Comparative transcriptome analysis of a female-sterile mutant (*fsm*) in Chinese cabbage (*Brassica campestris* ssp. *pekinensis*)

HUANG Shengnan, LIU Zhiyong, LI Chengyu, YAO Runpeng, LI Danyang, LI Xiang, HOU Li, FENG Hui

(Department of Horticulture, Shenyang Agricultural University, 110866)

Abstract: In this study, we identified the female-sterile mutant *fsm* in Chinese cabbage. This mutant, which exhibits stable inheritance, was derived from Chinese cabbage DH line 'FT' using a combination of isolated microspore culture and ethyl methanesulfonate (EMS) mutagenesis. Genetic analysis indicated that the phenotype of *fsm* is controlled by a single recessive nuclear gene. Morphological observations revealed significant differences between the floral organs of *fsm* and wild-type line 'FT'. Parts of the pistils of *fsm* are smaller and shorter than those of 'FT', especially the ovary, which may directly cause the female sterility of the mutant. Comparative transcriptome analysis of 'FT' and *fsm* using RNA-Seq revealed a total of 1,872 differentially expressed genes (DEGs) between 'FT' and *fsm*. Of these, a number of genes involved in ovule development were identified, such as PRETTY FEW SEEDS 2 (PFS2) and Temperature-Induced Lipocalin (TIL), which were upregulated in *fsm* vs. 'FT'. In addition, qRT-PCR analysis of the expression patterns of 18 DEGs confirmed the accuracy of the RNA-seq data. These results shed light on the molecular mechanisms underlying pistil development in Chinese cabbage.

Key words: Vegetable science; Chinese cabbage; Female sterility; Transcriptome analysis; RNA-Sequencing

0 Introduction

Floral organ development is the most obvious characteristic of the reproductive stage of flowering plants [1,2]. In recent years, a variety of floral organ mutants have been characterized, leading to the isolation of a series of floral development- and morphogenesis-related genes using various techniques [3,4,5,6].

Female sterility has been identified in plants [7,8,9,10,11,12]. Studies have been performed on the biological characteristics, development, genetic methods, and potential applications of female sterility [13,14,15,16,17,18,19]. Female-sterile mutants have been used to identify regulatory genes that influence ovule and female gametophyte development [20,21,22], the regulation of megasporogenesis [23], metabolism, division, and differentiation, and the developmental regulation of embryo sac cells [24,25,26,27,28,29,30]. Mutation of genes controlling female organ development, such as carpel development gene *DROPPING LEAF* (*LD*) and ovule development genes *FLORAL BINDING PROTEIN 7* (*FBP7*) and *SHATTERPROOF 1* (*SHP1*) [31,32,33,34], can lead to female sterility.. Luo et al. [35] found that the abnormal pistil development trait in rice mutant *dl(t)* is controlled by a single recessive gene, whose function may be similar to that of *SUPERMAN* (*SUP*), which regulates floral organ development in *Arabidopsis thaliana* [36,37].

Transcriptome analysis is an important component of functional genomics research [38,39]. RNA-sequencing (RNA-Seq) technology is widely used to study the transcriptomes of organism in plants [40,41,42,43,44,45]. RNA-Seq is mainly used to (1) detect new transcript [46]; (2) analyze gene expression patterns [47]; (3) analyze the regulation of gene expression by microRNA [48], RNA (lncRNA) [49], and RNA editing [50]; (4) study structural variations in transcripts,

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Brief author introduction:HUANG Shengnan (1987-), Female, Doctor, Chinese cabbage
Correspondance author: FENG Hui (1961-), Male, Professor, Chinese cabbage. E-mail: fenghuiaaa@263.net

such as alternative splicing (AS) [51,52] and gene fusion [53,54]; and (5) identify single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSR) [55].

Chinese cabbage (*Brassica campestris* ssp. *pekinensis* [Lour] Olsson), an economically important vegetable crop, is widely cultivated in Northeast Asia. With the completion of genome sequencing of this crop [56], transcriptome analysis of Chinese cabbage has become an important field of study. If female-sterile materials (male parents) and male sterile lines (female parents) are utilized for hybrid seed production, thus improving seed yield and purity [57,58,59,60,61].

In this study, we identified a female-sterile mutant (*fsm*) in Chinese cabbage. To help elucidate the molecular mechanism underlying pistil development, we conducted comparative transcriptome analysis using RNA-Seq technology to characterize the gene expression profiles in flower buds of *fsm* and the corresponding wild-type variety 'FT' on a global level. The main objective of this study was to identify differentially expressed genes (DEGs) and potential candidate genes related to pistil development. Our results provide a comprehensive view of the transcriptome of Chinese cabbage, which helps elucidate the regulatory mechanisms underlying pistil development in this crop.

1 Materials and Methods

1.1 Plant materials

The *fsm* mutant was derived from Chinese cabbage doubled-haploid (DH) line 'FT' via a combination of isolated microspore culture and ethyl methanesulfonate (EMS) mutagenesis. Based on our previous studies [62], the isolated microspores were treated with 0.08% EMS solution for 10 min.

1.2 Genetic analysis

To investigate the inheritance of *fsm*, 'FT' (P_1) and *fsm* (P_2) were used as the parents to develop the F_1, BC_1, and F_2 populations. Phenotypic data were obtained for each plant of the P_1, P_2, F_1, BC_1, and F_2 populations, and the segregation ratios of the BC_1 and F_2 populations were analyzed by a Chi-square (χ^2) test.

1.3 Morphological observation

In March 2015, morphological analyses of 'FT' and *fsm* plants were carried out at the full-bloom stage.

Floral organs observation: Mature floral organs were directly observed and photographed under a dissecting microscope (Nikon SMZ800, Japan).

Pollen viability detection: The anthers were removed from the stamens and the pollen from each anther was extruded onto a slide. The pollen was immersed in 0.1% TTC (2,3,5-triphenyltetrazolium chloride) dye solution, covered with a cover slip, and dyed for 15–20 min at 35–37 ℃ in an incubator. Pollen viability was observed under an optical microscope (Nikon ECLIPSE 80i, Japan).

Ovary and ovule development observation: Flowers on the flowering day of 'FT' and *fsm* were respectively marked and naturally pollinated. The length and width of the ovary were measured every other day; ovaries were measured six times, and each measurement was performed in three independent experiments. Ovule development in flowers on the day of and 5th day after natural pollination were observed and compared under a dissecting microscope (Nikon SMZ800, Japan).

1.4 RNA extraction

90 In December 2014, the seeds of ‘FT’ and *fsm* were sown in a greenhouse at Shenyang Agricultural University, China. In March 2015, developing flower buds were collected from ‘FT’ and *fsm* at the full-bloom stage. All samples were immediately frozen in liquid nitrogen and stored at -80 ℃. Mixed samples collected from five plants of ‘FT’ and *fsm* were used as a single biological replicate; three independent biological replicates were performed for ‘FT’ and *fsm*. 95 Total RNA from six samples of ‘FT’ and *fsm* (with three biological replicates) was extracted using TRIzol reagent (Invitrogen, USA) following the manufacturer’s instructions.

1.5 cDNA library construction and Illumina sequencing

Equal amounts of total RNA from the three replicates of ‘FT’ and *fsm* were pooled for RNA-Seq library construction, which were designated F1, F2, F4, M1, M2, and M3, respectively. 100 Oligo (dT)-coated magnetic beads were used to isolate mRNA, which was broken into small fragments by the addition of fragmentation buffer. First-strand cDNA was synthesized with these short fragments serving as templates, and second-strand cDNA was synthesized using the reaction system. The short fragments were purified and subjected to end repair and the addition of sequencing adapters. Following agarose gel electrophoresis, suitable fragments were selected as 105 templates for PCR amplification. Quantification and quality analysis of the constructed libraries were conducted using an Agilent 2100 Bioanalyzer and an ABI StepOnePlus Real-Time PCR System. The cDNA libraries were then sequenced on Illumina sequencing platform HiSeq^{TM} 2000.

1.6 Mapping of reads to the reference genome

Clean reads were obtained by removing reads containing adaptors, reads with more than 10% 110 unknown nucleotides, and low-quality reads with more than 50% bases with a quality value ≤ 10. Clean reads were mapped to the reference genome with SOAPaligner/SOAP2 [63], allowing no more than five base mismatches.

1.7 Assessment of differential gene expression

The expression levels of genes determined by RNA-Seq were normalized by the RPKM 115 (reads per kb per million mapped reads) method [64]. DESeq was applied to identify DEGs based on the RPKM-derived baseMean for each gene between samples [65]. The false discovery rate (FDR) was used as the threshold of P-value in multiple tests [66]. In the current study, genes with FDR ≤ 0.001 and the absolute value of $\log_2$ Ratio ≥ 4 were defined as DEGs [67].

1.8 Functional enrichment analysis of DEGs

120 To characterize the biological functions and metabolic pathways of the DEGs, the DEGs were subjected to Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis [68,69,70].

1.9 Quantitative real-time PCR (qRT-PCR) analysis

The *Actin* gene was used as an internal reference control, and gene-specific primers were 125 designed using Primer Premier 5.0 software. The qRT-PCR analysis was carried out using SYBR Green as a fluorescent detection dye and performed on a Bio-Rad IQ5 real time PCR detection system (Bio-Rad, USA). The $2^{-\Delta\Delta Ct}$ method was employed to calculate the relative expression levels of the target genes [71]. All reactions were performed with three biological replicates.

2 Results

130 ## 2.1 Identification and genetic analysis of *fsm*

The *fsm* mutant exhibited the same visible phenotype as wild-type line 'FT' in the M_0 generation. After selfing, segregation of characters appeared in the M_1 generation (segregation ratio of 196:54): a few plants showed female sterility characters. Plants with the same phenotype as 'FT' were further selfed, revealing that character segregation continued in the offspring
135 (segregation ratio of 95:5). These results suggest that the mutation may have occurred during the diploid period rather than during the haploid period during the process of microspore culture and that the mutant gene *fsm* is recessive.

Therefore, to further investigate the inheritance of the mutant *fsm*, 'FT' (P_1) and *fsm* (P_2) were used as the parents. As shown in Tab. 1, the 'FT': *fsm* ratio among the BC_1 progenies
140 produced from the F_1 × *fsm* backcross was approximately 1:1 ($\chi^2 = 2.07 < \chi^2_{0.05} = 3.84$). Of the 226 F_2 plants, 178 and 48 individuals showed the 'FT' and *fsm* phenotypes, respectively. The segregation ratios in the F_2 population conformed to the expected ratio of 3:1 ($\chi^2 = 1.81 < \chi^2_{0.05} = 3.84$). The results indicate that the *fsm* mutant trait is controlled by a single recessive nuclear gene.

145 Tab.1 Genetic analysis of *fsm* and progeny from crosses between wild-type line 'FT' and *fsm* in Chinese cabbage

Generation	'FT'	*fsm*	Total	Segregation Ratio	Expected Ratio	χ^2
P_1 ('FT')	105	0	105			
P_2 (*fsm*)	0	57	57			
F_1 (P_1 × P_2)	170	0	170			
F'_1 (P_2 × P_1)	0	0	0			
BC_1 (F_1 × 'FT')	206	0	206			
BC_1 (F_1 × *fsm*)	97	78	175	1.24: 1	1:1	2.07
F_2	178	48	226	3.71: 1	3:1	1.81

2.2 Morphological comparison of floral organs of 'FT' and *fsm*

The *fsm* plants exhibited female sterility and their floral organs exhibited abnormal morphology, which were significantly different from the traits of wild-type line 'FT' (Fig. 1A, B). Compared to 'FT', the majority of flower buds in *fsm* were smaller and the stigmas were exposed
150 (Fig. 1C). In addition, the sepals were significantly smaller (Fig. 1D, E), the number of petals and stamens was reduced (Fig. 1B, G), the petals were smaller and curled (Fig. 1F), and the anthers were smaller (Fig. 1H). The filaments and pistil parts were also shorter, especially the ovaries, and the stigmas were flat (Fig. 1I, J).

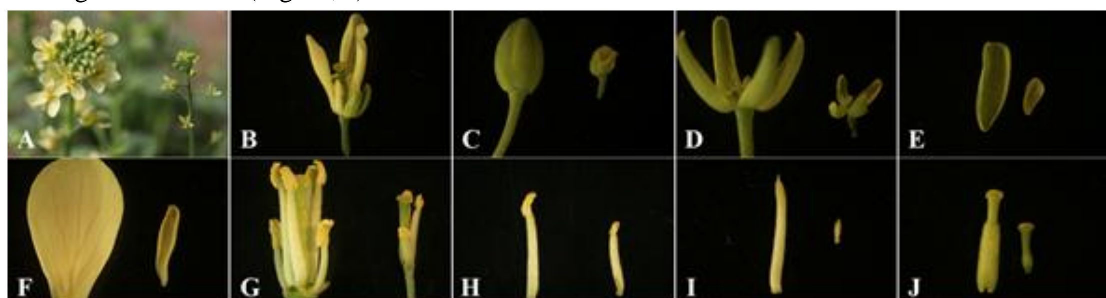

Fig. 1 Floral organ morphology of *fsm* and wild-type line ‘FT’

(A) Phenotype of ‘FT’ (left) and *fsm* (right); (B) floral organs of *fsm*; (C) flower buds of ‘FT’ (left) and *fsm* (right); (D, E) sepals of ‘FT’ (left) and *fsm* (right); (F) petals of ‘FT’ (left) and *fsm* (right); (G) stamens of ‘FT’ (left) and *fsm* (right); (H) anthers of ‘FT’ (left) and *fsm* (right); (I) filaments of ‘FT’ (left) and *fsm* (right); (J) pistils of ‘FT’ (left) and *fsm* (right).

2.3 Pollen viability observation

The pistils of *fsm* were completely sterile; however, there were small amounts of pollen in the stamens. As shown in Fig. 2, although there were few pollen grains in *fsm*, the pollen was viable. Therefore, the fertility of *fsm* stamens was normal.

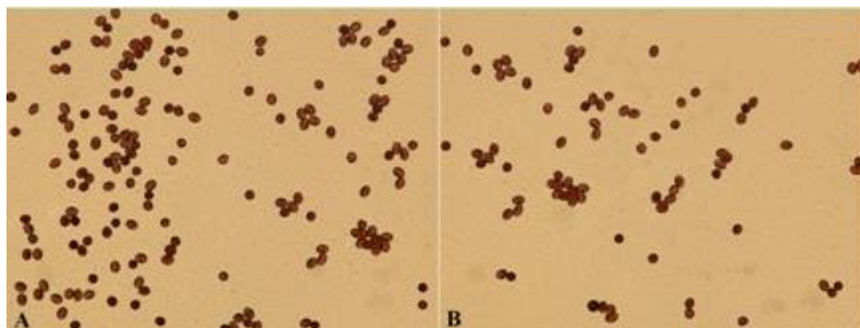

Fig. 2 Pollen viability of *fsm* and wild-type line ‘FT’

(A) Mature pollen microspores of ‘FT’; (B) mature pollen microspores of *fsm*.

2.4 Ovary and ovule development of ‘FT’ and *fsm*

Under natural pollination conditions, ovary development in *fsm* stopped at the end of flowering. The ovaries gradually became atrophied and yellow, ultimately leading to abscission. By contrast, ‘FT’ ovaries were elongated and widened at the end of flowering, and they developed rapidly (Fig. 3).

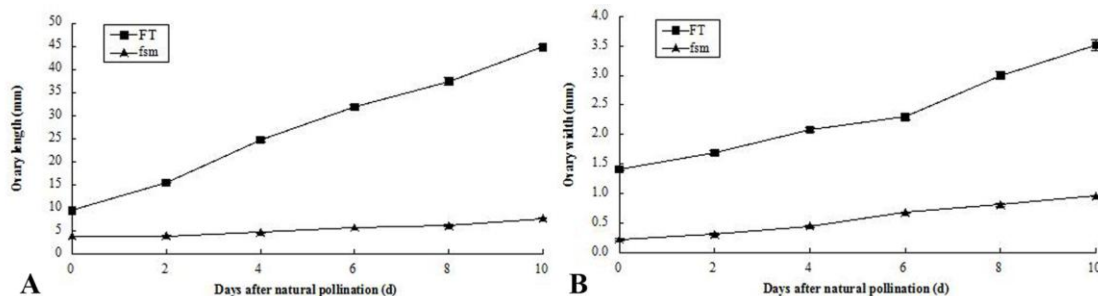

Fig. 3 Dynamic changes in the length and width of ovaries in *fsm* and ‘FT’ on different days after natural pollination

(A) Ovary length; (B) ovary width.

We observed the structural characteristics of ovule development in ‘FT’ and *fsm* under a dissecting microscope. Compared with ‘FT’, *fsm* ovaries were short, with fewer, smaller ovules. After natural pollination, FT’ ovules expanded and eventually developed into seeds, whereas *fsm* ovules degenerated and did not develop into seeds, ultimately leading to female sterility (Fig. 4).

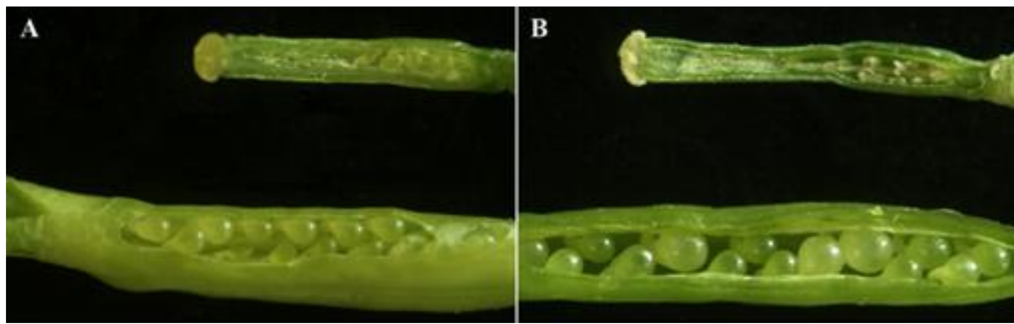

Fig. 4 Comparison of ovule development in *fsm* and 'FT'

(A) Structural characteristics of ovules in flowers on the flowering day in *fsm* (above) and 'FT' (below); (B) structural characteristics of ovules of the 5th day after natural pollination in *fsm* (above) and 'FT' (below).

2.5 Illumina sequencing and mapping reads to the reference genome

Based on Illumina sequencing, a total of 130,216,688 and 134,955,118 clean reads were generated from the three biological replicates of 'FT' and *fsm*, respectively. As shown in Tab. 2, of the total clean reads, the number of reads that could be mapped to the reference genome ranged from 23.3 to 28.3 million, and the percentage of cleans reads ranged from 53.95 to 61.52% in the six libraries.

Tab. 2 Summary of reads statistics from RNA-Seq of six libraries from *fsm* and wild-type line 'FT'

Summary	F1	F2	F4	M1	M2	M3
Total clean reads	43,518,072	43,433,878	43,264,738	46,054,444	44,645,322	44,255,352
Total base pairs	5,439,759,000	5,429,234,750	5,408,092,250	5,756,805,500	5,580,665,250	5,531,919,000
Total mapped reads	23,522,184 (54.05%)	23,450,417 (53.99%)	23,339,692 (53.95%)	28,329,921 (61.51%)	27,464,883 (61.52%)	27,164,647 (61.38%)
Perfect match reads	13,181,506 (30.29%)	13,175,823 (30.34%)	13,074,384 (30.22%)	15,992,485 (34.73%)	15,568,851 (34.87%)	15,373,270 (34.74%)
≤5bp mismatch reads	10,340,678 (23.76%)	10,274,594 (23.66%)	10,265,308 (23.73%)	12,337,436 (26.79%)	11,896,032 (26.65%)	11,791,377 (26.64%)
Unique match reads	22,821,592 (52.44%)	22,606,654 (52.05%)	22,581,156 (52.19%)	27,684,983 (60.11%)	26,845,981 (60.13%)	26,548,687 (59.99%)
Multi-position match reads	700,592 (1.61%)	843,763 (1.94%)	758,536 (1.75%)	644,938 (1.40%)	618,902 (1.39%)	615,960 (1.39%)
Total unmapped reads	19,995,888 (45.95%)	19,983,461 (46.01%)	19,925,046 (46.05%)	17,724,523 (38.49%)	17,180,439 (38.48%)	17090705 (38.62%)

2.6 Identification of SNPs

SNPs are widely used for genetic mapping and genetic diversity analysis in plants [72,73,74], and we identified SNPs using SOAPsnp software [75]. A total of 167,837 and 155,448 SNPs were identified in 'FT' and *fsm*, respectively. As shown in Tab. 3, the most common base substitutions were A/G and C/T, and the least common was C/G. A total of 43,661 and 31,272 SNPs were specifically detected in 'FT' and *fsm*, respectively.

Tab. 3 Summary of single nucleotide polymorphism (SNP) types identified in *fsm* and wild-type line 'FT'

SNP Type	'FT'	*fsm*
Transition	93,291 (55.58%)	89,372 (57.49%)
A/G	46,987 (28.00%)	43,980 (28.29%)
C/T	46,304 (27.59%)	45,392 (29.20%)
Transversion	74,546 (44.42%)	66,076 (42.51%)
A/C	19,933 (11.88%)	18,010 (11.59%)
A/T	19,551 (11.65%)	16,648 (10.71%)
C/G	15,990 (9.53%)	14,282 (9.19%)
G/T	19,072 (11.36%)	17,136 (11.02%)
Total	167,837	155,448

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2.7 Global analysis of differential gene expression

We detected a total of 1,872 DEGs, including, 1,021 upregulated and 851 downregulated genes in the *fsm* vs. 'FT' comparison, respectively. Therefore, the number of upregulated DEGs in *fsm* is dramatically higher than the number of downregulated DEGs. We also detected a number of specifically expressed genes (SEGs) in this study [76]. A total of 178 SEGs were identified
210 between 'FT' and *fsm*, including 49 SEGs in 'FT' and 129 in *fsm*.

2.8 DEGs related to pistil development

The morphological characterization suggested that the female sterility phenotype of *fsm* is related to pistil development (especially the presence of degenerated ovules). Among the DEGs, a number of ovule development-related genes were identified, including genes for *PRETTY FEW*
215 *SEEDS 2* (*PFS2*; *Bra026791*) and *Temperature-Induced Lipocalin* (*TIL*; *Bra020391*). In the *fsm* vs. 'FT' comparison, all of these genes were upregulated, with relatively high expression levels.

The mutant *fsm* not only exhibited female sterility, but the differences in other floral organs between 'FT' and *fsm* were also significant (Fig. 1). Consequently, numerous DEGs involved in floral development and flowering were also identified, such as genes encoding F-box family
220 protein, JASMONATE-ZIM-DOMAIN PROTEIN (JAZ), MYB family transcription factors, *AGAMOUS-LIKE* (*AGL*), flowering locus T (FT) and Auxin-Regulated Gene Involved In Organ Size (ARGOS). Most of these genes were highly expressed in the *fsm* vs. 'FT' comparison.

2.9 Functional enrichment analysis of DEGs using GO classification and KEGG pathway analysis

225 For the three main GO categories, the terms "cellular process" (GO: 0009987) and "metabolic process" (GO: 0008152), with 648 genes (59.8%) and 669 genes (61.7%), respectively, were dominant in the biological process category. In the molecular function category, the terms "binding" (GO: 0005488; 677, 62.9%) and "catalytic activity" (GO: 0003824; 638, 59.2%) were the most highly represented. In the cellular component category, "cell" (GO: 0005623; 780,
230 81.2%), "cell part" (GO: 0044464; 780, 81.2%), and "intracellular" (GO:0005622; 606, 63.1%) were the most highly represented groups (Fig. 5).

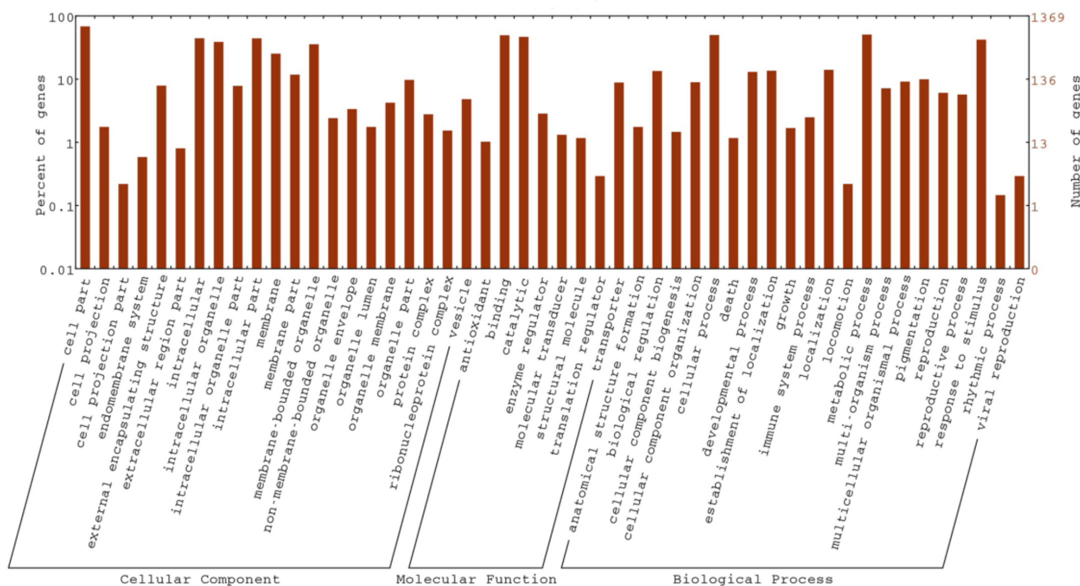


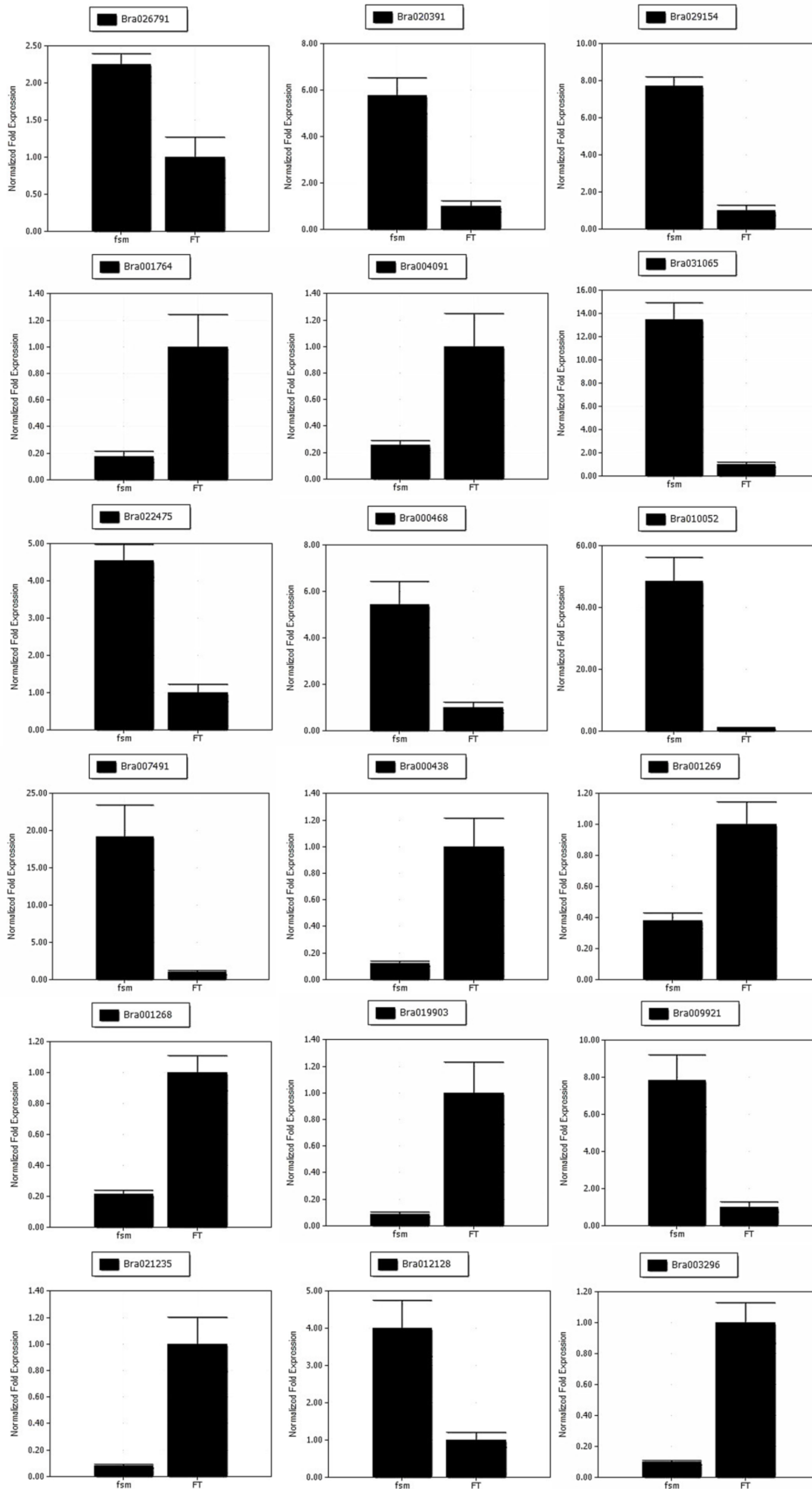
Fig. 5 GO functional classification of DEGs in the *fsm* vs. 'FT' comparison

In addition, GO analysis revealed a number of GO terms related to floral organ development, including gynoecium development, pollen tube growth, floral organ morphogenesis, pollen tube development, floral organ formation, floral organ development, floral whorl development, pollen development, and flower development. These results indicate that multiple biological processes are involved in the pistil development of *fsm*.

A total of 949 DEGs were mapped to 226 KEGG pathways. Metabolic pathways (218, 22.97%) was the largest pathway, followed by biosynthesis of secondary metabolites (131, 13.80%), plant-pathogen interaction (126, 13.28%), and plant hormone signal transduction (103, 10.85%). These results indicate that a variety of genetic and active metabolic pathways are involved in pistil development.

2.10 Analysis of the gene expression patterns by qRT-PCR

To help confirm the differential expression patterns of the DEGs detected by RNA-Seq, a total of 18 DEGs, including two ovule development-related genes (*Bra026791* and *Bra020391*), and sixteen floral development and flowering-related genes (*Bra029154*, *Bra001764*, *Bra004091*, *Bra031065*, *Bra022475*, *Bra000468*, *Bra010052*, *Bra007491*, *Bra000438*, *Bra001269*, *Bra001268*, *Bra019903*, *Bra009921*, *Bra021235*, *Bra012128*, and *Bra003296*) were selected for qRT-PCR analysis. As shown in Fig. 6, the gene expression patterns obtained by qRT-PCR are consistent with the RNA-Seq data, thus supporting the reliability of our transcriptome analysis.



Note: The relative expression levels of 18 DEGs identified by RNA-Seq analysis are shown.

Fig. 6 qRT-PCR analysis of gene expression patterns

255 # 3 Discussion

In this study, we identified the *fsm* mutant in Chinese cabbage, which exhibited stable inheritance. This mutant was derived from DH line 'FT' using a combination of isolated microspore culture and EMS mutagenesis. Based on a comparison with 'FT', we speculate that the mutant gene in *fsm* influences ovule development, which further affects pistil development, 260 eventually leading to female sterility. Comparative transcriptome analysis of 'FT' and *fsm* showed that a number of DEGs are related to ovule development. Further investigating these DES may increase our understanding of the molecular mechanisms underlying female sterility.

Morphological observations suggested that the degenerated ovules in the ovary might directly result in the female sterility of *fsm*. Therefore, identifying genes involved in ovule development 265 would facilitate analysis of female sterility. *PRETTY FEW SEEDS2* (*PFS2*) is primarily expressed in developing primordia, and its transcripts are most abundant in developing ovules [77]. *PFS2* encodes a homeodomain protein that regulates ovule development in *Arabidopsis thaliana*. This protein plays a prominent role during ovule patterning by regulating the differentiation of megaspore mother cells and cell proliferation of maternal integuments [78]. In *Arabidopsis* 270 *thaliana*, *Temperature-Induced Lipocalin* (*TIL*), which is mainly expressed in the embryo sacs of ovules, plays an essential role in female gametophyte development. Mutation of *TIL* causes ovule abortion and sometimes seed abortion, ultimately leading to low seed set [79]. Therefore, the upregulation of *PFS2* (*Bra026791*) and *TIL* (*Bra020391*) detected in *fsm* vs. 'FT' may affect ovule development, ultimately resulting in the female sterility phenotype of *fsm*.

275 Some female-sterile mutants (with abnormal ovule and embryo sac development) have recently been produced through mutagenesis, including chemical mutagenesis, transposon mutagenesis, and T-DNA insertion mutagenesis [80,81]. These mutants represent important materials for exploring floral organ-specific gene regulation and function. These mutants would be useful for studying female sterility-related proteins, to clone the related genes, and to further 280 analyze their structures and functions, shedding light on the molecular mechanisms underlying sex differentiation and development in floral organs.

The *fsm* mutant is different from previously reported female-sterile mutants [8,10,12], as it is impaired not only in pistil development, but also in the development of other floral organs. Our results indicate that *fsm* is controlled by a single recessive nuclear gene; however, fine mapping of 285 *fsm* remains to be performed. The results of gene mapping and transcriptome analysis could be used to further investigate candidate DEGs in *fsm*, especially DEGs identified between 'FT' and *fsm*, as well as SNPs specifically detected in *fsm* based on the RNA-Seq results. Female sterile lines can be used to improve seed yield and purity. Therefore, further investigating *fsm* may help reveal the molecular mechanism involved in female sterility, which would greatly facilitate 290 varietal improvement in Chinese cabbage.

In conclusion, we performed a systematic morphological investigation of *fsm*, followed by comparative transcriptome analysis between 'FT' and *fsm*. The results provide a comprehensive view of the expression profiles of genes involved in pistil development, which may help uncover the molecular mechanisms determining the phenotypic differences between these lines. Further 295 studies of the functions of DEGs involved in ovule development should increase our understanding of female sterility. Our results provide a solid foundation for uncovering the molecular mechanisms underlying female sterility in Chinese cabbage.

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大白菜雌性不育突变体 *fsm* 的转录组分析

黄胜楠，刘志勇，李承彧，姚润鹏，李丹杨，李想，侯莉，冯辉

500 （园艺学院，沈阳农业大学，110866）

摘要：雌性不育突变体 *fsm* 的表现特征是雌蕊败育，但是雄蕊正常可育，其它花器官表型也发生了较大变异。遗传分析结果表明，该突变性状由一对隐性核基因控制。与野生型 ‘FT’ 相比，突变体 *fsm* 的胚珠发育异常。利用 RNA-Seq 技术，对野生型‘FT’和突变体 *fsm* 的花蕾进行比较转录组分析，共检测到 1,872 个差异表达基因。经 GO 和 KEGG pathway 析，筛选

505 到一些参与胚珠发育的差异表达基因，包括 PRETTY FEW SEEDS 2（PFS2）和温度诱导的脂质运载蛋白（temperature-induced lipocalin; TIL），同时鉴定出多个与花发育和开花相关的基因。利用 qRT-PCR 技术，分析了其中的 18 个差异表达基因的表达模式，包括 2 个与胚珠发育相关的基因和 16 个与花发育和开花相关的基因，推测突变基因 *fsm* 可能影响了胚珠的发育，使之不能完成正常的受精过程。

510 **关键词**：蔬菜学；大白菜；雌性不育；转录组分析；RNA-Seq

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