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Effect of Exogenous Substance K_2SO_4 on the Nutritional Quality of Broccoli and Its Metabolic Regulation Mechanism

Meng Liu [1,2,†], Wei Huang [2,†], Junhua Zhang [2], Zixuan Zhao [2], Yaqin Wang [1], Nazim S. Gruda [3], Guangmin Liu [1,*] and Hongju He [1,*]

[1] Institute of Agri-Food Processing and Nutrition, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China; liumeng1357@126.com (M.L.); wangyaqin@iapn.org.cn (Y.W.)
[2] College of Agriculture and Forestry Science and Technology, Hebei North University, Zhangjiakou 075000, China; nlkjxyyy@163.com (W.H.); zhangjunhua196911@163.com (J.Z.); ash0517@126.com (Z.Z.)
[3] Institute of Plant Sciences and Resource Conservation, Division of Horticultural Sciences, University of Bonn, 53012 Bonn, Germany
* Correspondence: liuguangmin@iapn.org.cn (G.L.); hehongju@iapn.org.cn (H.H.)
† These authors contributed equally to this work.

Abstract: The impact of exogenous sulfate components on the nutritional quality of vegetables has been well documented. In this study, we examined the effects of adding K_2SO_4 to broccoli on its nutritional quality, active components, and the genes involved in glucosinolate synthesis. Different concentrations of exogenous K_2SO_4 of 25, 75, and 150 g·m^{-2} were applied to the "Naihan Youxiu" broccoli cultivar, while the control treatment received no potassium sulfate. Our primary objective was to gain insights into strategies for enhancing broccoli's nutritional and active components. The results showed that broccoli's vitamin C content in each treatment was lower than that in the control treatment. The contents of soluble protein, soluble sugar, polyphenols, and total flavonoids in the treatment of 150 g·m^{-2} K_2SO_4 were the highest. They increased by 23.51%, 87.49%, 146.00%, and 22.73% more than the control, respectively. MDA was significantly inhibited after the 75 g·m^{-2} treatment, whereas SOD had the highest activity under the 75 g·m^{-2} treatment. Therefore, the 150 g·m^{-2} treatment was beneficial in improving the nutritional quality and antioxidant capacity of broccoli. The contents of PRO, SIN, NAP, GBC, 4ME, NEO, total indole glucosinolates, and total glucosinolates reached the peak at the 150 g·m^{-2} K_2SO_4 treatment, RAA and total aliphatic glucosinolates reached the peak at the 75 g·m^{-2} K_2SO_4 treatment, and ERU and 4OH reached the highest at the 25 g·m^{-2} K_2SO_4 treatment. The sulforaphane content was the highest in the 150 g·m^{-2} treatment, and myrosinase activity was the highest in the 75 g·m^{-2} treatment. It can be seen that the 150 g·m^{-2} treatment significantly increased the content of glucosinolates, total indole glucosinolates, total glucosinolates, and sulforaphane in broccoli. *CYP79B2*, *CYP83B1*, *CYP83A1*, *AOP2*, *UGT74B1*, and *MYB34* were significantly up-regulated under 150 g·m^{-2} K_2SO_4 treatment and reached the peak value. *CYP79F1*, *CYP81F4*, and *MAM1* showed significant inhibitory effects when treated with 150 g·m^{-2} of K_2SO_4. The expression levels of *BCAT4*, *CYP81F1*, *ST5a*, *ST5c*, and *SUR1* were down-regulated under the 150 g·m^{-2} K_2SO_4 treatment, but not significantly. In summary, the K_2SO_4 150 g·m^{-2} treatment had the best effect on nutritional quality, antioxidant activity, the content of glucosinolates, total glucosinolates, sulforaphane, and expression of *CYP79B2*, *CYP83B1*, *CYP83A1*, *FMO2*, *UGT74B1*, *AOP2*, and *MYB34* genes.

Keywords: potassium sulfate; glucosinolate; gene expression

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1. Introduction

Broccoli (*Brassica oleracea var. italica*) is an important economic crop with rich nutritional value [1]. Because of its high content of glucosinolates (GS) and secondary sulfur-containing compounds, it has become the research object of many researchers [2]. In addition, the

degradation products of glucosinolate have a strong antibacterial effect and play a role in preventing prostate, gastrointestinal, and lung cancers [3].

The basic structure of all glucosinolates consists of β-glucosinolates, sulfonated oxime, and a branched R chain [4]. According to the structure of variable side chain R, glucosinolates can be divided into three categories: aliphatic glucosinolates, aromatic glucosinolates, and indole glucosinolates [5]. The enzymes *BCAT4*, *MAM1*, *MAM2*, and *MAM3* play a crucial role in the initial stage of glucosinolate synthesis. *MYB28* regulates *MAM1* and *MAM3*, while the *BCAT4* catalytic chain's first growth reaction occurs in the cytosol [6]. Cytochrome P450 of the *CYP79* family converts precursor amino acids into acetaldoxime [7,8]. Next, cytochrome P450 of the *CYP83* family oxidizes acetaldoxime into tempting compounds, such as nitrile oxides or acyl nitro compounds [9,10]. These compounds continue the central structure of glucosinolate synthesis under the catalysis of other enzymes.

Different genes are involved in the biosynthesis of the core structure of glucosinolates [11], resulting in different kinds of glucosinolates. For example, *ST5B*, *plST5C*, *FMO*, *AOP2*, *AOP3*, and *GSL-OH* are involved in the biosynthesis of aliphatic glucosinolates, while *MYB34*, *CYP83B1*, *ST5A*, *CYP81*, and *IGM* are involved in the biosynthesis of indole glucosinolates [12]. Therefore, it is important to study which specific genes are involved in enhancing glucosinolate content induced by exogenous substances.

The results showed that the combined treatment of 4 mM $ZnSO_4$ and 0.1 mM Na_2SeO_3 up-regulated the expression of *UGT74B1* and *BoHMTI* genes in broccoli sprouted 6 days after germination, and the content of total glucosinolates was significantly higher than that of the control [13]. Yang et al. [14] found that $ZnSO_4$ significantly increased the expression of *FMOGS-OX* and *MYR* but significantly decreased the expression of *AOP2* and *ESP*. K_2SO_4 and Met increased the expression of *AOP2* and *ESP*, while K_2SO_4 down-regulated the expression of *FMOGS-OX*. After $ZnSO_4$ and K_2SO_4 treatment, the glucosinolate content in broccoli increased by 58.2% and 20%, respectively. Mao Shuxiang [15] found that at 1 mM sulfur concentration, the expressions of *CYP79F1*, *CYP83A1*, *SUR1*, and *UGT74C1* were significantly up-regulated after 50 μM of selenium treatment, but at 4 mM sulfur concentration, the expressions of *CYP79F1*, *CYP83A1*, *SUR1*, and *UGT74C1* were significantly inhibited after the 50 μM selenium treatment but reached the peak at the 150 μM selenium treatment.

The application of exogenous substances can not only induce the effect of glucosinolate gene expression but also affect the nutritional quality. The results of Chengkai [16] showed that the content of soluble sugar and soluble protein could be significantly increased by applying nutrient solution containing 1 mmol·L^{-1} of sulfur and 10 μmol·L^{-1} of selenium. Still, the content of soluble sugar and soluble protein in broccoli seedling leaves could be reduced by applying nutrient solution containing 5 mmol·L^{-1} of sulfur and 20 μmol·L^{-1} of selenium, 10 mmol·L^{-1} of sulfur, and 20 μmol·L^{-1} of selenium. Therefore, in this study, "excellent cold-resistant" broccoli varieties were used as test materials to study the effects of different concentrations of potassium sulfate on the nutritional quality and metabolic regulation mechanism of broccoli to provide a reference for strengthening the nutritional quality and metabolic regulation mechanism of broccoli.

2. Materials and Methods

2.1. Test Materials and Design

The field experiments were carried out from July to October 2020 at the Dongshengfangyuan Vegetable Research Station (39°53′ N, 116°03′ E,) at 43.5 m altitude in Beijing, China. The soil type is sandy loam, with a pH of 7.0. The variety of broccoli that was tested was "Naihan Youxiu". The seeds were sown using a 96-hole plug on July 3rd for seedling raising. On August 1st, seedlings with three leaves and one heart were planted on a flat border with a plant-to-plant spacing and row spacing of 40 cm × 45 cm, respectively. On August 29th, K_2SO_4 fertilizer was applied for the first time, and on September 15th, for the second time. A randomized block design was adopted in the experiment. According to the concentration of sulfur fertilizer, three treatments were set up, which were named S25

(25 g·m^{-2}), S75 (75 g·m^{-2}), and S150 (150 g·m^{-2}). The length × width = 9 m × 4. 5 m, the area is 40.5 m^2, and the total area of the three treatment cells was 121.5 m^2. K_2SO_4 fertilizer was applied to broccoli roots and then watered. Other farming operations were carried out according to the technical measures of high-quality broccoli production. A total of 5 broccoli were harvested for each treatment on September 29th. After harvest, laboratory experiments were conducted at the Vegetable Research Institute of the Beijing Academy of Agriculture and Forestry Sciences.

2.2. Test Reagents

The following materials were used in our research: K_2SO_4 (SDIC Xinjiang Lop Nur Potassium Salt Co., Ltd. (Xinjiang, China), with a sulfur content of at least 17.5%); a standard for propenyl glucosinolates (D1347, from Shanghai Baoman Biotechnology Co., Ltd. (Shanghai, China)); a kit for extracting RNA from polysaccharide polyphenol plants (model RC4-11, from Nanjing Nuoweizan Biotechnology Co., Ltd. (Nanjing, China)); HiScript® III All-in-one RT SuperMix Perfect for qPCR Kit (model R333-01, from Nanjing Nuoweizan Biotechnology Co., Ltd. (Nanjing, China)); and Taq Pro Universal SYBR qPCR Master Mix Kit (models Q712-02/03, from Nanjing Nuoweizan Biotechnology Co., Ltd. (Nanjing, China)).

2.3. Test Instruments

UV-2550 dual-beam ultraviolet-visible spectrophotometer (Shimadzu Corporation (Kyoto, Japan)); Infinite M1000 PRO multifunctional microplate reader (TECAN Group of Companies Beijing Representative Office (Beijing, China)); 762075 enzymes labeled plate (Greiner Bio-One Company (Nittingen, Germany)); and Roche LightCyler 480II Real-time Fluorescence Quantitative PCR Instrument (Roche (Shanghai, China)).

2.4. Item Determination

The determination of Vitamin C content was conducted using the Guo [17] method. The Bradford [18] method was utilized for measuring soluble protein content, while the Pramanik [19] method was used for determining soluble sugar content. The determination of polyphenol content was carried out using the Singleton [20] method, and the Wang [21] method was used for measuring the content of total flavonoids. The content of malondialdehyde was determined by Antonios [22]. The Tang [23] method was used for determining the activity of superoxide dismutase. The content of glucosinolates was determined by Wang [24], and the content of sulforaphane was measured using the method developed by Matusheski [25]. Myrosinase activity was measured with reference to the method developed by Guo [26].

To extract genomic RNA, use the RNA extraction kit from Nanjing Nuoweizan with the designated plate number. Next, reverse cDNA using the R333-01 reverse transcription kit from the same company. To perform fluorescence quantitative PCR, use the Q712-02/03 kit from Nanjing Nuoweizan. Then, synthesize specific primers for the internal reference gene and target gene using Premier 5 primer design software from the Beijing Ruibo Xingke Biotechnology Co., Ltd. (Table 1). Finally, calculate the results using the $2^{-\Delta\Delta CT}$ method [27].

2.5. Data Analysis and Processing

Unless otherwise specified, the data in the chart are the average of three repetitions, and the error bar shows the standard deviation. Duncan's analysis of variance was used to analyze the difference ($p < 0.05$). SPSS 22.0 was used to analyze the difference and Origin Pro 8.0 was used to map the difference.

Table 1. Gene-specific primer sequences.

Gene	Sense Primer (5′-3′)	Anti-Sense Primer (5′-3′)
Actin	CTGTTCCAATCTACGAGGGTTTC	GCTCGGCTGTGGTGGTGAA
MYB28	AGACTGCGATGGACTAACTACCT	CCGACCACTTGTTTCCACGA
MYB34	CGGGACGAACTGACA	CGACCGAGTATTTGCT
CYP79B2	CGGAGATGGTAAACAA	ATGGAGACGGAAAGC
CYP83B1	AGCAGACGCAAAGATA	ACCCGAAAGGTAGGA
CYP79F1	TAGACGAAGTGGTGGGA	GGCTACCTTTGGGAAT
CYP83A1	TCCTTATCCCTCGTGC	ACTCGTAGTCCGTGCC
BCAT4	TAGCAGAGGCGAAAG	TTGTAGCCGAAATCAC
MAM1	GCCGAGGATAGTCATA	ATCTTCGCAACCAAA
FMO2	GACCGTGGTTACGGGAGACTTG	GTAGCCATTGTATAACAAGCAACCC
UGT74B1	TCCACAGATTCACCCAT	AAGCCACGGACGAGA
ST5a	CAATGGAACCAACCACGAC	TGGGAGGGAAGCGATG
ST5b	CCGACACTACCTTACCGAACCA	CGTGAGGAAAAGAGGCGATG
ST5c	CCACGCCCAAAACTTCTTCA	TGAGTGGAGAAGAGCGTGTT
AOP2	GAGTAACGGAAAGAAAGAAGACAAGG	ATAAGCGTGAAGAGTAGAACGAGGT
AOP3	AGGTGAAGACCAAAGAGGGGAA	TCGGTGATACGGTGAAGGGA
SUR1	GCGGTTCGGTGGAGCTGATAAG	GCGGAAGCAAGGATAGACGGAAG
GSTF	GAGTCTTTCCTATCCCACA	TCTTCGGCAACAACG
CYP81F1	AAGCAGAGCGGTTCAAGAAG	GCGTGACCATTGTGTTACCA
CYP81F4	CGGTGGAGGAGAAGGAGAAA	CTGACACATGGCTCGTAACG
GSL-OH	CCAGGAAGTGAGAAGTGGGT	TAGCACCATCACCAGCATCA

3. Results and Analysis

3.1. Effects of Exogenous K_2SO_4 Treatment on Nutritional Components and Antioxidant Activity of Broccoli

3.1.1. Effect of Exogenous K_2SO_4 Treatment on Vitamin C Content in Broccoli

Figure 1A reveals that as the K_2SO_4 dosage increases gradually, the Vc content initially decreases and then increases afterwards. The lowest value of 75.90 mg·100g^{-1} was observed in the S25 treatment, indicating a 14.34% decrease compared with the control. On the other hand, the highest value of 88.61 mg·100 g^{-1} was recorded in the S0 treatment, with significant differences evident among all treatments. The highest dosage of K_2SO_4 resulted in a decrease of 3.75% compared with the control.

3.1.2. Effect of Exogenous K_2SO_4 Treatment on Soluble Protein Content in Broccoli

As the dosage of K_2SO_4 increased, the soluble protein content in broccoli showed a gradual increase. The differences among the treatments were significant, as shown in Figure 1B. In comparison to the control treatment, the soluble protein content experienced a rise of 11.32%, 20.01%, and 23.51% in S25, S75, and S150, respectively. The results indicate that the application of K_2SO_4 was beneficial to the increase in soluble protein content in broccoli.

3.1.3. Effect of Exogenous K_2SO_4 Treatment on Soluble Sugar Content in Broccoli

From Figure 1C, it is evident that the amount of soluble sugar in broccoli escalates as the dosage of K_2SO_4 increases. Moreover, this pattern of change is congruent with the alteration in the soluble protein content. The soluble sugar content of S25, S75, and S150 increased by 4.07%, 23.63%, and 87.49%, respectively, compared with S0. The results indicate that the application of K_2SO_4 was beneficial to the increase in soluble sugar content in broccoli.

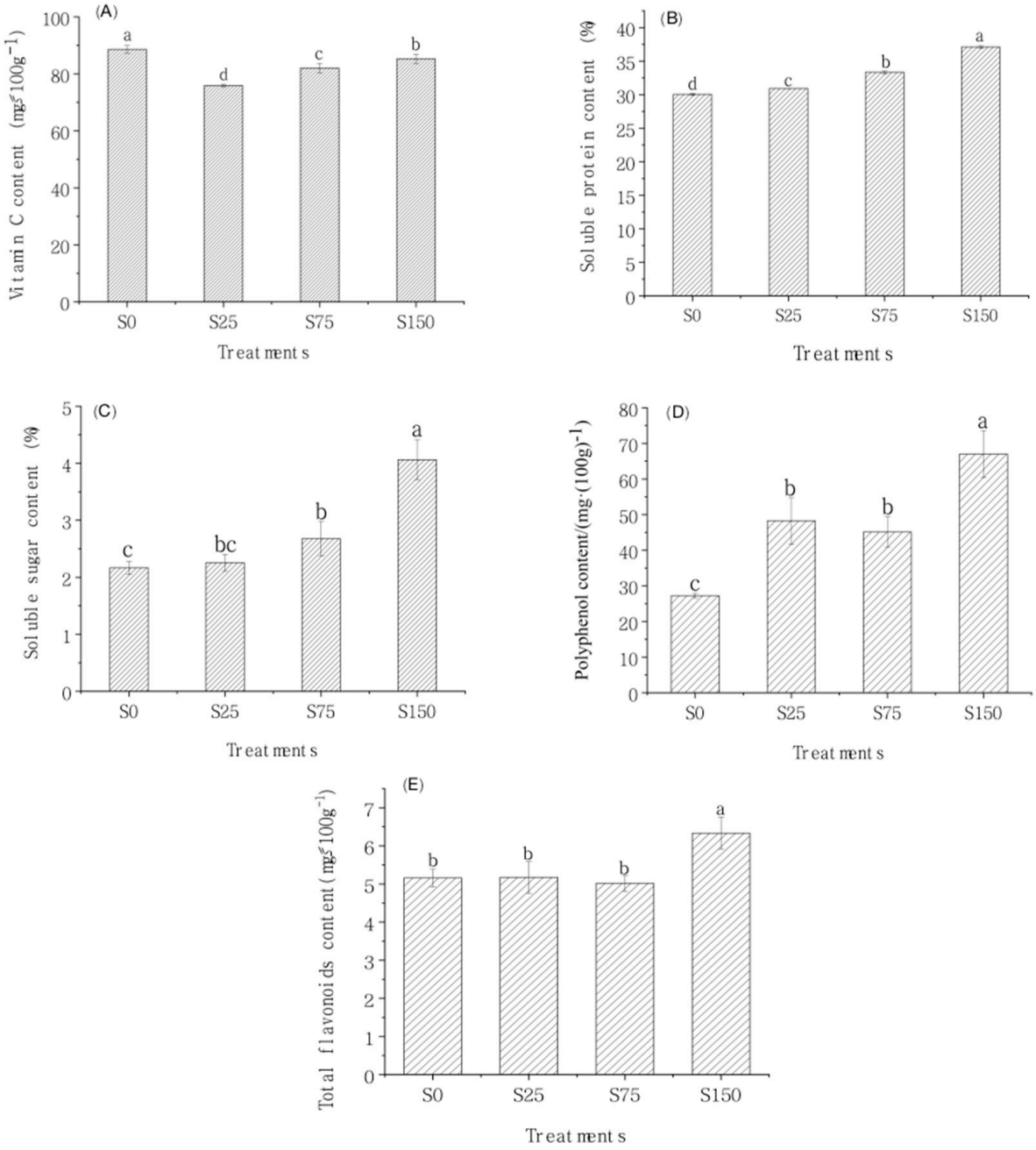

Figure 1. Effect of exogenous substance K_2SO_4 treatment on vitamin C content (**A**), soluble protein content (**B**), soluble sugar content (**C**), polyphenol content (**D**), and total flavonoids content (**E**) in broccoli. **Note:** Significant differences among different samples are indicated by different lowercase letters (a–d).

3.1.4. Effect of Exogenous K_2SO_4 Treatment on Polyphenol Content in Broccoli

As can be seen from Figure 1D, the polyphenol content in broccoli gradually increased with the increase in K_2SO_4 dosage, and there was a significant difference between S150 and other treatments, but there was no significant difference between S25 and S75 treatments. The polyphenol content in S150 was 2.5 times higher than S0, and 38.85% and 48.43% higher than S25 and S75, respectively. The results showed that K_2SO_4 could increase the content of polyphenols in broccoli and improve the antioxidant capacity.

3.1.5. Effect of Exogenous K_2SO_4 Treatment on the Content of Total Flavonoids in Broccoli

Figure 1E displays that as the K_2SO_4 dosage increases, the broccoli's total flavonoid content reaches its peak at S150, measuring at 6.33 mg·100g^{-1}. There is a notable difference between S150 and S0, S25, and S75, but no significant difference among S0, S25, and S75. Although S75 shows a slight decrease, it is not significant. In comparison to S0, S25, and S75, S150 saw an increase of 22.73%, 22.40%, and 26.24%, respectively.

3.1.6. Effect of Exogenous K_2SO_4 Treatment on MDA Content in Broccoli

It can be seen from Figure 2A that the content of malondialdehyde in broccoli decreased first and then increased with the increase in K_2SO_4 dosage. The content of MDA (malondialdehyde) can directly reflect the damage to plants in adversity [28]. Therefore, it can be seen that broccoli is the least damaged in adversity under the S75 treatment.

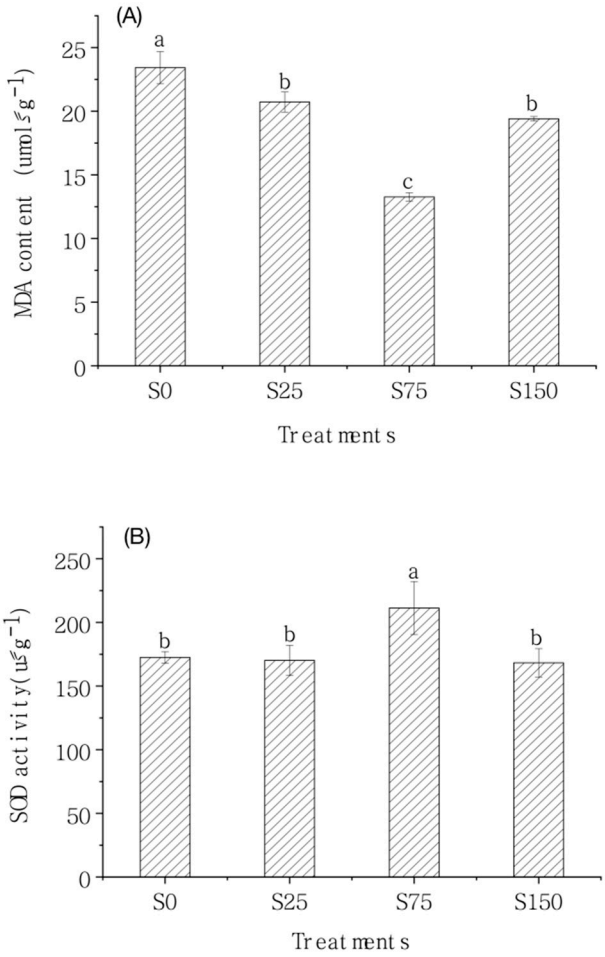

Figure 2. Effect of exogenous substance K_2SO_4 treatments on malondialdehyde content (**A**) and superoxide dismutase activity (**B**) in broccoli. **Note:** Significant differences among different samples are indicated by different lowercase letters (a–c).

3.1.7. Effect of Exogenous K_2SO_4 Treatment on Superoxide Dismutase Activity in Broccoli

From Figure 2B, it can be seen that SOD (superoxide dismutase) activity increased first and then decreased with the gradual increase in K_2SO_4 dosage, and the highest activity was 211.31 μ·g^{-1} at S75, which was significantly different from the other three treatments, and increased by 22.51% compared with the control.

3.2. Effect of Exogenous Substance K_2SO_4 Treatment on Active Substances in Broccoli

3.2.1. Effect of Exogenous K_2SO_4 Treatment on Glucosinolate Content in Broccoli

Nine glucosinolates were detected in broccoli in our experiment (Table 2). Table 2 reveals that the level of total aliphatic glucosinolate is greater in the K_2SO_4 treatments than in the control treatment. The difference between all treatments is significant, with the highest S75 content showing a 9.10% increase compared with the control. With the increase in K_2SO_4 concentration, the contents of PRO and SIN increased by 39.53% and 26.32%, respectively. The content of RAA was increased by 6.68%, 13.33%, and 8.97%, respectively, compared with the control. The changes in NAP and ERU were similar. The content of NAP and ERU increased by 36.36% and 4.31%, respectively, in the S25 treatment.

Table 2. Effect of exogenous substance K_2SO_4 treatment on glucosinolate content in broccoli (μmol·g^{-1}DW).

	Aliphatic Glucosinolate					Tol Aliphatic	Indole Glucosinolates				Tol Indole	Total
	PRO	SIN	RAA	NAP	ERU	Gs	4OH	GBC	4ME	NEO	Gs	
S0	0.129 d	0.057 c	2.933 d	0.011 b	0.510 b	3.639 d	0.478 b	3.568 c	0.987 c	2.089 c	7.123 c	10.762 c
S25	0.140 c	0.057 c	3.129 c	0.015 a	0.532 a	3.873 c	0.513 a	3.458 d	0.894 d	1.631 d	6.496 d	10.368 d
S75	0.145 b	0.061 b	3.324 a	0.010 b	0.430 d	3.970 a	0.304 d	4.933 b	0.994 b	3.842 b	10.072 b	14.042 b
S150	0.180 a	0.072 a	3.196 b	0.014 a	0.474 c	3.935 b	0.470 c	6.464 a	1.103 a	4.884 a	12.922 a	16.857 a

Note: PRO for 2-hydroxy-3-butenyl glucoside; RAA for 4-methylthiooxybutylthioside; NAP for 3-Butenylthioglycoside; SIN for 2-propenyl thioglycoside; 4OH for 4-hydroxyindolyl-3-methylthioglycoside; ERU for 4-methylthiobutenyl thioglycoside; GBC for 4-methylthiobutenyl thioglycoside; 4ME for 4-methylindolyl-3-methylthioglycoside; and NEO for 1-methylindolyl-3-methylthioglycoside (similarly hereinafter). Significant differences among different samples are indicated by different lowercase letters (a–d).

The content of glucosinolates in the total indole group accounted for 62.65–76.66% of total glucosinolates. In the total indole group, the glucosinolate content was highest in the S150 treatment, measuring 12.92 μmol·g^{-1}. This was significantly greater than the other treatments and marked an 81.42% increase compared with the control. Compared with the control, only the S25 treatment promoted the accumulation of 4OH, and the other treatments inhibited the accumulation of 4OH. The changes in GBC, 4ME, and NEO were similar. With the increase in exogenous K_2SO_4 dosage, the changing trend of GBC, 4ME, and NEO decreased first and then increased. The inhibition phenomenon was most obvious in the S25 treatment, and the maximum accumulation was found in the S150 treatment.

The content of total glucosinolates also decreased first and then increased. Under S25 treatment, the content of total glucosinolates reached a minimum value of 10.37 μmol·g^{-1}, which is 3.66% less compared with the control. The total glucosinolate content showed the highest increase of 56.63% compared with the control when the sulfur amount was raised to 150 g·m^{-2}. In general, except ERU and 4OH, the content of glucosinolates and total glucosinolates increased the most under the S150 treatment.

3.2.2. Effect of Exogenous K_2SO_4 Treatment on Sulforaphane Content in Broccoli

As can be seen from Figure 3A, the greater the amount of K_2SO_4, the higher the sulforaphane content in broccoli. At a dosage of 150 g·m^{-2} of K_2SO_4, the sulforaphane content was 21.32 μg·mL^{-1}, which was notably higher than all other treatments and doubled the amount of the control. Compared with S25 and S75, S150 increased by 104.10% and 39.61%, respectively.

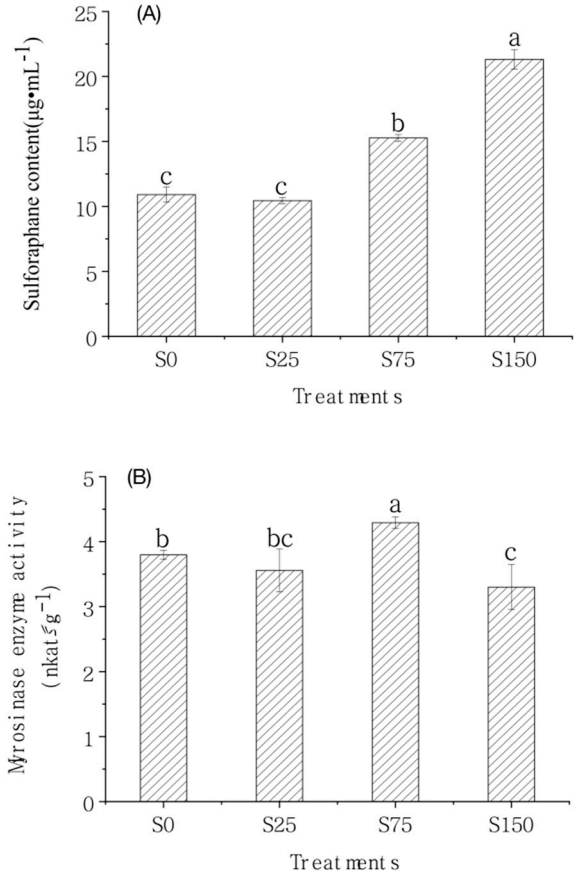

Figure 3. Effect of exogenous substance K_2SO_4 treatment on sulforaphane content (**A**) and myrosinase activity (**B**) in broccoli. **Note:** Significant differences among different samples are indicated by different lowercase letters (a–c).

3.2.3. Effect of Exogenous K_2SO_4 Treatment on Myrosinase Activity in Broccoli

Figure 3B clearly indicates that the S75 treatment exhibits the highest myrosinase activity among all other treatments. Conversely, the S150 treatment showed the lowest myrosinase activity, with a reduction of 23.10% compared with S75 and 13.08% compared with the control.

3.3. Effect of Exogenous Substance K_2SO_4 Treatment on the Expression of Key Genes of Glucosinolate Synthesis in Broccoli

3.3.1. Effect of Exogenous K_2SO_4 Treatment on the Expression of Key Genes for Aliphatic Glucosinolate Synthesis in Broccoli

The treatment with the highest total glucosinolate content and the control (S0, S150) were selected to detect the key synthesis and regulatory genes of the aliphatic glucosinolate synthesis pathway in broccoli. As shown in Figure 4, the expression patterns of *MYB28*, *CYP83A1*, *GSTF*, *ST5b*, *FMO2*, *AOP3*, *AOP2*, and *GSL-OH* were similar and increased gradually. Except for *CYP83A1* and *AOP2*, *MYB28*, *GSTF*, *ST5b*, *FMO2*, *AOP3*, and *GSL-OH* were up-regulated under S150 treatment, but their expression levels did not change significantly. However, *CYP83A1* and *AOP2* were significantly up-regulated under S150 treatment and reached the peak value. The expression levels of *BCAT4*, *SUR1,* and *ST5C* were down-regulated slightly but not significantly under S150 treatment. Therefore, S150 treatment significantly inhibited the expression of *CYP79F1* and *MAM1.*

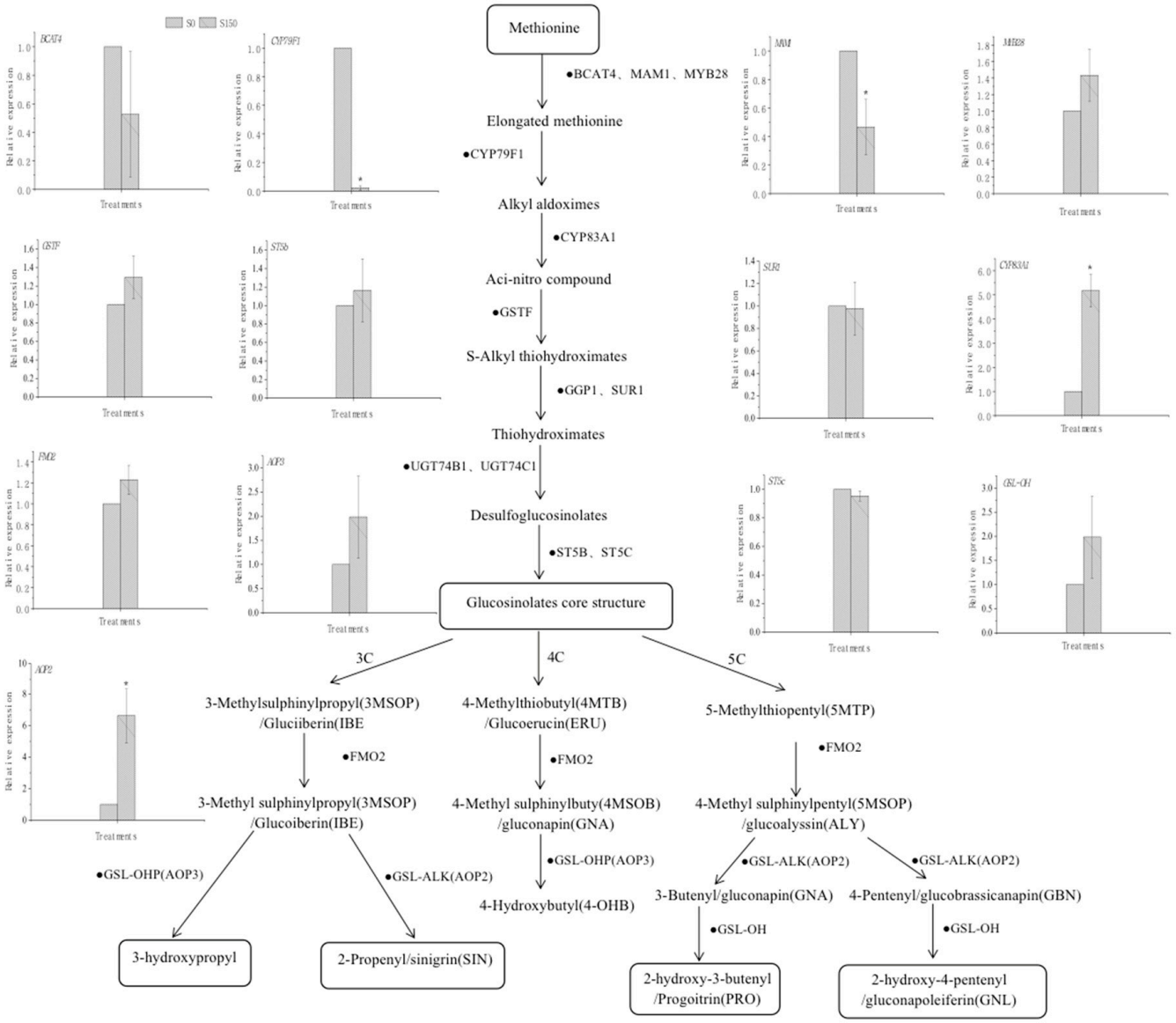

Figure 4. Effect of exogenous substance K_2SO_4 treatment on the expression of key genes in aliphatic glucosinolate synthesis in broccoli. Note: Expression was normalized to that of actin and the values in control plants were set to 1. Each data point is the average for each of the three biological replicates with three technical replicates against each. Note: * indicates significant differences between treatments ($p < 0.05$).

3.3.2. Effect of Exogenous K_2SO_4 Treatment on the Expression Level of Key Genes for Synthesis of Indole Glucosinolates in Broccoli

We detected the levels of expression of seven crucial genes involved in the regulation and synthesis pathway of indole glucosinolates in broccoli. As shown in Figure 5, the expression patterns of *MYB34*, *CYP79B2*, *CYP83B1*, *UGT74B1*, and *CYP81F1* were similar under different concentrations of K_2SO_4. The expression levels of *MYB34*, *CYP79B2*, *CYP83B1*, *UGT74B1*, and *CYP81F1* were higher than those of the control, except *CYP81F1*. The expression of *CYP81F4* and *ST5a* decreased with the increase in K_2SO_4 concentration, and the expression of *CYP81F4* was significantly inhibited at S150.

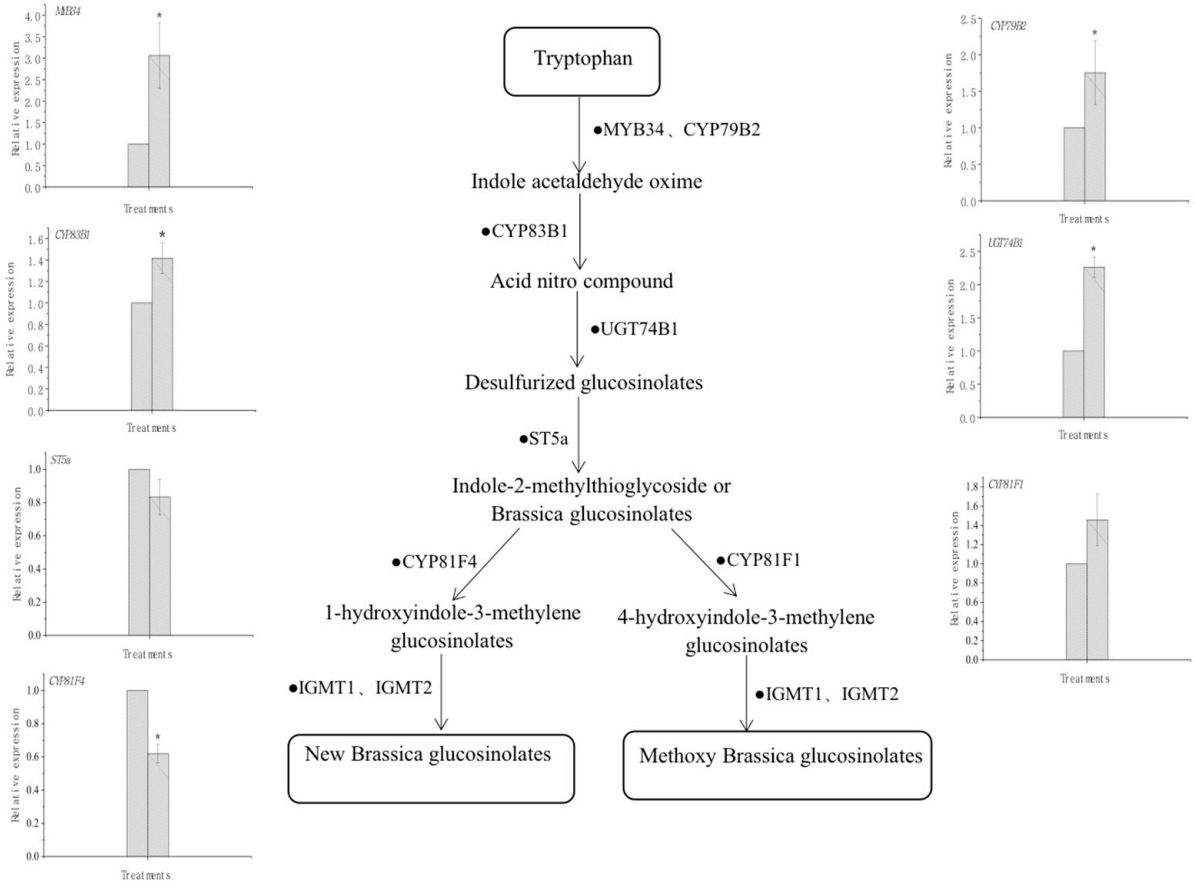

Figure 5. Effect of exogenous substance K_2SO_4 treatment on the expression of key genes for indole glucosinolate synthesis in broccoli. Note: * indicates significant differences between treatments ($p < 0.05$).

4. Correlation Analysis between Glucosinolate Content, Enzyme Activity, and Gene Expression Treated with Exogenous Substance K_2SO_4

To examine the uniformity of glucosinolate content, enzyme activity, and key gene expression in the glucosinolate synthesis pathway when treated solely with exogenous K_2SO_4, a correlation analysis was conducted using the Pearson correlation coefficient. The findings, shown in Table 3, reveal that sulforaphane exhibited a highly significant positive correlation with RAA and total aliphatic, total indole, and overall glucosinolate contents. It also displayed a very significant positive correlation with GBC. *MYB34* and *CYP83A1* were positively correlated with RAA, GBC, total aliphatic glucosinolates, total indole glucosinolates, and total glucosinolates, respectively. *MYB34* was positively correlated with sulforaphane, while *CYP83A1* was positively correlated with sulforaphane. *CYP79B2* and *FMO2* were positively correlated with RAA, GBC, total indole glucosinolates, and total glucosinolates, while *CYP79B2* was positively correlated with total aliphatic glucosinolates and sulforaphane. There was a very significant negative correlation between *FMO2* and myrosinase. *MAM1* was negatively correlated with RAA, GBC, total aliphatic glucosinolates, total indole glucosinolates, and total glucosinolates, but negatively correlated with sulforaphane. There was a significant negative correlation between *UGT74B1* and myrosinase.

Table 3. Correlation analysis between glucosinolate content, enzyme activity, and gene expression treated with exogenous substance K_2SO_4.

Pearson	RAA	GBC	TOL	Tol Aliphatic Gs	Tol Indole Gs	Sulfor-Aphane	Myrosi-Nase	*MYB34*	*CYP79B2*	*CYP83A1*	*MAM1*	*FMO2*
GBC	1.000 **											
TOL	1.000 **	1.000 **										
Tol aliphatic Gs	0.999 **	1.000 **	1.000 **									
Tol indole Gs	1.000 **	1.000 **	1.000 **	1.000 **								
Sulforaphane	0.993 **	0.994 **	0.994 **	0.995 **	0.994 **							
Myrosinase	−0.782	−0.774	−0.773	−0.755	−0.774	−0.734						
MYB34	0.922 **	0.921 **	0.921 **	0.921 **	0.921 **	0.888 *	−0.719					
CYP79B2	0.826 *	0.830 *	0.831 *	0.844 *	0.830 *	0.824 *	−0.375	0.897 *				
CYP83A1	0.981 **	0.983 **	0.983 **	0.986 **	0.983 **	0.993 **	−0.677	0.853 *	0.828 *			
MAM1	−0.920 **	−0.922 **	−0.922 **	−0.928 **	−0.922 **	−0.904 *	0.59	−0.976 **	−0.969 **	−0.890 *		
FMO2	0.827 *	0.820 *	0.819 *	0.802	0.820 *	0.788	−0.989 **	0.75	0.424	0.733	−0.633	
UGT74B1	0.992 **	0.991 **	0.990 **	0.986 **	0.991 **	0.979 **	−0.851 *	0.908 *	0.758	0.957 **	−0.881 *	0.891 *

N = 6. * Significant correlation at 0.05 level; ** The correlation was significant at the level of 0.01. Note: Red colors indicate positive correlations; blue indicates negative correlations.

5. Discussion

Sulfur is found in amino acids such as cysteine, methionine, and homocysteine and in some common enzymes. Therefore, sulfur is indispensable for proteins, enzymes, and physiologically active substances in crops. Sulfur fertilizer can promote protein synthesis, increase the oil content of oil crops, and enhance the nitrogen fixation ability of soybean crops. Applying sulfur fertilizer can also improve crop yield and quality. The results showed that the content of polyphenols in broccoli increased gradually with the increase in K_2SO_4 concentration. In contrast, the content of Vc in broccoli was lower than that in control under all sulfur treatments, which may be due to the stress effect of K_2SO_4. These results are similar to Chu Ting [29]. She treated cauliflower sprouts with four different concentrations of $MgSO_4$ (0, 25, 50, and 75 mmol·L^{-1}). It was also found that with the increase in $MgSO_4$ concentration, the Vc content decreased, and the total phenol content increased.

In this study, the contents of soluble protein, soluble sugar, and total flavonoids increased gradually with the increase in K_2SO_4 concentration. The content of MDA was the lowest at 75 g·m^{-2} K_2SO_4, but the activity of SOD was the highest. The results indicate that with the increase in K_2SO_4 concentration, the stress effect became more and more obvious and the antioxidant capacity of broccoli became stronger and stronger. In a study conducted by Ding Yi [30], a similar conclusion was drawn. With the increase in sulfur concentration, the content of flavonoids in pakchoi gradually increased, and the antioxidant capacity also gradually increased.

Glucosinolate can be divided into aliphatic glycosinolate, indole glycosinolate, and aromatic glycosinolate. It is generally believed that aliphatic glycosinolate plays a significant role in the overall adaptability of plants. In contrast, indole glycosinolate is considered the critical determinant of pest and disease resistance [31]. RAA belongs to aliphatic glucosinolates, while GBC belongs to indole glucosinolates. RAA degrades to produce sulforaphane, the most potent anticancer substance found in vegetables so far [32]; therefore, it has been widely discussed. Broccoli, as a vegetable containing 14 kinds of glucosinolates and a high content of glucosinolates, has become a research hotspot. Researchers have been experimenting with increasing the content range of glucosinolate, a sulfur-containing substance, by adding sulfur fertilizer. The results showed that the content of glucosinolates and total glucosinolates in broccoli treated with K_2SO_4 increased, and the content of sulforaphane increased significantly compared with the control, which was similar to that of Runqiang Yang [14]. After $ZnSO_4$ treatment, the content of total glucosinolates and sulforaphane in broccoli increased significantly. The reason may be that K_2SO_4 caused a stress effect on broccoli and accumulated glucosinolate content or that K_2SO_4 as a sulfur source promoted glucosinolate synthesis; however, this conclusion is related to a study conducted by Aires [33]. When KNO_3, K_2SO_4, and KCl were applied to broccoli sprouts, it was found that broccoli sprouts did not need any S and N fertilization to increase glucosinolate levels because there were different conclusions drawn regarding whether this would have adverse effects. This difference may be due to the specifics of the study conducted by Aires [33]. Broccoli sprouts were used as the experimental material, but the results of the experiment

were influenced by the different contents of glucosinolates in broccoli bulbs and broccoli in different periods.

Glucosinolate content will accumulate when plants are stressed, e.g., by high temperatures and hypoxia, or when exogenous substances such as methyl jasmonate and jasmonic acid are applied. However, the range of secondary metabolites will not continue to increase when the stress is to a certain extent [17,34–37]. Isothiocyanates are produced by the hydrolysis of glucosinolates by myrosinase, and epidermal-specific thionin exists in broccoli, which can make glucosinolates hydrolyze in the direction of nitriles, thus reducing the content of isothiocyanates. Still, nitriles have no anticancer activity [38]. Therefore, glucosinolate content and myrosinase activity are significant for forming isothiocyanate. The change in enzyme activity of black mustard is closely related to plant species, varieties, Vc content, and temperature [39]. It has been found that Vc can change the central conformation of myrosinase and affect its activity. When the concentration of Vc is low, it can increase the activity of myrosinase, but when the concentration of Vc is too high, it will inhibit its activity [39,40]. In this study, myrosinase activity was the highest under the 75 $g \cdot m^{-2}$ K_2SO_4 treatment, which may be due to the low content of Vc at this time. And the sulforaphane change trend is consistent with the change trend of total glucosinolate content. This result is the same as that found by Liping Guo [35]. It was found that the content of total glucosinolates and sulforaphane in broccoli treated by JA on the 3rd day of germination were similar.

In this study, *CYP79B2*, *CYP83B1*, *CYP83A1*, *FMO2*, *UGT74B1*, *GSTF*, *ST5b*, *AOP3*, *AOP2*, *GSL-OH*, *CYP81F1*, *MYB28*, and *MYB34* were all up-regulated or significantly up-regulated under S150 treatment. Guo et al. [35] found that broccoli buds formed on the 5th and 7th days of germination. The expressions of *ST5b*, *AOP2*, *UGT74B1*, *MYB28,* and *CYP83A1* were also up-regulated after 100 μM of JA treatment. The results indicate that the key genes in the glucosinolate anabolic pathway in broccoli had different responses to different concentrations of exogenous K_2SO_4, and exogenous K_2SO_4 treatment could regulate the synthesis and accumulation of glucosinolates in broccoli by regulating the expression of key genes in the glucosinolate anabolic pathway. However, the results of Guo [35] showed that the expression of *CYP79F1* was up-regulated, and the expression of *CYP79F1* was down-regulated after S150 treatment, which may be due to different experimental materials. In Guo's study [35], broccoli sprouts were used, and in this study, mature broccoli bulbs were used, which may contain different exogenous substances. Similarly, Aghajanzadeh [11] et al. showed up-regulation of *CYP79B2* in rapeseed stems, and the expressions of *CYP83B1* and *MYB34* were also up-regulated after 50 mM of Na_2SO_4 treatment.

The results of correlation analysis showed that *CYP79B2*, *CYP83A1*, *CYP81F1*, *FMO2*, *UGT74B1*, *AOP2*, and *MYB34* had a significant or extremely significant positive correlation with RAA, GBC, total indole glucosinolates, total aliphatic glucosinolates, and total glucosinolates, respectively, which indicated that *CYP79B2*, *CYP83A1*, *CYP81F1*, *FMO2*, *UGT74B1*, *AOP2*, and *MYB34* affected the components of glucosinolates and total glucosinolates. As a precursor of sulforaphane, RAA affected the accumulation of sulforaphane in broccoli. The contents of GBC, total indole glucosinolates, total glucosinolates, and sulforaphane were all at a high level under S150 treatment. Although the contents of RAA and total aliphatic glucosinolates did not reach the maximum, they increased significantly compared with the control. The expression levels of *CYP79F1*, *BCAT4*, *ST5a*, *ST5c*, *CYP81F4*, *SUR1*, and *MAM1* were all down-regulated or significantly down-regulated under S150 treatment. Mao Shuxiang [15] showed that the expression levels of *CYP79F1*, *BCAT4*, and *MAM1* were down-regulated with an increase in sulfur concentration. *MAM1* and *CYP81F4* were negatively correlated with the contents of RAA, GBC, total indole glucosinolates, total aliphatic glucosinolates, and total glucosinolates, respectively, which indicated that exogenous K_2SO_4 treatment could inhibit the contents of RAA, GBC, total indole glucosinolates, total aliphatic glucosinolates, and total glucosinolates by down-regulating the expression of *MAM1*. The results showed that the gene responses related to glucosinolate synthesis in broccoli were different under different K_2SO_4 treatments.

6. Conclusions

According to the comprehensive nutritional indexes, different dosages of K_2SO_4 treatment had different effects on the nutritional quality and antioxidant capacity of broccoli. The content of Vc in broccoli of all treatments was lower than that of the control. The content of soluble protein, soluble sugar, polyphenols, and total flavonoids in broccoli receiving the 150 $g \cdot m^{-2}$ treatment was the highest, which was significantly higher than that of the control. MDA was significantly inhibited after the 75 $g \cdot m^{-2}$ treatment, but SOD was the highest under the 75 $g \cdot m^{-2}$ treatment. We conclude that the treatment with 150 $g \cdot m^{-2}$ was beneficial in improving the nutritional quality and antioxidant capacity and significantly increased the content of glucosinolates, total indole glucosinolates, total glucosinolates, and sulforaphane in broccoli. The way certain genes were expressed, specifically *CYP79B2*, *CYP83B1*, *CYP83A1*, *FMO2*, *UGT74B1*, *GSTF*, *ST5b*, *AOP3*, *AOP2*, *GSL-OH*, *CYP81F1*, *MYB28*, and *MYB34* showed similarities. Notably, under the 150 $g \cdot m^{-2}$ K_2SO_4 treatment, *CYP79B2*, *CYP83B1*, *CYP83A1*, *AOP2*, *UGT74B1*, and *MYB34* were significantly up-regulated and reached their peak value. *CYP79F1*, *CYP81F4*, and *MAM1* showed significant inhibitory effects when treated with 150 $g \cdot m^{-2}$ of K_2SO_4. The expression levels of *BCAT4*, *CYP81F1*, *ST5a*, *ST5c*, and *SUR1* were down-regulated under the 150 $g \cdot m^{-2}$ K_2SO_4 treatment, but not significantly. According to our results, the change in glucosinolate content is consistent with the trend of gene expression of key enzymes in biosynthesis.

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References

1. Han, J.; Tao, W.; Hao, H.; Zhang, B.; Jiang, W.; Niu, T.; Li, Q.; Cai, T. Physiology and Quality Responses of Fresh-cut Broccoli Flores Pretreated with Ethanol Vapor. *J. Food Sci.* **2006**, *71*, S385–S389. [CrossRef]
2. Del Carmen Mart í Nez-Ballesta, M.; Moreno, D.A.; Carvajal, M. The physiological impact of glucosinolates on plant response to abiotic stress in Brassica. *Int. J. Mol. Sci.* **2013**, *14*, 11607–11625. [CrossRef] [PubMed]
3. Shapiro, T.A.; Fahey, J.W.; Wade, K.L.; Stephenson, K.K.; Talalay, P. Human Metabolism and Excretion of Cancer Chemoprotective Glucosinolates and Isotheocyanates of Cruciferous Vegetables. *Cancer Epidemiol. Biomark. Prev.* **1998**, *7*, 1091.
4. Hanschen, F.S.; Rohn, S.; Mewis, I.; Schreiner, M.; Kroh, L.W. Influence of the chemical structure on the thermal degradation of the lucosinolates in broccoli sprouts. *Food Chem.* **2012**, *130*, 1–8. [CrossRef]
5. Lin, L.Z.; Sun, J.; Chen, P.; Zhang, R.W.; Fan, X.E.; Li, L.W.; Harnly, J.M. Profiling of Glucosinolates and Flavonoids in *Rorippa indica* (Linn.) Hiern. (Cruciferae) by UHPLC-PDA-ESI/HRMSn. *J. Financ. Food Chem.* **2014**, *62*, 6118–6129. [CrossRef] [PubMed]
6. Schuster, J.; Knill, T.; Reichelt, M.; Gershenzon, J.; Binder, S. Branched-chain aminotransferase 4 is part of the chain elongation path in the biosynthesis of methodine-derived glucosinolates in Arabidopsis. *Plant Cell* **2006**, *18*, 2664–2679. [CrossRef]
7. Mikkelsen, M.D.; Hansen, C.H.; Wittstock, U.; Halkier, B.A. Cytochrome P450 CYP79B2 from Arabidopsis Catalyzes the Conversion of Tryptophan to Indole-3-acetaldoxime, a Precursor of Indole Glucosinolates and Indole-3-acetic Acid. *J. Biol. Chem.* **2000**, *275*, 33712–33717. [CrossRef]
8. Hull, A.K.; Vij, R.; Celenza, J.L. Arabidopsis cytochrome P450s that catalyst the first step of tryptophan-dependent indole-3-acetic acid biosynthetic. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2379–2384. [CrossRef]
9. Hemm, M.R.; Ruegger, M.O.; Chapter, C. The Arabidopsis ref2 mutant is defective in the gene encoding CYP83A1 and shows both phenylpropanoid and glucosinolate phenotypes. *Plant Cell* **2003**, *15*, 179–194. [CrossRef]

10. Hansen, C.H.; Du, L.; Naur, P.; Olsen, C.E.; Axelsen, K.B.; Hick, A.J.; Halkier, B.A. CYP83b1 is the oxime-metabolizing enzyme in the lucosinolate path in Arabidopsis. *J. Biol. Chem.* **2001**, *276*, 24790–24796. [CrossRef]
11. Aghajanzadeh, T.A.; Reich, M.; Kopriva, S.; De Kok, L.J. Impact of chloride (NaCl, KCl) and sulphate (Na_2SO_4, K_2SO_4) salinity on lucosinolate metabolism in Brassica rapa. *J. Agron. Crop Sci.* **2018**, *204*, 137–146. [CrossRef]
12. Yi, G.E.; Robin, A.H.K.; Yang, K.; Park, J.I.; Hwang, B.H.; Nou, I.S. Exogenous Method Jasmonate and Salicylic Acid Index Subspecies-Specific Patterns of Glucosinolate Accumulation and Gene Expression in *Brassica learned* L. *Molecules* **2016**, *21*, 1417. [CrossRef] [PubMed]
13. Cheng, Y. *Enrichment of Isothiocyanate in Western Blue Flower Buds by Selenium and Sulfur Interaction and Its Mechanism*; Yangzhou University: Yangzhou, China, 2020.
14. Yang, R.; Guo, L.; Jin, X.; Shen, C.; Zhou, Y.; Gu, Z. Enhancement of glucosinolate and sulforaphane formation of broccoli sprouts by zinc sulphate via its stress effect. *J. Funct. Foods* **2015**, *13*, 345–349. [CrossRef]
15. Mao, S. *Effect of Selenium and Sulfur Interaction on Sulforaphane Synthesis in Broccoli*; Hunan Agricultural University: Changsha, China, 2019.
16. Cheng, K. *Effects of Selenium and Sulfur Interaction on Physiological and Photosynthetic Characteristics of Broccoli Seedlings*; Sichuan Agricultural University: Chengdu, China, 2016.
17. Guo, L.; Yang, R.; Wang, Z.; Guo, Q.; Gu, Z. Effect of NaCl stress on health-promoting compounds and antioxidant activity in the sprouts of three broccoli cultivars. *Int. J. Food Sci. Nutr.* **2014**, *65*, 476–481. [CrossRef]
18. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantifications of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
19. Pramanik, B.K. Changes in acid invertase activity and sugar distribution during postharvest senescence in broccoli. *Pak. J. Biol. Sci.* **2004**, *7*, 679–684. [CrossRef]
20. Orthofer, R.L.V.S. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1998**, *299*, 152–178.
21. Wang, L.I.; Shan, T.; Xie, B.; Ling, C.; Shao, S.; Jin, P.; Zheng, Y. Glycine betaine reduces chilling injection in peach fruit by enhancing phenolic and sugar metabolisms. *Food Chem.* **2019**, *272*, 530–538. [CrossRef]
22. Papastergiadis, A.; Mubiru, E.; Van Langenhove, H.; De Meulenaer, B. Malondialdehyde Measurement in Oxidized Foods: Evaluation of the Spectrophotometric Thiobarbituric Acid Reactive Substances (TBARS) Test in Various Foods. *J. Agric. Food Chem.* **2012**, *60*, 9589–9594. [CrossRef]
23. Tang, B.; Wang, Y.; Ma, L. Simple and rapid catalytic spectrophotometric determination of superoxide anion radical and superoxide dismutase activity in natural medical vegetables using phenol as the substrate for horseradish peroxidase. *Anal. Bioanal. Chem.* **2004**, *378*, 523–528. [CrossRef]
24. Wang, Y.-Q.; Hu, L.-P.; Liu, G.-M.; Zhang, D.-S.; He, H.-J. Evaluation of the Nutritional Quality of Chinese Kale (*Brassica alboglabra* Bailey) Using UHPLC-Quadrupole-Orbitrap MS/MS-Based Metabolomics. *Molecules* **2017**, *22*, 1262. [CrossRef] [PubMed]
25. Matusheski, N.V.; Juvik, J.A.; Jeffery, E.H. Heating decisions epithiospecifier protein activity and increments sulforaphane formation in broccoli. *Phytochemistry* **2004**, *65*, 1273–1281. [CrossRef] [PubMed]
26. Guo, R.; Yuan, G.; Wang, Q. Effect of sucrose and mannitol on the accumulation of health-promoting compounds and the activity of metabolic enzymes in broccoli sprouts. *Sci. Hortic.* **2011**, *128*, 159–165. [CrossRef]
27. Czechowski, T.; Bari, R.P.; Stitt, M.; Scheible, W.R.; Udvardi, M.K. Real-Time RT-PCR profiling of over 1400 Arabidopsis translation factors: Unpreceded sensitivity reveals novel root-And shoot-Specific genes. *Plant J. Cell Mol. Biol.* **2004**, *38*, 366–379. [CrossRef] [PubMed]
28. Zhang, Y.; Lv, C.; Sun, J.; Song, X.; Makaza, N.; Wu, Y. Protective effects of broccoli extracts and sulforaphane against hydrogen peroxide induced oxidative stress in B16 cells. *J. Funct. Foods* **2021**, *87*, 104833. [CrossRef]
29. Chu, T.; Peng, C.; Guo, L. $MgSO_4$ Effects of treatment on physiological active substances and antioxidant capacity of broccoli sprouts. *Food Sci.* **2018**, *39*, 53–59.
30. Ding, Y. *Effects of Selenium and Sulfur Interaction on Growth and Antioxidant Quality of Pakchoi Plants*; Zhejiang Agriculture and Forestry University: Hangzhou, China, 2014.
31. Augustine, R.; Bisht, N.C. Biofortification of oilseed *Brassica juncea* with the anti-cancer compound glucoraphanin by supporting GSL-ALK gene family. *Sci. Rep.* **2015**, *5*, 18005. [CrossRef]
32. Juge, N.; Mithen, R.F.; Traka, M. Molecular basis for chemoprevention by sulforaphane: A comprehensive review. *Cell. Mol. Life Sci.* **2007**, *64*, 1105–1127. [CrossRef]
33. Aires, A.; Rosa, E.; Carvalho, R. Effect of nitrogen and sulfur fertilisation on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. *italica*). *J. Sci. Food Cult.* **2006**, *86*, 1512–1516. [CrossRef]
34. Guo, L.; Yang, R.; Zhou, Y.; Gu, Z. Heat and hypoxia stresses enhance the accumulation of aliphatic glucosinolates and sulforaphane in broccoli sprouts. *Eur. Food Res. Technol.* **2015**, *242*, 107–116. [CrossRef]
35. Guo, L.; Yang, R.; Gu, Z. Cloning of genes related to aliphatic glucosinolate metabolism and the mechanism of sulforaphane accumulation in broccoli sprouts under jasmonic acid treatment. *J. Sci. Food Agric.* **2016**, *96*, 4329–4336. [CrossRef] [PubMed]
36. Baenas, N.; Garcia-Viguera, C.; Moreno, D.A. Biotic elicitors effectually increase the glucosinolates content in *Brassicaceae sprouts*. *J. Agric. Food Chem.* **2014**, *62*, 1881–1889. [CrossRef] [PubMed]

37. Guo, R.; Hou, Q.; Yuan, G.; Zhao, Y.; Wang, Q. Effect of 2, 4-epibrassinolide on main health-promoting compounds in broccoli sprouts. *LWT-Food Sci. Technol.* **2014**, *58*, 287–292. [CrossRef]
38. Angelino, D.; Jeffery, E. Glucosinolate hydrolysis and bioavailability of resulting isotheocyanates: Focus on glucoraphanin. *J. Funct. Foods* **2014**, *7*, 67–76. [CrossRef]
39. Guo, Q.; Guo, L.; Wang, Z.; Zhuang, Y.; Gu, Z. Response surface optimization and identification of isotheocyanates produced from broccoli sprouts. *Food Chem.* **2013**, *141*, 1580–1586. [CrossRef]
40. Gu, Z.-X.; Guo, Q.-H.; Gu, Y.-J. Factors Influencing Glucoraphanin and Sulforaphane Formation in Brassica Plants: A Review. *J. Integr. Agric.* **2012**, *11*, 1804–1816. [CrossRef]