Cloning and Expression Analysis of the BjGSTF12 Gene in Brassica juncea (Postprint)

Authors: Zhu Yunna, Chen Fengmei, Li Zhixian, Wang Bin, Huimin Feng, Hu Fang, Li Haibo

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Abstract

To investigate the role of glutathione S-transferase encoding genes (GST) in anthocyanin accumulation in mustard, this study employed purple-stem and green-stem mustard near-isogenic lines as materials to clone a GST gene associated with anthocyanin accumulation, designated as BjGSTF12. Bioinformatic analysis was performed on the BjGSTF12-encoded protein and its promoter, and the expression levels in green-stem and purple-stem mustard and their correlation with anthocyanin content were analyzed. The results demonstrated: (1) The full-length genomic DNA and cDNA of BjGSTF12 were 808 bp and 651 bp, respectively, encoding 216 amino acids containing GST_N-terminal and GST_C-terminal conserved domains. However, no sequence variation in BjGSTF12 was observed between green-stem and purple-stem mustard. (2) BjGSTF12 exhibited the closest phylogenetic relationship with Arabidopsis thaliana AtGSTF12, both belonging to the phi subfamily. (3) Four base mutations/insertions were identified in the BjGSTF12 promoter sequences between the two mustard lines, yet both possessed identical types and numbers of cis-acting elements, including nine MYB binding sites, one gibberellin response element, and three abiotic stress response elements. (4) The anthocyanin content in purple-stem mustard was significantly higher than that in green-stem mustard, and the expression level of BjGSTF12 displayed a similar pattern of variation to anthocyanin content. (5) Protein-protein interaction network analysis indicated that BjGSTF12 interacts with proteins involved in key anthocyanin synthesis enzymes, glycosylation modification, and transport. Therefore, BjGSTF12 may play a significant role in anthocyanin accumulation in mustard stems, and it is hypothesized that BjGSTF12 may influence anthocyanin accumulation by regulating anthocyanin synthesis, modification, and transport through interacting proteins. In conclusion, this study establishes a theoretical foundation for further in-depth investigation of the function and mechanism of GST in anthocyanin accumulation in mustard stems.

Full Text

Preamble

Cloning and Expression Analysis of *BjGSTF12* in *Brassica juncea*

Yunna Zhu, Fengmei Chen, Zhixian Li, Bin Wang, Huimin Feng, Fang Hu, Haibo Li*
(Guangdong Provincial Key Laboratory of Utilization and Conservation of Food and Medicinal Resources in Northern Region / College of Biology and Agriculture, Shaoguan University, Shaoguan 512005, Guangdong, China)

Abstract

To investigate the role of glutathione S-transferase (GST) genes in anthocyanin accumulation in mustard (*Brassica juncea*), we cloned a GST gene associated with anthocyanin accumulation from near-isogenic lines of purple-stalk and green-stalk mustard, designated as *BjGSTF12*. Bioinformatics analysis was performed on the encoded protein and its promoter, and expression levels were analyzed in relation to anthocyanin content in both green-stalk and purple-stalk mustard lines. The results showed: (1) The full-length genomic and cDNA sequences of *BjGSTF12* were 808 bp and 651 bp, respectively, encoding 216 amino acids with conserved GST_N and GST_C terminal domains. However, no sequence differences were observed in *BjGSTF12* between the green-stalk and purple-stalk mustard lines. (2) *BjGSTF12* showed the closest phylogenetic relationship with *Arabidopsis AtGSTF12*, both belonging to the phi (ϕ) subfamily. (3) Four base mutations/insertions were identified in the *BjGSTF12* promoter sequences between the two mustard lines, but both contained identical types and numbers of cis-acting elements, including nine MYB binding sites, one gibberellin response element, and three abiotic stress response elements. (4) Anthocyanin content was significantly higher in purple-stalk mustard than in green-stalk mustard, and *BjGSTF12* expression patterns mirrored these changes in anthocyanin content. (5) Protein interaction network analysis revealed that BjGSTF12 interacts with key enzymes involved in anthocyanin synthesis, glycosylation modification, and transport proteins. Therefore, BjGSTF12 likely plays an important role in anthocyanin accumulation in mustard stalks, possibly by regulating anthocyanin synthesis, modification, and transport through protein interactions. In summary, this study provides a theoretical foundation for further investigation of GST function and the mechanisms of anthocyanin accumulation in mustard stalks.

Keywords: *Brassica juncea*, GST, bioinformatics analysis, expression analysis, anthocyanin accumulation

Introduction

Glutathione S-transferase (GST, EC 2.5.1.18) is a multifunctional superfamily enzyme widely distributed across living organisms. It primarily catalyzes the

conjugation of reduced glutathione (GSH) with hydrophobic and electrophilic substrates, sequestering potentially toxic endogenous compounds and xenobiotics in vacuoles or transporting them to the apoplast for detoxification (Cummins et al., 2011; Chen et al., 2013; Zhang et al., 2017). Based on gene structure, amino acid sequence similarity, and substrate specificity, the GST gene family is classified into seven subfamilies: phi (F-type), tau (U-type), zeta (Z-type), theta (T-type), lambda (L-type), dehydroascorbate reductase (DHAR), and tetrachlorohydroquinone dehalogenase (TCHQD), with phi and tau being plant-specific (Mohsenzadeh et al., 2011). All GST proteins contain N-terminal and C-terminal domains; the N-terminal domain serves as the GSH-specific binding site (G-site) and is relatively conserved, while the C-terminal domain contains the hydrophobic substrate binding site (H-site) and shows greater variability (Xing et al., 2020).

GST genes have been isolated and identified from various species including *Arabidopsis* (Mohsenzadeh et al., 2011), rapeseed (Wei et al., 2019), and pomegranate (Liu et al., 2021). Their functions are manifested in three main aspects: (1) catalyzing GSH conjugation with toxic xenobiotics or oxidative products to promote their metabolism, sequestration, or elimination, thereby reducing cellular damage (Chen et al., 2013); (2) regulating plant resistance to abiotic stresses such as salt and heavy metal stress (Lallement et al., 2014; Xing et al., 2020; Zhang et al., 2022); and (3) participating in the transmembrane transport and localization of secondary metabolites like anthocyanins (Marrs et al., 1995). Maize *Bronze-2* was the first GST gene reported to play an important role in anthocyanin accumulation (Marrs et al., 1995). Homologous genes such as *Arabidopsis TT19 (GSTF12)* (Sun et al., 2012), kiwifruit *AcGST1* (Liu et al., 2019), cotton *GhGSTF12* (Shao et al., 2021), and radish *RsGSTF12* (Niu et al., 2022) have been subsequently reported, indicating that GST function in anthocyanin accumulation is highly conserved across plants. Thus, GST family members play crucial roles in plant anthocyanin accumulation.

Mustard (*Brassica juncea*) is a cruciferous crop of the *Brassica* genus and an important vegetable, oilseed, and condiment crop worldwide. In China, mustard is classified into four major categories (root, stem, leaf, and stalk) comprising 16 varieties (Liu, 1996) and is cultivated across the country except in high-altitude cold regions like Tibet (Wan et al., 2020). With improving living standards and changing environmental conditions, new demands have emerged for mustard quality and variety resistance (Fu et al., 2022). Breeding purple vegetable varieties holds significant importance for enhancing both economic and nutritional value. Our research group developed a purple-stalk/green-stalk near-isogenic line (201-402) through multiple generations of backcrossing and selfing between purple-stalk *Aijie* mustard and *Bangcai* mustard. Using this near-isogenic line as material, we cloned the mustard *BjGSTF12* gene and its promoter through homology-based cloning. Bioinformatics software was employed for homology analysis, phylogenetic analysis of the encoded protein sequence, and cis-acting element analysis of the promoter sequence. Expression analysis was performed in both purple-stalk and green-stalk near-isogenic lines to examine its relationship

with anthocyanin content. This study aimed to address: (1) whether sequence differences exist in the *BjGSTF12* gene and promoter between purple-stalk and green-stalk mustard; (2) analysis of anthocyanin content in stalks and expression patterns of *BjGSTF12* in purple-stalk versus green-stalk mustard; and (3) analysis of BjGSTF12-interacting proteins. The results will provide information for understanding differences in anthocyanin content between purple-stalk and green-stalk mustard, lay a foundation for further elucidating the role of BjGSTF12 in anthocyanin accumulation in mustard stalks, and offer theoretical support for the effective utilization and conservation of mustard germplasm resources.

Materials and Methods

1.1 Plant Materials

The experimental material was the purple-stalk/green-stalk near-isogenic line (201-402) developed by our research group.

1.2 Plant Cultivation and Sampling

Mustard seeds were sterilized with 7.5% NaClO for 10 minutes and rinsed 4–5 times with sterile water before being sown in a peat:vermiculite (3:1) substrate for seedling cultivation. When seedlings reached the three-leaf stage, they were transplanted with uniform water and fertilizer management. Compound fertilizer (N-P_2O_5-K_2O = 15-5-25) was applied once at 50 kg/667 m^2 before transplanting and land preparation. After 40 days of transplanting, when plants began to bolt, fresh stalks were collected from uniformly growing purple-stalk and green-stalk mustard plants. Epidermal tissues were peeled, cut into small pieces, snap-frozen in liquid nitrogen, and stored at –80°C. Each sample consisted of three biological replicates, with each replicate comprising 4–6 plants.

1.3 Determination of Anthocyanin Content in Mustard Stalks

Approximately 0.05 g of epidermal tissue from purple-stalk and green-stalk mustard was chopped and extracted overnight at room temperature in the dark with 5 mL of 1% HCl-methanol solution. Anthocyanin content was determined by measuring absorbance at 530 nm using a UV-2600 spectrophotometer (Li et al., 2016).

1.4 Extraction of Genomic DNA and Total RNA, and cDNA Synthesis

Genomic DNA was extracted using the Tiangen Biotech novel genomic DNA extraction kit. Total RNA was extracted using the Promega Eastep® Super total RNA extraction kit, and cDNA was synthesized using the GoScript™ Reverse Transcription System.

1.5 Gene Cloning and Positive Clone Identification

Primers were designed based on the *BjGSTF12* gene sequence (BjuVB05G21730) from the *Brassicaceae* Database (Braju_{{{tum}}_{{V2}}}.0) (Table 1). Using primers GSTF12-F/R (Table 1) and DNA or cDNA samples from purple-stalk mustard stalks as templates, the *BjGSTF12* gene was cloned. PCR products were purified, ligated into the pMD20-T vector, and transformed into *E. coli* DH5α competent cells. Positive clones were identified by PCR and sequenced by Guangzhou Tsingke Biotechnology Co., Ltd.

1.6 Bioinformatics Analysis

Bioinformatics software and web servers listed in Table 2 were used to analyze the *BjGSTF12* gene and its encoded amino acid sequence.

1.7 Expression Analysis of *BjGSTF12* in Purple-Stalk and Green-Stalk Mustard Stalks

Real-time quantitative PCR was performed using SYBR Green® Premix Ex Taq™ with *Actin2* as the reference gene. Relative gene expression levels were calculated using the 2^(-ΔΔCt) method (Livak & Schmittgen, 2002).

1.8 Cloning and Cis-Acting Element Analysis of the *BjGSTF12* Promoter

Using genomic DNA from purple-stalk and green-stalk mustard as templates and primers GSTF12Pro-F/R (Table 1), the *BjGSTF12* promoter sequences were cloned, ligated into pMD20-T vector, and transformed into *E. coli* DH5α. Positive clones were sequenced. Promoter sequences from the two varieties were aligned using DNAMAN software. Cis-acting elements were predicted using the PlantCare web server and visualized with TBtools (Chen et al., 2020).

1.9 Statistical Analysis and Graphing

Data were processed using Microsoft Excel 2010. Duncan's significance analysis was performed using SPSS 19.0, and graphs were generated using SigmaPlot 11.0.

Results

2.1 Cloning of the Mustard *BjGSTF12* Gene

Genomic DNA and total RNA were extracted from purple-stalk mustard stalks, and total RNA was reverse-transcribed into cDNA. Using primers GSTF12-F/R (Table 1), the mustard *BjGSTF12* gene was cloned. PCR amplification yielded target bands as shown in Figure 1 [Figure 1: see original paper]. Sequencing revealed that the genomic and open reading frame sequences of *BjGSTF12* were 808 bp and 651 bp, respectively. The gene structure was analyzed using

Gene Structure Display Server 2.0 (Figure 2 [Figure 2: see original paper]), showing that *BjGSTF12* contains two introns located at positions 148–232 bp and 282–353 bp. The full-length CDS of *BjGSTF12* was 651 bp, encoding 216 amino acids. BLAST analysis showed that the *BjGSTF12* nucleotide sequence shared 94%, 94%, and 88% identity with *BnGSTF12* from rapeseed (*Brassica napus*, XM_{013794351}), *BrGSTF12* from Chinese cabbage (*B. rapa*, XM_{009127922}.3), and *AtGSTF12* from *Arabidopsis* (*Arabidopsis thaliana*, NM_{121728}.4), respectively. Therefore, this mustard gene was designated as *BjGSTF12*.

2.2 Bioinformatics Analysis of the Mustard BjGSTF12 Protein

ProtParam analysis of the BjGSTF12 amino acid sequence revealed that it encodes 216 amino acids with a molecular weight of 24.80 kDa and atomic composition of $C_{1121}H_{1765}N_{297}O_{313}S_{12}$. The protein is primarily composed of leucine (Leu, 11.6%), valine (Val, 9.7%), alanine (Ala, 8.8%), and glutamic acid (Glu, 8.8%). The theoretical isoelectric point (pI) is 5.73, the average hydrophobicity is –0.075, the aliphatic index is 98.38, and the instability coefficient is 38.30, indicating that BjGSTF12 is a stable protein.

Conserved domain analysis using the NCBI Conserved Domain Database revealed that BjGSTF12 contains GST_{NTERHE} and GST_{CTER} domains at positions 1–75 and 91–207 amino acids, respectively (Figure 3 [Figure 3: see original paper]:A). This confirms that BjGSTF12 possesses the typical GST-N and GST-C conserved domains and belongs to the GST gene family.

Hydrophilicity/hydrophobicity prediction using ExPASY ProtScale showed that the maximum positive score (2.522) occurs at amino acid position 116, while the minimum negative score (–2.256) occurs at position 40, with more hydrophilic than hydrophobic amino acids throughout the peptide chain, suggesting that BjGSTF12 is a hydrophilic protein (Figure 3 [Figure 3: see original paper]:B).

Secondary structure prediction using SOPMA indicated that BjGSTF12 contains 46.30% α-helix, 15.28% extended strand, 7.41% β-turn, and 31.02% random coil, with α-helices being the dominant structural component (Figure 3 [Figure 3: see original paper]:D).

Phosphorylation site analysis using NetPhos 3.1 identified several potential phosphorylation sites: four threonine (Thr) sites, three tyrosine (Tyr) sites, and one serine (Ser) site, suggesting that BjGSTF12 may be regulated by threonine, tyrosine, and serine kinases (Figure 3 [Figure 3: see original paper]:C). SignalP 6.0 analysis detected no signal peptide sequence, indicating that BjGSTF12 is a non-secreted protein.

Subcellular localization prediction using Plant-PLoc server indicated cytoplasmic localization. TMHMM Server 2.0 analysis revealed no transmembrane structures. Tertiary structure prediction using SWISS-MODEL showed a monomeric structure dominated by α-helices with scattered extended strands, β-sheets, and

random coils, consistent with secondary structure predictions (Figure 3 [Figure 3: see original paper]:E).

2.3 Homology Alignment and Phylogenetic Analysis of Mustard BjGSTF12 Amino Acid Sequence

Multiple sequence alignment of BjGSTF12 with GST proteins from rapeseed (BnGSTF12, XP_{048602386}.1), *Arabidopsis* (AtGSTF12/AtTT19, NP_{197224}.1), Chinese cabbage (BrGSTF12, XP_{009126170}.1), radish (RsGSTF12, XP_{018444305}.1), cotton (GhGSTF12, XP_{016744877}.2), and kiwifruit (AcGST1, PSS21435.1) showed amino acid similarity ranging from 52.07% to 95.31% (Figure 4 [Figure 4: see original paper]). The lowest similarity (52.07%) was with kiwifruit AcGST1, while the highest (95.31%) was with rapeseed BnGSTF12.

Conserved motif analysis using MEME identified 10 conserved motifs (Motif 1–Motif 10) (Figure 5 [Figure 5: see original paper]). BjGSTF12 and GST members from rapeseed, Chinese cabbage, *Arabidopsis*, and radish shared six conserved motifs (Motif 1–Motif 6). Kiwifruit AcGST1 and rapeseed BnGSTF12 additionally contained Motif 8 and Motif 10, while AcGST1 and cotton GhGSTF12 contained Motif 7, which were absent in other species. All seven species contained Motif 1, Motif 2, Motif 4, and Motif 5.

Phylogenetic analysis with 50 *Arabidopsis* GST family members showed that *Arabidopsis* GSTs cluster into seven subfamilies: tau (28 members), phi (11 members), DHAR (3 members), zeta (2 members), theta (2 members), lambda (2 members), and TCHQD (1 member). Mustard BjGSTF12 clustered first with *Arabidopsis* GSTF12 and then with ATGSTF11, belonging to the phi subfamily and being distantly related to other subfamilies (Figure 6 [Figure 6: see original paper]).

2.5 Anthocyanin Content and *BjGSTF12* Expression Analysis in Near-Isogenic Lines

To investigate the role of *BjGSTF12* in anthocyanin synthesis in stalk epidermis, we analyzed anthocyanin content and *BjGSTF12* expression in our previously developed near-isogenic lines. As shown in Figure 7 [Figure 7: see original paper]:A and B, green-stalk mustard exhibited green leaves, stalks, and stalk leaves, while purple-stalk mustard showed green leaves but distinct purple coloration in stalks and young stalk leaves. Anthocyanin content was significantly higher in purple-stalk mustard than in green-stalk mustard ($P<0.01$) (Figure 7 [Figure 7: see original paper]:C). *BjGSTF12* showed weak expression in green-stalk mustard but significantly higher expression in purple-stalk mustard (Figure 7 [Figure 7: see original paper]:D). These results demonstrate that *BjGSTF12* expression patterns correlate with anthocyanin content differences between the two stalk-color mustard lines.

2.6 Cis-Acting Element Prediction of the Mustard *BjGSTF12* Promoter

Promoter sequences upstream of the *BjGSTF12* translation start site were amplified from green-stalk and purple-stalk mustard genomic DNA, yielding 1,855 bp and 1,857 bp sequences, respectively. Alignment using DNAMAN software revealed 99.78% similarity, with four base mutations/insertions in the purple-stalk mustard promoter: insertion of G and A at positions 130 and 1,333 bp, respectively, and T→A and T→G mutations at positions 937 and 980 bp (Figure 8 [Figure 8: see original paper]).

Cis-acting element prediction using PlantCare and visualization with TBtools showed identical element types and numbers in both promoters, including nine TATA-box core promoter elements, five CAAT-box elements, nine MYB transcription factor binding sites (six MYB elements and three MYB-like sequences), one gibberellin response element (GARE), one anaerobic induction element (ARE), two wound and defense response elements (WRE3), and eight unknown elements (one taTAAATATct element and seven CTCC elements) (Figure 9 [Figure 9: see original paper]).

2.7 Protein Interaction Network of Mustard BjGSTF12

The STRING database was used to construct a protein interaction network for mustard BjGSTF12 using *Arabidopsis* as the model plant (Figure 10 [Figure 10: see original paper]). BjGSTF12 was predicted to interact with dihydroflavonol-4-reductase (DFR), chalcone synthase (TT4/CHS), leucoanthocyanidin dioxygenase (LDOX), anthocyanidin 3-O-glucosyltransferase (UF3GT), malonyl-CoA:anthocyanidin 5-O-glucoside-6”-O-malonyltransferase (AT5MAT), MATE transporter (TT12), autoinhibited H+-ATPase isoform 10 (AHA10), UDP-glycosyltransferase superfamily member (AT4G14090), and HXXXD-type acyltransferase family protein (AT1G03495). DFR, TT4, LDOX, and UF3GT are key enzymes in anthocyanin biosynthesis; AT5MAT, AT4G14090, and AT1G03495 are involved in anthocyanin glycosylation and acylation; TT12 and AHA10 are responsible for proanthocyanidin transport to vacuoles. These interactions suggest that BjGSTF12 may participate in anthocyanin biosynthesis, modification, and transport through protein-protein interactions.

Discussion and Conclusion

Glutathione S-transferases are multifunctional enzymes that play important roles in cellular detoxification (Chen et al., 2013), stress resistance (Lallement et al., 2014), and secondary metabolite transport such as anthocyanins (Marrs et al., 1995; Sun et al., 2012). However, few studies have reported on GST gene function in mustard and its relationship with stalk color formation. In this study, we isolated the genomic and open reading frame sequences of *BjGSTF12* from purple-stalk mustard. The gene contains three exons and two introns

(Figure 2) and encodes a protein with GST_{NTERHE} and GST_{CTER} domains, confirming its membership in the GST gene family (Figure 3:A). Phylogenetic analysis clustered mustard BjGSTF12 with *Arabidopsis* phi subfamily GST members, particularly *AtGSTF12*, indicating that it belongs to the phi subfamily (Figure 5).

Anthocyanins are flavonoid compounds that provide a range of colors from red, pink, and orange to blue in plants, serving as important indicators for evaluating organ color and nutritional quality. Anthocyanins are synthesized in the cytoplasm through the coordinated action of phenylalanine ammonia-lyase (PAL), TT4/CHS, flavanone 3-hydroxylase (F3H), DFR, UF3GT, and LDOX (Hou et al., 2017), and are sequestered in vacuoles through GST, multidrug and toxin extrusion (MATE), and ATP-binding cassette (ABC) transporters (Shao et al., 2021). We cloned *BjGSTF12* from both purple-stalk and green-stalk mustard, finding identical nucleotide and amino acid sequences that matched perfectly with the mustard genome BjuVB05G21730. The 651 bp CDS encodes 216 amino acids showing high homology (84.33%–95.31%) with GSTF12 proteins from rapeseed, Chinese cabbage, *Arabidopsis*, and radish. In addition to stress regulation, GST proteins participate in anthocyanin transport (Marrs et al., 1995; Kitamura et al., 2004). *GSTF12* has been reported to mediate anthocyanin transport from the cytoplasm to vacuoles in *Arabidopsis* (Sun et al., 2012), cotton (Shao et al., 2021), and radish (Niu et al., 2022). Furthermore, *GST1* functions as an anthocyanin carrier in kiwifruit and potato (Liu et al., 2019; Zhang et al., 2023). These findings demonstrate the conserved function of plant GSTF genes in anthocyanin accumulation. Our study revealed weak *BjGSTF12* expression in green-stalk mustard but significantly elevated expression in purple-stalk mustard (Figure 7D), with expression patterns correlating with anthocyanin content differences.

Although anthocyanin content and *BjGSTF12* expression differed markedly between the two lines, the cloned *BjGSTF12* gene sequences were identical, suggesting that the effect on anthocyanin accumulation may occur at the transcriptional level. We isolated the *BjGSTF12* promoter sequences from both lines, finding 99.78% similarity with four single-base mutations/insertions in the purple-stalk mustard promoter (Figure 8). Cis-acting element prediction revealed identical element types and numbers in both promoters, including MYB binding sites, gibberellin response elements, anaerobic induction elements, and wound/defense response elements (Figure 9). Previous studies have shown that GST genes are regulated by MYB transcription factors to promote anthocyanin accumulation (Hu et al., 2016; Zhang et al., 2023). In rapeseed, *GSTF12* co-expresses with multiple MYB transcription factors including MYB5, MYB56, MYB61, and MYB118 (Wei et al., 2019). Goswami et al. (2018) demonstrated that *TT19 (GSTF12)* co-regulates anthocyanin synthesis and accumulation with MYB111 and TT8 in high-anthocyanin resynthesized *Brassica napus*. Additionally, the *BjGSTF12* promoter contains hormone and environmental response elements besides MYB binding sites. However, the relationship between these promoter mutations/insertions and expression differences between green-stalk

and purple-stalk mustard requires further investigation.

STRING database analysis of the BjGSTF12 interaction network revealed interactions with key anthocyanin biosynthetic enzymes (DFR, TT4/CHS, LDOX, UF3GT), transport proteins (TT12, AHA10), and modification enzymes (AT5MAT, AT4G14090, AT1G03495). However, GST itself does not directly catalyze anthocyanin biosynthesis but rather functions as a carrier for vacuolar transport (Sun et al., 2012). Thus, BjGSTF12 may regulate anthocyanin biosynthesis, modification, and transport through protein interactions, thereby influencing anthocyanin accumulation. Further experimental evidence is needed to support this hypothesis. Future research will focus on subcellular localization, functional validation, and screening of upstream transcription factors through yeast library construction to elucidate the biological function and mechanism of GST in anthocyanin accumulation in mustard stalks.

In summary, we cloned and analyzed the *BjGSTF12* gene and promoter sequences from green-stalk and purple-stalk mustard. The results indicate that *BjGSTF12* belongs to the phi subfamily of plant GST genes. Although the promoter sequences contain only a few base mutations/insertions and identical cis-acting elements, *BjGSTF12* expression differs significantly between green-stalk and purple-stalk mustard, correlating with anthocyanin content differences. This suggests that *BjGSTF12* is a functional gene that may play an important role in anthocyanin accumulation in mustard stalks, providing a candidate gene for future studies on anthocyanin accumulation mechanisms in mustard. However, the specific regulatory mechanism of BjGSTF12 in anthocyanin accumulation requires further investigation.

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Note: Figure translations are in progress. See original paper for figures.