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ARTICLE

# Expression and Interaction Analysis of *FAZ1 Protein* in *Brassica oleracea*

**Hecui Zhang[1,#], Xiaoping Lian[2,#], Yizhong Zhang[1], Tonghong Zuo[1], Chongmo Yuan[1], Qinqin Xie[1] and Liquan Zhu[1,*]**

[1]College of Agronomy and Biotechnology, Southwest University, Chongqing, 400715, China

[2]State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, School of Agriculture, Yunnan University, Kunming, 650091, China

[*]Corresponding Author: Liquan Zhu. Email: zhuliquan@swu.edu.cn
[#]Contributed equally to this work and are co-first authors

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## ABSTRACT

To identify and characterize genes involved in reproductive tissue abscission in *Brassica oleracea*, the transcript data of pollinated pistil was analyzed. A differentially expressed gene, named *BoFAZ1(FLOWER ABSCISSION ZONE1)* was identified, which contains one exon and encompass a 139aa. Furthermore, a T-DNA insertion mutant (SALK_302_G01) (*faz1* mutant) was obtained from *Arabidopsis thaliana* mutant library. Floral organ shedding from mutants was delayed and a V-shaped structure in the boundary region between the stalk and torus of the sepal abscission zone was obtained in f*az1* mutant. The cell density of this structure was lower than that of the corresponding region in the wild-type control. In the transgenic plants, the normal development of the stalk zone of *faz1* was recovered completely by transforming a 1919-bp DNA fragment of *BoFAZ1* into the *faz1* mutant. In Addition, our data showed that *BoFAZ1* was expressed in mature pollen grains, but not in the bracts, roots, stems, leaves, and sepals. Its expression in the filaments, stigma, and pistil exfoliation layer gradually increased after pollination. Subcellular localization experiments showed that BoFAZ1 was located in the cell membrane. A myristoylation site was found at the N-terminus of *BoFAZ1*. Removal of this site resulted in protein dislocation in the cytoplasm, cell membrane and nucleus. Finally, a yeast two-hybrid test indicated that BoH3.2 (histone H3.2), a protein involved in abscission zone development, interacted with BoFAZ1. This interaction was verified by a GST pull-down assay. In summary, our data indicated that *BoFAZ1* was involved in the formation of the pistil abscission zone in *B. oleracea*.

## KEYWORDS

Abscission; AZ; *B. oleracea*; flower development organ boundary

## 1 Introduction

Abscission in plants is a physiological process of shedding unwanted organs, such as leaves, floral organs, fruits, and seeds at specific points in their life cycle or in response to environmental cues [1]. This process occurs in a developmentally defined region of cells called the abscission zone (AZ), which is established at the base of the organs to be shed. The abscission zones (AZs) consisting of 1–50 small, undifferentiated, or meristematic cell layers are critical mediators to make the organs of various plant parts detached properly from the main body [2–4]. Most studies on abscission have focused on the roles

of plant hormones on the development and activation of AZs. Most of the classical plant hormones have been shown to affect the timing of abscission. In general, the accepted concept is that ethylene promotes the establishment of AZs, whereas auxin has the opposite effect [5].

Besides plant hormones, multiple transcription factors, small signaling peptides, and membrane traffic regulators have been found to play essential roles in the development of AZs and the shedding of plant organs in many species. For example, the transcriptional activators BLADE-ON-PETIOLE1 (BOP1) and BLADE-ON-PETIOLE2 (BOP2) redundantly controlled the patterning of the proximal regions of developing leaves and floral organs. In *Arabidopsis thaliana* leaves, BOP1 and BOP2 were involved in AZ formation. It was revealed that the double mutants of *bop1/bop2* failed to produce normal AZ in leaves [6,7]. The tobacco *BOP2* homologue was associated with regulating the corolla (petal) AZ [8]. In rice, the SUPERNUMERARY BRACT (SNB) promoted the expression of two abscission-related genes, *qSH1* and *SH5*, which participated in seed abscission via regulation of lignin deposition and organ detachment in specific areas [9]. In addition, mutations in the *REPLUMLESS* gene, which is a homolog of *BELL1* (*BEL1*) in *A. thaliana*, resulted in a lack of AZs in temperate *indica* rice cultivars [10,11]. In wheat, the transcription factor APETALA2 (AP2) had impacts on flowering, glume shape, and seed shedding [12,13]. The development of floral AZs was disrupted when *BREVIPEDICELLUS* (BP, also known as KNAT1) was knocked out in *A. thaliana* [14]. The *A. thaliana ZINC FINGER PROTEIN2* (*AtZFP2*) gene expressed in the AZ of stamens, petals, and sepals affected the structure of AZs in the Arabidopsis flower stalk [15]. *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*) was required for the formation of the stamen AZ through inhibiting the growth of floral organ receptacle boundaries [16]. The transcription factor, ASYMMETRIC LEAVES1 *(AS1*), played an important role in the morphology and structure of the AZ boundary in flowers [17]. Additionally, *JOINTLESS*, *MACROCALYX* (*MC*), and *SLMBP21*, two members of the MADS-box gene family, were required in the development of the tomato flower AZ [18]. 1-aminocyclopropane-1-carboxylate oxidase (*ACO*) family and *LeLX* genes were expressed in the tomato petiole. All these three genes had implications in AZ development [19]. The Tomato Hybrid Proline-rich Protein (*THyPRP*) gene was specifically expressed in the flower AZ substantially inhibited tomato pedicel abscission following flower removal [20]. HAWAIIAN SKIRT (HWS), a F-box protein, prevented the fusion of adjacent sepals and affected the timing of their abscission [21]. However, the molecules and mechanism regulating floral organ abscission in *Brassica* is still largely unknown.

To explore potential regulators of floral abscission in *Brassica*, a differentially expressed gene, designated as *FLOWER ABSCISSION ZONE1* (*BoFAZ1*) was identified from the pollinated pistil of *Brassica oleracea* for the first time by analyzing the transcriptome data from databases. A T-DNA insertion mutant of *BoFAZ1* in *Arabidopsis thaliana* exhibited the procrastination of floral organ shedding from the stalk. The expression of *BoFAZ1* in the filaments, stigma, and pistil exfoliation layer was gradually increased after pollination. Subcellular localization data indicated that *BoFAZ1* is a cell membrane protein. Finally, a yeast two-hybrid test revealed that *BoFAZ1* interacted with BoH3.2 (histone H3.2), a protein involved in abscission zone development. In summary, our data suggested that *BoFAZ1* is involved in the formation of pistil abscission zone in *Brassica*. The data presented herein may be useful for future studies on the development of the pistil AZ and the shedding of flower organs from *B. oleracea* plants.

## 2 Materials and Methods

### *2.1 Growth Conditions of Plant Materials*

*Brassica oleracea* var. *capitata* L. seeds, provided by the Chongqing Key Laboratory of Olericulture, were planted and grown in a glasshouse in the Xiema Cruciferous Research Institute, Southwest University, Chongqing, China. To investigate the potential regulator during *Brassica* floral organ

abscission, the pistils were collected at 0, 15, 30, 60 min after self- and cross-pollination and stored at −80°C freezer until total RNA extraction.

The *FAZ1* mutants SALK_302_G01 were from the mutant library and *Pro$^{FAZ1}$: GUS* was in the *A. thaliana* Columbia ecotype background. The FAZ1/Pro35S:BoFAZ1 plants were generated with natural crosses. The T-DNA insertions of *FAZ1* homozygous lines were identified by polymerase chain reaction (PCR) genotyping with the primers listed in Tab. S1. Seeds were germinated and grown on half-strength Murashige and Skoog (MS) medium containing 1% sucrose and 0.7% agar. All plants were grown at 23°C under long-day conditions (16-h light/8-h dark).

### *2.2 Bioinformatics Analysis and Data Analysis*

The ClutsalW program was used for multiple protein sequence alignments and MEGA X was used to construct the Bar chart.

### *2.3 RNA Extraction and qRT-PCR Analysis*

Total RNA was extracted from floral tissues at different time intervals after pollination with the RNAprep Pure Plant Kit, Tiangen, Beijing, China. First-strand cDNA was synthesized from 1 μg total RNA with the PrimeScript RT Reagent Kit with gDNA Eraser. A quantitative real-time (qRT)-PCR assay was performed with the SYBR Premix Ex Taq Kit using the 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with the following PCR program: 95°C for 30 s; 40 cycles of 95°C for 10 s, 56°C for 10 s, and 72°C for 10 s. Gene expression analyses were conducted with three replicates, and expression levels were normalized against that of *Actin2* according to the $2^{-\Delta\Delta Ct}$ method [22]. Details regarding the qRT-PCR primers are provided in Tab. S1.

### *2.4 Plasmid Construction and Transformation*

A 1.5-kb promoter region upstream of the *FAZ1* start codon was amplified by PCR with the primers listed in Tab. S1. The amplified fragments were inserted into the *Sal*I/*Eco*RI sites of the pCAMBIA1391 vector to generate the pBoFAZ1: GUS recombinant plasmid. For subcellular localization analysis, *FAZ1* cDNA was cloned into the p35S: GFP vector. GST pull-down assay was according to [23]. The primer sequences used are listed in Supplementary Tab. S1.

### *2.5 Scanning Electron Microscopy (SEM)*

Mutant and wild type flower organs were examined with a stereo microscope and photographed. The sepals and petals were removed with tweezers, and the stamens and pistils were placed on conductive gels before they were analyzed with a scanning electron microscope. Experimental flowers were fixed in formaldehyde-acetic acid (45% ethanol, 2.5% acetic acid, and 2.5% formalin) at room temperature overnight, dehydrated in an ethanol series, and critical-point dried (DCP-1). Samples were mounted on aluminum stubs, silver painted, sputter-coated with Au/Pd, and imagined under a SEM microscope.

### *2.6 Genetic Complementation Test*

The cDNA fragment of *BoFAZ1*, the homologous gene of *AtFAZ1* in Arabidopsis, was amplified from *Brassica oleracea*. A recombinant plasmid with *FAZ1* coding sequence fused to the CaMV 35S promoter, and was generated and transformed into the mutant *faz1 of A. thaliana* according to a previously described protocol.

### *2.7 GUS Staining*

Plant materials were stained with a solution comprising 1 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronide, 50 mM $NaPO_4$ (pH 7.0), 0.4 mM $K_3Fe(CN)_6$, and 0.1% (v/v) Triton X-100 at 37°C for 6 h. After the GUS staining, chlorophyll was removed with a 4-h treatment with 70% ethanol. Samples were then analyzed, and images were captured with an MVX10 stereomicroscope (Olympus, Tokyo, Japan).

### *2.8 Subcellular Localization of BoFAZ1 Protein*

A recombinant plasmid with *FAZ1* coding sequence fused to a GFP-encoding gene driven by the CaMV 35S promoter was generated and transfected into *A. thaliana* according to a previously described protocol [24]. An empty vector (i.e., GFP-encoding gene only) was used as a control. Confocal fluorescent images of the transformed *A. thaliana* cells were acquired with the LSM 510 Meta inverted laser scanning confocal microscope (Carl Zeiss, Jena, Germany).

### *2.9 Yeast Two-Hybrid Screening*

The Matchmaker Gold Yeast Two-Hybrid System (Clontech, Tokyo, Japan) was used to detect proteins interacting with BoFAZ1. To identify interacting proteins, we used BoFAZ1d as the bait to screen a *B. oleracea* cDNA library. Competent yeast cells were prepared with LiAc following the manufacturer's instructions. The pGBKT7-BoFAZ1d recombinant plasmid was transfected into Y2HGold yeast cells, whereas the pGADT7-Library recombinant plasmid was inserted into Y187 yeast cells. Bait toxicity and recombinant plasmid activation tests were performed at 30°C on synthetic defined (SD/−Leu and SD/−Trp) media in Petri plates. The Y187 [pGADT7-T] × Y2Hgold [pGBKT7-Lam] transformation was used as a negative control, whereas the Y187 [pGADT7-T] × Y2H Gold [pGBKT7-53] transformation served as a positive control. The screening of protein interactions was performed for each pGBKT7-PUB7 derivative and pGADT7-Library on DDO (SD/−Leu/−Trp) and QDO/A (SD/−Ade/−His/−Leu/−Trp/AbA) media in Petri plates following a 3-day incubation period.

### *2.10 GST Pull-Down Assays*

GST pull down assay was conducted to study the interaction between BoFAZ1 and BoH3.2. BoFAZ1d was cloned into pGEX-4T-1vector, while BoH3.2 was cloned into pET-15b vector. The pET- BoH3.2 and pGEX- BoFAZ1 fused proteins were isolated and purified from E. *coli* (Transetta DE3). The pGEX4T-1 vector and pGEX-BoFAZ1d fusion proteins were incubated with glutathione-agarose beads, which were then used to pull down 6×His tagged pET-BoH3.2. The protein solutions after pull down were analyzed by western blot analysis.

## 3 Results

### *3.1 BoFAZ1 was Differentially Expressed in the Pistils after Self-Pollinated*

According to GO term classifications, one of the differentially expressed genes (DEG) was selected, which was significantly up-regulated expressed after self-pollination [25]. qRT-PCR assays further confirmed that the expression level of *BoFAZ1* displayed an 11, 20 and 65-fold increase at 15, 30, and 60 min after self-pollination (SP), respectively, and *BoFAZ1* expression was slowly increased after cross-pollination (CP) (Fig. 1).

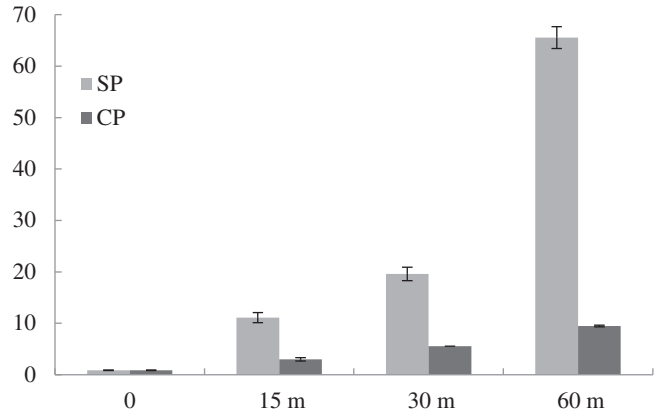


**Figure 1:** Expression analysis and gene amplification of *BoFAZ1*. mRNA expression of *BoFAZ1* at 0, 15, 30, and 60 min after pollinations obtained from transcriptomic data analysis. SP: Self-pollination; CP: Cross pollination

To obtain the genomic and encoding full-length sequence of *BoFAZ1* from *B. oleracea*, polymerase chain reactions (PCR) with individual gDNA and cDNA as amplification templates were carried out by using a specific primer pair. This was designed based on the transcriptome sequence data. The cDNA and gDNA sequencing results revealed that *BoFAZ1* consisted of 420 bp, with only one exon. The deduced amino acid sequence analysis suggested that *BoFAZ1* encoded a 139 aa peptide, with a molecular weight of approximately 17 kDa. An analysis from the NCBI database indicated that *BoFAZ1* was located on chromosome C3. Additionally, the TMHMM server showed that a signal peptide was missing on *BoFAZ1*, whereas the ScanProsite online program revealed a myristoylation site at the amino acid positions 2–7 of BoFAZ1 (i.e., N-terminus).

### *3.2 Homologous Analysis of the BoFAZ1 Gene in Brassicaceae*

To characterize this novel gene in *Brassicaceae*, a multiple protein sequence alignment was performed to reveal the amino acid similarity among *BoFAZ1*, *BnFAZ1*, *BrFAZ1* and *AtFAZ1* from *B. oleracea*, *B. napus*, *B. rapa* and *A. thaliana,* respectively (Fig. 2A). The alignment showed that the full length of the BoFAZ1 protein shared substantial identity within these proteins. For example, the BoFAZ1 and BnFAZ1 sequences had only one amino acid difference, whereas the BoFAZ1 and BrFAZ1 sequence had three amino acid variation. To further analyze the phylogenetic relationships between FAZ1 in *B. oleracea*, a phylogenetic tree was constructed using the Gramene online tool (http://www.gramene.org/) (Fig. 2B). The tree indicated that the highest similarity and collinearity were observed between *BoFAZ1* (accession number: Bo3g109530) and *BnFAZ1* (accession number: BnaCnng41410D). The similarity between Bo3g109530 and Bo8g077850 was very low. Furthermore, a synteny comparison was conducted to analyze the conservation of genes surrounding *FAZ1*. As shown in Fig. 2C, genes belonging to similar gene families were located at both sides of the *FAZ1* gene in *B. oleracea*. In summary, the *FAZ1* gene was highly conserved in *Brassicaceae*.

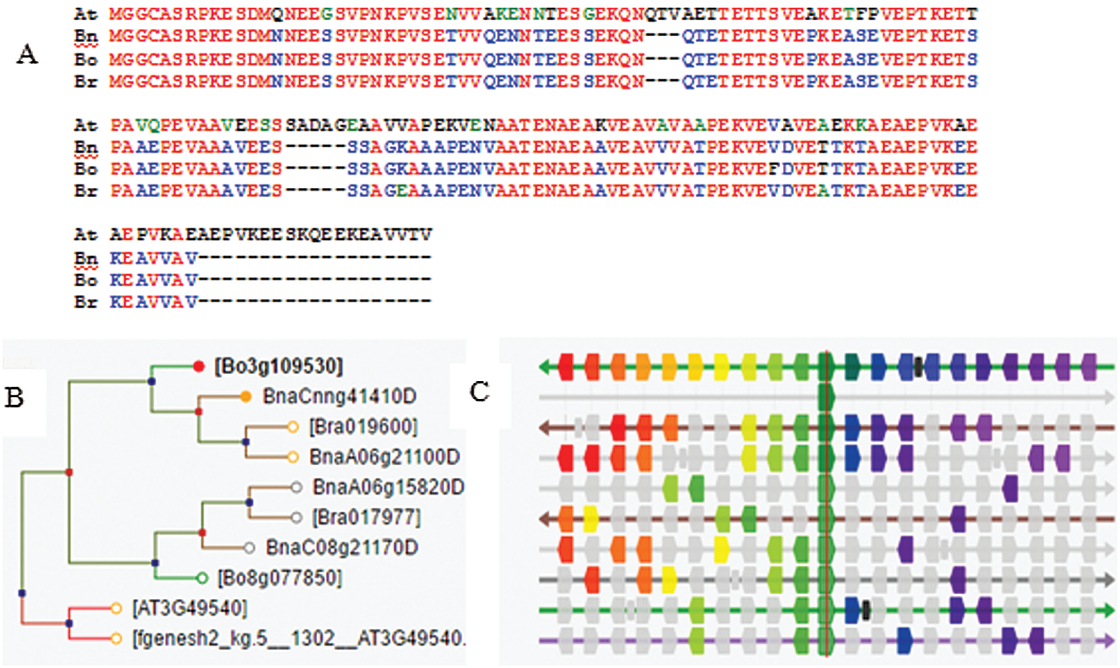


**Figure 2:** *FAZ1* sequence alignment and phylogenetic relationships. A, Alignment of protein sequences of multiple species. At: *Arabidopsis*, Bo: *Brassica oleracea*, Bn: *Brassica napus*, Br: *Brassica rape*. B, Phylogenetic analysis of FAZ1 of *B. oleracea*. C, Gene conservation analysis surrounding *FAZ1*. The 10 genes flanking either side of *FAZ1* are color-coded based on the gene family

### *3.3 Phenotypic Analysis of the at FAZ1 Mutant*

To investigate the potential biological function of *BoFAZ1* in *B. oleracea*, T-DNA insertion mutants were generated in *A thaliana* given the fact that *A thaliana* is one of the classical model plants and a single-copy homolog of *BoFAZ1* in *A. thaliana*. *A thaliana* T-DNA insertion mutant (SALK_302_G01) (i.e., *faz1* mutant) was analyzed to characterize the potential effects of *FAZ1* on the flower AZ. The sequencing results revealed that the T-DNA was inserted in the second exon of the *FAZ1* locus in this mutant (Fig. 3A). To our surprise, a phenotypic comparison suggested that the abscission of the petals and sepals in the *faz1* mutants was delayed around 4–5 days in comparison to that in the wild-type controls (Fig. 3B). To take a closer look, scanning electron microscopy (SEM) images displayed that the boundary between the sepal and torus was smooth in the wild-type controls, whereas it formed a V-shaped structure in the mutants (Figs. 3E and 3F). Additionally, the cell density of this V-shaped structure was around 30% lower than that of the corresponding region in the wild-type controls (Figs. 3C, 3D and 3I). To confirm whether the mutation of (SALK_302_G01) resulted in the mutant phenotype, we performed a complementation experiment by transforming a 1919-bp DNA fragment into the *Atfaz1* mutant that contained the cDNA of *BoFAZ1*. *BoFAZ1*, the homologous gene of *AtFAZ1*, after transformation to *faz1* showed that the mutant *faz1* restored the characteristics of the wild type (Figs. 3C, 3D and 3G–3I).

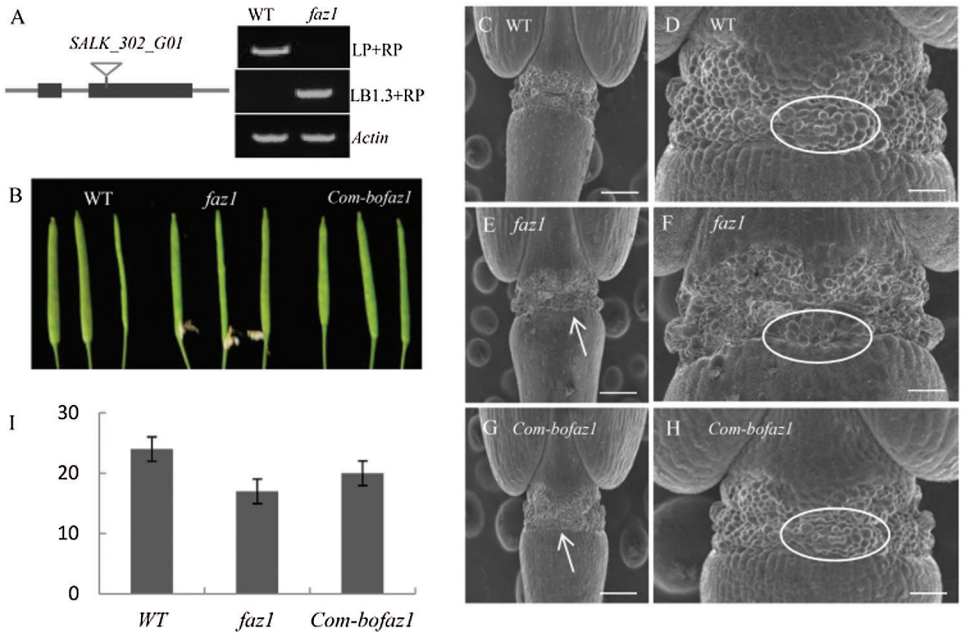


**Figure 3:** *FAZ1* regulated floral organ abscission in *Arabidopsis thaliana*. A, Diagram of the genomic region flanking the T-DNA insertion site (left) and genotype *faz1* mutants by PCR (right). LP: left primer for *FAZ1*; RP: right primer for *FAZ1*; LB1.3: T-DNA left border primer; LP + RP: detection of wild-type (WT); LB1.3 +RP: detection of the homozygous *faz1* mutant. Actin: Actin of Genome sequence. B, Representative siliques from wild type (WT), *faz1* T-DNA insertion mutant (*faz1*) and complementary experimental plant by *Com-bofaz1*. Flowers of the WT had abscised, but the mutant flowers delayed abscission. The phenotype was recovered by *Com-bofaz1*. C-E, Scanning electron microscope (SEM) of floral AZ of WT. (C), *faz1* mutants (E) and *Com-bofaz1* (G). D, F, H, SEM higher magnification of floral AZ of WT (D), *faz1* mutant (F) and *Com-bofaz1*(H). I, Cell numbers in the AZ of WT control, *faz1* mutant plants, and *Com-bofaz1* plants. Scale bar: 500 μm

### *3.4 FAZ1 Expression Profile*

The novel *FAZ1* gene may associate with floral organ abscission. This triggered us to study its expression pattern during the flowering of *B. oleracea*. To this end, the promoter region approximately 1.5 kb upstream of the start codon from *B. oleracea* was amplified by PCR and fused to the β-glucuronidase (GUS) reporter vector. This construct was inserted into *A. thaliana* plants. Three independent transgenic lines were obtained and examined to confirm that the GUS expression activity in these lines were consistent with each other. The GUS staining results disclosed a lack of positive signals in the cotyledons, hypocotyls, and roots, indicating that *BoFAZ1* was not expressed in these tissues. During the reproduction stage, GUS signals were detected in the mature pollen grains as well as in the filament, stigma, and exfoliation layer (Figs. 4A−4H). Thus, *BoFAZ1* was apparently expressed in immature pollen grains, but the expression level gradually decreased as the flower buds developed. In contrast, *BoFAZ1* expression levels gradually increased in the filament, stigma, and exfoliation layer.

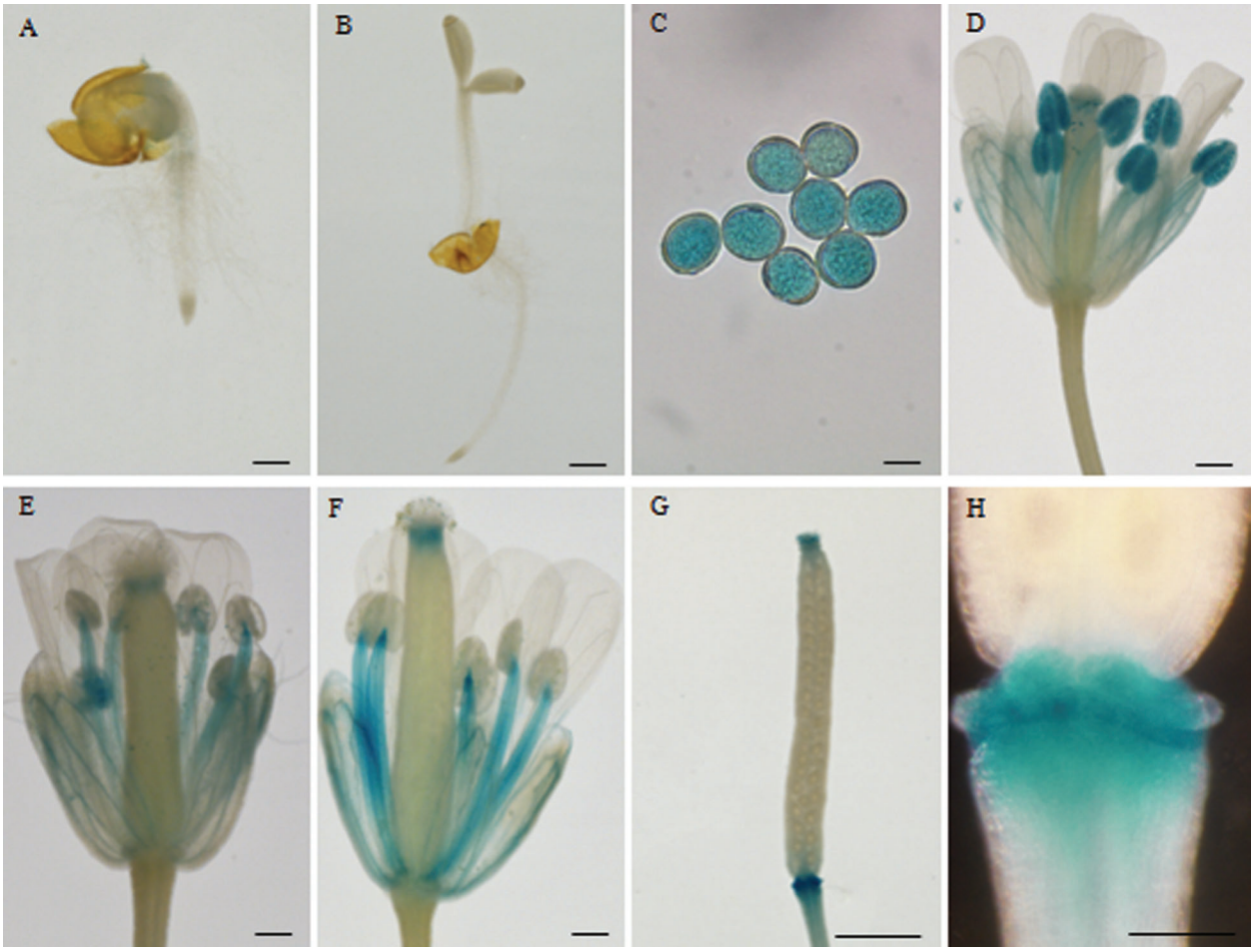


**Figure 4:** *BoFAZ1* promoter-driven GUS gene expression patterns in transgenic *Arabidopsis thaliana*. A–B, no GUS activity in seedlings. C, GUS staining in pollen grains. D–F, GUS staining in flowers at developmental stages. G, GUS staining in style and flower AZ. H, Strong GUS staining in flower AZ. Scale bar: (A−F) 500 µm, (G) 100 µm, (H) 1 mm

To further elucidate the subcellular localization of the BoFAZ1 protein, a BoFAZ1: green fluorescent protein (GFP) fusion protein as well as GFP alone (as a control) were transiently expressed in separate *A. thaliana* protoplasts. The green fluorescence coming from the Bo*FAZ1*:GFP fusion protein was detected in the cell membrane (Fig. 5). Another construct (BoFAZ1d:GFP) was also constructed and transiently transfected into *A. thaliana* protoplasts for the production of a GFP fusion protein without the seven amino acids, a possible myristoylation site at the N-terminus of BoFAZ1. An examination of *A. thaliana* protoplasts under fluorescent microscope indicated BoFAZ1d was localized in the cytoplasm, nucleus and cell membrane which was as same as the cellular location of the control GFP protein (Fig. 4). Accordingly, the myristoylation site is likely required for regulating the cellular localization of BoFAZ1.

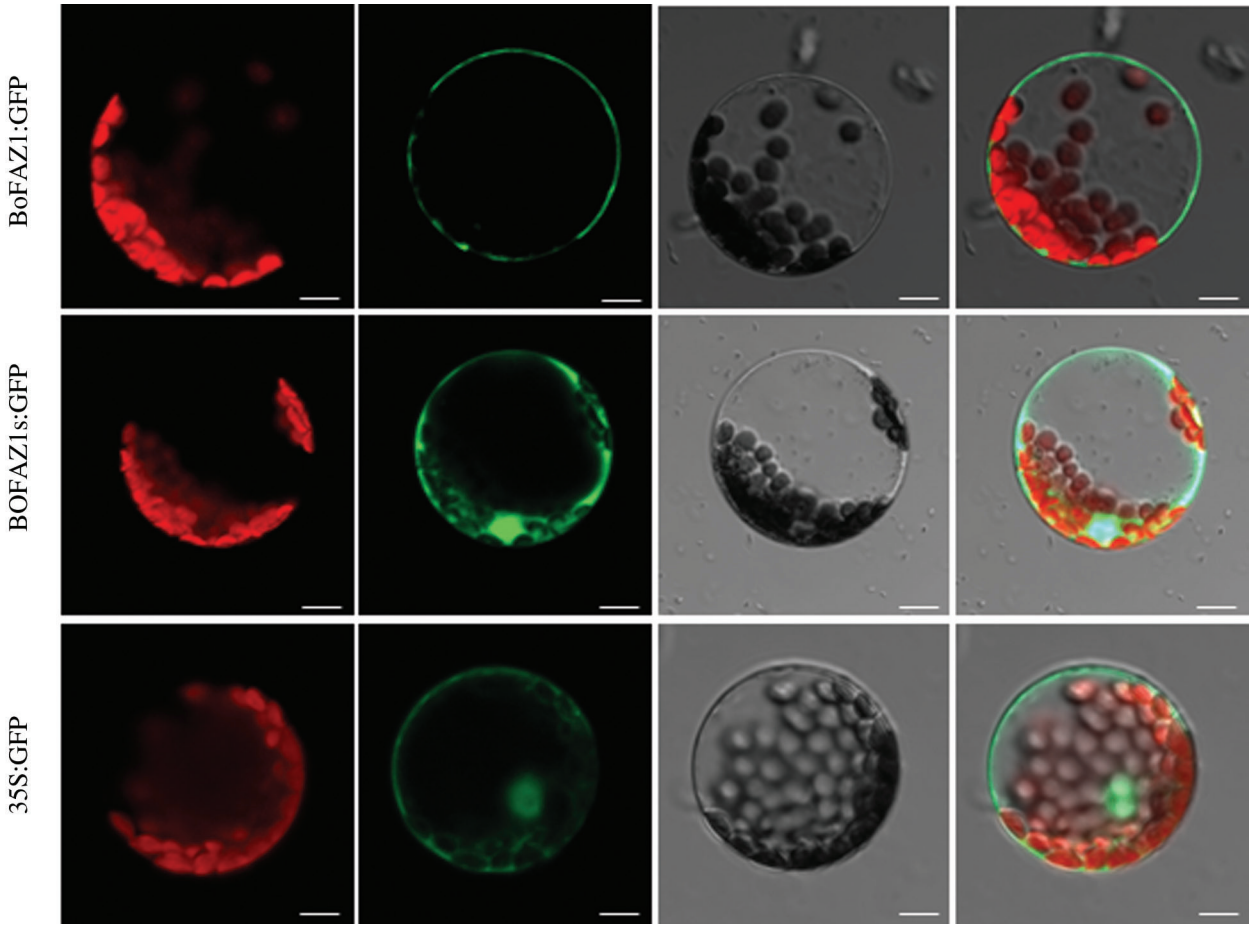


**Figure 5:** Subcellular localization of BoFAZ1. The BoFAZ1:GFP fusion protein was detected in the cell membrane, whereas the BoFAZ1d:GFP fusion protein was localized in the cytoplasm, nucleus, and cell membrane. The GFP alone (control) was primarily localized in the cytoplasm, nucleus and cell membrane. Chloroplast, chlorophyll auto-fluorescence; GFP:GFP fluorescence; Light: bright light field; Merge: combination of GFP fluorescence, bright light, and chlorophyll auto-fluorescence images. Scale bar, 10 μm

### *3.5 FAZ1 Interacts with Histone H3.2*

To investigate the mechanism by which BoFAZ1 affects the development of the flower AZ, one feasible approach was to examine the possible interacting proteins which may be associated with the flower AZ development or abscission process. To this purpose, a yeast two-hybrid system was applied in this study. *FAZ1d* sequence was fused to the BD domain sequence of the pGBK-T7 vector to generate the bait plasmid, while floral cDNA was inserted into pGADT7 vector to construct the prey plasmids. The yeast two-hybrid clones screening and DNA sequencing results showed that a clone harboring FAZ1d and BoH3.2 (histone H3.2) can grow on the selection QDO/A medium, whereas no growth was observed in the negative control (Fig. 6). These data suggested that BoFAZ1d can directly interact with the histone protein BoH3.2. To further confirm the interaction between FAZ1and BoH3.2, an *in-vitro* GST pull-down assay was performed. The BoFAZ1d sequence was fused to expressing vector pGEX4T-1 to generate the GST-BoFAZ1d fusion protein. The BoH3.2 fragment was cloned into another expressing vector pET43.1a to produce the $His_6$-tagged BoH3.2 protein. GST pull-down assay did verify the interaction of

BoFAZ1 and BoH3.2 (Fig. 6B). It is known that Histone H3 is one of the key factors responsible for the nucleosome formation. Moreover, the genes encoding histone H3 (*H3.1* and *H3.2*) were highly expressed in the meristematic tissues and abscission region. Therefore, BoFAZ1 may play an important role in floral abscission possibly via interaction with BoH3.2 in the *B. oleracea* floral organ.

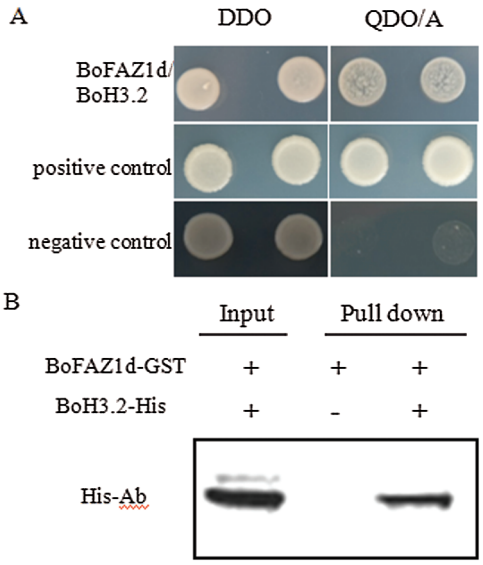


**Figure 6:** Interaction between BoFAZ1d and BoH3.2. A, Yeast two-hybrid assay for the interaction between BoFAZ1d and BoH3.2. Yeast cells were grown on agar-solidified DDO (SD/-Leu/-Trp) and QDO/A (SD/-Ade/-His/-Leu/-Trp/AbA) media, respectively. Yeast clones were formed on the positive control and cells transfected with BoFAZ1d and BoH3.2, but not the negative control. B, *In vitro* GST pull-down assay for the interaction of BoFAZ1 and BoH3.2. Purified BoH3.2-His tagged protein was pulled down by BoFAZ1d-GST fusion protein and was detected by western blot analysis using an His- antibody

## 4 Discussion

In this study, we first discovered that the *BoFAZ1* may be involved in the floral abscission in *B. oleracea* likely through the interaction with the histone BoH3.2 protein. As a single copy in *A. thaliana* and *B. oleracea* genomes, the membrane protein *BoFAZ1* was specifically expressed in reproductive organs including flower AZ accompanied with flowering and subsequent abscission of unwanted organs after pollination and fertilization. This discovery provides a new perspective in the molecular basis of the floral abscission in *B. oleracea*.

Gubert reported that instead of developing at the sepal base, AZs are usually formed as an inverted V-shape within the proximal regions of the medial and lateral sepals in the *A. thaliana as1* mutant [17]. In rice, the APETALA2-like transcription factor SUPERNUMERARY BRACT induces seed shattering by upregulating the *qSH1* and *SH5* expression levels, while altering the seed size by modulating the longitudinal cell elongation [9]. The delayed abscission of petals and sepals in the *A. thaliana faz1* mutants (Fig. 3) as well as our scanning electron microscopy results are consistent with the results of the Gubert et al. study [17]. In the transgenic plants, the phenotype of *faz1* was recovered completely by Com-*bofaz1*, indicating that BoFAZ1 has the same function as AtFAZ1 in *Arabidopsis*. These results implied that the loss-of-function mutation of *FAZ1* delayed the abscission of the flower organ due to changes in the structure of the flower AZ. Consequently, we believe that FAZ1 influences the abscission of flower organs and eventually affects the shedding of fruits, seeds, and stems.

A previous study reported that N-terminal myristoylated proteins have diverse biological effects that influence protein subcellular localization, protein–protein interactions, and signal transduction [26]. Proteins with their myristoylation site modification are generally localized in the plasma membrane. The demyristylation via a myristoylation inhibitor or a mutation to the Gly2 of a myristoylation site prevented the resulting protein from localizing in the cell membrane [27,28]. Recent studies concluded that myristoylation modifications regulate protein stability by modulating the ubiquitination pathway through a changing activity of the c-Src kinase [29]. In the current study, BoFAZ1 was found to localize in the cell membrane, whereas BoFAZ1d, which lack a myristoylation site, was detected in the cytoplasm and nucleus. These results indicated that the supposing myristoylation site did control the cellular localization of BoFAZ1, which is consistent with the known effects of N-myristoylation sites in many proteins.

We observed the interaction between BoFAZ1 and BoH3.2 in yeast two-hybrid and GST pull-down assays. The histone genes *H1*, *H2A*, *H2B*, *H3*, and *H4* are often expressed in eukaryotes, wherein they have important effects on the eukaryotic chromatin and are responsible for the formation of nucleosomes [30]. Additionally, various post-translational modifications of histone H3, such as methylation, acetylation, and phosphorylation, are important for regulating gene transcription. The histone H3 genes (*H3.1* and *H3.2*) are highly expressed in plant meristematic tissues [31]. The H3.2 protein is highly acetylated in meristematic tissues [32]. An earlier investigation of rice proved that *H3.2* expression was responsive to abscisic acid (ABA) treatment [33]. Another study revealed that ABA accumulated in the AZ after the floral organ was manually removed in *Lupinus luteus* [34]. Therefore, H3.2 may affect the development of the AZ through the ABA signaling pathway. The interaction between FAZ1 and H3.2 suggests that FAZ1 may affect the development of the pistil AZ by modulating the ABA signaling pathway via its interaction with H3.2.

## 5 Conclusion

BoFAZ1 was first identified in *Brassica oleracea*. The loss-of-function mutation of FAZ1 delayed the abscission of the flower organ and changes the structure of the flower AZ. In the transgenic plants, the phenotype of faz1 was recovered completely by Com-bofaz1. The possible mechanism may be that BoFAZ1 participates in the formation of the pistil abscission zone through the BoH3.2 (histone H3.2), a protein involved in the abscission zone development. Further studies are needed to find the possible mechanism of the pistil abscission zone in *Brassica* as noted in the present study.

**Author Contributions:** ZHC, LXP and ZLQ designed the experiments. ZHC, LXP, ZTH and LQY performed the experiments. ZHC, LXP and ZYZ analyzed the data. HDK and XQQ contributed analysis tools. ZHC and LXP wrote the manuscript. ZHC and ZLQ revised the manuscript. All authors have read and agreed to the submitted version of the manuscript.

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## Appendix

**Table S1:** Details regarding the primers used in this study

| Primer | Sequence (5′ to 3′) | Remarks |
|---|---|---|
| FAZ1-GUS-F | CAAGCTTGGCTGCAGGTCGACTCCCCAATTTCTACTACACTCATC | GUS |
| FAZ1-GUS-R | GGTGGACTCCTCTTAGAATTCTCATGTCTGATTCCTTGGGTC | |
| FAZ1-GFP-F | AAGTCCGGAGCTAGCTCTAGAATGGGTGGTTGTGCGAGTAG | Subcellular localization |
| FAZ1-GFP-R | GCCCTTGCTCACCATGGATCCAACAGCGACAACCGCTTCT | |
| FAZ1d-GFP-F | AAGTCCGGAGCTAGCTCTAGACCCAAGGAATCAGACATGAAC | Subcellular localization |
| FAZ1d-GFP-R | GCCCTTGCTCACCATGGATCCAACAGCGACAACCGCTTCT | |
| FAZ1-PGBKT7-F | TCAGAGGAGGACCTGCATATGCCCAAGGAATCAGACATGAAC | Yeast two-hybrid |
| FAZ1-PGBKT7-R | ATGCCCACCCGGGTGGAATTCAACAGCGACAACCGCTTCT | |
| Bo FAZ1d-GST-F | GGATCCATGGCCCAAGGAATCAGACATGAAC | BoFAZ1d/pGEX-4T |
| Bo FAZ1d-GST-R | GTCGACCTGGCGAGAACAGCGACAACCGCTTCT | BoFAZ1d/pGEX-4T |
| BoH3.2-His-F | ATGGCTCGTACCAAGCAAACC | *BoH3.2*b/pET-15b |
| BoH3.2-His-R | TCAAGCTCTTTCACCACGGATTC | *BoH3.2*-/pET-15b |