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PbZAT12, Independent of PbMYB10, Activates Structural Genes to Promote Anthocyanin Biosynthesis in 'Red Zaosu' Pear Fruit (*Pyrus bretschneideri* Rehd.)

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Abstract: The red color of the pear peel in red-skinned European pear is due to the accumulation of anthocyanins. Numerous transcription factors play pivotal roles in anthocyanin biosynthesis, with zinc finger proteins frequently implicated in the regulation of this process via MYB10, as documented in earlier studies. In this article, we identified a zinc finger protein gene, named PbZAT12, that does not rely on PbMYB10, to regulate anthocyanin biosynthesis. The PbZAT12 protein was localized within the nucleus and exhibited a positive influence on the accumulation of anthocyanins in the peel of 'Red Zaosu' pears. Moreover, overexpression of *PbZAT12* resulted in a significant up-regulation of *PbDFR*, *PbANS*, and *PbUFGT* expression levels in pear fruitlets. Y1H assays demonstrated a direct binding ability of PbZAT12 to *proPbDFR*, *proPbANS*, and *proPbUFGT*, which was supported by a dual luciferase assay, indicating its potential to activate the transcriptional activity of these promoters. However, in contrast to its effect on the aforementioned promoters, PbZAT12 did not exhibit an activation of *PbMYB10*. In summary, our findings suggest that a zinc finger transcription factor, PbZAT12, exerts a positive influence on anthocyanin biosynthesis in pear fruit through direct upregulation of the expression levels of *PbDFR*, *PbANS*, and *PbUFGT*.

Keywords: 'Red Zaosu' pear; PbZAT12; anthocyanin

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1. Introduction

Anthocyanin, a vital secondary metabolite, imparts vibrant red, blue, and purple hues to diverse tissues [1]. These pigments give plants special colors that attract pollinators and animals to help with seed dispersal [2]. Additionally, they function as natural antioxidants, contributing significantly to human health and well-being [3,4]. Anthocyanin accumulation is a complex process regulated by various factors, including light exposure [5,6], temperature variations [3,7,8], sugar availability, and plant hormone signaling [9,10]. The biosynthesis process of anthocyanin in plants is closely related to the light signal transduction pathway, and sunlight up-regulates anthocyanin accumulation [11–14]. Light exerts a pivotal regulatory influence on the anthocyanin biosynthesis pathway by modulating the expression of both structural and regulatory genes, thereby facilitating the enhanced accumulation of anthocyanins [15]. Overexpression of PAP1 in Arabidopsis thaliana seedlings promotes anthocyanin accumulation and significantly increases DFR transcription levels [16]. Light enhances the anthocyanins in apple through the promotion

of expression of key anthocyanin transcription factors, specifically MdMYB1 (MdMYBA) and MdMYB10 [11,17], in pear, by PbMYB10 and PbMYB114 [18,19].

A combination of enzymatic processes and transcriptional regulators regulates the biosynthesis of anthocyanin [20–23]. Enzymes involved in this process include flavonol synthase (FLS), dihydroflavonol reductase (DFR), leucoanthocyanidin dioxygenase/anthocyanidin synthase (LDOX/ANS), anthocyanidin reductase (ANR), and UDP-glucose: flavonoid 3-glucosyltransferase (UFGT) and other related enzymes [24,25]. Meanwhile, the accumulation of anthocyanin is primarily mediated by MYBs, basic Helix-Loop-Helix (bHLHs), and WD40, which can form a MYB-bHLH-WD40 (MBW) protein complex in plants [25,26].

Zinc finger proteins, as a group of transcription factors, are involved in diverse physiological and biochemical processes spanning across a wide array of organisms [27]. Based on their structural characteristics, these proteins can be categorized into various types, including C8, C6, C4, C2H2, C3HC4, C4HC3, C2HC, and LIM (C2HC5) [28]. Within the diverse range of zinc finger proteins, C2H2-type (cysteine2/histidine2-type) zinc finger proteins are most closely associated with stress-related transcription factors [27]. As a subfamily of zinc finger proteins, BBX family members have been extensively implicated in the regulation of anthocyanin biosynthesis [29,30]. The zinc finger protein ZAT12, which is the same as a member of the zinc finger proteins family, was initially identified as a responsive gene to HL (RHL41, also named ZAT12) [31]. RHL41 plays a role in modulating the expression of multiple light- and oxidative-stress-responsive genes in Arabidopsis [32], and it has been shown to increase anthocyanin levels [31]. However, the specific molecular mechanism by which it regulates anthocyanin biosynthesis has not been studied yet.

We identified a light-responsive gene, *PbZAT12*, through transcriptome analysis of 'Red Zaosu' pear fruit peel reddening under high temperature and high light. PbZAT12 contains the conserved C2H2 domain, and we propose that it enhances anthocyanin biosynthesis in red skin pear. In this study, we identified *PbZAT12* through differential gene screening in the transcriptome of 'Red Zaosu' pear, which showed a correlation with anthocyanin content in the pear peel. We validated the promotion of anthocyanin accumulation by PbZAT12 through transient injection in pear peel. In *PbZAT12*-OE, we screened for some structural genes that exhibited similar expression levels to *PbZAT12* by qRT-PCR. Furthermore, we confirmed the direct binding of PbZAT12 to the promoter regions of these genes and its enhancement of promoter activity through Y1H experiments and the dual-luciferase reporter assay system. Therefore, we demonstrated that *PbZAT12* positively regulates anthocyanin biosynthesis in pear peel by directly modulating the expression of structural genes involved in anthocyanin biosynthesis, and we tentatively inferred that this regulation occurs independently of *PbMYB10*. These findings contribute to enriching the regulatory network of anthocyanin biosynthesis in pear peel and are of significant importance in uncovering the regulatory differences of different transcription factors in anthocyanin biosynthesis.

2. Materials and Methods

2.1. Plant Materials

In 2018, for our study, we carefully chose the fruit of 'Red Zaosu' (*Pyrus bretschneideri* Rehd.) as our primary experimental material. These fruits were obtained from a well-established commercial orchard situated in Mei County, Shaanxi Province, China. The peels of fruitlets overexpressing *PbZAT12* were harvested 5 days post injection in 35 DAFB (Days after full bloom), and the harvested fruit samples were rapidly frozen in liquid nitrogen to preserve their biochemical composition and enzymatic activity. Subsequently, they were stored at −80 °C to maintain their integrity during long-term storage. This freezing and storage process allowed for accurate measurements of anthocyanin concentrations and facilitated the extraction of RNA for further analysis.

The dual luciferase assay was conducted using *Nicotiana benthamiana* seedlings, which were cultivated in a controlled environment. The seedlings were grown in a light incubator

set at a temperature of 22 °C, with a photoperiod consisting of 16 h of light followed by 8 h of darkness.

2.2. Transcriptome Sequencing

Three types of samples were collected from the fruit of 'Red Zaosu': red-striped peel (GS), green-striped peel (RS), and all-red peel (AR). Three replicates were collected for each sample type. RNA was extracted from the peel samples and transcriptome libraries were constructed. Illumina HiSeq 2000 (Illumina, San Diego, CA, USA) sequencing was performed on the libraries. For specific details regarding transcriptome construction and data analysis, please refer to Zhai et al. [24].

2.3. Anthocyanin Concentration Measurements

In this study, we implemented a slightly modified version of a previously established method [33] to extract and determine total anthocyanin from our samples. The absorbance values were measured simultaneously at 520 nm and 700 nm using a Microporous plate spectrophotometer (Multiskan GO; Thermo Scientific, Waltham, MA, USA). The buffer liquid was used as a negative control.

2.4. Sequence Alignment and Phylogenetic Analysis of Proteins

To align the ZAT proteins from different species, we used the DNAMAN program from LynnonBiosoft, CA, USA. For the phylogenetic analysis, we used the MEGA 5.0 program (Mega Limited, Auckland, New Zealand) and applied the NJ method with the JTT model.

2.5. RNA Extraction and Expression Analysis

The qRT-PCR reactions were conducted using SYBR Premix Ex Taq II (TaKaRa, Dalian, China) following the manufacturer's instructions and performed on an Icycler iQ5 instrument (Bio-Rad, Berkeley, CA, USA). Each sample was subjected to three biological replicates, and each biological replicate was run with three technical replicates. The specific method of RNA extraction and setting up the QRT-PCR system is based on the method in Yao et al.'s article [19]. The resulting qRT-PCR data were analyzed using the $2^{-\Delta\Delta CT}$ method, with each biological replicate undergoing three separate analyses.

Please refer to Supplemental Table S1 for the primer sequences used for actin, anthocyanin biosynthetic genes, and *PbZAT12*.

2.6. Subcellular Localization Experiments of Gene

The complete coding sequence of *PbZAT12* was amplified using PCR from cDNA extracted from the peel of 'Red Zaosu'. Subsequently, it was inserted into the plant binary expression vector pCambia 2300 along with a GFP tag, driven by the CaMV35S promoter, resulting in the creation of the construct p35S::GFP-*PbZAT12*. These constructs were then transformed into Agrobacterium tumefaciens strain EHA105 (ToloBio, Shanghai, China) for the purpose of introducing them into the cells of *Nicotiana benthamiana* leaves.

The p35S::GFP-PbZAT12 plasmid-carrying Agrobacterium tumefaciens strain EHA105 was cultured on LB solid medium at 28 °C for 48 h. Subsequently, the Agrobacterium cells were resuspended in an infiltration buffer containing 10 mM $MgCl_2$, 10 mM MES, and 200 μM acetosyringone and incubated at room temperature until reaching an optical density (OD_{600}) of 0.8. The resulting suspension was then used to infiltrate the cells of *Nicotiana benthamiana* leaves using the methods described by Jiang et al. [34]. Following infiltration, the *Nicotiana benthamiana* leaf samples were kept in shaded conditions at room temperature for 48 h, and the resulting GFP fluorescence was observed using an Olympus BX63 microscope. The primer sequences used for subcellular localization can be found in Supplemental Table S1.

2.7. Transient Expression Assay in Skin of Pear Fruitlets

The complete coding DNA sequence (CDS) of *PbZAT12* was fully cloned into the pBI121 binary vector, resulting in the generation of *PbZAT12*-OE plasmids. The plasmids were then introduced into Agrobacterium tumefaciens strain EHA105 for a transient expression assay in the skin of pear fruitlets. The same protocol for Agrobacterium incubation as the subcellular localization experiments was followed. For the injection of the skin of pear fruitlets, the method outlined in Spolaore et al. [35] was utilized, while the injection volume was carried out according to Zhai et al. [24]. To visualize GUS staining, the plant materials were stained with 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) as described in Spolaore et al. [35].

2.8. Yeast One-Hybrid (Y1H) Assay

The Y1H assays were conducted following the instructions provided by the Matchmaker Gold Yeast One-Hybrid System Kit (Clontech, Mountain View, CA, USA). First, 800 bp fragments of the promoters of the structural genes were inserted into pAbAi to create the pAbAi-baits. Subsequently, the complete CDS of *PbZAT12* was introduced into the pGADT7 vector, resulting in the generation of the AD-*PbZAT12* vector. The pAbAi-bait vectors were then linearized and individually transformed separately into the Y1H Gold cell. Transformants were selected on SD/-Ura agar plates that lacked uracil, and successful integration into the Y1HGold genome was confirmed by Colony PCR analysis using 630495 (Clontech). After determining the minimum inhibitory concentration of Aureobasidin A (AbA) for the bait reporter yeast strains, the AD-*PbZAT12* vector was introduced into the bait yeast strains. Subsequently, the transformed yeast strains were screened on an SD/-Leu/AbA plate. The experiments were conducted in triplicate, with each experiment repeated three times.

2.9. Dual Luciferase Assay

To obtain the promoter sequences of *PbANS*, *PbDFR*, and *PbUFGT*, genomic DNA isolated from 'Red Zaosu' was used as the template for PCR amplification. The primers used for amplification were designed based on the R045Q (TaKaRa, Dalian, China) and gene-specific sequences (Supplementary Table S1). The amplified promoter sequences were ligated into the HindIII and BamHI restriction sites of the pGreenII 0800-LUC vector, which were named *proPbANS*, *proPbDFR*, and *proPbUFGT*, respectively [36]. The full-length coding sequences of *PbZAT12* were inserted into the pGreenII 0029 62-SK vector [36].

Subsequently, the recombinant plasmids containing the *PbZAT12* gene and the pSoup helper plasmid were separately introduced into the Agrobacterium tumefaciens strain EHA105 [36]. Agrobacterium tumefaciens cells carrying the *PbZAT12*-SK construct were mixed individually with the *proPbANS*, *proPbDFR*, or *proPbUFGT* constructs in a 1:1 ratio. The bacterial mixture was then injected into leaves of 4-week-old *Nicotiana benthamiana* plants. After injection, the plants were grown in a growth chamber for 3 days. The specific method is based on the method in Yao et al.'s article [19].

3. Results

3.1. Identification and Sequence Analysis of PbZAT12 TF in Pear

We discovered a ZAT transcription factor (TF) (GenBank accession number: XP_009337587.1) through a transcriptome study, which exhibited the greatest difference in RS vs. GS expression, and FPKM (Fragments Per Kilobase Million) > 0 (Figure 1a). To further identify zinc finger transcription factors in pear, we examined the conserved domains of the Arabidopsis thaliana AtZAT12 protein (NP_200790.1) in the GenBank database. This analysis revealed 73 *ZAT* genes from white pear genomic genes in the NCBI database. Phylogenetic analysis indicated that the two candidate ZAT proteins clustered together with AtZAT12 proteins (Figure 1b). Consequently, we designated the ZAT TF (XP_009337587.1) as PbZAT12. Sequence alignment and phylogenetic tree analysis revealed that PbZAT12 had the highest protein sequence identity with AtZAT12 (Figure 1c).

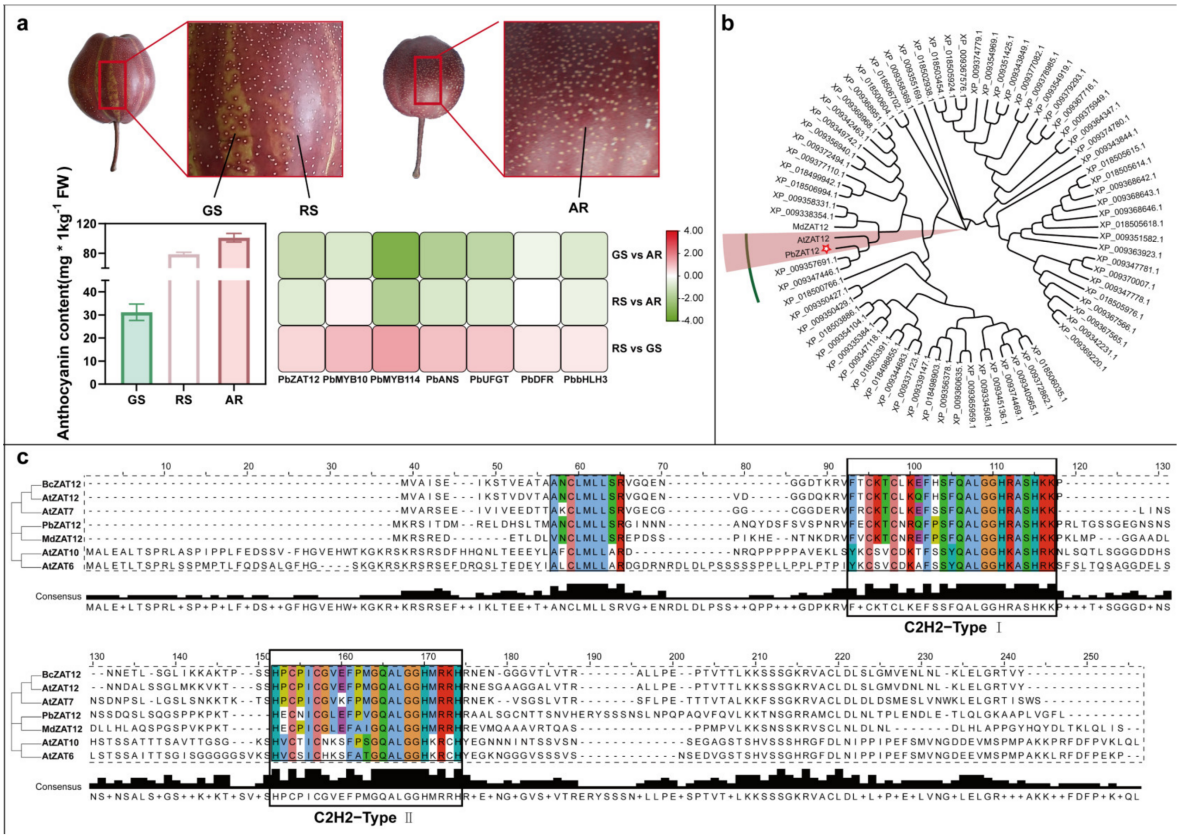

Figure 1. Phylogenetic Analysis of Pear Genes from Different Plant Species. (**a**) RS, GS, and AR phenotypes of 'Red Zaosu' pear; anthocyanin content of PbZAT12; and log2.Fold_change values of anthocyanin-biosynthesis-related genes in the transcriptome (RS: Red stripes peel of 'Red Zaosu'; GS: Green stripes peel of 'Red Zaosu'; AR: Peel of all-red 'Red Zaosu'). (**b**) Phylogenetic analysis of MdZAT12 and AtZAT12 in pear, apple, and Arabidopsis. The five-pointed stars represent *PbZAT12*, the red shaded areas represent the closest homologous genes to *PbZAT12*, and the green outlined areas represent multiple homologous genes clustered together with *PbZAT12* in the phylogenetic tree. (**c**) Phylogenetic analysis of ZAT proteins in different plants and multiple sequence alignment of different ZAT proteins. The accession numbers of the proteins obtained from NCBI are listed in Supplementary Table S2. The C2H2 conserved domain is highlighted with a box in (**c**). Note: We named XP_009337587.1 as PbZAT12.

3.2. PbZAT12 Plays an Active Role in Anthocyanin Accumulation in Pear Fruitlets

To investigate whether the expression of *PbZAT12* affects anthocyanin accumulation, we performed transient expression of *PbZAT12*-OE constructs on pear fruitlet peels using agrobacterium infiltration. Our results showed that compared to fruitlets injected with an empty vector, fruitlets with *PbZAT12*-OE constructs had significantly higher levels of anthocyanin accumulation on their peels (Figure 2a). To confirm successful infection on the fruitlet peels, we monitored the GUS signals (Figure 2b). Moreover, the expression level of *PbZAT12* was significantly increased in the peel of *PbZAT12*-OE 'Zaosu' fruitlets (Figure 2c). These findings suggest that PbZAT12 plays a role in promoting anthocyanin accumulation in the skin of pear fruitlets.

3.3. PbZAT12 Accumulates Anthocyanin by up-Regulating Anthocyanin-Related Genes in Skin of Pear Fruitlets

To identify potential downstream genes regulated by PbZAT12, we performed real-time PCR analysis of structural genes and transcription factors involved in anthocyanin biosynthesis in the peel of *PbZAT12*-OE pear fruitlets. The findings revealed a substantial increase in the expression levels of *PbDFR*, *PbANS*, and *PbUFGT* in the peel of *PbZAT12*-OE

fruitlets compared to those injected with an empty vector (Figure 3). These findings suggest that PbZAT12 promotes anthocyanin accumulation in the peel of pear fruitlets by regulating the expression of genes involved in anthocyanin biosynthesis.

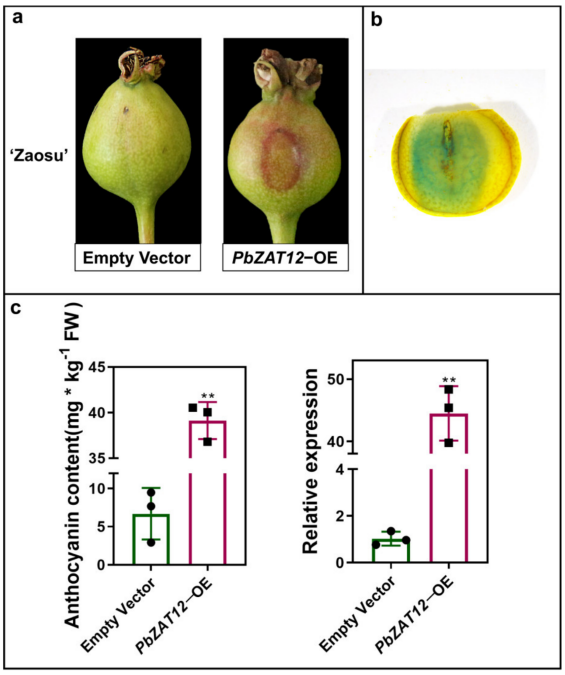

Figure 2. Anthocyanin content and gene expression level around transient expression in 'Zaosu' pear fruit peel. (**a**) Pear fruit peel coloration around overexpressed PbZAT12 fruitlets. (**b**) The validity of infection of the skin of GUS-stained 'Zaosu' fruitlets. (**c**) The expression levels of *PbZAT12* and anthocyanin concentration of overexpressed *PbZAT12* fruitlets. Significant differences were analyzed with Student's *t*-test: ** $p < 0.01$.

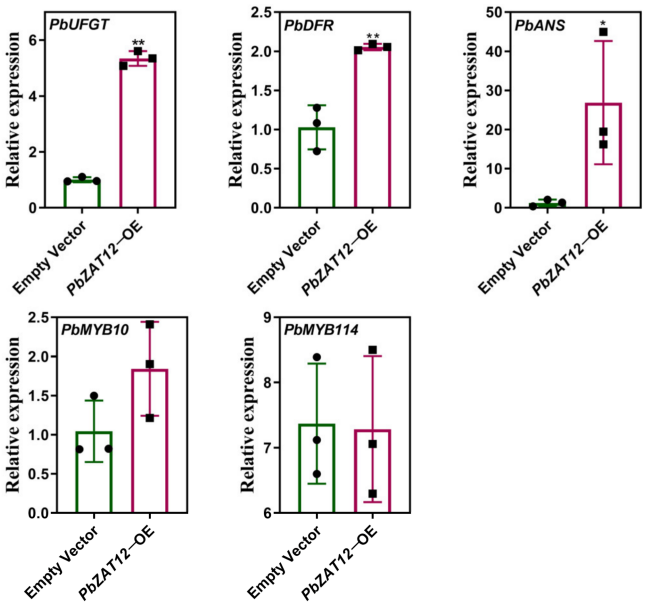

Figure 3. The expression of anthocyanin-biosynthesis-related genes and MYB transcription factors in overexpressed *PbZAT12* fruitlets. The promoters of expression analyses are listed in Supplementary Table S1. The accession numbers of genes for NCBI are presented in Supplementary Table S2. Statistical analysis was performed using Student's *t*-test, and significance was indicated as follows: * $p < 0.05$, ** $p < 0.01$.

3.4. Subcellular Localization of PbZAT12

According to Uniprot (http://www.uniprot.org/, accessed on 23 August 2022), the subcellular localization prediction suggests that AtZAT12 is predominantly localized in the nucleus. To determine the localization of PbZAT12, localization analyses were performed using transient assays in *Nicotiana benthamiana* leaf cells. The results revealed that the GFP fluorescence of 35S: PbZAT12-GFP was localized to the nucleus of *Nicotiana benthamiana* leaf cells (Figure 4), indicating that PbZAT12 is also a nuclear protein. In contrast, the positive control 35S: GFP exhibited GFP fluorescence in both the cytomembrane and nucleus of *Nicotiana benthamiana* leaf cells (Figure 4).

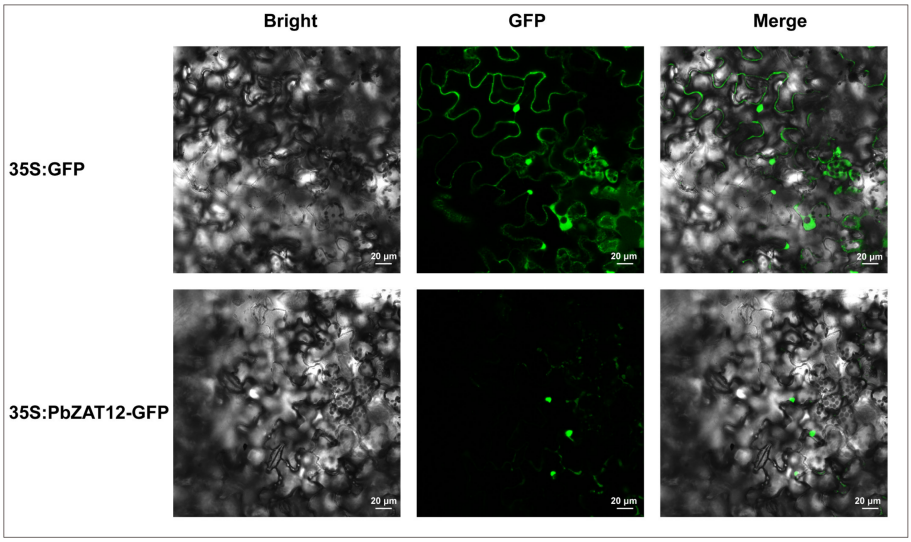

Figure 4. Subcellular location of PbZAT12. The localization of PbZAT12 protein in *Nicotiana benthamiana* leaf cell. Bars = 0.01.

3.5. PbZAT12 Regulates the Promoter Activities of PbDFR, PbANS, and PbUFGT in Anthocyanin Biosynthetic Pathway

To confirm the impact of PbZAT12 on the promoters of anthocyanin synthesis structural genes, we performed both a Y1H assay and dual-luciferase analysis. The Y1H assay revealed that PbZAT12 was capable of directly binding to the promoters of *PbDFR*, *PbANS*, and *PbUFGT* (Figure 5a). We then analyzed the activation of PbZAT12 on the promoters of anthocyanin-related structural genes in leaves of *Nicotiana benthamiana*. Co-infiltration of PbZAT12 with *PbDFR*, *PbANS*, or *PbUFGT* in *Nicotiana benthamiana* leaves resulted in a significant increase in luciferase activities compared to the control group (Figure 5b), indicating that PbZAT12 is capable of both binding directly and activating *proPbDFR*, *proPbANS*, and *proPbUFGT*.

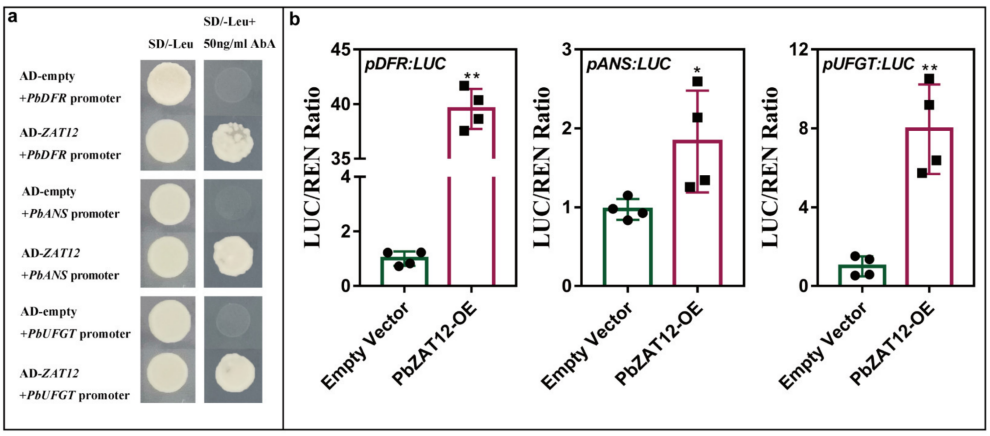

Figure 5. Influence of PbZAT12 on transcriptional activity of anthocyanin biosynthetic structural genes.

(**a**) The direct binding between *PbZAT12* and structural genes in yeast one-hybrid assays. (**b**) The activation effect of *PbZAT12* on *proPbDFR, proPbANS,* and *proPbUFGT* was detected using dual luciferase assay in *N. benthamiana* leaves. The ratio of luciferase activity of the structural gene (LUC) to that of 35S Renilla (REN) was normalized using the LUC/REN value obtained from the empty vector. The results are presented as the means of five biological replicates, and significant differences were determined by comparing with the control. Statistical analysis was performed using Student's *t*-test, and significance was indicated as follows: * $p < 0.05$, ** $p < 0.01$.

4. Discussion

The accumulation of anthocyanins is regulated by light activation through the light signaling pathway [37–40]. The BBX subfamily of zinc finger proteins can respond to the light pathway, activate the expression of downstream genes, and thus promote the biosynthesis of anthocyanins [29,30]. As a member of zinc finger proteins, the *ZAT* gene family has been studied in Arabidopsis in previous studies [38]. In this study, the obtained *PbZAT12* showed high similarity to its homologous genes (Figure 1b,c) [41]. ZAT12 has been reported to assist plants in stress responses across various species, including abiotic stresses such as salt stress and high light stress, as well as biotic stresses [42]. Although the involvement of ZAT12 in stress responses is often associated with anthocyanin accumulation, the specific mechanisms underlying this process are not yet well understood. Overexpression of *AtZAT12* enhances anthocyanin accumulation and improves plant tolerance to biotic and abiotic stresses [31,32]. RHL41 (also known as ZAT12) can promote anthocyanin biosynthesis when overexpressed in Arabidopsis, which is related to its induction by high light, and has thick and dark green leaves [31,43]. In the comparative transcriptomic data of two tropical water lilies under cold stress, it was observed that the expression level of *NlZAT12* is highly correlated with anthocyanin accumulation. However, the study did not describe the expression changes of regulatory genes involved in anthocyanin synthesis in *NlZAT12*-OE [44]. These findings are consistent with the results of our study, where overexpression of *PbZAT12* promotes anthocyanin accumulation in pear peel (Figure 2a,b). However, previous studies did not specifically elucidate the molecular pathways involved in anthocyanin biosynthesis during this process.

Studies on anthocyanin biosynthesis focus on both the structural genes and regulatory genes, as well as the MYB (MYB10)-bHLH-WD40 (MBW) complex [23,45–47]. *PyPIF5*, a light-responsive gene, is involved in anthocyanin biosynthesis through the PymiR156a-PySPL9-PyMYB114/MYB10 module [48]. Even though transcription factor PbWRKY75 can directly regulate the structural genes involved in anthocyanin biosynthesis, they also have an impact on *PbMYB10b* (also named *PbMYB114*) [49]. Some external factors, such as H_2S, can induce the persulfidation of PyMYB10 at Cys218, thereby affecting the biosynthesis of anthocyanins [19]. The pathway through which zinc finger proteins promote anthocyanin biosynthesis was primarily focused on MYB10 too. As a member of the zinc finger structure protein family, BBX proteins 1, 17, 15, 35, 51, and 54 were found to directly act as activators of the MYB10 promoter in apple [29]. In pear peel, PpBBX16 is a positive regulator of light-induced anthocyanin accumulation by activating MYB10 [50]. PpBBX18 was found to form a heterodimer with PpHY5 through two B-box domains, with PpHY5 binding to the G-box motif of *PpMYB10* and *PpBBX18* inducing its transcription, while PpZAT5 represses *PpBBX18* expression and suppresses anthocyanin biosynthesis by binding to its CAAT motif [51,52]. Zinc finger transcription factors were found to activate the expression of *MYB10*, rather than directly activating the expression of genes involved in anthocyanin biosynthesis in previous studies. However, in this study, there was no significant difference in the expression levels of *PbMYB10* and *PbMYB114* genes between *PbZAT12*-OE and WT, indicating that PbZAT12 might not regulate anthocyanin biosynthesis in pear peel through PbMYB10 (Figure 3), which is different from other zinc finger proteins that regulate anthocyanin biosynthesis in pear or apple. No significant inhibition of *PbMYB10* and *PbMYB114* expression was observed in our experiments.

The coloring mechanism of 'Red Zaosu' pear, an oriental red pear, has been reported. The accumulation and coloration of anthocyanins in the red stripes of 'Red Zaosu' pear under light conditions are known to be influenced by various genes, such as *PpPIF8* [53], *PpHY5* [54], and *PpBBX14* [55]. There are also some studies on the reasons for the formation of red and green stripes in 'Red Zaosu'. For example, the demethylation of the *PpMYB10* promoter region has been implicated in the development of red coloration and red stripes in 'Red Zaosu' pears [56]. Furthermore, the methylation level of the PbGA2ox8 promoter region affects the expression of *PbMYB10*, *PbUFGT1*, and *PbGSTF12*, revealing the reason for the red and green stripes in 'Red Zaosu' pear [57]. However, our findings demonstrate that overexpression of *PbZAT12* in pear peel significantly increased the expression levels of *PbDFR*, *PbANS*, and *PbUFGT* (Figure 3). Our study confirms the correlation between PbZAT12 and anthocyanin biosynthesis through direct regulation of the structural genes involved in the pathway. But our study revealed that overexpression of PbZAT12 did not affect the expression of *PbMYB10*, which is different to transcriptomic data, indicating that the underlying mechanism of red and green stripes in 'Red Zaosu' pear is complex. This pathway provides another possibility for the formation of red and green stripes in 'Red Zaosu' pear (Figure 6).

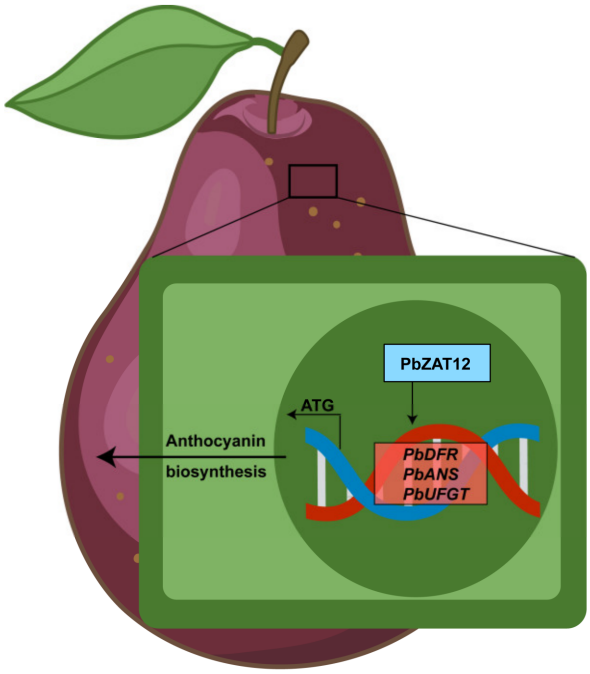

Figure 6. Model of the molecular mechanism by which PbZAT12 promotes anthocyanin biosynthesis. PbZAT12 directly binds to the promoter regions of structural genes (*PbDFR*, *PbANS*, and *PbUFGT*) involved in anthocyanin biosynthesis, thereby enhancing their transcriptional activity and promoting anthocyanin biosynthesis in pear peel.

5. Conclusions

In this study, PbZAT12 was found to regulate anthocyanin accumulation by directly binding to and activating *proPbDFR*, *proPbANS*, and *proPbUFGT*. Overall, zinc-finger transcription factor PbZAT12 can enhance anthocyanin biosynthesis by increasing the expression levels of *PbDFR*, *PbANS*, and *PbUFGT* in pear.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9070775/s1, Table S1: List of primers of *PbZAT12* and FPKM of *PbZAT12* in the transcriptome. Table S2: Gene/protein name and accession number.

Author Contributions: L.X. (Lingfei Xu) and Z.W. designed the experiments. Y.L., H.C. and X.L. performed the experiments. R.Z., C.Y., L.X. (Lijuan Xiao) and Y.X. analyzed the data. Z.W., L.X. (Lingfei Xu), Z.Z. and H.C. wrote and revised this manuscript. Z.W. and H.C. participated in the research and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be made available on request.

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Conflicts of Interest: The authors confirm that there are no known conflicts of interest associated with this publication.

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