Scientia Horticulturae 301 (2022) 111089

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Involvement of three ABRE-binding factors in the gametophytic self-incompatibility reaction in pear

Lei Wu [a,1], Ying Xu [a,1], Min He [a], Xue-Ting Jiang [b], Kai-Jie Qi [a], Chao Gu [a,*], Shao-Ling Zhang [a,*]

[a] Centre of Pear Engineering Technology Research, State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China
[b] College of Life Sciences, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

ARTICLE INFO

Keywords:
Pyrus bretschneideri
Self-incompatibility
ABRE-binding factor
Pollen tube growth
Transcriptional regulation

ABSTRACT

Self-incompatibility (SI) facilitates the rejection of pollen by self S-RNase. It is believed that a large number of genes are responsive to SI reactions; however, little is known about the transcriptional regulation of these genes. Herein, we explored the role of ABRE-binding factor (ABF) in gametophytic self-incompatibility (GSI). Phylogenetic analysis showed that eight *ABF* genes in pear clustered with five *ABF* genes in *Arabidopsis*. Of these *ABF* genes, *PbABF.E.1* and *PbABF.E.2* were expressed in all tissues, and *PbABF.B* was expressed in most tissues. These three *ABF* genes were also detected in the self- and cross-pollinated styles. Additionally, RNA-Seq analysis revealed 11,476 genes that were differentially expressed between the self- and cross-pollinated styles at 24, 48, and/or 72 h after pollination. Of these differentially expressed genes (DEGs), 278 were differentially expressed between the self- and cross-pollinated styles at any stage and thus are responsive to the GSI reaction. Notably, abscisic acid-responsive elements were detected in the promoter sequences of the 1213 DEGs including the six genes negatively correlated with pollen tube growth. The dual-luciferase assay showed that the promoter of these six genes was activated by *PbABF.B, PbABF.E.1*, and/or *PbABF.E.2*. Therefore, *PbABF.B, PbABF.E.1*, and *PbABF.E.2* were involved in the GSI reaction by mediating the expression of genes responsive to the GSI reaction.

1. Introduction

Self-incompatibility (SI) is a widespread mechanism that prevents inbreeding by enabling the pistil to reject pollen from genetically related individuals, thereby out-crossing in flowering plants. As a result of the different molecular and genetic control mechanisms, SI is divided into sporophytic SI (SSI) and gametophytic SI (GSI). SSI has been detected in Brassicaceae (Hiscock and Mclnnis, 2003) and Oleaceae (Breton et al., 2014), while GSI has been widely studied in Rosaceae (Wu et al., 2013a), Solanaceae (Luu et al., 2000), Plantaginaceae (Kubo et al., 2010), and Rutaceae (Liang et al., 2020). Of these two SI systems, GSI is controlled by a single multi-allelic locus (*S*-locus) containing at least two genes that determine stylar and pollen specificities (Wu et al., 2013a). The pistil determinant is known to encode an S-RNase (Anderson et al., 1986; Sassa et al., 1996; Xue et al. 1996), while the pollen determinant is a single *S*-locus (haplotype) *F-box* gene (SLF or SFB) in *Prunus* species but is likely controlled by multiple *F-box* genes in Solanaceae and some Rosaceae species (Kakui et al., 2011; Kubo et al., 2010).

In addition to pistil-*S* and pollen-*S* determinants, numerous modifiers have been identified to be involved in the GSI reaction. In tobacco, HT-B, which is stabilized by the interaction between S-RNase and SLF in an *S*-locus, can induce RNA degradation to prevent pollen tube growth (Goldraij et al., 2006). A 120-kDa glycoprotein has a direct role in *S*-specific pollen rejection (Hancock et al., 2005). In apple, MdABCF, a cytomembrane protein, transports S-RNase from the outside to inside of the pollen tube (Meng et al., 2014). MdMVG and MdPPa are inactivated by interacting with S-RNase to inhibit pollen tube growth (Li et al., 2018; Yang et al., 2018). MdD1, which is induced by jasmonic acid signal transduction, can directly interact with, and inhibit the activity of self and non-self S-RNase to promote pollen tube growth (Gu et al., 2019). In pear, PbrPLDδ1, a phospholipase D protein, increases phosphatidic acid levels to prevent depolymerization of the actin

Abbreviations: SI, Self-incompatibility; GSI, Gameametophytic self-incompatibility; ABF, ABRE-binding factor; ABA, Abscisic acid; HAP, Hours after pollination; RPKM, Reads per kilo-base per million; DEG, Differentially expressed gene; ABRE, ABA-responsive element; qRT-PCR, Quantitative real-time PCR.

* Corresponding authors.
E-mail addresses: guchao_lw@tom.com (C. Gu), slzhang@njau.edu.cn (S.-L. Zhang).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scienta.2022.111089
Received 11 December 2021; Received in revised form 21 March 2022; Accepted 22 March 2022
Available online 4 April 2022
0304-4238/© 2022 Elsevier B.V. All rights reserved.

cytoskeleton elicited by self S-RNase (Chen et al., 2018). VHA-a1, a member of V-ATPase family, can induce cytoplasmic acidification to reduce the deposition of diacylglycerol kinase 4, thus resulting in vacuolar morphological changes and nuclear DNA degradation in the pollen tube (Kong et al., 2021). Of these modifiers, PbrPLDδ1 is positively responsive to self S-RNase (Chen et al., 2018), while VHA-a1 is negatively responsive to self S-RNase (Kong et al., 2021). MdD1 is induced by both self and non-self S-RNases (Gu et al., 2019). These results indicate that self S-RNase inhibits pollen tube growth by affecting a series of modifiers.

The expression of these modifiers in the pollen tube must be regulated by various transcription factors. However, one study reported that the *MdD1* expression is induced by a bHLH transcription factor MdMYC2 (Gu et al., 2019). MdMYC2 is a downstream factor of jasmonic acid signal transduction (Gu et al., 2019) that plays an important role in the defense response to stress (Wasternack and Hause, 2013). Abscisic acid (ABA) is one of the most important phytohormones in stress defense (Yoshida et al., 2019). It is reported that exogenous ABA promotes pollen germination or tube growth in *Petunia hybrid* (Kovaleva et al., 2016), *Tradescantia paludosa* (Malik et al., 1976), and *Arachis hypogea* (Malik and Chhabra, 1976). However, the opposite result was detected in *Torenia fournieri* (Wu et al., 2008), *Nicotiana tabacum* (Chibi et al., 1995), and *Arabidopsis* (Zhou et al., 2010). Interestingly, exogenous ABA promotes pollen germination but inhibits pollen tube growth in *Zea mays* (Dhingra and Varghese, 1985) and *Pyrus pyrifolia* (Zhang et al., 2003). It is suggested that low concentration of ABA increases pollen germination and tube growth, but high concentration of ABA decreases both traits (Frascaroli and Tuberosa, 1992). Whether by promoting or inhibiting, ABA affects pollen germination and tube growth by activating down-stream factors via the Receptor-PP2C (Protein phosphatase 2C)-SnRK2 (Sucrose nonfermenting-1-related protein kinase 2) signal pathway (Umezawa et al., 2010). ABRE-binding factor (ABF) is a positive effector of ABA responses and modulates the expression of abiotic stress-responsive genes (Hossain et al., 2010a, 2010b). However, little has been reported on the role of ABF in the GSI reaction.

Pear is one of the Rosaceae species that presents typical GSI. The SI severely influences fruit set and decreases fruit production. In the past decade, a series explored the mechanism of GSI in pear. For example, the breakdown of SI in 'Jinzhui', 'Yanzhuang', 'Zaoguan', 'Xinxue', and 'Sha 01′ was surveyed based on the inheritance of S-RNase alleles (Li et al., 2020; Qi et al., 2011a, 2011b; Shi et al., 2018; Wu et al., 2013b). S-RNase-directed signal transduction is widely believed to refine the process of programmed cell death (PCD) in the self-pollen tube (Chen et al., 2018; Kong et al., 2021; Wang et al., 2009, 2010; Wu et al., 2021). In a previous study, we confirmed that the ABA concentration was increased at 24 h after pollination (HAP) but decreased at 48 HAP (Shi et al., 2017). Therefore, as the positive effector of ABA responses, ABF may be involved in GSI by transcriptionally regulating modifiers in the pear pollen tube. In this study, by analyzing of the expression patterns of *ABF* genes in different tissues, three *ABF* genes, *PbABF.E.1, PbABF.E.2*, and *PbABF.B*, which had higher levels of expression in the pollen tube than the other *ABF* genes, were isolated from the pear genome. Subsequently, RNA-sequencing (RNA-Seq) analysis revealed the genes responsive to the GSI reaction. Of these genes, the expression of six was regulated by *PbABF.E.1, PbABF.E.2*, and/or *PbABF.B*. Therefore, *PbABF.E.1, PbABF.E.2*, and *PbABF.B* may be involved in the GSI reaction. This information provides insight into the transcriptional regulation network of modifiers in the GSI reaction in pear.

2. Materials and methods

2.1. Plant material and treatment

The pear cultivar Jinzhui (JZ; *Pyrus bretschneideri*) presents self-compatibility and is a spontaneous mutant of the self-incompatible cultivar Yali (YL; *Pyrus bretschneideri*). Using *Pyrus calleryana* Decne as rootstock, the 20-year-old cvs. JZ and YL trees were maintained at Jiangpu Orchard, Nanjing Agricultural University (Nanjing, Jiangsu Province, China). The anthers were collected on the day before flowering (March 27, 2019) and placed on dried paper at 27 ± 2 ℃ to disperse the pollen. The pollen grains were used to pollinate the style of cv. YL. Approximately 3000 flowers located on the fruiting branches were used for the self-/cross-pollination tests. These flowers were emasculated on March 27 and were pollinated and bagged at between 8 and 11 am on March 28 (a sunny day). The self-/cross-pollinated styles were collected at 24, 48, and 72 HAP. The collected styles were divided into two groups; one was immediately frozen in liquid nitrogen and stored at −80 ℃ until use, while the other was fixed in FAA (37% formaldehyde/glacial acetic acid/50% ethanol, 5:5:90) and stored at 4 °C until use. The pollen tubes growing in the style of cv. YL were detected as reported in a previous study (Wang et al., 2017).

2.2. Phylogenetic analysis of ABF genes

Eight eudicot species were selected for phylogenetic analysis of *ABF* genes, including four Rosaceae species, namely, pear (*Pyrus bretschneideri*), apple (*Malus domestica*), peach (*Prunus persica*), and strawberry (*Fragaria* × *ananassa*); one Vitaceae species, namely, grape (*Vitis vinifera*); one Solanaceae species, namely, tomato (*Lycopersicon esculentum*); two Caricaceae species, namely, papaya (*Carica papaya*) and orange (*Citrus sinensis*). *Arabidopsis ABF* genes were used as indexes to search homologous genes by blasting in the pear (http://peargenome.njau. edu.cn/), apple, peach, strawberry, orange, grape, and papaya genomes (https://phytozome.jgi.doe.gov/). The amnio acid sequences were aligned using Clustal W (Thompson et al., 1994) and were then used to construct a phylogenetic tree using MEGA software (version 6) with an equally weighted neighbor-joining method. Bootstrap values were determined from A total of 1000 replicates.

2.3. RNA-Seq

The RNA-Seq library was constructed from the self- and cross-pollinated styles at 24, 48, and 72 HAP. Total RNAs of the six samples were extracted using an RNAprep Pure Plant Kit of Polysaccharides & Polyphenolics-rich (Tiangen, Beijing, China). After attaching oligo-dT, the mRNAs were fragmented and used to synthesize the first- and second-strand cDNA with random hexamer primers. The double-stranded cDNA was supplemented with A-tails and then attached to a special adaptor for sequencing. Finally, PCR was performed to enrich the screened fragments using the adaptor primers. The library was prepared using the Illumina gene expression sample preparation kit (Illumina, San Diego, CA) and sequenced on an Illumina HiSeq2000 (Illumina) using 150-bp paired-end reads. The clean reads were selected by removing the low-quality raw reads and were mapped to the pear reference genome V 1.0 (http://peargenome.njau.edu.cn/) using TopHat v2.0.9 (Trapnell et al., 2009). Only 3-bp mismatches were allowed. The RNA sequencing data have been deposited in the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/Traces/sra_ sub/sub.cgi) under the accession number SRP234049.

2.4. Gene expression analysis

Gene expression levels were calculated based on the reads per kilobase per million (RPKM) mapped to the clean reads. The number of clean reads mapped to each gene was counted using HTseq v 0.5.4p3 (Anders et al., 2015). The differentially expressed genes (DEGs) were isolated based on a threshold of fold change of 2 and were annotated against the NCBI RefSeq nucleotide database and the Swiss-Prot and UniProt protein databases.

2.5. ABA-responsive elements

To identify the DEGs that may be mediated by ABF transcription factors, the 2500-bp sequences upstream of the initiation codons of all DEGs were isolated from the pear genome. The potential *cis*- or *trans*-acting elements in the promoter sequences was predicted using PlantCARE software (http://bioinformatics.psb. ugent.be/webtools/plantcare/html/). The number and position of the ABA-responsive elements (ABREs) were recorded and analyzed in Excel version 2016 (Microsoft Corp., Redmond, MA, USA).

2.6. Quantitative real-time RT-PCR

Total RNA was extracted using the RNAprep Pure Plant Kit of Polysaccharides & Polyphenolics-rich (Tiangen, Beijing, China). The first-strand cDNA was synthesized using the TransScript One-Step gDNA Removal and cDNA synthesis Supermix (TransGen, Beijing, China). Quantitative real-time RT-PCR (qRT-PCR) was carried out in a LightCycler 480®II/96 Thermal Cycler (Roche Diagostics, Rotkreuz, Switzerland). The PCR reaction mixture and cycling conditions were identical to a previous report (Gu et al., 2020). The *TUBULIN* gene was used as the internal control in pear (Chen et al., 2015). All of the primer sequences are listed in Table S1.

2.7. Dual-luciferase assay

The full-length sequences of *PbABF.E1, PbABF.E2*, and *PbABF.B* were amplified from the pollen grains of cv. YL and inserted into the pSAK277 vector to construct gene overexpression vectors. Approximately 2500-bp sequences were amplified from the upstream of initiation codons of target genes and were inserted into a pGreenII 0800-LUC vector. The recombined plasmids were subjected to luminescence assay using the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI). Firefly luciferase (Luc) and Renilla luciferase (Ren) activities were measured using a Cell Imaging Multi-Mode Reader Cytation 3 (BioTek, Santa Barbara, CA). At least six biological replicates were used in each assay. All of the primer sequences are listed in Table S1.

3. Results

3.1. Phylogenetic classification of ABF gene family members in pear

To classify the *ABF* gene family members in pear, *ABF* genes were identified from rosid, asterid, and Malvidae species, using *Arabidopsis ABF* genes as indexes. A total of 14, 16, 11, 8, 7, 7, 17, and 10 potential *ABF* genes were identified from the pear, apple, peach, strawberry, grape, papaya, and tomato genomes, respectively. These genes could be classified into four classes (from I to IV) based on the reliable bootstrap values (> 0.98; Fig. 1). However, all the *Arabidopsis ABF* genes were clustered into class I, indicating that the genes in class I belong to the *ABF* gene family. Class II had a relatively low bootstrap value (0.64) compared to class I, indicating that the genes in class II may be *ABF-like* genes. Classes III and IV had low bootstrap values (<0.5) compared to class I, indicating that the genes in these two classes do not belong to the *ABF* gene family. Moreover, class I was comprised of six groups, including ABF.A, ABF.B, ABF1/3/4, ABF.C, ABF.D, and ABF.E (Fig. 1). To facilitate the description of these *ABF* genes in pear, *Pbr040390.1* in group ABF.B was designated as *PbABF.B; Pbr017778.1* and *Pbr007589.1* in group ABF.C were, respectively, designated as *PbABF.C.1* and *PbABF.C.2; Pbr014592.1* and *Pbr003516.1* in group ABF.D were, respectively, designated as *PbABF.D.1* and *PbABF.D.2; Pbr010436.1* and *Pbr024746.1* in group ABF.E were, respectively, designated as *PbABF.E.1* and *PbABF.*

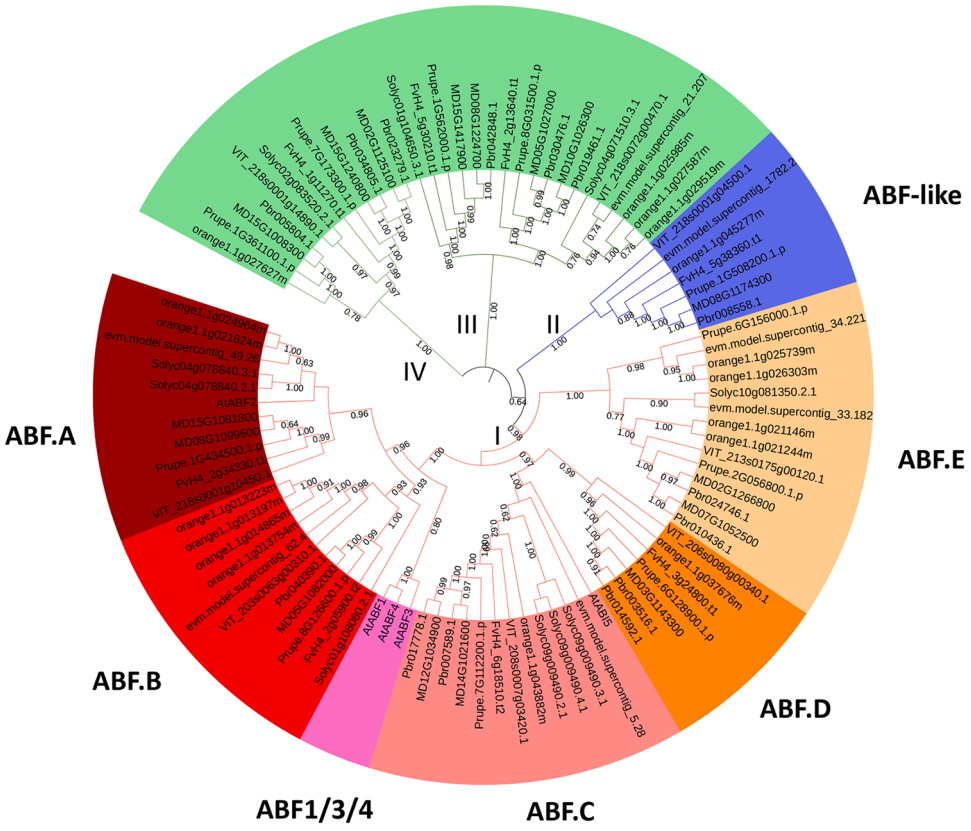

Fig. 1. Identification of *ABF* genes in pear. Phylogenetic analysis showed that the homologous genes of the *Arabidopsis ABF* genes could be classified into four classes I–IV. All five *Arabidopsis ABF* genes were clustered into class I, indicating that the genes in class I belong to the *ABF* gene family. Based on reliable bootstrap, class I was divided into six groups, five of which (excluding ABF1/3/4) contained at least one *ABF* gene in pear.

E.2; and Pbr008558.1 in group ABF-like was designated as *PbABFL*.

3.2. Expression pattern of ABF genes in different tissues

To characterize the expression pattern of *ABF* genes in pear, the transcriptome data from the pollen, leaf, petiole, peduncle, and flesh were obtained from previous studies (Gu et al., 2020, 2021; Zhou et al., 2016). In the pollen grains and pollen tubes, *PbABF.E.2* had the highest level of expression in the pollen tubes at six hours after culturing, indicating that *PbABF.E.2* may play an important role in pollen tube growth (Fig. 2A). Both *PbABF.E.1* and *PbABF.D.2* were also expressed in the pollen grains and pollen tubes, while the other four *ABF* genes were not detected (Fig. 2A).

In the leaves, petioles, and peduncles of the pear cultivar Cuiguan, *PbABF.E.1, PbABF.E.2*, and *PbABF.B* were detected at different stages, while the other five *ABF* genes were undetectable (Fig. 2A). However, *PbABF.B* had the highest level of expression in the petioles at 30 DAFB and in the peduncles at 75 DAFB, indicating that *PbABF.B* may play an important role in petiole and peduncle development. By contrast, *PbABF.E.2* had the highest level of expression in the leaves at 110 days after flower blooming (DAFB). Moreover, in the fruit flesh of cv. Cuiguan, *PbABF.E.1, PbABF.E.2, PbABF.C.2*, and *PbABF.B* were detected at different stages, whereas the other four *ABF* genes were not detected (Fig. 2A). Notably, *PbABF.C.2* had the highest level of expression in the flesh at 45 DAFB. These results suggest that *PbABF.E.2* and *PbABF.C.2* may, respectively, play more important roles in leaf and flesh development than *PbABF.B*.

In the post-harvested fruits, *PbABF.E.1, PbABF.E.2*, and *PbABF.B* were also detected in each sample, while the other five *ABF* genes were undetectable (Fig. 2B). Noteworthy, *PbABF.E.2* had higher levels of expression in the temperature- and ethylene-affected fruits than most of the other *ABF* genes (Fig. 2B), indicating that *PbABF.E.2* plays an important role in post-harvested fruit senescence. Moreover, *PbABF.B* had a lower level of expression in the ethephon-treated fruits (ET) than its control (ET-C) and had higher levels of expression in 1-MCP-treated fruits (1-MCP) than spontaneously senescent fruits (1-MCP-C; Fig. 2B), indicating that *PbABF.B* responds negatively to ethylene and fruit senescence.

3.3. RNA-Seq analysis of self- and cross-pollinated styles of cv. YL

To test whether *ABF* genes were expressed in compatible and incompatible pollen tubes, field pollination tests were conducted to investigate the dynamic growth of the pollen tubes. The pollen tubes had similar lengths in the self- and cross-pollinated styles at 24 HAP (Fig. 3A). However, pollen tube growth almost stopped in the self-pollinated styles after 24 HAP (YY24), while pollen tube growth continued in the cross-pollinated styles at 48 HAP (YJ48) and arrived at the bottom of the cross-pollinated styles at 72 HAP (YJ72). This result suggested that the style of cv. YL is compatible with the pollen grain of cv. JZ. Secondly, RNA-Seq was performed on the self- and cross-pollinated styles of cv. YL. A total of 13.14, 14.03, and 12.18 million raw reads were, respectively, generated from the cross-pollinated styles at 24 HAP (YJ24), YJ48, and YJ72, and a total of 11.19, 12.60, and 13.84 million raw reads were, respectively, generated from YY24 and the self-pollinated style at 48 and 72 HAP (YY48 and YY72; Table S2). After filtering the low-quality raw reads, over 68% of the raw reads could be mapped to the pear reference genome. A total of 30,814 genes were detected from all six samples. Of these genes, *PbABF.E.1, PbABF.E.2*, and *PbABF.B* had higher levels of expression than *PbABF.C.1* and *PbABF.C.2* in all samples, which were almost undetectable in any sample (Fig. 3B). The other three *ABF* genes, *PbABF.D.1, PbABF.D.2*, and *PbABFL* were not expressed in any sample. This result suggests that *PbABF.E.1, PbABF.E.2*, and *PbABF.B* maybe important role in the GSI

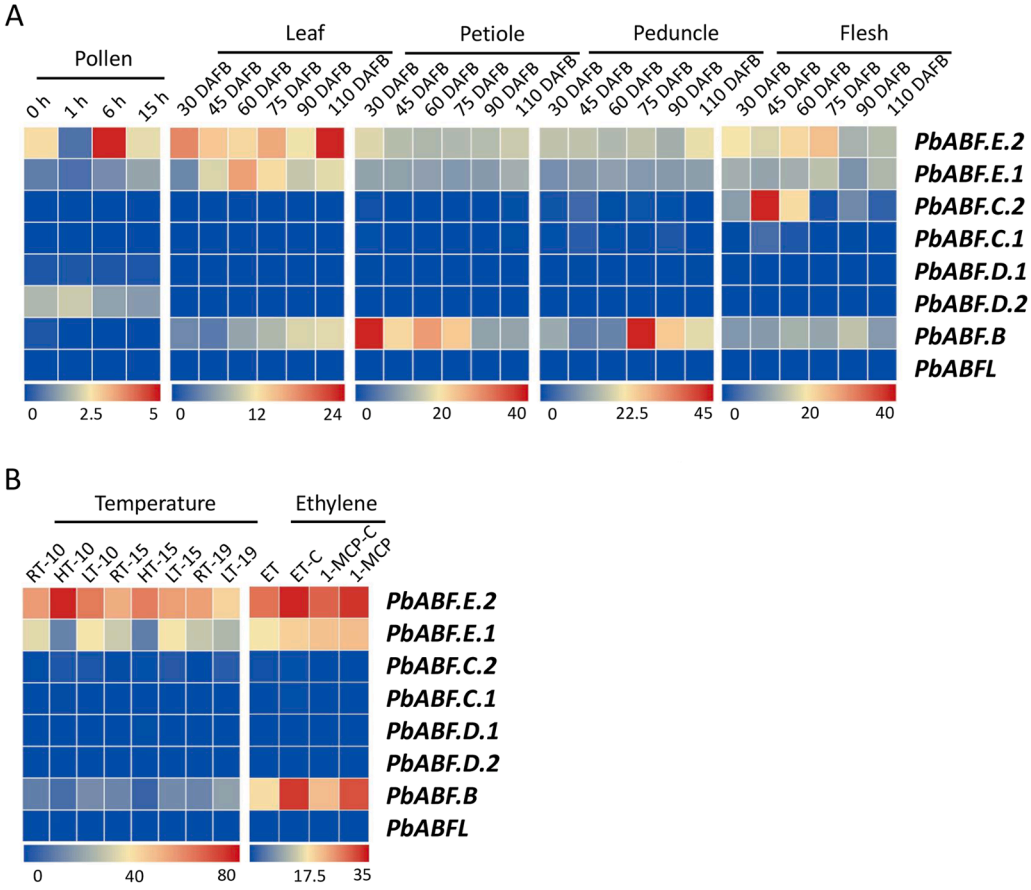

Fig. 2. Expression patterns of pear *ABF* genes in different tissues. (A) The expression patterns of pear *ABF* genes in the pollen tubes, leaves, petioles, peduncles, and flesh. The symbols, 0 h, 1 h, 6 h, and 15 h indicate the time of culture of pollen tube, while 30 DAFB, 45 DAFB, 60 DAFB, 75 DAFB, 90 DAFB, and 110 DAFB indicate the collection time of the leaf, petiole, peduncle, and flesh samples, respectively. (B) The expression patterns of the pear *ABF* genes were detected in the fruit under high-temperature (HT), room-temperature (RT), low-temperature (LT), ethylene (ET), and 1-MCP treatment. RT-10, RT-15, and RT-19 represent the fruits under room-temperature conditions at 10, 15, and 19 days after treatment, respectively. HT-10 and HT-15 represent the fruits under high-temperature conditions at 10 and 15 days after treatment, respectively. LT-10, LT-15, and LT-19 represent the fruits under low-temperature conditions at 10, 15, and 19 days after treatment, respectively. ET and ET-C represent the fruit with and without ethylene treatment, respectively. 1-MCP and 1-MCP-C represent the fruit with and without 1-MCP treatment, respectively.

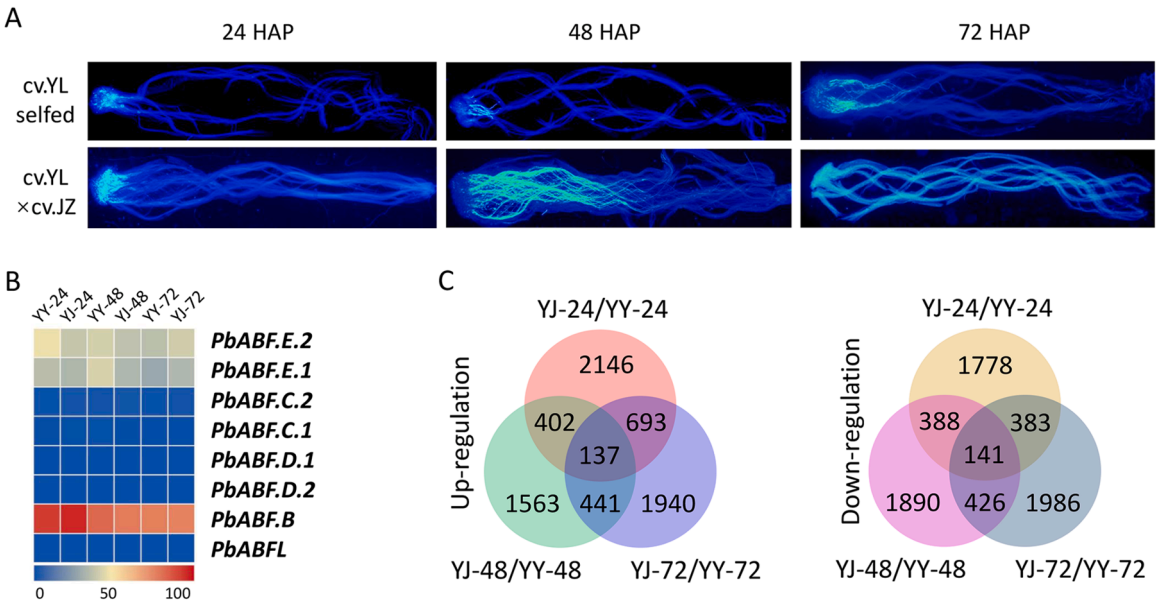

Fig. 3. Identification of the genes responsive to the GSI reaction. (A) The dynamic growth of the pollen tube of cv. YL or JZ was visualized in cv. YL styles at 24, 48, and 72 HAP. (B) Expression pattern of pear *ABF* genes in the self- and cross-pollinated styles. (C) Identification of the genes differentially expressed between the self- and cross-pollinated styles.

reaction in pear.

To identify the genes responsive to the GSI reaction, differentially expressed analysis was performed between self- and cross-pollinated styles at different stages. The result showed that 6068, 5388, and 6147 genes were differentially expressed (DEGs) between YJ24 and YY24, between YJ48 and YY48, and between YJ72 and YY72, respectively (Fig. 3C and Tables S3–S5). After removing the repeated genes, a total of 11,476 DEGs were identified from all three comparisons. Compared to the self-pollinated styles, 137 DEGs were upregulated but 141 DEGs were downregulated in the cross-pollinated styles at all three stages (Fig. 3C). Functional annotation showed that *pectate lyase* (Pbr010756.1 and Pbr036221.1), *cell division control protein* (Pbr012821.1), *pectinesterase inhibitor* (Pbr041880.2 and Pbr012745.1), and *cyclin* (Pbr021971.1 and Pbr015329.1) were included in the upregulated DEGs (Table S6). Interestingly, the proteins encoded by these genes are necessary for pollen tube growth. Obviously, these 278 genes that were distributed on all 17 chromosomes and 33 scaffolds (Table S6) are responsive to the SI reaction.

3.4. Distribution of ABREs in the promoters of DEGs

To explore the potential role of *PbABF.E.1, PbABF.E.2*, and *PbABF.B* in the SI reaction, ABREs were predicted in the promoters of all 11,476 DEGs. The result showed that the promoter sequences of 1213 DEGs included at least one ABRE (Table S6). Further analysis revealed that the number of ABREs was less than six in the promoter sequences of 817 DEGs, ranged from seven to 10 in the promoter sequences of 268 DEGs, ranged from 11 to 14 in the promoter sequences of 93 DEGs, and ranged from 15 to 22 in the promoter sequences of 35 DEGs (Fig. 4A and Table S6). Overall, with the increasing ABRE elements, the number of

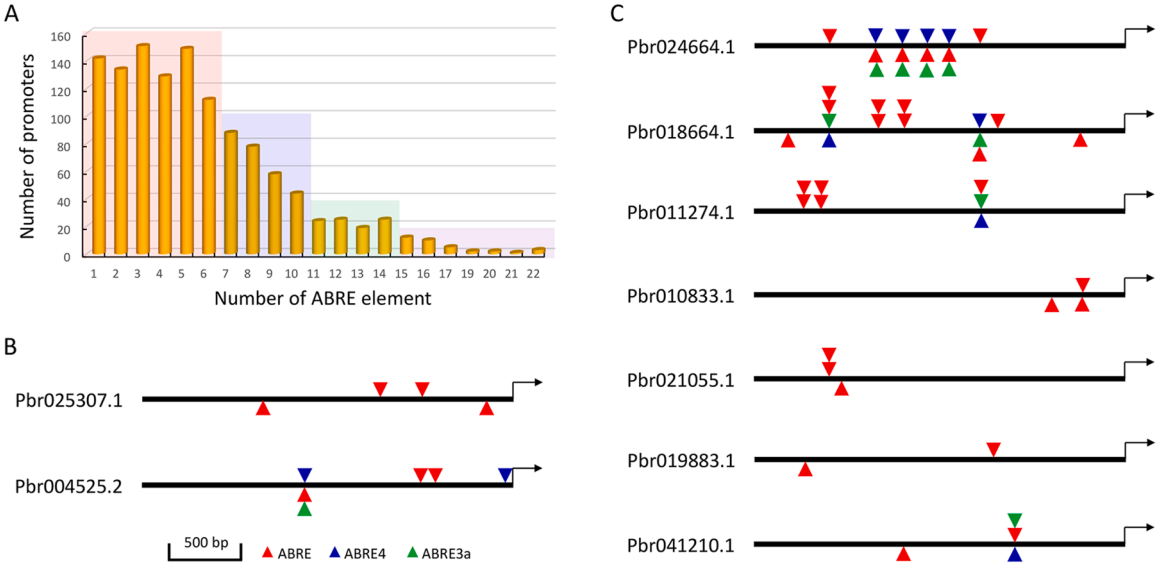

Fig. 4. Identification of the promoters possessing ABRE in the DEGs. (A) Number of ABRE(s) in the promoters of the DEGs. (B) The position of ABRE(s) in the promoters of the genes responsive to the GSI reaction.

promoters with ABREs gradually decreased. Of these 1213 DEGs, nine were differentially expressed between the self- and cross-pollinated styles at all three stages (Fig. 4B and C). The promoters of *Pbr025307.1* and *Pbr004525.2* (two upregulated DEGs in the cross-pollinated styles) included four and six ABREs, respectively (Fig. 4B). The promoters of *Pbr024664.1, Pbr018664.1, Pbr011274.1, Pbr010833.1, Pbr021055.1, Pbr019883.1*, and *Pbr041210.1* (seven upregulated DEGs in the cross-pollinated styles) included 14, 14, 7, 3, 3, 2, and 4 ABREs, respectively (Fig. 4C). This result suggests that *PbABF.E.1, PbABF.E.2*, and *PbABF.B* may be involved in the SI reaction by binding to ABREs to activate the corresponding promoters of the DEGs.

3.5. Interaction of ABF transcription factors with promoters possessing ABRE

To validate the expression pattern of nine DEGs, qRT-PCR was performed in the self- and cross-pollinated styles. The result showed that *Pbr024664.1, Pbr018664.1, Pbr011274.1, Pbr010833.1, Pbr021055.1*, and *Pbr041210.1* exhibited lower expression in the cross-pollinated styles than in the self-pollinated styles at 24, 48, and 72 HAP (Fig. 5). This result is consistent with the RNA-Seq analysis results. By contrast, both *Pbr025307.1* and *Pbr004525.2* showed lower expression in the cross-pollinated styles than in the self-pollinated styles at 24 and 48 HAP. *Pbr019883* had similar levels of expression in the self- and cross-pollinated styles at 24 and 72 HAP. Evidently, the expression patterns of *Pbr025307.1, Pbr004525.2*, and *Pbr019883* tested by qRT-PCR is differed from the result of the RNA-Seq analysis.

To test whether PbABF.E.1, PbABF.E.2, and PbABF.B activated the promoters of *Pbr024664.1, Pbr018664.1, Pbr011274.1, Pbr010833.1, Pbr021055.1*, and *Pbr041210.1*, a dual-luciferase assay was performed between three ABF transcription factors and six promoters. As indicated in Fig. 6, compared to the leaves transformed with an empty vector, *LUC* activity driven by the *Pbr018664.1* or *Pbr010833.1* promoter was increased in the leaves overexpressing *PbABF.E.1* (35S::PbABF.E.1). *LUC* activity driven by the *Pbr018664.1, Pbr010833.1*, or *Pbr041210.1* promoter was increased in leaves overexpressing *PbABF.E.2* (35S::PbABF.E.2), and *LUC* activity driven by any promoter was increased in leaves overexpressing *PbABF.B* (35S::PbABF.B). Therefore, *PbABF.B* could enhance the activity of all six promoters, but both PbABF.E.1 and PbABF.E.2 stimulated the activity of some promoters.

4. Discussion

During the GSI reaction, the process by which pollen tube growth is arrested by self S-RNase exhibits the typical feature of PCD, which include swollen mitochondria, nuclear DNA degradation, and cytoskeleton depolymerization (Liu et al., 2007; Wang et al., 2009, 2010). PCD in pollen tube is likely mediated by a series of the genes responsive to the GSI reaction. In a previous study, transcriptome analysis of self- and cross-pollinated styles at 48 HAP revealed 520 genes responsive to the GSI reaction (Shi et al., 2017). Herein, we isolated 278 genes that were differentially expressed between the self- and cross-pollinated styles at any stage (Fig. 3C). Many of these DEGs overlapped to those from a previous study (Shi et al., 2017), such as *pectate lyase* (Pbr010756.1 and Pbr036221.1), *cell division control protein* (Pbr012821.1), *pectinesterase inhibitor* (Pbr041880.2 and Pbr012745.1), and *cyclin* (Pbr021971.1 and Pbr015329.1). High-throughput sequencing is an efficient approach for isolating DEGs responsive to the GSI reaction, but little is known about the molecular pathway by which self S-RNase influences the expression of these DEGs.

The ABF transcription factor, which belongs to the subfamily of bZIP-type transcription factors (Jakoby et al., 2002), has been widely studied in various physiological processes including stomatal density (Wang et al., 2021) and abiotic stress tolerance (Huang et al., 2010; Liu et al., 2019; Tang et al., 2012). The overexpression of SlAREB1 enhances the tolerance to a series of abiotic stresses and promotes the expression of biotic and abiotic stress-related genes in tomato (Orellana et al., 2010; Yáñez et al., 2009). In this study, to survey the potential roles in different tissues, the expression patterns of all eight *ABF* genes were characterized in the pollen, leaf, petiole, peduncle, and flesh. *PbABF.C.1, PbABF.D.1*, and *PbABFL* were almost undetectable in any tissue, while *PbABF.C.2* and *PbABF.D.2* were, respectively, expressed in pollen and flesh (Fig. 2). By contrast, *PbABF.E.1* and *PbABF.E.2* were expressed in all tissues, and *PbABF.B* was expressed in most tissues (Fig. 2). Similar results were also found in the self- and cross-pollinated styles (Fig. 3B). These results indicate that *PbABF.B, PbABF.E.1*, and *PbABF.E.2* are likely involved in regulating pollen tube growth and tissue development, and thus may affect the expression of the DEGs responsive to the GSI reaction.

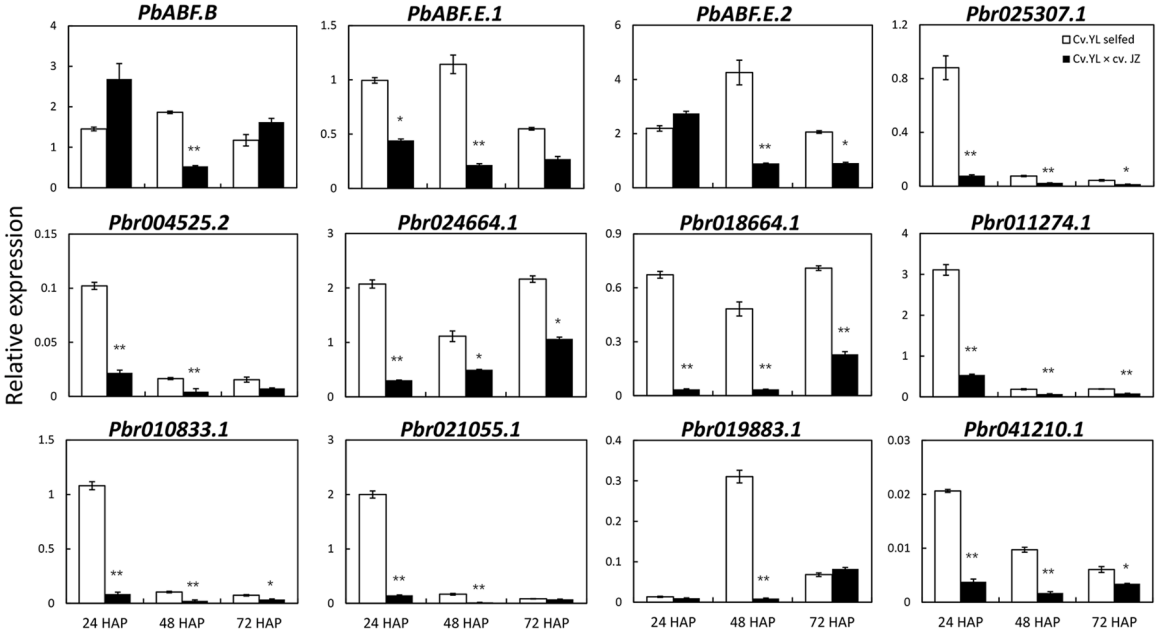

Fig. 5. Validation of gene expression pattern by qRT-PCR. Standard errors and analysis of variance were calculated by student's *t*-test. Single and double asterisks represent the levels of significance at *P*-values of < 0.05 and < 0.01, respectively.

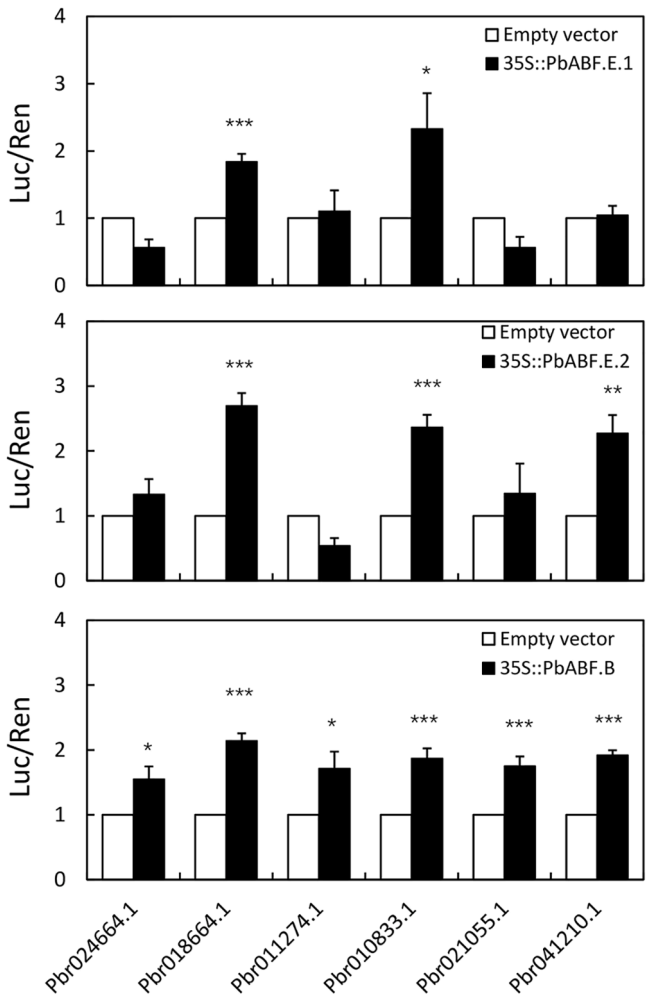

Fig. 6. Interaction of three ABF transcription factors with the promoters of the genes responsive to the GSI reaction. Standard errors and analysis of variance were calculated by student's *t*-test. Single, double, and triple asterisks represent the levels of significantce at *P*-values of < 0.05, < 0.01 and < 0.001, respectively.

It is reported that ABF transcription factors can bind to the ABREs in the promoter to activate the expression of downstream genes (Choi et al., 2000). These ABREs were detected in the promoter of the 1213 DEGs (Table S6). To test the transcriptional activation of *PbABF.B, PbABF.E.1*, and *PbABF.E.2*, the promoters of six DEGs responsive to the GSI reaction were selected to drive the reporter gene. PbABF.E.1, as well as PbABF.E.2 and PbABF.B, enhanced the activity of the *Pbr018664.1* and *Pbr010833.1* promoters (Fig. 6). PbABF.E.2 and PbABF.B enhanced the activity of the *Pbr041210.1* promoter, while PbABF.B specifically enhanced the activity of the *Pbr024664.1, Pbr011274.1*, and *Pbr021055.1* promoters. Therefore, both *PbABF.B, PbABF.E.1*, and *PbABF.E.2* affect the expression of the DEGs responsive to the GSI reaction, but their roles differed slightly from one other. This difference may result from the temporal expression of these three *ABF* genes.

To characterize the expression pattern of the three *ABF* genes and six target DEGs, qRT-PCR was performed in the self- and cross-pollinated styles at different stages. The result showed that the expression levels of *Pbr018664.1, Pbr010833.1, Pbr041210.1, Pbr024664.1, Pbr011274.1*, and *Pbr021055.1* were decreased in the cross-pollinated style compared to the self-pollinated style at any stage (Fig. 5), indicating that the expression of these genes was negatively correlated with pollen tube growth. By contrast, compared to the self-pollinated style, all three *ABF* genes were downregulated in the cross-pollinated styles at 48 HAP, and *PbABF.E.1* and *PbABF.E.2* were also downregulated in the cross-pollinated styles at 24 and 72 HAP, respectively (Fig. 5). Three *ABF* genes had obviously different expression patterns. Due to *PbABF.B, PbABF.E.1*, and *PbABF.E.2* positively affecting the activity of the *Pbr018664.1, Pbr010833.1, Pbr041210.1, Pbr024664.1, Pbr011274.1*, and/or *Pbr021055.1* promoters (Fig. 6), these three *ABF* genes may be involved in the negative mediation of pollen tube growth.

In the self-pollinated styles, the pollen tube growth was arrested by self S-RNase (Fig. 3A); a process that is associated with abiotic stress. In *Arabidopsis*, the expression of *ABF* genes was enhanced by abiotic stress, including ABA, salt, cold, and/or drought (Choi et al., 2000). It is reasonable to speculate that the expression of *PbABF.B, PbABF.E.1*, and *PbABF.E.2* is induced by self S-RNase stress. Taken together, these results suggest that an ARF-introduced molecular pathway of self S-RNase that affects the expression of the DEGs emerged as follows: in the self-incompatible pollen tubes, self S-RNase initially induced the expression of *PbABF.E.1*, leading to the slow growth of the pollen tubes at the time from 0 to 24 HAP. Subsequently, self S-RNase induced the expression of *PbABF.B, PbABF.E.1*, and *PbABF.E.2*, leading to the inhibition of pollen tube growth from 24 to 48 HAP. However, the canceled induction of self S-RNase on the expression of both *PbABF.B* and *PbABF.E.1* resulted in a short window for pollen tube growth from 48 to 72 HAP. Eventually, the pollen tubes were subjected to PCD under self S-RNase stress.

5. Conclusion

Based on RNA-Seq analysis of the self- and cross-pollinated styles, three *ABF* genes were isolated together with 11,476 DEGs. Notably, the promoter sequences of the 1213 DEGs included at least one ABRE. Moreover, in the promoters of the 278 DEGs responsive to the GSI reaction, six included at least three ABREs and were activated by *PbABF.B, PbABF.E.1*, and/or *PbABF.E.2*. These six DEGs were negatively correlated with pollen tube growth, suggesting that *PbABF.B, PbABF.E.1*, and *PbABF.E.2* were involved in the GSI reaction by positively affecting the expression of the six DEGs. These results provide possible guidance to overcome the SI barrier through exogenous ABA treatment of the self-pollinated styles in pear.

CRediT authorship contribution statement

Lei Wu: Formal analysis, Writing – original draft, Writing – review & editing. **Ying Xu:** Formal analysis, Writing – review & editing. **Min He:** Data curation, Resources, Writing – review & editing. **Xue-Ting Jiang:** Data curation, Writing – review & editing. **Kai-Jie Qi:** Resources, Writing – review & editing. **Chao Gu:** Visualization, Writing – original draft, Writing – review & editing. **Shao-Ling Zhang:** Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript. This work supported by the Bioinformatics Center of Nanjing Agricultural University, the National Natural Science Foundation of China (32172511), the Jiangsu Agricultural Science and Technology Innovation Fund (CX(20)2020), the Fundamental Research Funds for the Central Universities (KYYJ202116), and the Earmarked Fund for China Agriculture Research System (CARS-28).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2022.111089.

References

Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq-a python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169.

Anderson, M.A., Cornish, E.C., Mau, S.L., Williams, E.G., Hoggart, R., Atkinson, A., Bonig, I., Grego, B., Simpson, R., Roche, P.J., 1986. Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. Nature 321, 38–44.

Breton, C.M., Farinelli, D., Shafig, S., Heslop-Harrison, J.S., Sedgley, M., Bervillé, S.J., 2014. The self-incompatibility mating system of the olive (Olea europaea L.) functions with dominance between S-alleles. Tree Genet. Genomes 10, 1055–1067.

Chen, J., Li, X., Wang, D., Li, L., Zhou, H., Liu, Z., Wu, J., Wang, P., Jiang, X., Fabrice, M. R., Zhang, S., Wu, J., 2015. Identification and testing of reference genes for gene expression analysis in pollen of *Pyrus bretschneideri*. Sci. Hortic. 190, 43–56.

Chen, J., Wang, P., de Graaf, B.H.J., Zhang, H., Jiao, H., Tang, C., Zhang, S., Wu, J., 2018. Phosphatidic acid counteracts S-RNase signaling in pollen by stabilizing the actin cytoskeleton. Plant Cell 30, 1023–1039.

Chibi, F., Angosto, T., Matilla, A., 1995. Variations of the patterns of abscisic acid and proline during maturation of *Nicotiana tabacum* pollen grains. J. Plant Physiol. 147, 355–358.

Choi, H.I., Hong, J.H., Ha, J.O., Kang, J.Y., Kim, S.Y., 2000. ABFs, a family of ABA-responsive element binding factors. J. Biol. Chem. 275, 1723–1730.

Dhingra, H.R., Varghese, T.M., 1985. Effect of growth regulators on the *in vitro* germination and tube growth of maize (*Zea mays* L.) pollen from plants raised under sodium chloride salinity. New Phytol. 100, 563–569.

Frascaroli, E., Tuberosa, R., 1992. Effect of abscisic acid on pollen germination and tube growth of Maize genotypes. Plant Breed. 110, 250–254.

Goldraij, A., Kondo, K., Lee, C.B., Hancock, C.N., Sivaguru, M., Vazquez-Santana, S., Kim, S., Phillips, T.E., Cruz-Garcia, F., McClure, B., 2006. Compartmentalization of S-RNase and HT-B degradation in self-incompatible *Nicotiana*. Nature 439, 805–810.

Gu, C., Xu, H.Y., Zhou, Y.H., Yao, J.L., Xie, Z.H., Chen, Y.Y., Zhang, S.L., 2020. Multiomics analyses unveil the involvement of microRNAs in pear fruit senescence under high- or low-temperature conditions. Hortic. Res. 7, 196.

Gu, C., Wu, R.F., Yu, C.Y., Qi, K.J., Wu, C., Zhang, H.P., Zhang, S.L., 2021. Spatio-temporally expressed sorbitol transporters cooperatively regulate sorbitol accumulation in pear fruit. Plant Sci. 303, 110787.

Gu, Z., Li, W., Doughty, J., Meng, D., Yang, Q., Yuan, H., Li, Y., Chen, Q., Yu, J., Liu, C.S., Li, T., 2019. A gamma-thionin protein from apple, MdD1, is required for defence against S-RNase-induced inhibition of pollen tube prior to self/non-self recognition. Plant Biotechnol. J. 17, 2184–2198.

Hancock, C.N., Kent, L., McClure, B.A., 2005. The stylar 120 kDa glycoprotein is required for S-specific pollen rejection in *Nicotiana*. Plant J. 43, 716–723.

Hiscock, S.J., Mclnnis, S.M., 2003. Pollen recognition and rejection during the sporophytic self-incompatibility response: brassica and beyond. Trends Plant Sci. 8, 606–613.

Hossain, M.A., Cho, J.I., Han, M., Ahn, C.H., Jeon, J.S., An, G., Park, P.B., 2010a. The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice. J. Plant Physiol. 167, 1512–1520.

Hossain, M.A., Lee, Y., Cho, J.I., Ahn, C.H., Lee, S.K., Jeon, J.S., Kang, H., Lee, C.H., An, G., Park, P.B., 2010b. The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. Plant Mol. Biol. 72, 557–566.

Huang, X.S., Liu, J.H., Chen, X.J., 2010. Overexpression of PtrABF gene, a bZIP transcription factor isolated from *Poncirus trifoliata*, enhances dehydration and drought tolerance in tobacco via scavenging ROS and modulating expression of stress-responsive genes. BMC Plant Biol. 10, 230.

Jakoby, M., Weisshaar, B., Droge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T., Parcy, F., 2002. bZIP transcription factors in Arabidopsis. Trends Plant Sci. 7, 106–111.

Kakui, H., Kato, M., Ushijima, K., Kitaguchi, M., Kato, S., Sassa, H., 2011. Sequence divergence and loss-of-function phenotypes of *S* locus F-box brothers genes are consistent with non-self recognition by multiple pollen determinants in self-incompatibility of Japanese pear (*Pyrus pyrifolia*). Plant J. 68, 1028–1038.

Kong, X.X., Mei, J.W., Zhang, J., Liu, X., Wu, J.Y., Wang, C.L., 2021. Turnover of diacylglycerol kinase 4 by cytoplasmic acidification induces vacuole morphological change and nuclear DNA degradation in the early stage of pear self-incompatibility response. J. Integr. Plant Biol. https://doi.org/10.1111/jipb.13180.

Kovaleva, L., Voronkov, A., Zakharova, E., Minkina, Y., Timofeeva, G., Andreev, I., 2016. Regulation of petunia pollen tube growth by phytohormones: identification of their potential targets. J. Agric. Sci. Technol. 6, 239–254.

Kubo, K., Entani, T., Takara, A., Wang, N., Fields, A.M., Hua, Z., Toyoda, M., Kawashima, S., Ando, T., Isogai, A., Kao, T.H., Takayama, S., 2010. Collaborative non-self recognition system in S-RNase-based self-incompatibility. Science 330, 796–799.

Li, W., Meng, D., Gu, Z., Yang, Q., Yuan, H., Li, Y., Chen, Q., Yu, J., Liu, C., Li, T., 2018. Apple S-RNase triggers inhibition of tRNA aminoacylation by interacting with a soluble inorganic pyrophosphatase in growing self-pollen tubes *in vitro*. New Phytol. 218, 579–593.

Li, Y., Wu, J., Wu, C., Yu, J., Liu, C., Fan, W., Li, T., Li, W., 2020. A mutation near the active site of S-RNase causes self-compatibility in S-RNase-based self-incompatible plants. Plant Mol. Biol. 103, 129–139.

Liang, M., Cao, Z., Zhu, A., Liu, Y., Tao, M., Yang, H., Xu, Q., Wang, S., Liu, J., Li, Y., Chen, C., Xie, Z., Deng, C., Ye, J., Guo, W., Xu, Q., Xia, R., Larkin, R.M., Deng, X., Bosch, M., Franklin-Tong, V.E., Chai, L, 2020. Evolution of self-compatibility by a mutant S m-RNase in citrus. Nat. Plants 6, 131–142.

Liu, J., Chu, J., Ma, C., Jiang, Y., Ma, Y., Xiong, J., Cheng, Z.M., 2019. Overexpression of an ABA-dependent grapevine bZIP transcription factor, VvABF2, enhances osmotic stress in arabidopsis. Plant Cell Rep. 38, 587–596.

Liu, Z.Q., Xu, G.H., Zhang, S.L., 2007. *Pyrus pyrifolia* stylar S-RNase induces alterations in the actin cytoskeleton in self-pollen and tubes in viro. Protoplasma 232, 61–67.

Luu, D.T., Qin, X., Morse, D., Cappadocia, M., 2000. S-RNase uptake by compatible pollen tubes in gametophytic self-incompatibility. Nature 407, 649–651.

Malik, C.P., Chhabra, N., 1976. Hormonal regulation of pollen germination and pollen tube elongation in *Arachis hypogea* Reitz. Proc. Indian Acad. Sci. Sect. B 84, 101–108.

Malik, C.P., Chhabra, N., Vermani, S., 1976. Cyclic AMP-induced elongation of pollen tubes in *Tradescantia paludosa*. Biochem. Physiol. Pflanz. 169, 311–315.

Meng, D., Gu, Z.Y., Li, W., Wang, A.D., Yuan, H., Yang, Q., Li, T.Z., 2014. Apple MdABCF assists in the transportation of S-RNase into pollen tubes. Plant J. 78, 990–1002.

Orellana, S., Yañez, M., Espinoza, A., Verdugo, I., González, E., Ruiz-Lara, S., Casaretto, J.A., 2010. The transcription factor SlAREB1 confers drought, salt stress tolerance and regulates biotic and abiotic stress-related genes in tomato. Plant Cell Environ. 33, 2191–2208.

Qi, Y.J., Wang, Y.T., Han, Y.X., Qiang, S., Wu, J., Tao, S.T., Zhang, S.L., Wu, H.Q., 2011a. Self-compatibility of 'Zaoguan' (*Pyrus bretschneideri* Rehd.) is associated with style-part mutations. Genetica 139, 1149–1158.

Qi, Y.J., Wu, H.Q., Cao, Y.F., Wu, J., Tao, S.T., Zhang, S.L., 2011b. Heteroallelic diploid pollen led to self-compatibility in tetraploid cultivar 'Sha 01' (*Pyrus sinkiangensis* Yü). Tree Genet. Genomes 7, 685–695.

Sassa, H., Nishio, T., Kowyama, Y., Hirano, H., Koba, T., Ikehashi, H., 1996. Self-incompatibility (*S*) alleles of the Rosaceae encode members of a distinct class of the T2/S ribonuclease superfamily. Mol. Gen. Genet. 250, 547–557.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Shi, S.L., Cheng, H.Y., Wu, L., Xie, Z.H., Gu, C., Zhang, S.L., 2018. Identification of S-genotypes in 18 pear accessions and exploration of the breakdown of self-incompatibility in the pear cultivar Xinxue. Sci. Hortic. 238, 350–355.

Shi, D., Tang, C., Wang, R., Gu, C., Wu, X., Hu, S., Jiao, J., Zhang, S., 2017. Transcriptome and phytohormone analysis reveals a comprehensive phytohormone and pathogen defence response in pear self-/cross-pollination. Plant Cell Rep. 36, 1785–1799.

Tang, N., Zhang, H., Li, X., Xiao, J., Xiong, L., 2012. Constitutive activation of transcription factor OsbZIP46 improves drought tolerance in rice. Plant Physiol. 158, 1755–1768.

Trapnell, C., Pachter, L., Salzberg, S.L., 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25, 1105–1111.

Umezawa, T., Nakashima, K., Miyakawa, T., Kuromori, T., Tanokura, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2010. Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. Plant Cell Physiol. 51, 1821–1839.

Wang, C.L., Xu, G.H., Jiang, X.T., Chen, G., Wu, J., Wu, H.Q., Zhang, S.L., 2009. S-RNase triggers mitochondrial alteration and DNA degradation in the incompatible pollen tube of *Pyrus pyrifolia in vitro*. Plant J. 57, 220–229.

Wang, C.L., Wu, J., Xu, G.H., Gao, Y.B., Chen, G., Wu, J.Y., Wu, H.Q., Zhang, S.L., 2010. S-RNase disrupts tip-localized reactive oxygen species and induces nuclear DNA degradation in incompatible pollen tubes of *Pyrus pyrifolia*. J. Cell. Sci. 123, 4301–4309.

Wang, G.M., Gu, C., Qiao, X., Zhao, B.Y., Ke, Y.Q., Guo, B.B., Hao, P.P., Qi, K.J., Zhang, S. L., 2017. Characteristic of pollen tube that grew into self style in pear cultivar 727 and parent assignment for cross-pollination. Sci. Hortic. 216, 226–233.

Wang, Y.H., Que, F., Li, T., Zhang, R.R., Khadr, A., Xu, Z.S., Tian, Y.S., Xiong, A.S., 2021. DcABF3, an ABF transcription factor from carrot, alters stomatal density and reduces ABA sensitivity in transgenic Arabidopsis. Plant Sci. 302, 110699.

Wasternack, C., Hause, B., 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in annals of botany. Ann. Bot. 111, 1021–1058.

Wu, C., Gu, Z., Li, T., Yu, J., Liu, C., Fan, W., Wang, B., Jiang, F., Zhang, Q., Li, W., 2021. The apple MdPTI1L kinase is phosphorylated by MdOXI1 during S-RNase-induced reactive oxygen species signaling in pollen tubes. Plant Sci. 305, 110824.

Wu, J., Gu, C., Khan, M.A., Wu, J., Gao, Y., Wang, C., Korban, S.S., Zhang, S., 2013a. Molecular determinants and mechanisms of gametophytic self-incompatibility in fruit tress of Rosaceae. CRC Crit. Rev. Plant Sci. 32, 53–68.

Wu, J., Qin, Y., Zhao, J., 2008. Pollen tube growth is affected by exogenous hormones and correlated with hormone changes in styles in *Torenia fournieri* L. Plant Growth Regul. 55, 137–148.

Wu, J., Li, M., Li, T., 2013b. Genetic features of the spontaneous self-compatible mutant, 'Jin Zhui' (*Pyrus bretschneideri* Rehd.). PLoS One 8, e76509.

Xue, Y., Carpenter, R., Dickinson, H.G., Coen, E.S., 1996. Origin of allelic diversity in *Antirrhinum S* locus RNases. Plant Cell 8, 805–814.

Yang, Q., Meng, D., Gu, Z., Li, W., Chen, Q., Li, Y., Yuan, H., Yu, J., Liu, C., Li, T., 2018. Apple S-RNase interacts with an actin-binding protein, MdMVG, to reduce pollen tube growth by inhibiting its actin-severing activity at the early stage of self-pollination induction. Plant J. 95, 41–56.

Yáñez, M., Cáceres, S., Orellana, S., Bastías, A., Verdugo, I., Ruiz-Lara, S., Casaretto, J.A., 2009. An abiotic stress-responsive bZIP transcription factor from wild and cultivated tomatoes regulates stress-related genes. Plant Cell Rep. 28, 1497–1507.
Yoshida, T., Christmann, A., Yamaguchi-Shinozaki, K., Grill, E., Fernie, A.R., 2019. Revisiting the basal role of ABA-roles outside of stress. Trends Plant Sci. 24, 625–635.
Zhang, S., Gao, F., Chen, D., Gu, Z., 2003. The effects of plant growth regulating substances on pollen germ ination and tube gowth in Fengshui pear (*pyrus serotina*). Acta Bot. Boreal. Occident. Sin. 23, 586–591.
Zhou, H., Yin, H., Chen, J., Liu, X., Gao, Y., Wu, J., Zhang, S., 2016. Gene-expression profile of developing pollen tube of *Pyrus bretschneideri*. Gene Expr. Patterns 20, 11–21.
Zhou, Y., Bai, L., Gao, L., Ma, X., Song, C., 2010. Effects of exogenous ABA on Arabidopsis pollen growth. J. Henan Univ. 40, 274–277.