase active site ASLLRLhFHDC in the N segment, and VALSGGHTI, the sub-heme ligand site closest to peroxidase, in the C segment, within almost all amino acid sequences.



(**a**)

Figure 6. *Cont.*



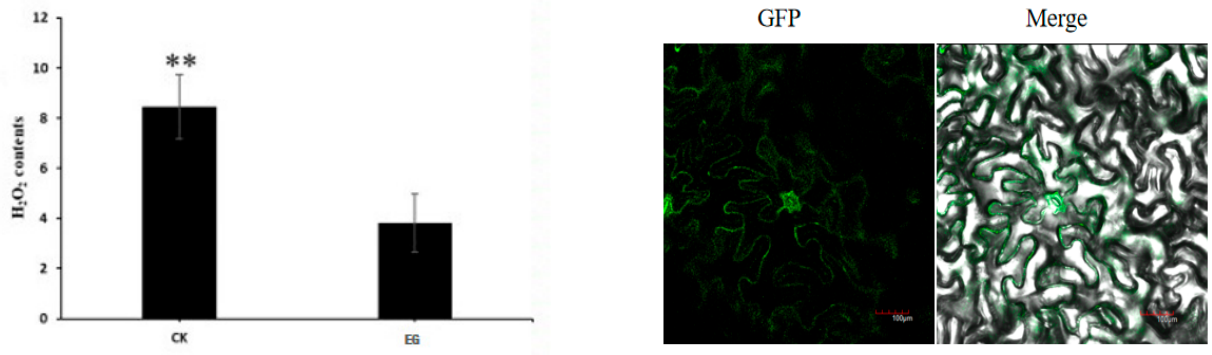
(**b**)

Figure 6. Phylogenetic tree for *PpPOD4-like* protein from different plant species (**a**) and cis-acting element of promoter (**b**).

The prediction of promoter-acting elements indicated that, alongside core elements such as CAAT-box and TATA-box, the *PpPOD4-like* promoter encompassed numerous hormone response elements, light response elements, defence and stress response elements, anaerobic induction elements, and MYB binding sites associated with drought induction and specific expressions (Figure 6b). Among the hormone response elements, the most abundant were ABRE response elements (5 ABRE elements), followed by gibberellin response elements (3 P-box elements) and auxin response elements (2 TGA-element elements).

3.6. Subcellular Localization and H_2O_2 Content in Overexpressed N. benthamiana

Prediction results from Cello v.2.5 demonstrated that the PpPOD4-like protein predominantly localizes to the cell membranes. The green fluorescence signal from GFP, following the infection of the lower epidermis of *N. benthamiana* leaves with the carrier p1304-GFP-*PpPOD4-like*, is depicted in Figure 7a. Subsequent examination of the fluorescent protein's luminous position revealed the localization of *PpPOD4-like* protein in the cell membranes (Figure 7a). Additionally, using un-transfected *N. benthamiana* leaves as the CK control, the H_2O_2 content in overexpressed *N. benthamiana* leaves was measured (EG). The results indicated that the average H_2O_2 content in *N. benthamiana* with the *PpPOD4-like* gene was reduced by 54.86% compared to the CK group, suggesting that *PpPOD4-like* exerts a regulatory effect on H_2O_2 (Figure 7b).



(**a**) (**b**)

Figure 7. (**a**) Subcellular localization results of PpPOD4-like protein overexpression *N. benthamiana*. (**b**) The change of H_2O_2 content (μmol/g) in *PpPOD4-like* overexpression *N. benthamiana*, ** indicate significant difference ($p < 0.01$). Scale bars, 100 μm.

3.7. Expression Analysis of PpPOD4-like during Pear Bud Dormancy

In the dormancy stage spanning from 0 to 21 days after treatment at 4 °C, the expression pattern of *PpPOD4-like* exhibited an initial increase followed by a subsequent decrease. The upregulation of *PpPOD4-like* expression was notably induced by short-term exposure to low temperatures and gradually declined during the progression of endodormancy, reaching a valley during the deep endodormancy stage. The expression of *PpPOD4-like* significantly increased between days 21 and 28, coinciding with the transition from endodormancy to ecodormancy. This suggests that *PpPOD4-like* plays a pivotal role in regulating H_2O_2 during this stage, and may serve as a marker gene for the shift from endodormancy to ecodormancy. Subsequently, in the ecodormancy stage after 28 days, *PpPOD4-like* maintained a stable and elevated expression level, indicating its primary involvement in alleviating excess H_2O_2 during this phase. Additionally, on day 28, following the release of bud endodormancy, the H_2O_2 content decreased, and POD activity exhibited a sudden increase followed by a decrease (Figure 2a,b).

3.8. Correlation Analysis

The results of correlation analysis are presented in Table 2. The H_2O_2 content demonstrated a correlation with *PpPOD4-like* gene expression on days 14, 21, 35, 42, and 49 following low-temperature treatment, with a particularly high correlation observed between days 21 and 35 after treatment. Furthermore, a correlation between POD activity and *PpPOD4-like* gene expression was evident on days 0, 14, 28, 35, 42, and 49 of low-temperature treatment, with a notably high correlation on days 0 and 35. These findings indicate that H_2O_2 content and POD activity are associated with the *PpPOD4-like* gene at various durations of low-temperature treatment, especially after 21 days of low-temperature treatment in endodormancy and 35 days of low-temperature treatment during the ecodormancy stage. The results underscore the role of *PpPOD4-like* in regulating H_2O_2 content through the modulation of POD activity, contributing to the regulation of the pear bud dormancy process.

Table 2. Correlation between content of H_2O_2, activity of POD and *PpPOD4-like* gene.

	Chilling Days	Content of H_2O_2	Activity of POD	*PpPOD4-like*
Content of H_2O_2	0	1	0.949	0.045 *
	7	1	0.019 *	0.050 *
	14	1	0.987	0.468
	21	1	0.889	0.883
	28	1	0.682	0.087
	35	1	0.166	0.800
	42	1	0.576	0.682
	49	1	0.877	0.306
Activity of POD	0		1	0.904
	7		1	0.031 *
	14		1	0.519
	21		1	0.227
	28		1	0.595
	35		1	0.966
	42		1	0.742
	49		1	0.571

Note: * means significantly (bilateral) at 0.05 level.

4. Discussion

The regulation of bud endodormancy is pivotal for the phenology and yield of various plants, particularly temperate fruit trees. Existing studies on bud dormancy primarily focus on hormone metabolism, water metabolism, respiratory metabolism, and antioxidant metabolism [11,33,34]. In the realm of antioxidant metabolism, Reactive Oxygen Species (ROS), notably Hydrogen Peroxide (H_2O_2), assumes a crucial role in plant dormancy.

Numerous investigations have demonstrated H_2O_2 as a key signalling molecule influencing various aspects of plant growth and development [35], it serves to activate signalling molecules such as ABA, ethylene, and NO [36–38]. Furthermore, exogenous H_2O_2 has been shown to alter the dormancy processes of pear [8] and dormant grapes [12], underscoring the significant role of H_2O_2 in plant dormancy. In our study, the H_2O_2 content in pear floral buds exhibited an initial increase followed by a decrease during the dormancy process under low-temperature treatment at 4 °C. The peak H_2O_2 concentration coincided with the release of internal dormancy, aligning with findings by Gao et al. [14] and Shao et al. [13]. Moreover, exogenous H_2O_2 was found to enhance the cold resistance of tomato plants by inducing the expression of *SlMAPK 1/2/3* and modulating the activities of plant hormones and antioxidant enzymes [39]. According to the study, SOD expressions were repressed to maintain high levels of O_2^-, and peroxidases were activated to reduce the accumulation of H_2O_2 in stem cells [4]. These results collectively affirm the role of H_2O_2 as a signalling molecule in the regulation of pear bud dormancy.

It is known that Peroxidase (POD)-mediated H_2O_2 is a major contributor to the oxidative burst of extracellular ROS [40]. POD activity increased throughout the bud dormancy process in pear, reaching its peak upon the release of endodormancy and subsequently declining with dormancy release. Intriguingly, analogous patterns were observed in grape [41], nectarine [42], blueberry [43], and other fruit trees, where POD activity exhibited a sudden surge following the release of bud endodormancy. Consequently, we postulate that the release of bud endodormancy might be linked to the abrupt alteration in POD activity. Notably, the trend in POD activity mirrored the changes in H_2O_2 content during bud dormancy, suggesting that POD regulates the bud dormancy process by modulating the balance of ROS, particularly H_2O_2.

Members of the Peroxidase (*POD*) gene family play diverse roles in various plant biological processes. For example, overexpression of *POD* genes (*AtPrx22*, *AtPrx39*, *AtPrx69*) in *Arabidopsis thaliana* enhances its cold tolerance [44]. The *GhPOX1* gene in *Gossypium* influences cell growth by mediating Reactive Oxygen Species (ROS) production [45]. In *N. benthamiana*, the overexpression of *Vinca minor* peroxidase genes *CrPrx* and *CrPrx1* improves cold tolerance and germination rate [46]. Conversely, exogenous abscisic acid (ABA) application enhances POD activity and induces the expression of related genes, such as the ROS clearance system [47]. Studies have demonstrated improved POD activity in grape dormant buds following exogenous ABA treatment [48]. Furthermore, ABA induces the expression of peroxidase genes in *Tamarix* (*ThPrx1* and *ThPrx2*) [49] and *tomato* (*tap1* and *tap2*) [50]. Previous research by Li et al. [10] found a close relationship between ABA and the expression of the *PpPP2C1* gene in pear bud dormancy. These studies collectively suggest that ABA may play a crucial role in plant growth and development by inducing the expression of *POD* genes. In our study, the *PpPOD4-like* gene was cloned from pear buds, encoding a stable, basic, hydrophilic protein. This gene, classified within Class III secreted *POD*, shared similarities with the *POD4* gene related to mature fruit stone cells in pear, hinting at a potential role in lignin synthesis [51]. *PpPOD4-like*, localized in cell membranes, exhibited regulatory capabilities over H_2O_2 content. Its expression pattern during dormancy indicated a close association with pear bud dormancy, suggesting involvement in the regulation of extracellular ROS.

Moreover, H_2O_2 within ROS possesses the ability to easily diffuse into cells, activating downstream signals through reversible protein phosphorylation. It can also act on transcription factors, activating nuclear signalling and regulating gene expression [52]. Promoter analysis of *PpPOD4-like* revealed numerous hormone response elements, including ABA-responsive elements, photoresponse elements, and cis-acting elements involved in defence and stress response. ABA response elements are particularly associated with stress and signal transduction of corresponding hormones related to gene regulation [53]. ABA has been found to inhibit the *MAPK* cascade pathway-mediated ROS during endodormancy in pear buds [54]. Additionally, studies have shown that ABA upregulates H_2O_2 levels by inhibiting Protein Phosphatase 2Cs (*PP2Cs*) [10]. Results indicated that the

expression of *PpPOD4-like* in pear buds might be induced by ABA, influencing the bud dormancy process. Notably, *PpPOD4-like* exhibited high expression during both endodormancy and ecodormancy stages, significantly decreasing upon endodormancy release, implying a robust regulatory effect in promoting floral bud break. However, given the diverse isoenzymes constituting POD, *PpPOD4-like* might also participate in defence and stress response. Consequently, the expression pattern of *PpPOD4-like* did not entirely align with the trend of POD activity. Our laboratory's constructed differentially expressed miRNAs [22] and lncRNAs [23] during pear bud dormancy suggested that *PpPOD4-like* expression is negatively regulated by miRNA (un-conservative_scaffold192.0_14068) and lncRNA (TCONS_00019077). Thus, the expression of *PpPOD4-like* may be governed by low temperature, ABA, and corresponding miRNA and lncRNA. Throughout the pear bud dormancy process, low temperature induces the expression of *PpPOD4-like*, and it regulates H_2O_2 content through POD, actively participating in dormancy release regulation. Additionally, we propose that low temperature promotes ABA formation, inducing *PpPOD4-like* expression, while the interaction between miRNA and lncRNA inhibits *PpPOD4-like* expression. Further investigation is essential to elucidate the intricate regulatory mechanisms of *PpPOD4-like* in pear bud dormancy.

5. Conclusions

In this work, we utilised quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) to screen seven Peroxidase (*POD*) genes in pears (*Pyrus pyrifolia*), identifying the most significantly expressed gene, denoted as *PpPOD4-like*. The screening process, coupled with expression analysis, revealed a substantial up-regulation of *PpPOD4-like*, exhibiting an overall upward trajectory in response to hydrogen peroxide (H_2O_2) treatment. Furthermore, during the dormancy process, the expression of *PpPOD4-like* was induced by low temperatures. Concurrently, the H_2O_2 content exhibited a rapid increase throughout endodormancy, followed by a swift decrease during ecodormancy. The observed pattern of Peroxidase (POD) activity mirrored that of H_2O_2 content. Our findings imply that *PpPOD4-like* regulates H_2O_2 levels through POD activity, actively participating in the modulation of dormancy release.

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References

1. Nan, W.; Jing, Z.; Lei, Y.U.; Qi, Z.O.U.; Zhang-Wen, G.U.O.; Zuo-Lin, M.A.O.; Yi-Cheng, W.; Sheng-Hui, J.; Hong-Cheng, F.; Hai-Feng, X.U.; et al. Progress on the Resource Breeding of Kernel Fruits I: Progress on the Germplasm Resources, Quality development and Genetic Breeding of Apple in China. *Plant Genet. Resour.* **2019**, *20*, 801–812.
2. Salama, A.-M.; Ezzat, A.; El-Ramady, H.; Alam-Eldein, S.M.; Okba, S.K.; Elmenofy, H.M.; Hassan, I.F.; Illés, A.; Holb, I.J. Temperate Fruit Trees under Climate Change: Challenges for Dormancy and Chilling Requirements in Warm Winter Regions. *Horticulturae* **2021**, *7*, 86. [CrossRef]
3. Prudencio, A.S.; Díaz-Vivancos, P.; Dicenta, F.; Hernández, J.A.; Martínez-Gómez, P. Monitoring the transition from endodormancy to ecodormancy in almond through the analysis and expression of a specific class III peroxidase gene. *Tree Genet. Genomes* **2019**, *15*, 44. [CrossRef]
4. Zeng, J.; Dong, Z.; Wu, H.; Tian, Z.; Zhao, Z. Redox regulation of plant stem cell fate. *EMBO J.* **2017**, *36*, 2844–2855. [CrossRef]
5. Swanson, S.; Gilroy, S. ROS in plant development. *Physiol. Plant.* **2010**, *138*, 384–392. [CrossRef] [PubMed]
6. Petrov, V.; Hille, J.; Mueller-Roeber, B.; Gechev, T.S. ROS-mediated abiotic stress-induced programmed cell death in plants. *Front. Plant Sci.* **2015**, *6*, 69. [CrossRef]

7. Turkan, I. ROS and RNS: Key signalling molecules in plants. *J. Exp. Bot.* **2018**, *69*, 3313–3315. [CrossRef]
8. Kuroda, H.; Sugiura, T.; Sugiura, H. Effect of hydrogen peroxide on breaking endodormancy in flower buds of Japanese pear (Pyrus pyrifolia Nakai). *J. Jpn. Soc. Hortic. Sci.* **2005**, *74*, 255–257. [CrossRef]
9. Dangl, J.L.; Jones, J.D.G. Plant pathogens and integrated defence responses to infection. *Nature* **2001**, *411*, 826–833. [CrossRef]
10. Li, L.; Liu, J.; Liang, Q.; Feng, Y.; Wang, C.; Wu, S.; Li, Y. Downregulation of lncRNA *PpL-T31511* and *Pp-miRn182* Promotes Hydrogen Cyanamide-Induced Endodormancy Release through the PP2C-H_2O_2 Pathway in Pear (*Pyrus pyrifolia*). *Int. J. Mol. Sci.* **2021**, *22*, 11842. [CrossRef]
11. Pérez, F.J.; Vergara, R.; Rubio, S. H_2O_2 is involved in the dormancy-breaking effect of hydrogen cyanamide in grapevine buds. *Plant Growth Regul.* **2008**, *55*, 149–155. [CrossRef]
12. Ito, A.; Tuan, P.A.; Saito, T.; Bai, S.; Kita, M.; Moriguchi, T. Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. *Tree Physiol.* **2011**, *41*, 529–543. [CrossRef]
13. Shao, H.; Ma, F.-W. Relationship between breaking of dormancy and reactive oxygen species metabolism in flower buds of pear. *J. Plant Physiol. Mol. Biol.* **2004**, *30*, 660–664.
14. Gao, D.; Shu, H.; Li, X. The relationship of H_2O_2 Content in Buds with the Endodormancy of Fruit Trees. *Acta Hortic. Sin.* **2002**, *29*, 209–213.
15. Lee, C.-J.; Park, S.-U.; Kim, S.-E.; Lim, Y.-H.; Ji, C.Y.; Kim, Y.-H.; Kim, H.S.; Kwak, S.-S. Overexpression of IbLfp in sweetpotato enhances the low-temperature storage ability of tuberous roots. *Plant Physiol. Biochem.* **2021**, *167*, 577–585. [CrossRef]
16. Passardi, F.; Cosio, C.; Penel, C.; Dunand, C. Peroxidases have more functions than a Swiss army knife. *Plant Cell Rep.* **2005**, *24*, 255–265. [CrossRef]
17. Kawano, T. Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. *Plant Cell Rep.* **2003**, *21*, 829–837. [CrossRef]
18. Foyer, C.H.; Descourvières, P.; Kunert, K.J. Protection against oxygen radicals: An important defence mechanism studied in transgenic plants. *Plant Cell Environ.* **1994**, *17*, 507–523. [CrossRef]
19. Li, L.; Liu, J.; Liang, Q.; Zhang, Y.; Kang, K.; Wang, W.; Feng, Y.; Wu, S.; Yang, C.; Li, Y. Genome-wide analysis of long noncoding RNAs affecting floral bud dormancy in pears in response to cold stress. *Tree Physiol.* **2021**, *41*, 771–790. [CrossRef]
20. Li, W.; Zhang, S.; Tang, H.; He, L. Effect of Exogenous Gibberellic Acid on Paeonia rockii Seeds Germination. *Acta Bot. Boreali-Occident. Sin.* **2019**, *39*, 1819–1826.
21. Noriega, X.; Burgos, B.; Pérez, F.J. Short day-photoperiod triggers and low temperatures increase expression of peroxidase RNA transcripts and basic peroxidase isoenzyme activity in grapevine buds. *Phytochemistry* **2007**, *68*, 1376–1383. [CrossRef]
22. Amaya, I.; Botella, M.A.; de la Calle, M.; Medina, M.I.; Heredia, A.; Bressan, R.A.; Hasegawa, P.M.; Quesada, M.A.; Valpuesta, V. Improved germination under osmotic stress of tobacco plants overexpressing a cell wall peroxidase. *FEBS Lett.* **1999**, *457*, 80–84. [CrossRef]
23. Xie, Z.; Rui, W.; Yuan, Y.; Song, X.; Liu, X.; Gong, X.; Bao, J.; Zhang, S.; Shahrokh, K.; Tao, S. Analysis of *PRX* Gene Family and Its Function on Cell Lignification in Pears (*Pyrus bretschneideri*). *Plants* **2021**, *10*, 1874. [CrossRef]
24. Liu, F.; Liang, D.; Wang, J.; Lv, X.; Gong, M. The Effect of Promoting Sprouting Process by Cyanamide on the Growth of Grape Leaves. *J. Biobased Mater. Bioenergy* **2017**, *11*, 649–654. [CrossRef]
25. Wang, Z.; Ma, R.; Zhao, M.; Wang, F.; Zhang, N.; Si, H. NO and ABA Interaction Regulates Tuber Dormancy and Sprouting in Potato. *Front. Plant Sci.* **2020**, *11*, 311. [CrossRef] [PubMed]
26. Ma, X.; Li, L.; Liu, J.; Yang, M.; Chen, J.; Liang, Q.; Wu, S.; Li, Y. Identification and Differentially Expressed Analysis of microRNA Associated with Dormancy of Pear Flower Buds. *Acta Hortic. Sin.* **2018**, *45*, 2089–2105. [CrossRef]
27. Yamane, H.; Ooka, T.; Jotatsu, H.; Hosaka, Y.; Sasaki, R.; Tao, R. Expressional regulation of PpDAM5 and PpDAM6, peach (*Prunus persica*) dormancy-associated MADS-box genes, by low temperature and dormancy-breaking reagent treatment. *J. Exp. Bot.* **2011**, *62*, 3481–3488. [CrossRef] [PubMed]
28. Liu, G. Molecular Physiological Mechanism of Pear Dormancy. Ph.D. Thesis, Zhejiang University, Hangzhou, China, 2013.
29. Lang, G.A.; Early, J.D.; Martin, G.C.; Darnell, R.L. Endo-, Para-, and Ecodormancy: Physiological Terminology and Classification for Dormancy Research. *HortScience* **1987**, *22*, 371–377. [CrossRef]
30. Wu, J.; Wang, Z.; Shi, Z.; Zhang, S.; Ming, R.; Zhu, S.; Khan, M.A.; Tao, S.; Korban, S.S.; Wang, H.; et al. The genome of the pear (*Pyrus bretschneideri* Rehd.). *Genome Res.* **2013**, *23*, 396–408. [CrossRef]
31. Gong, B.; Yi, J.; Wu, J.; Sui, J.; Khan, M.A.; Wu, Z.; Zhong, X.; Seng, S.; He, J.; Yi, M. LlHSFA1, a novel heat stress transcription factor in lily (*Lilium longiflorum*), can interact with LlHSFA2 and enhance the thermotolerance of transgenic Arabidopsis thaliana. *Plant Cell Rep.* **2014**, *33*, 1519–1533. [CrossRef]
32. Yan, P.; Zeng, Y.; Shen, W.; Tuo, D.; Li, X.; Zhou, P. Nimble Cloning: A Simple, Versatile, and Efficient System for Standardized Molecular Cloning. *Front. Bioeng. Biotechnol.* **2020**, *7*, 460. [CrossRef]
33. Arora, R.; Rowland, L.J.; Tanino, K. Induction and release of bud dormancy in woody perennials: A science comes of age. *HortScience* **2003**, *38*, 911–921. [CrossRef]
34. Alves, G.; Decourteix, M.; Fleurat-Lessard, P.; Sakr, S.; Bonhomme, M.; Améglio, T.; Lacointe, A.; Julien, J.-L.; Petel, G.; Guilliot, A. Spatial activity and expression of plasma membrane H+-ATPase in stem xylem of walnut during dormancy and growth resumption. *Tree Physiol.* **2007**, *27*, 1471–1480. [CrossRef]

35. Desikan, R.; A.-H.-Mackerness, S.; Hancock, J.T.; Neill, S.J. Regulation of the arabidopsis transcriptome by oxidative stress. *Plant Physiol.* **2001**, *127*, 159–172. [CrossRef]
36. Choudhary, R.; Saroha, A.E.; Swarnkar, P.L. Effect of abscisic acid and hydrogen peroxide on antioxidant enzymes in Syzygium cumini plant. *J. Food Sci. Technol.* **2012**, *49*, 649–652. [CrossRef]
37. Song, X.G.; She, X.P.; Wang, J. Inhibition of darkness-induced stomatal closure by ethylene involves a removal of hydrogen peroxide from guard cells of Vicia faba. *Russ. J. Plant Physiol.* **2012**, *59*, 372–380. [CrossRef]
38. Wendehenne, D.; Durner, J.; Klessig, D.F. Nitric oxide: A new player in plant signalling and defence responses. *Curr. Opin. Plant Biol.* **2004**, *7*, 449–455. [CrossRef]
39. Wang, L.; Chen, L.; Li, R.; Zhao, R.; Yang, M.; Sheng, J.; Shen, L. Reduced Drought Tolerance by CRISPR/Cas9-Mediated *SlMAPK3* Mutagenesis in Tomato Plants. *J. Agric. Food Chem.* **2017**, *65*, 8674–8682. [CrossRef]
40. O'Brien, J.A.; Daudi, A.; Butt, V.S.; Paul Bolwell, G. Reactive oxygen species and their role in plant defence and cell wall metabolism. *Planta* **2012**, *236*, 765–779. [CrossRef]
41. Zhang, A.; Zheng, Y.; Chen, T.; Geng, W.; Fang, Y.; Wang, H. Variation of Active Oxygen Metabolism, An-ti-oxidation System in Grapevine Buds Tissue during Bud Dormancy Inducing and Releasing. *Acta Bot. Bore-Ali-Occident. Sin.* **2012**, *32*, 2075–2081.
42. Tian, L.; Fang, J.; Wang, L.; Niu, L. Changes of several physiological indexes during dormancy releasing in nectarine cultivar Huaguang. *J. Fruit Sci.* **2006**, *23*, 121–124.
43. Li, B.; Xia, X.; Liu, S. Changes in Physiological and Biochemical Properties and Variation in DNA Methylation Patterns during Dormancy and Dormancy Release in Blueberry (*Vaccinium corymbosum* L.). *Plant Physiol. J.* **2015**, *51*, 1133–1141. [CrossRef]
44. Kim, B.H.; Kim, S.Y.; Nam, K.H. Genes encoding plant-specific class III peroxidases are responsible for increased cold tolerance of the brassinosteroid-insensitive 1 mutant. *Mol. Cells* **2012**, *34*, 539–548. [CrossRef]
45. Mei, W.; Qin, Y.; Song, W.; Li, J.; Zhu, Y. Cotton GhPOX1 encoding plant class III peroxidase may be responsible for the high level of reactive oxygen species production that is related to cotton fiber elongation. *J. Genet. Genom.* **2009**, *36*, 141–150. [CrossRef]
46. Kumar, S.; Jaggi, M.; Sinha, A.K. Ectopic overexpression of vacuolar and apoplastic Catharanthus roseus peroxidases confers differential tolerance to salt and dehydration stress in transgenic tobacco. *Protoplasma* **2012**, *249*, 423–432. [CrossRef]
47. Bright, J.; Desikan, R.; Hancock, J.T.; Weir, I.S.; Neill, S.J. ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. *Plant J.* **2006**, *45*, 113–122. [CrossRef]
48. Li, X.; Yang, Y.; Zhang, L.; Zuo, S.; Li, L.; Jiao, J.; Li, J. Regulation of Endogenous Hormone Content and Asr1 Gene Expression in Maize Seedlings by Exogenous ABA under Low Temperature Stress. *Chin. J. Crops* **2017**, *43*, 141–148. [CrossRef]
49. Li, H.; Dong, J.; Jiang, J.; Liu, G. Cloning and expression analysis of two novel Prxs genes from Tamarix hispida. *J. Beijing For. Univ.* **2012**, *34*, 48–52. [CrossRef]
50. Sherf, B.A.; Bajar, A.M.; Kolattukudy, P.E. Abolition of an Inducible Highly Anionic Peroxidase Activity in Transgenic Tomato. *Plant Physiol.* **1993**, *101*, 201–208. [CrossRef] [PubMed]
51. Li, W.; Feng, J.; Tang, Z.; Dong, S.; Xu, L.; Zhang, S.; Zhang, Y. Correlation Analysis of Solid Stone Cell Content and Expression of POD4 Gene in Korla Fragrant Pear. *Xinjiang Agric. Sci.* **2017**, *54*, 60–65.
52. Fujita, Y.; Fujita, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J. Plant Res.* **2011**, *124*, 509–525. [CrossRef] [PubMed]
53. Kumar, M.; Kesawat, M.S.; Ali, A.; Lee, S.-C.; Gill, S.S.; Kim, H.U. Integration of Abscisic Acid Signaling with Other Signaling Pathways in Plant Stress Responses and Development. *Plants* **2019**, *8*, 592. [CrossRef] [PubMed]
54. Liang, Q.; Lin, X.; Liu, J.; Feng, Y.; Niu, X.; Wang, C.; Song, K.; Yang, C.; Li, L.; Li, Y. Genome-Wide Identification of MAPKK and MAPKKK Gene Family Members and Transcriptional Profiling Analysis during Bud Dormancy in Pear (*Pyrus* × *bretschneideri*). *Plants* **2022**, *11*, 1731. [CrossRef] [PubMed]