

## Article

# Comparative Analysis of *SUS* in Chinese Cabbage, Black Mustard, and Cabbage, and Their Diurnal Expression Response to Light

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## Highlights

### What are the main findings?

- We identified the members of the *SUS* gene family in *Brassica rapa*, *B. nigra*, and *B. oleracea*, and revealed the differences among the three in terms of protein physicochemical properties, phylogeny, subfamily conserved domains, interspecies collinearity, as well as KEGG and GO analyses, using bioinformatics analysis techniques.
- We have revealed that in the Yu's triangle, the AA genome of the *SUS* gene family has a higher similarity with the CC genome, while the similarity with the BB genome is lower.
- We analyzed the response of *Brassica rapa SUS* to different light intensities and found that high light intensity enhanced the rhythmic expression of the *SUS* gene family in *Brassica rapa*.

### What are the implications of the main findings?

- The comparative analysis of the *SUS* gene in *Brassica rapa*, *Brassica nigra*, and *Brassica oleracea* has laid a theoretical foundation for in-depth studies on the evolutionary relationship of the *SUS* gene in cruciferous plants.
- This will lay the foundation for deciphering the molecular regulatory mechanism by which the *SUS* gene affects flowering through its influence on sucrose synthesis.



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## Abstract

Sucrose synthase plays a crucial role in sucrose metabolism and flower regulation. By using bioinformatics techniques, 13, 16 and 13 *SUS* members were identified in *Brassica rapa*, *Brassica nigra*, and *Brassica oleracea*, respectively. The *SUS* family can be divided into three subfamilies. The *SUS II* subfamily has three *B. nigra* genes with significantly reduced motif types. The *SUS III* subfamily has fewer motif 2 in *Arabidopsis thaliana* compared to the other species. The interspecific collinearity analysis revealed a “one-to-one, many-to-one” relationship between species. KEGG revealed that *Brassica nigra* showed significantly higher enrichment in the metabolism pathway, while *Brassica rapa* and *Brassica oleracea*

exhibited higher significance in carbohydrate metabolism. GO indicated that *Brassica nigra* had more enriched biological process pathways than the other two species. It reveals that *SUS* has a higher degree of similarity between the AA genome and the CC genome, while its similarity to the BB genome is lower. The analysis of the response of *BraSUSs* to different light intensities revealed that the differentially expressed genes *BraA07g028740.3C* and *BraA07g037520.3C* had enhanced expression rhythms under high light intensity. Using three diploid basal species with different flowering traits (Chinese cabbage, cabbage, and black mustard) as experimental materials, we conducted a comparative analysis of the *SUS* gene family. This will lay the foundation for deciphering the molecular regulatory mechanism by which the *SUS* gene affects flowering through its influence on sucrose synthesis.

**Keywords:** *Brassica*; Chinese cabbage; comparative genomics; light intensity; sucrose synthase gene family

## 1. Background

SUS (Sucrose synthase) is a glycosyltransferase widely present in plants, where it exerts a central role in sucrose synthesis and decomposition [1–3]. Notably, SUS is the only reversible key enzyme in sucrose metabolism [4,5], catalyzing the conversion of UDP (uridine diphosphate) and sucrose into UDP-Glc (uridine diphosphate glucose) and fructose [6]. Beyond this catalytic role, SUS is also involved in cellulose, callose, and starch synthesis, sucrose transport, regulation of flowering, and responses to oxidative stress and abiotic stresses such as drought, low temperature, and high salinity [1,7,8]. In plants, *SUS* genes generally occur as small gene families [9]. Previous studies reported that most fruits, vegetables, and crops contain five to six *SUS* members [10–14]; and 14 family members were identified the black mustard (*Brassica juncea*) within the *Brassicaceae* family [15].

The circadian clock is an endogenous timing mechanism that enables plants to “anticipate” periodic changes in external environmental factors like light, temperature, and humidity, maintaining synchronization between internal and external life processes [16–18]. The plant biological clock consists of three parts: the input pathway, the core oscillator, and the output pathway [19]. Light, as a major external environmental factor in the input pathway, not only drives photosynthesis but also serves as a crucial cue for circadian regulation. Sugar metabolism, a downstream product of photosynthesis, is tightly regulated by circadian rhythms and, in turn, provides feedback to the clock [20]. Studies have shown that the rhythmicity of photosynthesis and endogenous sugar signals can regulate the expression of *PRR7* (*Pseudo-response regulator 7*) to modulate the circadian rhythm in *A. thaliana* [21]. Sucrose has been identified as a potential zeitgeber in the clock input pathway, directly regulating the expression of the circadian gene *GI* (*Gigantea*) [22]. Chincinska et al. found that *SUT4* (*Sucrose transporter 4*) influences diurnal gene expression by regulating sucrose export from leaves [23].

Light is characterized by attributes such as light intensity, light quality and light wavelength [19]. Studies have shown that in the light intensity range of  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  to  $900 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , the key gene *PnSPS* for sucrose–starch conversion in black pepper (*Piper nigrum* L.) is upregulated, while *PnSUS* is downregulated to maintain the homeostasis of sucrose/starch in leaves, and this homeostasis is related to the downregulation of the flowering-inhibiting gene *PnFLC* and the upregulation of the flowering gene *PnFT*, jointly promoting the induction of inflorescences [24]. Chen et al.’s research demonstrated that alternating red and blue light alters both the activity and gene expression of SPS (sucrose phosphate synthase) and SUS in butterhead lettuce, influencing carbohydrate accumulation

and growth [25]. Liu et al. reported that under continuous red-blue light, low blue-light intensity enhanced yield, starch, sucrose accumulation, and the activity of key enzymes such as SPS and SUS in lettuce cultivars Yidali and Zishan [26]. However, continuous illumination, regardless of spectrum, disrupted the diurnal oscillations of carbohydrate metabolism and associated enzyme activity. Previous studies have mostly focused on the effects of red and blue light on glucose metabolism enzymes, but the impact of light intensity on the circadian rhythm of sucrose synthase has not been systematically explored.

Chinese cabbage (*B. rapa* L. ssp. *pekinensis*, AA, n = 10) is a fundamental vegetable crop in the *Brassicaceae* family's *Brassica* genus "Yu's triangle", along with black mustard (*B. nigra*, BB, n = 8) and cabbage (*B. oleracea* var. *capitata*, CC, n = 9) as the three basic diploid species of *Brassica*. The three are rich in nutrients such as sugars, cellulose and various vitamins, and hold considerable nutritional and economic importance [27–29]. However, the flowering mechanisms of the three species are different, and the requirements for vernalization conditions and light conditions for inducing flowering are also different: black mustard can flower without vernalization; cabbage and Chinese cabbage both require vernalization to flower. Cabbage is a green body vernalization plant, while Chinese cabbage can sense vernalization as soon as its seed bud germinates [30]. Previous studies have found that in the *Brassica* genus, pak choi (*Brassica rapa* ssp. *chinensis* Makino) shows enhanced SPS and SUS activities during the flower transition period, and the increase in sucrose synthesis is greater than that in sucrose metabolism, indicating that the sucrose synthesis process is significantly enhanced during the flower transition [31]. However, the comparative study of the SUS gene family in the diploid basic species of the *Brassica* genus, such as Chinese cabbage, with black mustard and cabbage has not been reported yet.

This study adopted bioinformatics to identify members of the SUS gene family in Chinese cabbage, black mustard and cabbage. At the comparative genomic level, the evolutionary relationship of the *Brassica* SUS gene family was analyzed by comparing the physicochemical properties of SUS proteins in Chinese cabbage and black mustard and cabbage, the differences in conserved domains of SUS gene subfamilies, and the collinearity between Chinese cabbage and species. By subjecting Chinese cabbage to different light intensity treatments and analyzing the rhythmic expression patterns of the SUS gene family at the transcriptome level, this study lays a foundation for further investigating how SUS genes in the *Brassica* genus influence flowering mechanisms.

## 2. Materials and Methods

### 2.1. Data Sources and Research Materials

Genome and proteome sequences of Chinese cabbage, black mustard, and cabbage were downloaded from the BRAD database (<http://brassicadb.cn/>, accessed on 14 August 2024) [32–34], with Chinese cabbage represented by *B. rapa* L. ssp. *pekinensis*. The *A. thaliana* genome and proteome sequences were obtained from the TAIR database (<https://www.arabidopsis.org/>, accessed on 14 August 2024). The high-generation pure and self-crossing line Chinese cabbage 'A03' was selected as the research material for gene expression analysis. The research material was provided by the key laboratory of innovation and utilization of vegetable germplasm in Hebei province.

### 2.2. Identification of SUS Gene Family Members

The retrieved data were used to construct genome and proteome databases. SUS genes were screened using two complementary methods: (1) Using the domain of sucrose synthase (Sucrose-synth, PF00862) as the search model, the HMM Search function in the TBtools software (TBtools-II version 2.486) was used to search for sequences containing this domain in the protein database of the corresponding species. (2) The BLASTP program was

used with *AT4G02280* (*SUS3*) as the query sequence. Candidate sequences were merged, and redundancies were eliminated after verification in the InterPro database (version108; <https://www.ebi.ac.uk/interpro/>, accessed on 18 August 2024), yielding the final *SUS* gene family members.

### 2.3. Physicochemical Properties and Phylogenetic Analysis of *SUS* Gene Family Proteins

The physicochemical properties of the obtained *SUS* protein sequence were analyzed using “Protein Parameter Calc” in the TBtools software. Multiple sequence alignment was performed using “ClustalW” in MEGA11 (Version 11.0.13), and an evolutionary tree was constructed using “Phylogenetic Analysis” (evolutionary model: amino acid substitution model; statistical method: Maximum Likelihood; No. of Bootstrap Replications: 1000). Finally, the evolutionary tree was beautified using Figtree (Version 1.4.4).

### 2.4. Analysis of Conserved Domains of the *SUS* Gene Family

*SUS* protein sequences were submitted to the MEME suite (Version 5.5.9; <https://meme-suite.org/>, accessed on 3 September 2024) to identify conserved motifs, and use “Visualize Motif Pattern” in the TBtools software for visualization.

### 2.5. Collinearity Analysis Among Species of the *SUS* Gene Family

Collinearity analysis of the *SUS* gene family among Chinese cabbage, black mustard, cabbage, and *A. thaliana* was performed using the “MCscanX” function in the TBtools software.

### 2.6. Functional Enrichment Analysis of *SUS* Gene Families in Chinese Cabbage, Black Mustard and Cabbage

After performing gene function annotation for different species using eggNOG-mapper (<http://eggno-mapper.embl.de/>, accessed on 12 October 2024), the KEGG and GO functional enrichment analysis of the *SUS* gene family members in Chinese cabbage, black mustard, and cabbage was conducted using the “eggNOG-mapper Helper”, “KEGG Enrichment analysis” and “GO Enrichment” functions in the TBtools software. The “Plants.20211128.TBtoolsKeggBackEnd” file was used as the BackGround file for the KEGG analysis.

### 2.7. Transcriptome Analysis of Chinese Cabbage Under Low Light Intensity and High Light Intensity, and Analysis of the Expression Pattern of the *SUS* Gene

Light intensity settings: Low light intensity was  $125 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ; high light intensity was  $180 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

Sampling method: The seeds of the Chinese cabbage variety ‘A03’ (WT) were sown in  $2 \text{ cm} \times 4 \text{ cm}$  (diameter  $\times$  height) hollow cylindrical tubes filled with a substrate mixture (composed of imported peat, coconut coir, perlite, vermiculite and bio-fertilizer). The tubes were placed in a constant temperature environment of  $22^\circ\text{C}$ , with a humidity of 50%, and subjected to a 12 h dark and 12 h light daily cycle. The cultivation was carried out from the time when the cotyledons emerged, and different light intensity treatments were applied for one week. Then, the light intensity was changed to 24 h of full light. At 6 o’clock on the fourth day of full light, samples from the low-light-intensity group and the high-light-intensity group were collected, denoted as LW1 (WT under low light intensity) and HW1 (WT under high light intensity). Samples were taken every 6 h for a total of 8 times, each time including all leaves except the cotyledons. Three biological replicates were set up to obtain the RNA-Seq data of the Chinese cabbage.

The transcriptome data of this study was sequenced and libraryed by BioMarker Technologies Co., Ltd. (BioMarker Technologies Co., Ltd., Beijing, China) Total RNA

was extracted using the TianGen Automated RNA Extraction Kit (TianGen Biochemical Technology Co., Ltd., Beijing, China.) (DP862-T3 Automated Extraction—Reduced Volume). The purity, concentration, and integrity of the RNA samples were tested using advanced molecular biology equipment to ensure the use of qualified samples for transcriptome sequencing. Subsequently, a standard cDNA library was constructed. After the library construction was completed, the concentration and insert fragment size of the library were detected using Qubit 2.0 and Agilent 2100(BioMarker Technologies Co., Ltd., Beijing, China), and the effective concentration of the library was accurately quantified using the Q-PCR method to ensure the quality of the library. Sequencing was performed on the BGI T7 (BGI Genomics, Shenzhen, China) platform using 150 base pairs (PE150) for reading. Quality control of the raw reads was performed using an internal Perl script (BioMarker Technologies Co., Ltd., Beijing, China). Then, the reference genome ([www.bioinformatics.lab.cn/EMSMutation/home](http://www.bioinformatics.lab.cn/EMSMutation/home), accessed on 5 March 2025) was aligned using HISAT2 v2.1.0, and the gene expression level was quantified using the FPKM (Fragments Per Kilobase of exon model per Million mapped fragments) method. The differentially expressed genes (DEGs) were identified using the DESeq2R package v1.12.4 (with a *p*-adjustment < 0.05 and an absolute value of log2FoldChange > 1 as the two criteria). In this study, the expression abundance of the Chinese cabbage *SUS* gene was represented by FPKM values, and an expression heatmap of the Chinese cabbage *SUS* gene was made using the Tbttools software.

3. Results

3.1. Identification Results of SUS Gene Family Members

Through double screening, members of the *SUS* gene family were identified, 13 *SUS* genes in Chinese cabbage (*BraSUSs*), 16 in Black mustard (*BniSUSs*), 13 in Cabbage (*BolSUSs*), and 10 *SUS* genes in *A. thaliana*, which together give rise to 17 gene transcripts (*ATSUSs*) (Table 1).

Table 1. Members of the *SUS* gene family.

| <i>A. thaliana</i>            | Chinese Cabbage<br>( <i>B. rapa</i> L. ssp. <i>pekinensis</i> ) | Black Mustard ( <i>B. nigra</i> ) | Cabbage ( <i>B. oleracea</i> var. <i>capitata</i> ) |
|-------------------------------|-----------------------------------------------------------------|-----------------------------------|-----------------------------------------------------|
| <i>AT1G04920</i>              | <i>BraA02g028970.3C</i>                                         | <i>BniB01g051680.2N.1</i>         | <i>BolC02g041410.2J</i>                             |
| <i>AT1G73370</i> <sup>2</sup> | <i>BraA03g004560.3C</i>                                         | <i>BniB02g042090.2N.1</i>         | <i>BolC03g005210.2J</i>                             |
| <i>AT3G43190</i>              | <i>BraA03g008480.3C</i>                                         | <i>BniB02g042490.2N.1</i>         | <i>BolC03g010920.2J</i>                             |
| <i>AT4G02280</i>              | <i>BraA03g009830.3C</i>                                         | <i>BniB02g051170.2N.1</i>         | <i>BolC05g003020.2J</i>                             |
| <i>AT4G10120</i> <sup>3</sup> | <i>BraA05g015010.3C</i>                                         | <i>BniB02g070170.2N.1</i>         | <i>BolC05g058110.2J</i>                             |
| <i>AT5G11110</i>              | <i>BraA06g032610.3C</i>                                         | <i>BniB02g077870.2N.1</i>         | <i>BolC06g017370.2J</i>                             |
| <i>AT5G20280</i>              | <i>BraA06g034940.3C</i>                                         | <i>BniB03g038950.2N.1</i>         | <i>BolC06g032260.2J</i>                             |
| <i>AT5G20830</i>              | <i>BraA07g028740.3C</i>                                         | <i>BniB03g051760.2N.1</i>         | <i>BolC06g044870.2J</i>                             |
| <i>AT5G37180</i> <sup>3</sup> | <i>BraA07g037520.3C</i>                                         | <i>BniB05g043140.2N.1</i>         | <i>BolC07g038200.2J</i>                             |
| <i>AT5G49190</i>              | <i>BraA09g001880.3C</i>                                         | <i>BniB05g058730.2N.1</i>         | <i>BolC07g041050.2J</i>                             |
|                               | <i>BraA10g003470.3C</i>                                         | <i>BniB05g060610.2N.1</i>         | <i>BolC09g001700.2J</i>                             |
|                               | <i>BraA10g019840.3C</i>                                         | <i>BniB06g045830.2N.1</i>         | <i>BolC09g051640.2J</i>                             |
|                               | <i>BraA10g020290.3C</i>                                         | <i>BniB06g060740.2N.1</i>         | <i>BolC09g052140.2J</i>                             |
|                               |                                                                 | <i>BniB08g004970.2N.1</i>         |                                                     |
|                               |                                                                 | <i>BniB08g010650.2N.1</i>         |                                                     |
|                               |                                                                 | <i>BniB08g044840.2N.1</i>         |                                                     |

Note: *AT1G73370*<sup>2</sup> indicates that there are two transcripts of this gene; *AT4G10120*<sup>3</sup> indicates that there are three transcripts of this gene.

### 3.2. Comparative Analysis of Physicochemical Properties of Chinese Cabbage SUS with Black Mustard and Cabbage Proteins

A comparison of the physicochemical properties of Chinese cabbage SUS with that of black mustard, cabbage, and *A. thaliana* proteins reveals (Table 2): In terms of amino acid count, Chinese cabbage had the smallest number of amino acids, intermediate between black mustard and cabbage, and all smaller than *A. thaliana*. For example, *BraA03g008480.3C* encoded 181 amino acids, *BniB05g060610.2N.1* encoded 120, and *BolC05g058110.2J* encoded 368, while the smallest *A. thaliana* gene (*AT5G37180.3*) encoded 591. With respect to molecular weight, the smallest SUS protein in Chinese cabbage was intermediate between black mustard and cabbage, yet it remained lighter than the smallest *A. thaliana*. Specifically, *BraA03g008480.3C* weighed 21,205.62 ku, *BniB05g060610.2N.1* weighed 14,049.3 ku, *BolC05g058110.2J* weighed 42,742.68 ku, and the smallest *A. thaliana* SUS protein weighed 67,451 ku (*AT5G37180.3*). Regarding theoretical PI, most SUS proteins were acidic. However, black mustard harbored the highest number of alkaline SUS proteins. Only one alkaline SUS protein was detected in Chinese cabbage (*BraA03g008480.3C*) and in cabbage (*BolC06g032260.2J*); *A. thaliana* contained two (*AT5G37180* and *AT1G73370*). By contrast, four alkaline SUS proteins were found in black mustard (*BniB03g038950.2N.1*, *BniB01g051680.2N.1*, *BniB05g060610.2N.1*, *BniB02g051170.2N.1*). The instability index showed limited variation across the four species, ranging from 33.38 (*BniB03g038950.2N.1*) to 58.43 (*BniB05g060610.2N.1*). One exception was *BraA03g008480.3C*, which exhibited an exceptionally high instability index of 88.47. The aliphatic index of SUS proteins ranged from 81.93 (*BraA03g008480.3C*) to 98.17 (*BniB02g051170.2N.1*), all below 100, with no significant differences. The grand average of hydropathicity was negative for all SUS proteins, ranging from  $-0.575$  (*BolC05g058110.2J*) to  $-0.17$  (*AT5G49190*), indicating hydrophilicity.

**Table 2.** Physicochemical properties of SUS gene family members.

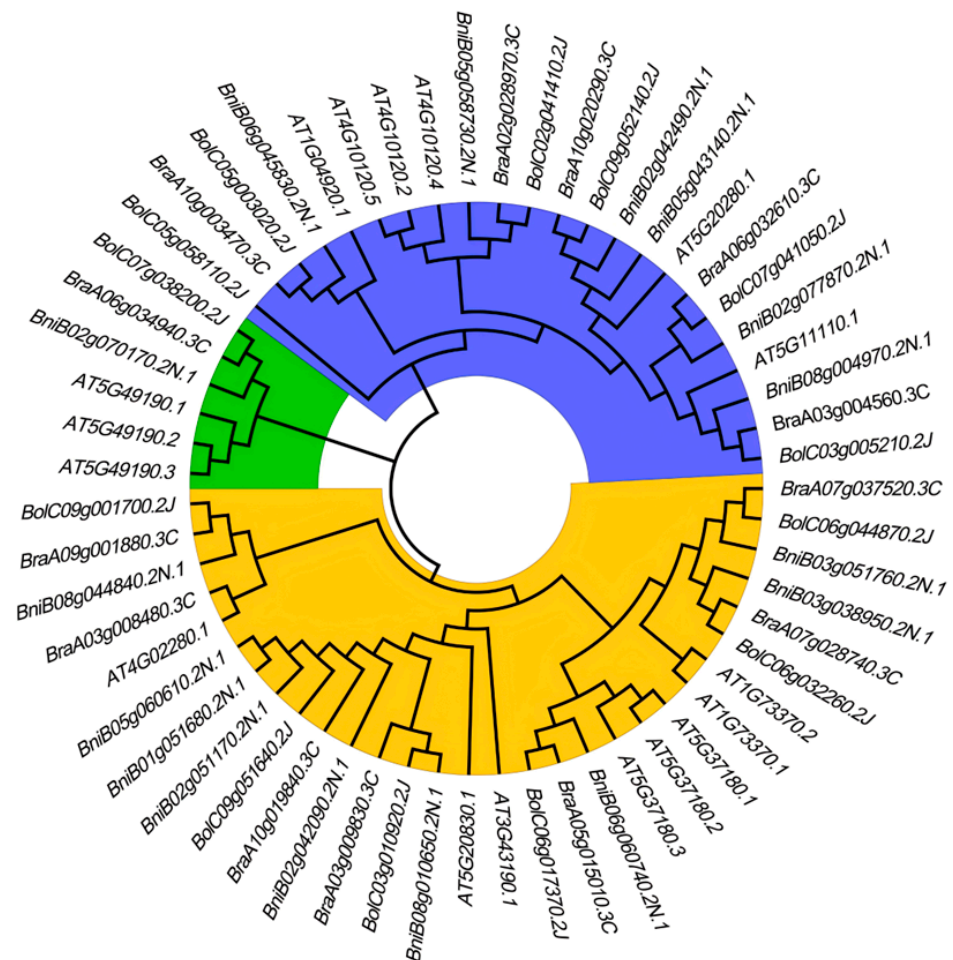
| Sequence ID             | Number of Amino Acid | Molecular Weight/Da | Theoretical PI | Instability Index | Aliphatic Index | Grand Average of Hydropathicity |
|-------------------------|----------------------|---------------------|----------------|-------------------|-----------------|---------------------------------|
| <i>AT1G04920.1</i>      | 1062                 | 119,475.78          | 5.91           | 46.21             | 85.83           | −0.478                          |
| <i>AT1G73370.1</i>      | 942                  | 106,875.57          | 8.13           | 36.91             | 84.56           | −0.391                          |
| <i>AT1G73370.2</i>      | 898                  | 102,026.91          | 6.98           | 34.26             | 86.2            | −0.384                          |
| <i>AT3G43190.1</i>      | 808                  | 93,002.67           | 6.12           | 33.51             | 92.67           | −0.3                            |
| <i>AT4G02280.1</i>      | 809                  | 92,002.08           | 5.85           | 42.06             | 88.31           | −0.276                          |
| <i>AT4G10120.2</i>      | 1050                 | 118,878.35          | 6.23           | 47.18             | 88.91           | −0.46                           |
| <i>AT4G10120.4</i>      | 876                  | 98,332.34           | 6.17           | 48.32             | 92.24           | −0.331                          |
| <i>AT4G10120.5</i>      | 849                  | 95,082.59           | 6.17           | 47.6              | 92.07           | −0.327                          |
| <i>AT5G11110.1</i>      | 1047                 | 116,966.02          | 6.18           | 42.81             | 86.98           | −0.438                          |
| <i>AT5G20280.1</i>      | 1043                 | 117,320.99          | 6              | 46.71             | 84.22           | −0.471                          |
| <i>AT5G20830.1</i>      | 808                  | 92,997.62           | 5.83           | 34.58             | 92.91           | −0.333                          |
| <i>AT5G37180.1</i>      | 837                  | 95,034.46           | 6.13           | 36.23             | 86.2            | −0.323                          |
| <i>AT5G37180.2</i>      | 649                  | 74,074.63           | 7.62           | 40.01             | 84.45           | −0.34                           |
| <i>AT5G37180.3</i>      | 591                  | 67,451              | 8.36           | 40.79             | 85.8            | −0.339                          |
| <i>AT5G49190.1</i>      | 807                  | 92,064.11           | 5.7            | 38.56             | 91.95           | −0.241                          |
| <i>AT5G49190.2</i>      | 797                  | 90,765.02           | 5.79           | 37.96             | 95.16           | −0.17                           |
| <i>AT5G49190.3</i>      | 741                  | 84,462.43           | 5.7            | 38.7              | 92.38           | −0.23                           |
| <i>BraA02g028970.3C</i> | 1025                 | 115,817.9           | 6.22           | 44.98             | 87.09           | −0.464                          |
| <i>BraA03g004560.3C</i> | 1039                 | 116,309.6           | 6.08           | 44.76             | 88.95           | −0.392                          |
| <i>BraA03g008480.3C</i> | 181                  | 21,205.62           | 9.95           | 88.47             | 81.93           | −0.318                          |
| <i>BraA03g009830.3C</i> | 806                  | 92,477.14           | 5.76           | 35.98             | 94.09           | −0.311                          |
| <i>BraA05g015010.3C</i> | 837                  | 95,075.54           | 6.15           | 35.78             | 87.49           | −0.337                          |
| <i>BraA06g032610.3C</i> | 1072                 | 119,930.4           | 6.55           | 45.84             | 87.39           | −0.433                          |

Table 2. Cont.

| Sequence ID        | Number of Amino Acid | Molecular Weight/Da | Theoretical PI | Instability Index | Aliphatic Index | Grand Average of Hydropathicity |
|--------------------|----------------------|---------------------|----------------|-------------------|-----------------|---------------------------------|
| BraA06g034940.3C   | 807                  | 91,991.01           | 5.94           | 37.93             | 90.84           | −0.237                          |
| BraA07g028740.3C   | 940                  | 106,392.89          | 6.94           | 33.46             | 83.7            | −0.376                          |
| BraA07g037520.3C   | 943                  | 107,044.64          | 6.42           | 38.5              | 83.01           | −0.37                           |
| BraA09g001880.3C   | 811                  | 92,336.53           | 5.83           | 41.26             | 88.82           | −0.274                          |
| BraA10g003470.3C   | 1049                 | 117,617.54          | 5.77           | 46.16             | 85.97           | −0.443                          |
| BraA10g019840.3C   | 805                  | 92,207.77           | 5.8            | 35.38             | 93.48           | −0.299                          |
| BraA10g020290.3C   | 1066                 | 119,980.32          | 6              | 43.74             | 86.87           | −0.407                          |
| BniB01g051680.2N.1 | 180                  | 21,155.6            | 8.4            | 51.75             | 95.17           | −0.331                          |
| BniB02g042090.2N.1 | 805                  | 92,318.94           | 5.85           | 34.71             | 94.32           | −0.3                            |
| BniB02g042490.2N.1 | 1043                 | 117,233.78          | 5.98           | 43.83             | 84.41           | −0.466                          |
| BniB02g051170.2N.1 | 230                  | 26,427.91           | 9.01           | 50.39             | 98.17           | −0.25                           |
| BniB02g070170.2N.1 | 807                  | 92,087.38           | 6.09           | 37.16             | 91.69           | −0.22                           |
| BniB02g077870.2N.1 | 1047                 | 116,901.08          | 6.69           | 43.82             | 87.34           | −0.437                          |
| BniB03g038950.2N.1 | 935                  | 105,934.8           | 8.11           | 33.38             | 84.47           | −0.353                          |
| BniB03g051760.2N.1 | 938                  | 106,506.87          | 6.21           | 38.5              | 82              | −0.383                          |
| BniB05g043140.2N.1 | 1042                 | 117,238.64          | 5.83           | 41.71             | 84.4            | −0.467                          |
| BniB05g058730.2N.1 | 1033                 | 116,950.16          | 5.98           | 45.12             | 88.38           | −0.448                          |
| BniB05g060610.2N.1 | 120                  | 14,049.3            | 8.67           | 58.43             | 83.58           | −0.377                          |
| BniB06g045830.2N.1 | 1065                 | 119,454.59          | 5.7            | 45.18             | 87.23           | −0.446                          |
| BniB06g060740.2N.1 | 846                  | 96,270              | 6.37           | 37.31             | 88.87           | −0.328                          |
| BniB08g004970.2N.1 | 1040                 | 116,345.65          | 6.25           | 44                | 89.06           | −0.404                          |
| BniB08g010650.2N.1 | 843                  | 96,939.72           | 6.26           | 35.06             | 95.75           | −0.252                          |
| BniB08g044840.2N.1 | 882                  | 100,558             | 6.08           | 39.86             | 87.74           | −0.3                            |
| BolC02g041410.2J   | 1032                 | 116,568.92          | 6.3            | 44.28             | 87.54           | −0.453                          |
| BolC03g005210.2J   | 1038                 | 115,963.29          | 6.21           | 42.58             | 88.95           | −0.389                          |
| BolC03g010920.2J   | 806                  | 92,470.04           | 5.67           | 34.45             | 94.09           | −0.308                          |
| BolC05g003020.2J   | 1065                 | 119,583.73          | 5.74           | 46.5              | 86.14           | −0.454                          |
| BolC05g058110.2J   | 368                  | 42,742.68           | 6.19           | 45.98             | 83.67           | −0.575                          |
| BolC06g017370.2J   | 837                  | 95,139.67           | 6.18           | 34.9              | 87.84           | −0.334                          |
| BolC06g032260.2J   | 940                  | 106,523.07          | 7.2            | 34.42             | 82.66           | −0.382                          |
| BolC06g044870.2J   | 934                  | 106,248.74          | 6.24           | 36.96             | 83.39           | −0.363                          |
| BolC07g038200.2J   | 893                  | 102,196.89          | 6.54           | 39.64             | 89.73           | −0.238                          |
| BolC07g041050.2J   | 1047                 | 116,762.8           | 6.33           | 44.13             | 87.6            | −0.428                          |
| BolC09g001700.2J   | 811                  | 92,412.63           | 5.85           | 41.52             | 88.69           | −0.281                          |
| BolC09g051640.2J   | 805                  | 92,300.94           | 5.77           | 36.34             | 94.09           | −0.296                          |
| BolC09g052140.2J   | 1042                 | 117,466.14          | 6              | 43.31             | 84.94           | −0.467                          |

### 3.3. Phylogenetic Analysis of the SUS Gene Family

A phylogenetic tree was constructed using 59 SUS protein sequences from Chinese cabbage, black mustard, cabbage and *A. thaliana*. The analysis divided the SUS members into three subfamilies (Figure 1): among them, SUS I included five Chinese cabbage SUS gene members, black mustard and cabbage each contained six SUS gene members, and four *A. thaliana* SUS gene members; SUS II includes seven cabbage SUS gene members, nine black mustard SUS gene members, six cabbage SUS gene members and five *A. thaliana* SUS gene members, with black mustard having the largest number of SUS gene family members; SUS III consists of one cabbage SUS gene member, one black mustard SUS gene member, one cabbage SUS gene member and three *A. thaliana* SUS gene members.



**Figure 1.** Phylogenetic tree of the *SUS* gene family. Note: *SUS I* is the blue area; *SUS II* is the yellow area; *SUS III* is the green area.

### 3.4. Analysis of Conserved Motifs Between *SUS* Gene Subfamilies

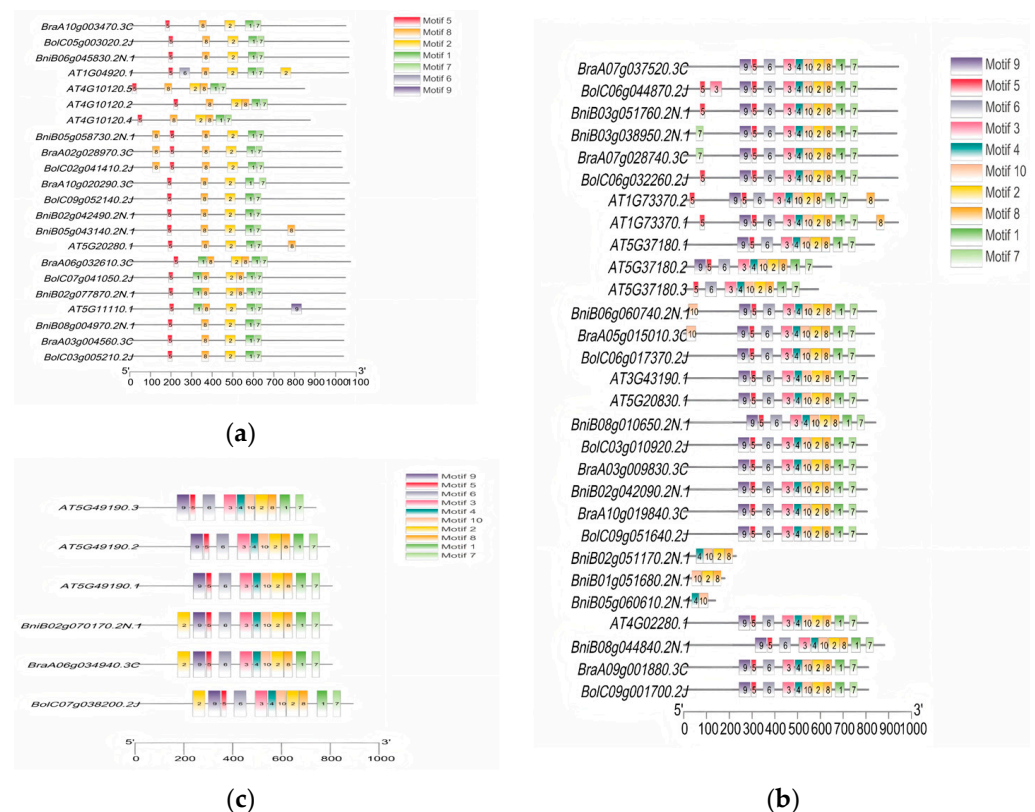
Conserved motifs of *SUS* proteins were identified using the MEME online tool, with the number of motifs constrained to 10 (Figure 2). The *SUS I* subfamily contained only seven motifs, lacking motif 3, motif 4 and motif 10 compared to the other two subfamilies. Comparative analysis across the three basic species and *A. thaliana* revealed that the *AT1G04920.1* carried an additional copy of motif 6 relative to other *SUS I* members, and the *AT5G1111.1* had more motif 9 than the other members of the *SUS I* subfamily (Figure 2a). Both Chinese cabbage and cabbage of the *SUS II* subfamily contain 10 motifs. But *AT5G37180.3* contains nine, while *BniB02g051170.2N.1*, *BniB01g051680.2N.1*, and *BniB05g060610.2N.1* had four, three, and two motifs, respectively (Figure 2b). Members of the *SUS III* subfamily all contained 10 motifs, but all *A. thaliana* *SUS III* subfamily members lacked a repetition of the motif 2 (Figure 2c).

### 3.5. Collinearity Analysis of the *SUS* Gene Family Between Chinese Cabbage and Other Species

Interspecies collinearity plots revealed the distribution of collinear relationships between Chinese cabbage and *A. thaliana*, Chinese cabbage and black mustard, and black mustard and cabbage, with *SUS* family members highlighted in blue (Figure 3). *SUS* genes were present on nearly all chromosomes, except for chromosome 2 of *A. thaliana* and chromosomes 1, 4, and 8 of Chinese cabbage. Thirteen collinear gene pairs were detected between Chinese cabbage and *A. thaliana*. Among them, *AT4G10120* and *AT5G37180* have no collinearity genes in Chinese cabbage, but there are still many cor-

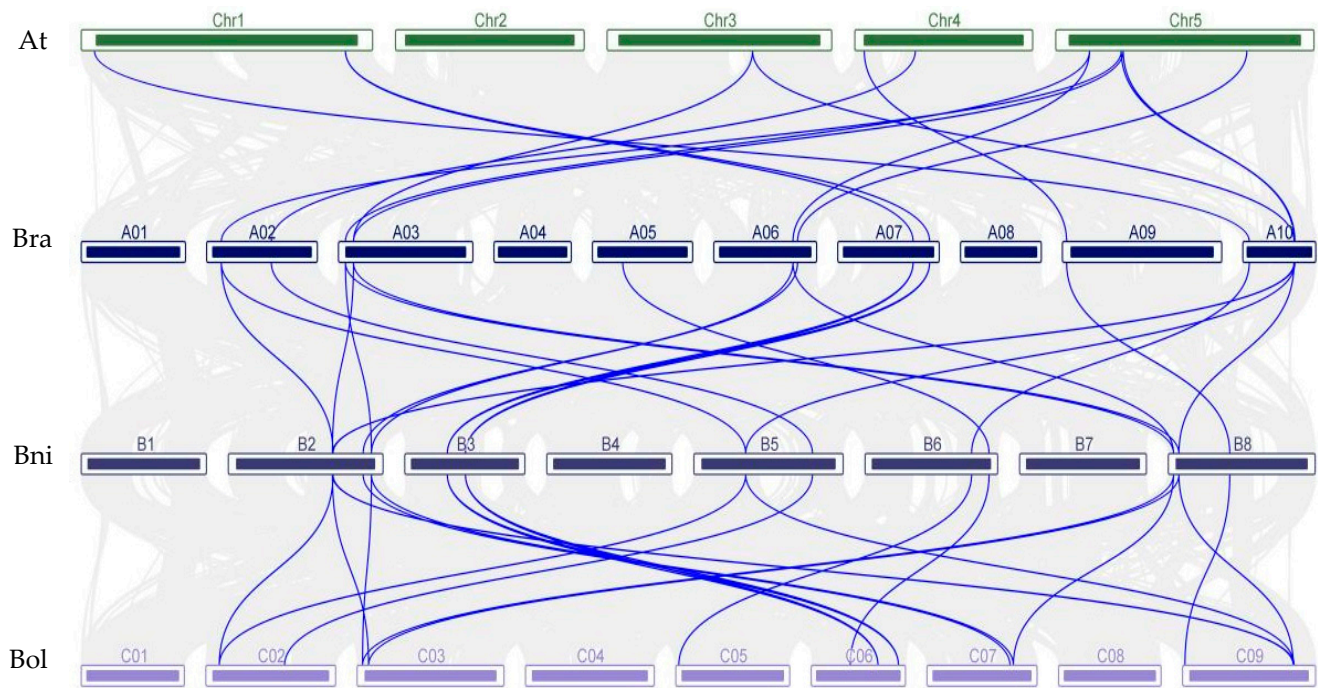
responding relationships. For example, *AT5G20280* has two pairs of collinearity relationships, *BraA02g00880.3C* and *BraA10g020290.3C*. Both *AT3G43190* and *AT5G20830* are collinear genes with *BraA10g019840.3C*, and *AT5G49190* and *BraA06g034940.3C* are collinear gene pairs.

An analysis of the collinearity between Chinese cabbage and black mustard revealed that black mustard has no *SUS* gene family members on chromosomes 4 and 7. There are 21 pairs of collinear genes between them, among which *BraA03g008480.3C*, *BniB02g051170.2N.1*, *BniB01g051680.2N.1* and *BniB05G06061.2N.1* have no corresponding collinear genes. There are multiple pairs of collinear genes: *BniB03g051760.2N.1* corresponds to two collinear genes, *BraA07g037520.3C* and *BraA07g028740.3C*. *BraA10g020290.3C* corresponds to two pairs of collinear relationships, *BniB02g042490.2N.1* and *BniB05g043140.2N.1*; *BniB05G05873.2N.1* and *BraA02g028970.3C* are collinear gene pairs.



**Figure 2.** Analysis of the conserved motifs of the *SUS* genes in *A. thaliana*, *B. rapa*, *B. nigra* and *B. oleracea*. (a) Conserved sequences of *SUS I* gene in *A. thaliana*, *B. rapa*, *B. nigra* and *B. oleracea*; (b) conserved sequences of *SUS II* gene in *A. thaliana*, *B. rapa*, *B. nigra* and *B. oleracea*; (c) conserved sequences of *SUS III* gene in *A. thaliana*, *B. rapa*, *B. nigra* and *B. oleracea*.

In addition, an analysis of collinearity between black mustard and cabbage reveals that cabbage has no *SUS* gene family members on chromosomes 1, 4, and 8. There are 21 collinear gene pairs between black mustard and cabbage. Among them, *BolC06g044870.2J* corresponds to two collinear genes, *BniB03g051760.2N.1* and *BolC09g051640.2J*. *BolC09g001700.2J* and *BniB08g044840.2N.1* are collinear gene pairs, and *BolC05g058110.2J* has no collinear gene found in black mustard. With black mustard as the mediator, there are multiple sets of collinear genes between Chinese cabbage and black mustard. For example, with *BniB03g051760.2N.1* as the mediator, there is a collinear relationship with the corresponding *BraA07g037520.3C*, *BraA07g02870.3C* and *BolC06g044870.2J*; Chinese cabbage *SUS* has homologous genes with *A. thaliana*, black mustard and cabbage.



**Figure 3.** Collinearity analysis of *B. rapa* and *A. thaliana*, *B. rapa* and *B. nigra*, and *B. nigra* and *B. oleracea* in the genome. Note: At represents *A. thaliana*; Bra represents *B. rapa*; Bni represents *B. nigra*; Bol represents *B. oleracea*. The gray lines represent the collinear relationships in the genome; the blue line represents the collinear relationship among members of the SUS gene family.

### 3.6. Functional Enrichment Analysis of the SUS Gene Family in Chinese Cabbage, Black Mustard, and Cabbage

The KEGG functional enrichment analysis of *SUS* genes in Chinese cabbage, black mustard and cabbage revealed that *SUS* genes were commonly enriched in five pathways, including starch and sucrose metabolism, carbohydrate metabolism and Glycosyltransferases (Figure 4a,c,e). However, enrichment patterns differed among species: Chinese cabbage and cabbage exhibited stronger associations with carbohydrate metabolism (Figure 4a,e), whereas black mustard showed higher significance in metabolism overall (Figure 4c).

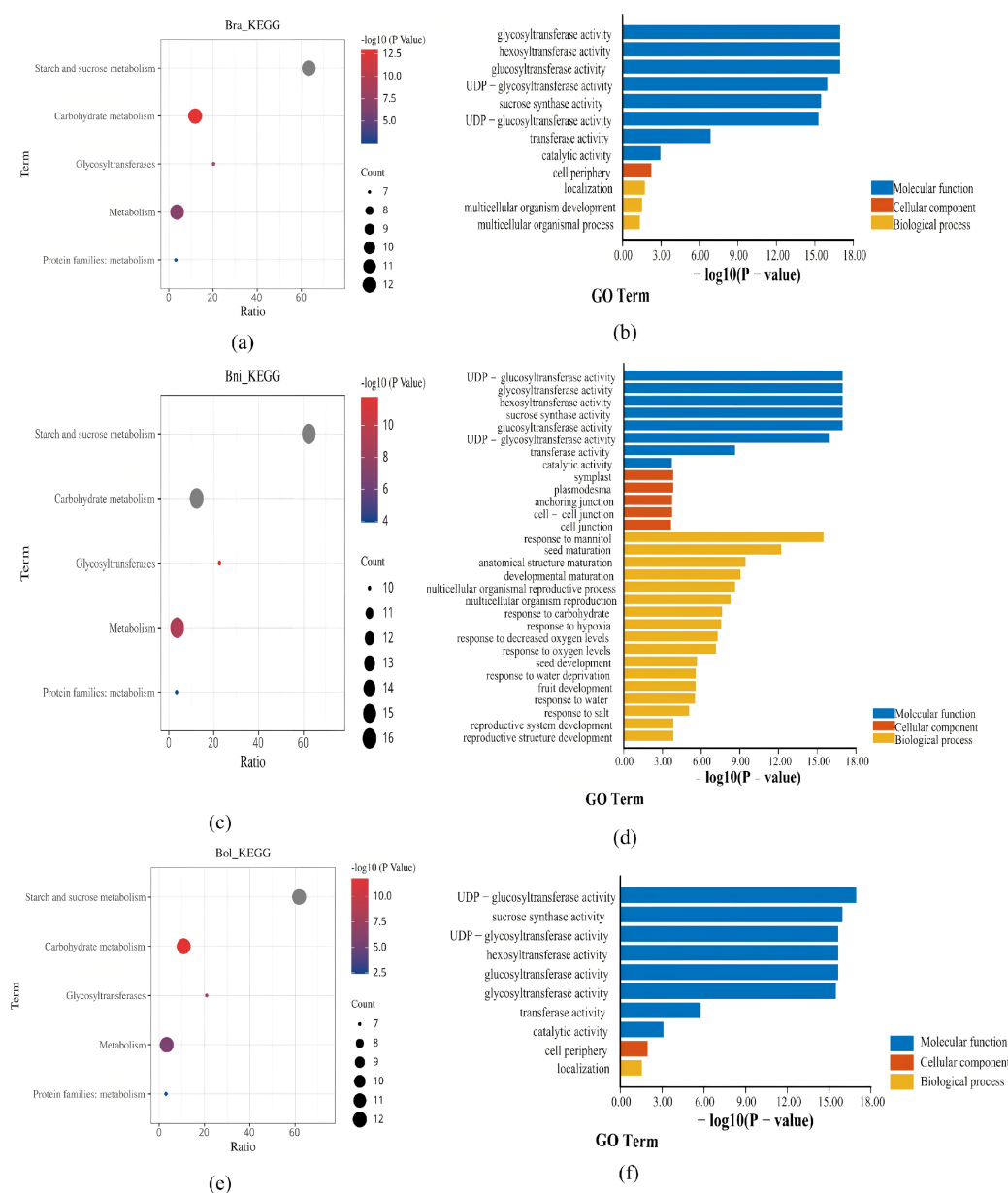
GO functional enrichment analysis of *SUS* genes in these three species indicated that black mustard had a larger number of enriched pathways in biological processes (17 pathways) compared to Chinese cabbage and cabbage (Figure 4d). By contrast, molecular function and cellular component enrichment showed little variation among the three species, with all being associated with categories such as glycosyltransferase activity and cell periphery (Figures 3 and 4b,d,f).

### 3.7. Diurnal Expression Patterns of SUS Gene in Chinese Cabbage Under Low and High Light Intensities

Analysis of the expression levels of the *SUS* gene family in Chinese cabbage under different light intensity conditions revealed the following: Under low light intensity conditions, *BraA02g028970.3C* and *BraA10g020290.3C* exhibited relatively high expression at LW3 (18:00 on day 1), LW4 (24:00 on day 1), and LW8 (24:00 on day 2), suggesting a certain degree of rhythmic expression pattern. In contrast, *BraA07g028740.3C*, *BraA05g015010.3C*, and *BraA07g037520.3C* showed similar expression profiles, with peaks at LW1 (06:00 on day 1), LW6 (12:00 on day 2), and LW8 (24:00 on day 2), though no consistent rhythmicity was observed (Figure 5a).

Under high light intensity conditions, *BraA02g028970.3C* and *BraA10g020290.3C* displayed pronounced rhythmic expression, with consistently high expression at HW3 (18:00

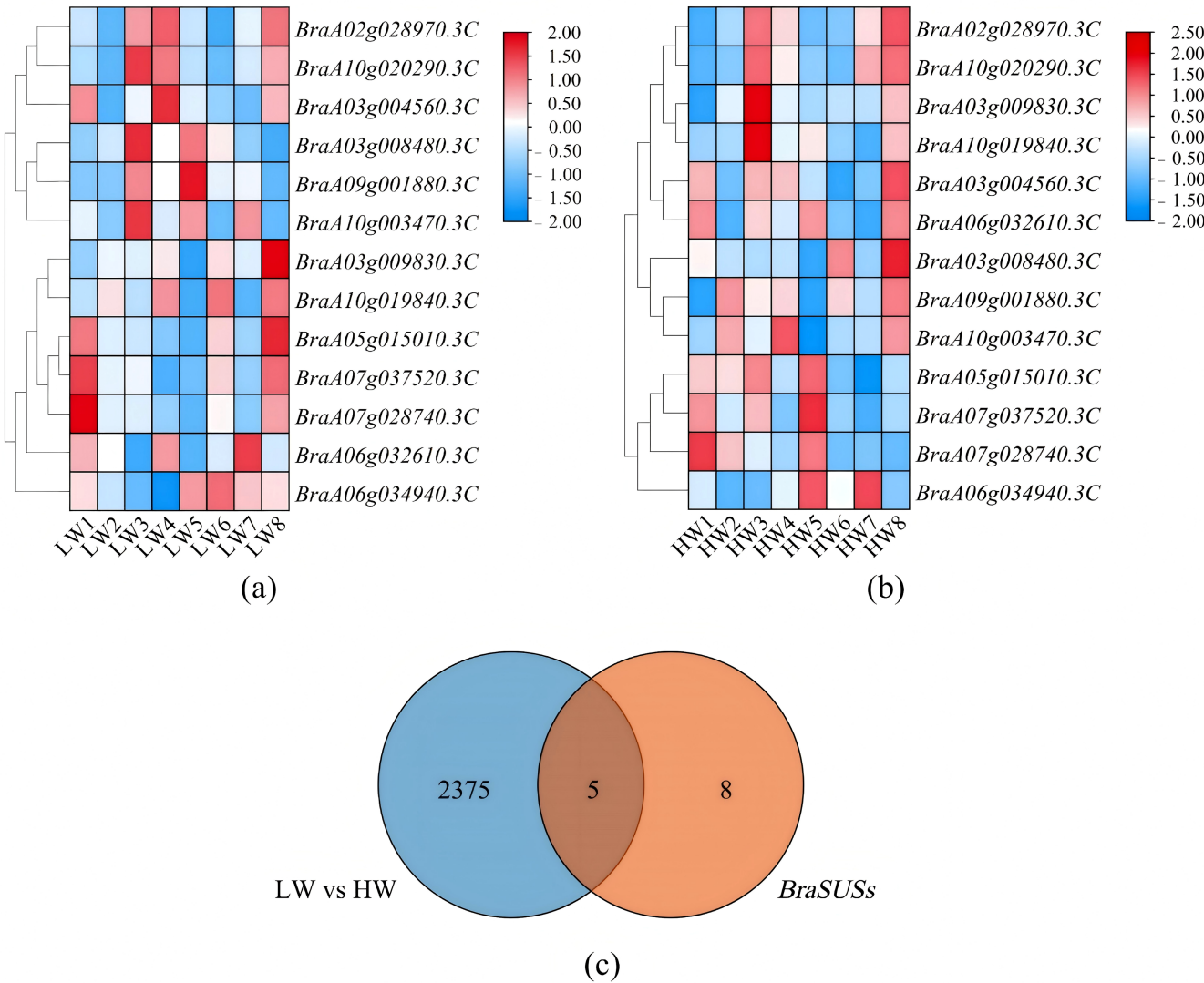
on day 1), HW4 (24:00 on day 1), HW7 (18:00 on day 2), and HW8 (24:00 on day 2). Meanwhile, *BraA07g028740.3C*, *BraA05g015010.3C*, and *BraA07g037520.3C* showed elevated expression at HW1 (06:00 on day 1) and HW5 (06:00 on day 2), indicating a rhythmic expression pattern under these conditions (Figure 5b). By analyzing the differentially expressed genes between LW and HW, it was found that among the 13 members of the *SUS* gene family identified, five showed differential expression, with HW showing upregulated expression; among them, and *BraA02g028970.3C*, *BraA07g028740.3C* and *BraA07g037520.3C* also exhibited differences in expression rhythms (Figure 5c and Table 3).



**Figure 4.** Functional enrichment analysis of the *SUS* gene family in *B. rapa*, *B. nigra* and *B. oleracea*. (a) KEGG enrichment analysis of the *SUS* gene family in *B. rapa*; (b) GO enrichment analysis of the *SUS* gene family in *B. rapa*; (c) KEGG enrichment analysis of the *SUS* gene family in *B. nigra*; (d) GO enrichment analysis of the *SUS* gene family in *B. nigra*; (e) KEGG enrichment analysis of the *SUS* gene family in *B. oleracea*; (f) GO enrichment analysis of the *SUS* gene family in *B. oleracea*.

**Table 3.** Comparison of Upregulation and Downregulation of *BraSUSs* Gene Expression Between LW and HW.

| BraID                   | HW1<br>vs.<br>LW1 | HW2<br>vs.<br>LW2 | HW3<br>vs.<br>LW3 | HW4<br>vs.<br>LW4 | HW5<br>vs.<br>LW5 | HW6<br>vs.<br>LW6 | HW7<br>vs.<br>LW7 | HW8<br>vs.<br>LW8 |
|-------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| <i>BraA02g028970.3C</i> | no   down         | no   up           | no   up           | no   down         | no   down         | yes   up          | no   up           | no   up           |
| <i>BraA03g009830.3C</i> | no   up           | yes   up          | yes   up          | yes   up          | yes   up          | no   up           | yes   up          | no   up           |
| <i>BraA07g028740.3C</i> | no   up           | no   up           | no   up           | no   up           | yes   up          | no   up           | no   up           | no   down         |
| <i>BraA07g037520.3C</i> | no   up           | no   up           | no   up           | no   up           | yes   up          | no   up           | no   up           | no   up           |
| <i>BraA10g019840.3C</i> | no   up           | no   down         | yes   up          | no   up           | no   up           | no   up           | no   up           | yes   up          |



**Figure 5.** Expression patterns of the *SUS* gene in Chinese cabbage under low light intensity and high light intensity, and analysis of differentially expressed genes between LW vs. HW, and *BraSUSs*. (a) Expression level of *SUS* gene in *B. rapa* under low light intensity; (b) expression level of *SUS* gene in *B. rapa* under high light intensity; (c) Venn diagram of the “differentially expressed genes between LW vs. HW” and the “*BraSUSs* genes”. Note: “LW” refers to “WT under low light intensity conditions”, while “HW” denotes “WT under high light intensity conditions”.

## 4. Discussion

### 4.1. Phylogenetic Analysis of the Brassica SUS Gene Family

The *SUS* gene family has been identified and studied in various plant species and is typically classified into three subfamilies [4,35,36]. Both *A. thaliana* and species within the genus *Brassica* belong to the *Brassicaceae* family, and Chinese cabbage, black mustard, and cabbage are believed to have originated from a whole-genome triplication of the *A. thaliana* genome. According to this model, each gene in *A. thaliana* would theoretically correspond to three orthologous copies in *Brassica* species [37]. However, Wang et al. reported that only the *AtAPX1* gene has three orthologs in both Chinese cabbage and cabbage [29]. Similarly, Zheng et al. demonstrated that the ratio of orthologous genes between *A. thaliana* and Chinese cabbage, or cabbage, exceeds the theoretical 1:3 ratio, indicating incomplete compliance with the triploidy hypothesis [38]. Consistent with these observations, the present study did not detect a strict 1:3 orthologous relationship between *AtSUSs* and their counterparts in Chinese cabbage (*BraSUSs*), black mustard (*BniSUSs*), or cabbage (*BolSUSs*). This result contradicts the expectations of the *Brassica* triploidy theory yet aligns with earlier empirical reports. Studying phylogenetics plays a crucial role in predicting the genetic and evolutionary relationships of gene families as well as their potential functions. Previous studies have shown that in mustard plants, there are 4 *BjuSUSs* genes belonging to the same subfamily that are highly expressed in the stems; and the *AtSUS 1* gene belonging to the same subfamily also shows a high transcriptional level in the stems, indicating that some genes with similar gene structures have similar functions [15]. Studying the phylogenetic and evolutionary relationships of *SUS* genes in three diploid basic species of *Brassica* lays the foundation for in-depth research on the functions of *SUS* genes in *Brassica* species.

### 4.2. Collinearity Analysis of the Brassica SUS Gene Family

As early as 20 million years ago, the *Brassicaceae* family's *Arabidopsis* and *Brassica* genera had already diverged [15]. The gene replication events that occurred within the plant body played a crucial role in the expansion of gene families and the diversification of functions [39]. Zhang et al. identified syntenic relationships of *OXR* (antioxidant genes) among Chinese cabbage, black mustard, cabbage, and *A. thaliana*: four syntenic gene pairs were found between Chinese cabbage and *A. thaliana*, five between Chinese cabbage and cabbage, and six between black mustard and cabbage [40]. In *B. juncea*, 29, 12, and 10 *SUS* homologous gene pairs were identified with *A. thaliana*, Chinese cabbage and black mustard, respectively [15]. In this study, collinearity analysis revealed 13 *SUS* syntenic pairs between Chinese cabbage and *A. thaliana*, and 21 syntenic pairs each between Chinese cabbage and black mustard, and between black mustard and cabbage. Additionally, studies have found that certain *SUS* genes in the *Brassica* species of mustard have paralogs, which are homologous to some genes in Chinese cabbage and black mustard, although some *BjuSUSs* have not been matched with corresponding genes [15]. Therefore, the *SUS* gene family in Chinese cabbage, cabbage, and black mustard has undergone varying degrees of gene fragment duplication or loss during evolution. The diversification of gene structure plays a certain role in gene evolution, and different gene structures can affect gene functional expression [40]. By comparing the collinearity of *SUS* genes among Chinese cabbage, cabbage, and black mustard, we can elucidate the evolutionary relationships between the AA, BB, and CC genomes within the *Brassica* genus, providing a basis for studying gene functions in polyploid crops such as *Brassica napus* (AACC) and *Brassica juncea* (AABB), thereby facilitating the development of higher-quality and higher-yielding vegetable varieties.

#### 4.3. Analysis of the Expression Response of the SUS Gene Family in Chinese Cabbage to Different Light Intensities

Light is an important environmental factor in the input pathways of the biological clock. Light can be further divided into factors such as light intensity and light quality [4]. Plants can perceive changes in light signals such as light intensity, quality, and photoperiod, thereby influencing the rhythmicity of their biological clocks and playing a crucial role in regulating life processes including vegetative and reproductive growth [41]. Sucrose, the principal product of photosynthesis, increases in concentration with rising light intensity within a suitable range, thereby enhancing photosynthetic efficiency [42]. Glowacz et al. reported that elevated light intensity led to significant increases in soluble sugars, cellulose, and vitamin C content in water spinach [43]. However, under combined high light and high temperature stress, accelerated chlorophyll breakdown has been shown to reduce chlorophyll a and b levels significantly [44]. Strong light stress can also cause over-reduction in components in the PETC (photosynthetic electron transport chain), and PSII (photosystem II) is particularly susceptible to inactivation under high light conditions [45]. Strong light leads to higher temperatures. Research has found that in corn under high-temperature and drought conditions, the activity of sucrose synthase decreases, and the expression of *SUS1* is downregulated, thereby inhibiting the transfer of sucrose to UDP-glucose and affecting the viability of pollen [46].

The requirements for vernalization and light conditions for inducing flowering in the three basic species of *Brassica* are different. This study analyzed the differentially expressed genes and rhythmic expression patterns of the *SUS* gene family in Chinese cabbage under low and high light intensities. The results showed that under high light conditions, the rhythmic expression of the differentially expressed genes *BraA07g028740.3C* and *BraA07g037520.3C* was enhanced, and the rhythmic expression of *BraA05g015010.3C* was also enhanced (Figure 5b). Studies have shown that during the flower transformation process of pak choi, the contents of sucrose, glucose and fructose in the stem tips significantly increased, and the differentially expressed genes were significantly enriched in pathways such as sugar metabolism, flower development and circadian rhythms [31]. Thus, it can be speculated that the high light intensity may have affected the endogenous circadian rhythm of the biological clock, thereby increasing the number of rhythmic expression of the *SUS* gene family members, changing the rhythm, and subsequently affecting the sucrose content and the flowering process. However, the research found that when black pepper was at a light intensity of  $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ – $450 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , *PnSPS* and *PnSWEET* were expressed at low levels, while *PnSUS* was expressed at high levels. The ratio of sucrose to starch in the leaves significantly increased, and this high sucrose/starch ratio was significantly negatively correlated with the number of inflorescences [24]. This might be due to the different sensitivity of different species to sucrose. In any case, the *SUS* gene is closely related to the flowering mechanism of plants. Studying the comparative analysis of the *SUS* gene family in the *Brassica* genus and the response of Chinese cabbage to different light intensities will lay a theoretical foundation for further research on the mechanism by which the *SUS* gene in the *Brassica* genus regulates sucrose and thereby affects flowering.

## 5. Conclusions

This study identified 13, 16, and 13 *SUS* gene family members in Chinese cabbage, black mustard, and cabbage, respectively, through bioinformatic approaches. By comparing the physicochemical properties, phylogenetic relationships, conserved motifs, interspecific specificity, and KEGG/GO functional enrichment of *SUS* genes, this study found that the *SUS* gene family shows higher similarity between Chinese cabbage and cabbage than

with black mustard. Furthermore, expression profiling of Chinese cabbage *SUS* genes under low and high light intensities demonstrated that high light conditions enhanced the rhythmic expression patterns of the *SUS* gene family. Using three diploid basal species with different flowering traits (Chinese cabbage, cabbage, and black mustard) as experimental materials, we conducted a comparative analysis of the *SUS* gene family. This will lay the foundation for deciphering the molecular regulatory mechanism by which the *SUS* gene affects flowering through its influence on sucrose synthesis.

**Author Contributions:** M.X. and D.F.: Data analysis, Writing—original draft. B.W.: experiments operation, data processing. F.J. and Z.G.: Results discussion. L.H.: Data processing. D.W., Y.L. and D.F.: Manuscript revision, Review and editing. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** All data and materials generated and analyzed during this study are included in this published article. The datasets generated and analyzed during the current study are available in the Sequence Read Archive (SRA) repository in the National Center for Biotechnology Information (NCBI) under the accession number PRJNA1243566.

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**Conflicts of Interest:** The authors declare no competing interest.

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