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The Study of Selenium Fertilizer on the Growth of Xiangzayou 787 and Related Molecular Mechanism

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Abstract: Rapeseed is the largest self-produced oil crop in China which plays an important role in ensuring the safety of edible oil. However, its current per unit yield is far below Canada and Europe. In this study, selenium fertilizer and other micro fertilizers were sprayed on Xiangzayou 787 at the seedling stage. The results showed that the yield per plant increased 24.3% with sprayed selenium compared to the control (CK). Compared with the CK, the chlorophyll content in leaves at the flowering stage was significantly increased by 20.8%, and the soluble sugar content in the silique wall and seeds at the maturity stage was significantly increased and increased by 62.1% during the budding stage. The functional leaves of Xiangzayou 787 with the sprayed selenium fertilizer and the CK were used as raw materials for absolute quantitative transcriptome sequencing analysis. Accompanied with bioinformatics analysis, six differential genes which affect growth were discovered. The expression level of the protein phosphatase 2C gene in the silique wall was significantly higher than that of the CK. The *PP2C78* gene was significantly positively correlated with the chlorophyll and soluble sugar content in leaves and the correlation coefficients were 0.539 and 0.547. According to gene expression levels, yield, and physiological indicators, *PP2C78* may be a key functional gene affecting rapeseed yield. In this study, selenium fertilizer was found to be an excellent foliar fertilizer for rapeseed; the *PP2C78* gene may be helpful for analyzing the yield increasing mechanism and used as a reference for screening new foliar fertilizers.

Keywords: selenium fertilizer; Xiangzayou 787; chlorophyll; soluble sugar; protein phosphatase 2C gene

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1. Introduction

Rapeseed (*Brassica napus* L.) is the largest edible oil crop in China. Hunan Province has the largest rapeseed planting area, with a total output of 2.438 million tons in 2022, ranking the third in China, and the average yield per unit area is 386 kg·hm^{-2} lower than the average yield of China [1]. The demand for edible oil has increased more and more, but the self-sufficiency rate is still low in China [2,3]. Therefore, improving the yield is the main goal of rapeseed production in China. The improvement of cultivation and management techniques may be the main way to increase rapeseed yield [3]. The application of fertilizers is one of the main factors affecting rapeseed yield. Currently, rapeseed cultivation in China uses compound fertilizer or little fertilization, and mainly focuses on nitrogen fertilizer (N), phosphorus fertilizer (P_2O_5), and potassium fertilizer (K_2O) [4,5]. So it is urgently necessary to find new fertilizers to improve rapeseed per unit yield.

Brassinolide plays an important role in plant growth, development and stress resistance [6–8]. Silicon can alleviate the effects of abiotic and biological stresses on plants and improve plant resistance [9], and spraying foliar silicon fertilizer in the seedling stage of rapeseed can improve its cold tolerance, yield, and 1000-grain weight [9]. The exogenous

application of selenium fertilizer with a reasonable concentration can promote plant growth, improve quality, and increase yield [10–12]. There has been relatively little Se research related to plant physiology and the effects of different combinations of the three types of foliar fertilizers mentioned above on the yield and quality of rapeseed. Appropriate selenium concentration can increase crop yield and improve crop quality. Zhang et al. [13] studied the effect of the foliar spraying of zinc, iron, and selenium on wheat yield, and found that only spraying selenium had a significant effect on wheat yield. Mu et al. [14]. found that by spraying different concentrations of selenium fertilizer at different growth stages of foxtail millet, appropriate selenium concentrations could improve crop yield and quality. Wang et al. [15] found that the foliar spraying of selenium fertilizer could increase soybean yield and selenium content by spraying the same concentration of selenium fertilizer at different stages of soybean development, but the concentration should not be too high. Huang et al. [16] studied the effects of foliar spraying and root spraying on the yield of rapeseed. It was found that the selenium content of rapeseed with the foliar spraying of selenium fertilizer was significantly higher than that with the root spraying of selenium fertilizer. Appropriate selenium could effectively promote the growth of rapeseed and increase the yield of rapeseed.

Selenium has an impact on the growth and development, stress resistance, photosynthesis, and respiration regulation of plants. Selenium can alleviate the damage caused by abiotic stress by absorbing beneficial substances, increasing the activity of antioxidant enzymes, and inhibiting the production of MDA. Wang et al. [17] used different concentrations of sodium selenate to affect tea seedlings and found that compared with the control, the contents of chlorophyll a, chlorophyll b, and carotenoids increased under low selenium concentration (0.15 and 0.3 mg/L). The appropriate concentration (0.3 mg/L) of selenium enhanced photosynthesis and root activity, and increased biomass. Liu et al. [18] conducted a pot experiment to study the effect of the foliar spraying of nano selenium fertilizer on the physiological characteristics of foxtail millet. They found that low selenium treatment was beneficial for improving seedling survival rate and promoting the growth of foxtail millet seedlings. Moreover, the low dosage of selenium treatment enhanced stress resistance and delayed the senescence of foxtail millet. Yang et al. [19] showed that compared with the control group, the foliar spraying of sodium selenite solution at a concentration of 60 g/hm^2 significantly increased POD activity and GSH content in the leaves, while reducing MDA content, effectively improving chlorophyll content in glutinous corn leaves. At this concentration, the antioxidant activity of glutinous corn leaves was enhanced, the degree of membrane lipid peroxidation was reduced, and the utilization of light energy by leaves was promoted. Ma et al. [20] found that compared with the control, when the concentration of Na_2SeO_3 was 0.200 g/kg, the proline and soluble sugar in the leaves of rapeseed seedlings increased by 377.21% and 47.38%, respectively. Low concentration selenium treatment could enhance the stress resistance of rapeseed seedlings, delay aging, and promote growth.

Absolute quantitative transcriptome sequencing [21,22] has a lower sample size and lower initial library size, more accurate quantitative analysis results, higher consistency between differential genes and qPCR verification, and a more accurate identification of variable splicing events than conventional transcriptome analysis [23,24]. At present, it is mainly applied in the field of animal medicine [21], with little application in plants [14]. In this study, brassinolide (BR), silicon (Si), and selenium (Se), and their different combinations were used as foliar fertilizers sprayed on rapeseed. Based on the agronomic and physiological indicators of the seedlings and mature stages, the optimal treatment was determined. Absolute quantitative transcriptome sequencing and bioinformatics analysis were used to identify potential differentially expressed genes that affect the growth of rapeseed seedlings, then the RT-qPCR method was used to study the expression patterns of these genes, which may be useful to provide a reference for screening suitable foliar fertilizers and molecular mechanism research.

2. Materials and Methods

2.1. Experimental Materials

The treatment agent was 0.01% 2,4-epibrassinolide (Hebei Lansheng Biotechnology Co., Ltd., Jinzhou, China), highly concentrated organic selenium $\geq$ 2 g·L^{-1} (Jiangxi Kenongwo Technology Co., Ltd., Yichun, China), and fly-proof liquid silicon $\geq$ 100 g·L^{-1} (Chuzhou Geli Fertilizer Technology Co., Ltd., Chuzhou, China).

Zhongshuang 11, Fengyou 958 and Xiangzayou 787 were provided by the College of Agriculture, Hunan Agricultural University (Changsha, China).

2.2. Experimental Methods

The experiment was conducted at the Yunyuan Base of Hunan Agricultural University (113°4′ E, 28°10′ N). Different treatments of rapeseed were sown on 1 October and harvested on 1 May of the following year.

The experiments were adopted as randomized block design, consisting of clean water as the CK BR, BR + Si, BR + Si + Se, BR + Se, Si, Si + Se, and Se. Each experiment was repeated 3 times, and the area of each experimental community was 5 m^2. The usage of BR, Se, and Si communities was 100 μL, 400 μL, and 250 μL, respectively, and then the volume was fixed to 200 mL, based on the dilution concentration provided by Jiangxi Kenongwo Technology Co., Ltd. company as a reference. On 27 October 2021, BR was sprayed on rapeseed [25], on 11 December 2021, Si fertilizer was sprayed on rapeseed [9], and on 2 December 2021 Se was sprayed on rapeseed [26].

2.2.1. Investigation on Agronomic Characters of Rapeseed

At the seedling stage (11 December 2021), 5 plants were selected for each treatment, and the number of green leaves on the main stem, total number of leaves on the main stem, length of the largest leaf, leaf width, root and stem thickness, and plant height were investigated [25]. During the harvest period (1 May 2022), 5 plants with moderate growth were selected for trait assessment in each treatment [27].

2.2.2. Physiological Indexes Measurement

Samples were collected one week after the foliar fertilizer spraying. At 20 days (10 April 2022), 25 days, 30 days, 35 days, and 40 days after pollination, the siliques were collected and used for physiological measurements and RNA extraction. Chlorophyll content was determined by the 95% alcohol extraction method [28], soluble sugar content was determined by the anthrone sulfuric acid method [29], and soluble protein content was determined by the Coomassie Brilliant Blue G-250 method [30].

Chlorophyll content extraction method: The fresh rape leaves were washed, dried, cut into pieces, and mixed well. A total of 1.00 g was weighed, and 3 portions were weighed, and placed in a 50 mL volumetric flask. Then, 95% alcohol was added, diluted to a constant volume, and the flask was placed in a dark place until the leaves were completely white. The absorbance values were measured and recorded at 645 nm and 663 nm by an ultraviolet–visible spectrophotometer.

$$\text{The total content of chlorophyll (mg/g)} = (20.2A645 + 8.02A663)V/W \times 1000.$$

In the formula, A645 represents the absorbance value at a wavelength of 645 nm; A663 represents the absorbance value at a wavelength of 663 nm; V is the volume of the extraction solution (mL); and W is the mass of the blade (g). This was repeated 3 times and the average value was taken.

Extraction method of soluble sugars: Take fresh plant leaves, wipe off surface dirt, cut, and mix well. Weigh 0.1–0.3 g for a total of 3 portions (or dry material), put them into 3 graduated test tubes, add 5–10 mL of distilled water, seal with plastic film, extract in boiling water for 30 min (2 extractions), filter the extract into a 25 mL volumetric flask, rinse the test tube and residue repeatedly, and determine capacity. Put 0.5 mL of the sample

solution into a test tube (repeat twice), add 1.5 mL of distilled water, and add 1 mL and 5 mL of phenol and concentrated sulfuric acid solution in order. The absorbance was measured and the content of sugar was found according to the standard curve.

2.2.3. Absolute Quantitative Transcriptome Sequencing

Samples (the third to last functional leaf) were conducted during the bud stage and sent to Wuhan Kangce Technology Co., Ltd. (Wuhan, China) for absolute quantitative transcriptome sequencing data analysis.

HISATA was used to align clean reads with the rapeseed reference genome. RSEM tools were used to detect genes and transcriptional expression levels, and DESeq2 was used to detect differentially expressed genes (p-value ≤ 0.05, $|\log2\text{fold change}| > 1$). The GO database (Gene Ontology, http://geneontology.org/, accessed on 15 March 2024) and KEGG database (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/, accessed on 15 March 2024) were used for functional annotation and classification statistics on differentially expressed genes.

2.2.4. Verification of Sequencing Results and Study on the Expression Patterns of Key Differentially Expressed Genes

Real-time fluorescence quantitative PCR technology was used to study the expression patterns of potential differentially expressed genes, and Actin was used as the internal reference gene. RNA was extracted by the Trans Zol Up Plus RNA Kit (Beijing GMO Biotechnology Co., Ltd., Beijing, China). RNA quality was measured by a Nanodrop2000 (Thermo Scientific, Waltham, MA, USA) and 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Hifair®III1st(TransGen Biotech Co., Ltd., Beijing, China) Strand cDNA Synthesis Super Mix for qPCR was used for cDNA synthesis with the gDNA digester plus reverse transcription kit. RT-qPCR was performed on the samples using the Hieff® qPCR SYBR®Green(Yeasen Biotechnology Co., Ltd., Shanghai, China) Master Mix kit. The reaction system and procedure referred to the method of Yan W [31]. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ formula [32].

2.3. Data Analysis and Statistics

SPSS 23.0 software was used for data analysis, and Excel 2016 was used to create charts.

3. Results

3.1. Selection of Varieties and Treatments

3.1.1. Effects of Different Treatments on the Growth of Rapeseed at Bud Stage

The results of the growth of rapeseed during the bud stage under different treatments are shown in Table 1. Compared with the CK, all treatments had a promoting effect on all three materials. Material C had the best effect, with a significant increase in the number of green leaves on the main stem, total leaves on the main stem, and plant height.

Table 1. Growth of rapeseed plants under different treatments at budding stage.

Materials	Green Leaves of Main Stem	Total Leaves of Main Stem	The Largest Leaf		Width of Rootstock (cm)	Plant Height (cm)
			Length (cm)	Width (cm)		
A1	10.67 ± 0.58 ab	11.00 ± 1.00 abc	16.17 ± 2.47 a	12.67 ± 1.26 a	1.53 ± 0.06 a	26.00 ± 2.00 ab
A2	12.00 ± 1.00 a	13.00 ± 1.00 a	17.67 ± 3.21 a	13.33 ± 2.31 a	1.73 ± 0.25 a	31.00 ± 1.00 a
A3	10.67 ± 0.58 ab	12.00 ± 1.00 ab	17.67 ± 2.08 a	12.00 ± 2.65 a	1.50 ± 0.30 a	30.00 ± 2.00 a
A4	9.67 ± 0.58 bc	11.00 ± 1.00 abc	18.00 ± 2.29 a	12.33 ± 1.04 a	1.63 ± 0.32 a	29.33 ± 3.00 a
A5	9.67 ± 1.53 bc	11.00 ± 1.00 abc	17.67 ± 3.21 a	12.00 ± 1.00 a	1.57 ± 0.51 a	26.00 ± 3.46 ab
A6	10.67 ± 1.53 ab	11.67 ± 1.53 abc	16.00 ± 2.65 a	10.17 ± 1.44 a	1.50 ± 0.50 a	27.00 ± 2.00 a
A7	8.33 ± 1.53 c	9.67 ± 2.08 c	16.47 ± 2.25 a	10.17 ± 1.76 a	1.27 ± 0.46 a	27.00 ± 2.65 a
A8	9.00 ± 1.00 bc	10.67 ± 0.58 bc	18.83 ± 2.47 a	12.50 ± 3.00 a	1.60 ± 0.36 a	22.00 ± 2.00 b

Table 1. *Cont.*

Materials	Green Leaves of Main Stem	Total Leaves of Main Stem	The Largest Leaf		Width of Rootstock (cm)	Plant Height (cm)
			Length (cm)	Width (cm)		
B1	15.67 ± 1.15 a	16.67 ± 1.53 a	26.30 ± 1.76 a	18.40 ± 1.22 a	2.13 ± 0.21 a	57.50 ± 0.50 b
B2	15.33 ± 0.58 a	17.00 ± 1.00 b	22.00 ± 3.00 b	18.17 ± 3.01 ab	2.27 ± 0.40 a	46.50 ± 1.50 d
B3	15.00 ± 1.73 ab	16.33 ± 1.53 a	27.33 ± 1.15 a	17.33 ± 0.58 abc	2.33 ± 0.29 a	43.50 ± 2.50 d
B4	15.67 ± 2.08 a	16.00 ± 0.00 a	25.33 ± 2.08 ab	16.00 ± 3.00 abc	2.00 ± 0.11 a	57.00 ± 1.00 b
B5	15.00 ± 1.73 ab	16.33 ± 1.53 a	29.00 ± 3.61 a	18.67 ± 1.53 a	2.17 ± 0.29 a	61.00 ± 1.00 a
B6	12.33 ± 2.52 bc	12.67 ± 0.58 a	24.67 ± 2.52 ab	15.00 ± 0.00 bc	2.000 ± 0.11 a	50.50 ± 2.50 c
B7	10.67 ± 0.58 c	12.00 ± 0.50 b	21.67 ± 2.08 b	14.33 ± 0.58 c	1.83 ± 0.29 a	54.50 ± 3.50 b
B8	12.00 ± 1.00 c	13.33 ± 1.53 b	22.67 ± 0.58 b	16.00 ± 1.00 abc	1.83 ± 0.29 a	56.00 ± 1.00 b
C1	11.00 ± 1.00 b	11.00 ± 1.00 b	27.50 ± 0.50 a	18.33 ± 1.54 a	2.20 ± 0.35 abc	21.33 ± 1.15 cd
C2	10.67 ± 0.57 b	11.00 ± 1.00 b	27.00 ± 2.00 a	18.67 ± 1.52 a	2.93 ± 0.11 a	22.00 ± 2.65 bcd
C3	13.00 ± 1.00 a	13.67 ± 0.57 a	27.67 ± 2.08 a	19.50 ± 0.50 a	2.67 ± 0.76 ab	24.00 ± 1.73 ab
C4	8.67 ± 0.57 c	9.50 ± 0.50 cd	21.00 ± 1.00 b	16.30 ± 0.57 ab	1.93 ± 0.11 bc	23.50 ± 0.50 abc
C5	11.67 ± 1.15 ab	11.67 ± 1.54 b	24.67 ± 4.61 ab	19.30 ± 2.31 a	2.57 ± 0.51 ab	23.50 ± 0.50 abc
C6	8.33 ± 1.15 c	9.50 ± 0.50 cd	24.67 ± 4.16 ab	14.30 ± 1.15 b	1.67 ± 0.29 c	24.67 ± 0.57 a
C7	10.33 ± 0.57 b	10.67 ± 0.57 bc	25.00 ± 3.00 ab	18.30 ± 3.21 a	2.33 ± 0.58 abc	20.00 ± 1.00 d
C8	8.00 ± 1.00 c	9.30 ± 0.57 d	21.50 ± 1.50 b	16.30 ± 1.15 ab	1.53 ± 0.58 c	17.00 ± 1.00 e

Note: A: Zhongshuang 11; B: Fengyou 958; C: Xiangzayou 787; 1–8: BR, BR + Si, BR + Si + Se, BR + Se, Si, Si + Se, Se, and CK. Statistical analysis: There were significant differences between different letters ($p < 0.05$).

3.1.2. Effects of Different Treatments on Yield and Yield Components of Rapeseed

The effects of different treatments on the yield and yield components are shown in Table 2. Compared with the CK, different treatments had a promoting effect on all three materials. Material C7 had the best effect, and the effective number of silique, 1000-grain weight, and yield per plant were significantly improved. Then, Xiangzayou 787 was used as a raw material in the following studies.

Table 2. Effects of different treatments on yield and yield components of rapeseed.

Materials	Effective Silique Number	Silique Length (cm)	Number of Seeds per Silique	Thousand-Grain Weight (g)	Yield per Plant (g)	Growth Rate (%)
A1	53.67 ± 8.50 ab	10.51 ± 0.97 a	20.60 ± 2.63 a	4.13 ± 0.03 b	13.03	−16.0
A2	65.33 ± 14.05 a	9.94 ± 0.87 a	19.48 ± 1.76 a	4.22 ± 0.04 b	18.54	19.3
A3	62.33 ± 3.78 a	9.68 ± 0.62 a	19.72 ± 4.06 a	4.16 ± 0.05 b	13.67	−12.4
A4	47.67 ± 4.62 b	9.17 ± 1.35 a	19.24 ± 3.26 a	4.00 ± 0.11 b	18.77	20.8
A5	44.00 ± 4.36 b	9.37 ± 0.82 a	20.64 ± 3.18 a	4.09 ± 0.46 b	12.15	−21.7
A6	29.67 ± 1.53 c	8.73 ± 1.33 a	17.16 ± 4.72 a	4.11 ± 0.04 b	9.60	−38.1
A7	44.00 ± 4.35 b	10.50 ± 0.31 a	22.64 ± 1.78 a	4.53 ± 0.07 a	17.90	15.2
A8	44.00 ± 4.35 b	9.87 ± 0.92 a	21.36 ± 2.03 a	4.15 ± 0.07 b	15.53	
B1	43.00 ± 2.65 f	6.21 ± 0.30 bc	16.84 ± 1.70 bc	3.60 ± 0.18 a	9.07	−43.9
B2	54.00 ± 2.00 ef	6.43 ± 0.32 abc	16.84 ± 1.92 bc	4.03 ± 0.03 a	9.79	−39.4
B3	71.00 ± 10.50 d	6.12 ± 0.32 c	15.56 ± 2.90 c	3.84 ± 0.05 a	9.90	−38.7
B4	74.67 ± 8.50 cd	6.57 ± 0.32 ab	18.56 ± 1.33 ab	3.58 ± 0.02 a	16.31	0.8
B5	61.67 ± 6.35 de	6.38 ± 0.35 abc	17.12 ± 2.15 bc	3.75 ± 0.04 a	11.46	−29.1
B6	87.33 ± 9.07 c	6.00 ± 0.33 c	15.74 ± 1.70 c	3.63 ± 0.04 a	14.15	−12.5
B7	122.33 ± 5.13 a	6.11 ± 0.25 c	16.33 ± 1.01 bc	3.59 ± 0.04 a	16.34	1.1
B8	107.00 ± 9.53 b	6.68 ± 0.12 a	19.88 ± 1.05 a	3.77 ± 0.09 a	16.17	
C1	85.30 ± 7.77 bc	7.60 ± 0.18 a	23.80 ± 0.11 a	3.72 ± 0.16 bc	21.82	0.0
C2	92.70 ± 7.02 b	6.50 ± 0.34 b	21.20 ± 0.22 b	3.79 ± 0.07 bc	26.54	21.6
C3	72.30 ± 5.77 cd	7.60 ± 0.16 a	23.70 ± 0.70 a	3.80 ± 0.08 bc	24.61	12.8
C4	70.30 ± 8.58 de	7.60 ± 0.04 a	22.70 ± 0.46 ab	3.66 ± 0.05 c	25.57	17.1
C5	53.00 ± 4.08 f	7.50 ± 0.05 a	23.50 ± 0.12 a	3.72 ± 0.04 bc	14.02	−35.7

Table 2. *Cont.*

Materials	Effective Silique Number	Silique Length (cm)	Number of Seeds per Silique	Thousand-Grain Weight (g)	Yield per Plant (g)	Growth Rate (%)
C6	57.70 ± 2.89 ef	7.60 ± 0.05 a	22.40 ± 1.06 ab	3.88 ± 0.17 b	16.47	−24.5
C7	126.00 ± 14.73 a	7.60 ± 0.22 a	23.40 ± 0.50 a	4.28 ± 0.13 a	27.13	24.3
C8	56.50 ± 4.27 ef	7.70 ± 0.12 a	23.70 ± 1.33 a	3.47 ± 0.03 d	21.82	

Note: A: Zhongshuang 11; B: Fengyou 958; C: Xiangzayou 787; 1–8: BR, BR + Si, BR + Si + Se, BR + Se, Si, Si + Se, Se, and CK. Statistical analysis: There were significant differences between different letters ($p < 0.05$).

3.1.3. Effects of Different Treatments on Physiological Indexes of Xiangzayou 787 at Different Growth Stages

Effects of Different Treatments on Chlorophyll Content of Xiangzayou 787 at Different Growth Stages

The changes in chlorophyll content under different treatments are shown in Table 3. Compared with the CK, different treatments had a significant impact on the chlorophyll content of leaves, flowers, and mature silique walls/seeds. Spraying Se treatment had the best effect, with a significant increase of 20.8% in chlorophyll content during the flowering period. From the initial flowering period to the final flowering period, there was a gradually decreasing trend with a significant increase of 50% during the initial flowering period. The chlorophyll content in silique wall and seeds gradually decreased, then increased by 45.5% 35 days after pollination.

Effects of Different Treatments on Soluble Sugar Content of Xiangzayou 787 at Different Growth Stages

Soluble sugar content under different treatments is shown in Table 3. Compared with the CK, different treatments had a significant impact on the soluble sugar content of leaves, mature silique wall, and seeds. Se treatment had the best effect with a significant increase of 62.1% in the bud stage. The trend of changes in the silique and seeds of the mature stage were consistent, both gradually decreasing 30 days after pollination, and the silique wall and seeds showed significant differences compared to the CK group 20 days after pollination.

Effects of Different Treatments on Soluble Protein Content of Xiangzayou 787 at Different Growth Stages

Soluble protein content under different treatments is shown in Table 3. Compared with the CK, different treatments had a significant impact on the soluble protein content of flower and mature silique wall/seeds. Se treatment had the best effect with a significant increase of 53.3% in the initial flowering stage. The content in the silique wall showed a gradually decreasing trend and significantly increased by 152% 30 days after pollination. The content in mature seeds reached its highest level as the growth period progressed, until 30 days after pollination, with an increased rate of 82%, and then gradually decreased.

The results in Tables 1–3 show that Xiangzayou 787 had the best effect with sprayed Se treatment. The bud stage was a period of simultaneous nutritional and reproductive growth in rapeseed. The growth status during the bud stage had a significant impact on rapeseed yield [33]. Therefore, samples sprayed with Se leaves during the bud stage were sent to the company for absolute quantitative transcriptome sequencing.

Table 3. Physiological indexes of Xiangzayou 787 under different treatments.

	Stage		Treatment								
			CK	BR	BR + Si	BR + Si + Se	BR + Se	Si	Si + Se	Se	Growth Rate (%) (Se)
Chlorophyll content	Leaf	T	0.45 ± 0.03 f	0.83 ± 0.03 b	0.65 ± 0.02 d	0.71 ± 0.03 c	1.01 ± 0.04 a	0.52 ± 0.01 e	0.66 ± 0.02 d	0.50 ± 0.02 e	11.1%
		H	1.20 ± 0.05 d	1.25 ± 0.05 cd	1.07 ± 0.06 e	2.11 ± 0.04 a	1.20 ± 0.04 d	1.31 ± 0.05 c	1.52 ± 0.05 b	1.45 ± 0.05 b	20.8%
	Flower	CH	0.08 ± 0.00 e	0.13 ± 0.00 abc	0.10 ± 0.01 cde	0.11 ± 0.00 bcd	0.09 ± 0.00 de	0.13 ± 0.01 ab	0.14 ± 0.00 a	0.12 ± 0.01 bcd	50%
		SH	0.08 ± 0.00 d	0.09 ± 0.01 c	0.12 ± 0.01 a	0.12 ± 0.00 a	0.12 ± 0.00 a	0.09 ± 0.01 c	0.08 ± 0.00 d	0.11 ± 0.00 b	37.5%
		ZH	0.06 ± 0.00 c	0.08 ± 0.00 b	0.06 ± 0.00 c	0.06 ± 0.00 c	0.09 ± 0.00 a	0.06 ± 0.01 c	0.05 ± 0.00 c	0.06 ± 0.00 c	0.0
	Thorn peel	20d	0.12 ± 0.01 c	0.14 ± 0.00 b	0.12 ± 0.01 c	0.12 ± 0.01 c	0.12 ± 0.00 c	0.13 ± 0.00 b	0.14 ± 0.00 b	0.15 ± 0.01 a	25%
		25d	0.18 ± 0.01 a	0.17 ± 0.00 a	0.14 ± 0.01 d	0.16 ± 0.00 c	0.16 ± 0.01 c	0.12 ± 0.00 f	0.16 ± 0.00 c	0.13 ± 0.01 e	−27.8%
		30d	0.14 ± 0.01 bc	0.16 ± 0.00 a	0.13 ± 0.00 de	0.14 ± 0.00 bc	0.14 ± 0.01 cd	0.13 ± 0.00 e	0.15 ± 0.01 d	0.11 ± 0.00 f	−21.4%
		35d	0.11 ± 0.00 d	0.15 ± 0.01 a	0.10 ± 0.00 de	0.12 ± 0.01 c	0.12 ± 0.01 c	0.09 ± 0.00 e	0.13 ± 0.01 b	0.16 ± 0.01 a	45.5%
		40d	0.11 ± 0.00 a	0.11 ± 0.00 ab	0.10 ± 0.00 cd	0.09 ± 0.00 e	0.10 ± 0.00 d	0.10 ± 0.00 d	0.11 ± 0.00 bc	0.09 ± 0.00 e	−18.2%
	Seed	20d	0.41 ± 0.02 c	0.53 ± 0.01 a	0.44 ± 0.01 b	0.42 ± 0.02 bc	0.53 ± 0.03 a	0.45 ± 0.00 b	0.46 ± 0.02 b	0.39 ± 0.00 c	−4.8%
		25d	0.44 ± 0.01 e	0.31 ± 0.00 f	0.45 ± 0.01 d	0.55 ± 0.01 b	0.45 ± 0.01 de	0.52 ± 0.01 c	0.66 ± 0.00 a	0.46 ± 0.01 d	4.5%
		30d	0.26 ± 0.01 e	0.33 ± 0.02 a	0.27 ± 0.02 de	0.22 ± 0.00 f	0.30 ± 0.00 b	0.25 ± 0.01 e	0.28 ± 0.01 cd	0.29 ± 0.00 bc	11.5%
		35d	0.27 ± 0.00 a	0.10 ± 0.00 e	0.15 ± 0.01 c	0.07 ± 0.00 f	0.11 ± 0.00 de	0.07 ± 0.01 f	0.20 ± 0.01 b	0.11 ± 0.02 cd	−59.2%
		40d	0.13 ± 0.00 b	0.08 ± 0.01 e	0.07 ± 0.01 a	0.10 ± 0.01 c	0.09 ± 0.01 de	0.13 ± 0.01 b	0.07 ± 0.00 f	0.09 ± 0.00 d	−30.8%
Soluble carbohydrate content	Leaf	T	44.6 ± 2.4 d	41.3 ± 2.5 d	45.7 ± 1.8 d	51.3 ± 1.7 c	41.7 ± 3.3 d	67.5 ± 6.5 b	75.2 ± 2.9 a	72.3 ± 3.7 ab	62.1%
		H	184.4 ± 2.1 d	188.5 ± 5.0 d	159.1 ± 0.3 f	210.5 ± 4.9 b	168.9 ± 5.6 e	201.4 ± 1.8 c	216.5 ± 6.9 a	199.8 ± 7.8 c	8.3%
	Flower	CH	226.1 ± 1.3 de	255.8 ± 9.5 cd	312.0 ± 7.6 b	217.6 ± 3.1 e	446.7 ± 9.6 a	268.3 ± 2.5 c	335.6 ± 9.5 b	222.6 ± 12.2 e	−1.5%
		SH	102.1 ± 5.7 cd	111.4 ± 4.3 bc	116.8 ± 4.4 ab	124.8 ± 7.8 a	112.4 ± 1.6 bc	106.0 ± 5.1 cd	105.8 ± 2.9 cd	98.6 ± 2.2 d	−3.4%
		ZH	49.5 ± 1.6 c	43.7 ± 1.6 e	48.7 ± 1.6 cd	38.1 ± 2.2 f	65.5 ± 1.9 a	65.8 ± 3.8 a	56.4 ± 1.3 b	46.4 ± 1.1 de	−6.2%
	Thorn peel	20d	118.6 ± 1.5 e	333.2 ± 3.8 b	241.3 ± 11.2 c	214.5 ± 5.6 c	241.9 ± 5.8 d	219.9 ± 8.4 c	293.7 ± 10.8 a	75.6 ± 2.7 f	−36.2%
		25d	141.2 ± 8.0 c	127.3 ± 4.3 d	145.9 ± 6.3 c	152.4 ± 5.9 c	145.1 ± 5.5 c	184.4 ± 7.0 a	168.3 ± 6.9 b	119.1 ± 4.2 d	−15.7%
		30d	59.3 ± 5.7 b	45.7 ± 2.2 c	79.5 ± 4.3 a	40.8 ± 4.1 c	60.5 ± 3.8 b	30.4 ± 1.8 d	55.5 ± 3.8 b	76.0 ± 4.0 a	28.1%
		35d	14.2 ± 0.2 d	18.0 ± 0.9 c	14.3 ± 0.67 d	17.6 ± 1.0 c	28.5 ± 1.5 a	18.1 ± 0.4 c	17.9 ± 1.0 c	22.8 ± 0.7 b	60.5%
		40d	50.0 ± 5.4 cd	49.1 ± 0.5 d	63.3 ± 2.7 b	42.2 ± 4.7 c	71.1 ± 2.1 a	50.5 ± 1.4 cd	39.9 ± 0.7 e	62.0 ± 4.0 b	24%
	Seed	20d	155.2 ± 1.1 c	171.5 ± 4.7 bc	126.2 ± 5.4 d	172.8 ± 5.3 bc	63.1 ± 6.7 e	120.8 ± 1.3 d	219.8 ± 8.4 a	178.2 ± 3.7 b	14.8%
		25d	70.1 ± 3.2 c	33.2 ± 2.9 e	56.2 ± 3.2 d	95.7 ± 6.9 a	73.9 ± 0.7 bc	90.1 ± 2.0 a	94.5 ± 0.6 a	78.9 ± 3.0 b	12.6%
		30d	49.2 ± 2.5 bc	51.1 ± 3.2 bc	45.9 ± 1.6 bc	45.6 ± 1.1 bc	44.6 ± 3.6 c	57.6 ± 5.4 a	52.1 ± 4.4 ab	49.3 ± 3.1 bc	0.2%
		35d	13.0 ± 0.1 f	19.6 ± 0.9 c	15.1 ± 0.7 e	19.7 ± 1.1 c	29.3 ± 1.5 a	17.5 ± 0.4 d	19.7 ± 1.1 c	23.9 ± 0.78 b	83.8%
		40d	35.5 ± 4.9 c	41.0 ± 0.4 bc	56.8 ± 2.4 ab	43.4 ± 3.6 bc	71.7 ± 2.1 a	45.4 ± 1.2 bc	35.8 ± 0.6 c	55.4 ± 3.6 ab	56%

Table 3. *Cont.*

	Stage		Treatment								
			CK	**BR**	**BR + Si**	**BR + Si + Se**	**BR + Se**	**Si**	**Si + Se**	**Se**	**Growth Rate (%) (Se)**
Soluble protein content	Leaf	T	0.07 ± 0.01 d	0.11 ± 0.01 b	0.08 ± 0.00 cd	0.15 ± 0.00 a	0.11 ± 0.00 bc	0.13 ± 0.01 ab	0.10 ± 0.00 bc	0.10 ± 0.01 bc	42.9%
		H	0.23 ± 0.01 a	0.24 ± 0.01 a	0.17 ± 0.01 c	0.17 ± 0.00 c	0.22 ± 0.01 ab	0.16 ± 0.01 c	0.19 ± 0.00 bc	0.22 ± 0.01 ab	−4.3%
	Flower	CH	0.15 ± 0.01 d	0.21 ± 0.01 bc	0.20 ± 0.01 bc	0.23 ± 0.01 b	0.17 ± 0.01 c	0.18 ± 0.01 c	0.27 ± 0.00 a	0.23 ± 0.00 b	53.3%
		SH	0.08 ± 0.01 d	0.12 ± 0.01 a	0.10 ± 0.00 c	0.10 ± 0.01 c	0.10 ± 0.00 c	0.12 ± 0.01 ab	0.11 ± 0.00 bc	0.10 ± 0.01 c	25%
		ZH	0.11 ± 0.01 c	0.14 ± 0.01 b	0.12 ± 0.01 bc	0.14 ± 0.0 ab	0.13 ± 0.01 b	0.14 ± 0.01 b	0.12 ± 0.01 bc	0.16 ± 0.00 a	45.4%
	Thorn peel	20d	78.4 ± 1.9 d	37.8 ± 4.7 e	100.7 ± 1.10 c	97.8 ± 2.7 c	102.6 ± 2.8 c	130.1 ± 6.9 b	39.1 ± 3.3 e	185.5 ± 5.6 a	136%
		25d	41.0 ± 5.8 bc	37.8 ± 4.7 c	57.3 ± 1.38 a	38.9 ± 3.0 c	43.9 ± 0.5 bc	53.8 ± 4.5 ab	39.1 ± 3.3 c	44.5 ± 1.0 abc	8.5%
		30d	11.1 ± 0.5 d	18.3 ± 1.4 cd	22.4 ± 2.1 b	22.1 ± 1.4 bc	11.3 ± 3.7 d	21.1 ± 1.1 c	20.5 ± 4.1 c	28.0 ± 2.4 a	152%
		35d	38.8 ± 0.7 a	37.0 ± 2.7 ab	23.9 ± 1.6 de	34.0 ± 1.0 abc	32.6 ± 5.8 bc	32.6 ± 2.1 bc	28.9 ± 3.9 cd	19.0 ± 0.9 e	−51%
		40d	25.1 ± 2.9 b	29.6 ± 1.4 b	26.1 ± 3.6 b	36.0 ± 2.2 a	27.4 ± 3.6 b	15.5 ± 2.0 c	25.9 ± 4.9 b	24.3 ± 0.3 b	−3.1%
	Seed	20d	208.4 ± 4.3 c	264.0 ± 11.1 b	266.7 ± 10.1 b	283.0 ± 6.4 b	127.2 ± 2.1 e	157.8 ± 6.1 d	266.1 ± 5.8 b	354.7 ± 6.5 a	70.2%
		25d	90.2 ± 6.3 d	118.3 ± 8.0 c	79.6 ± 7.7 d	163.0 ± 6.4 a	144 ± 4.8 ab	56.4 ± 2.1 e	137.8 ± 4.8 bc	119.1 ± 7.1 c	32%
		30d	45.9 ± 3.6 d	42.6 ± 2.7 d	51.3 ± 4.3 d	87.6 ± 1.2 a	42.4 ± 4.4 d	81.8 ± 4.0 ab	46.7 ± 3.1 d	63.0 ± 5.7 c	37.3%
		35d	41.1 ± 2.4 c	83.6 ± 2.7 a	36.6 ± 2.4 c	44.1 ± 2.0 bc	55.1 ± 4.4 b	39.1 ± 3.4 c	40.8 ± 2.5 c	74.8 ± 1.8 a	82%
		40d	215.5 ± 5.4 b	185.5 ± 1.2 c	93.4 ± 2.6 e	170.0 ± 4.7 c	236.6 ± 4.3 a	132.5 ± 4.6 d	208.7 ± 1.9 c	208.5 ± 1.6 c	−3.2%

Note: T, H: leaves at the bolting stage and flowering stage; CH, SH, and ZH: flowers at initial flowering, full flowering, and final flowering; 20d~40d: silique wall on the 20th, 25th, 30th, 35th, and 40th day after pollination. Statistical analysis: There were significant differences between different letters ($p < 0.05$).

3.2. Absolute Quantitative Transcriptomic Analysis and Verification

3.2.1. Quality Analysis and Sequence Comparison of Sequencing Data

In the absolute quantitative transcriptome sequencing analysis, 131.775 to 166.879 million different numbers of original sequences were detected in the treatment group and CK group (Table 4). The number of retained sequences after UID deduplication was 102.714–132.472 million, accounting for 86.13–90.12%, with Q30 greater than 96.03% and GC content ranging from 49.39 to 49.63%. After comparison with the reference genome of rapeseed, 94.773–121.544 million matching sequences were obtained with an alignment rate of 91.29–92.28%, and a single mapped sequence was above 91.52%. This indicated that the sequencing data had high reliability.

Table 4. UID-mRNA-seq data information.

Sample	Clean Reads Rate (%)	Q30 (%)	GC (%)	Reads with UIDs (%)	Dedup Reads	Total Mapped (%)	Uniquely Mapped (%)
CK1	85.98	96.03	49.45	117686572 (96.02%)	107785894 (87.94%)	121543682 (91.75)	111470355 (91.71)
CK2	87.76	96.43	49.59	130789468 (95.96%)	120718794 (88.57%)	111412178 (92.28)	101993415 (91.55)
CK3	86.87	96.4	49.39	109816312 (95.93%)	102714270 (89.72%)	111442216 (91.95)	102221860 (91.73)
T1	88.09	96.65	49.56	141190300 (96.05%)	132472412 (90.12%)	99141823 (91.98)	91097281 (91.89)
T2	88.17	96.72	49.63	134570288 (96.00%)	120727438 (86.13%)	110200647 (91.29)	100850923 (91.52)
T3	88.12	96.7	49.61	133096032 (95.98%)	121194130 (87.40%)	94773439 (92.27)	87438061 (92.26)

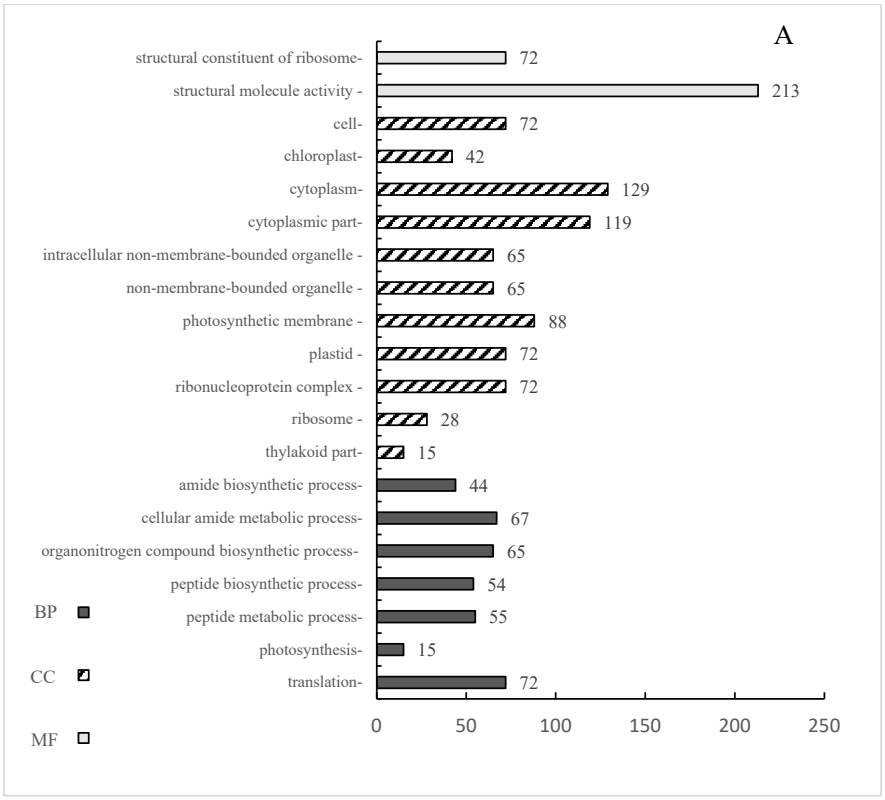
Note: T: Se treatment; CK: water spray; 1~3: three repetitions; Q30: percentage of bases with base recognition accuracy above 99.9%; GC: the proportion of guanine and cytosine in the four DNA bases (adenine, thymine, cytosine, and guanine); Reads with UIDs (%): number of reads with UID sequence and proportion of clean reads; Dedup Reads (%): the number and proportion of retained reads after UID deduplication; Total mapped (%): the total number and proportion of sequences to the reference genome; Uniquely mapped (%): the number and proportion of sequences with unique alignment positions on the reference sequence.

3.2.2. Expression Analysis of Differential Genes

DESeq2 was used to detect differentially expressed genes, and the conditions for screening differentially expressed genes were p-value ≤ 0.05 and $|\log2\text{fold change}| > 1$. A total of 1079 differential genes were obtained, including 923 up-regulated genes and 156 down-regulated genes.

Differential Gene GO Enrichment Analysis and KEGG Enrichment Analysis

GO enrichment analysis showed that DEGs mainly existed in three categories: biological processes, cell components, and molecular functions (Figure 1). In biological processes (BPs), the differentially up-regulated genes were concentrated in translation (72), cellular amide metabolic process (67), and organonitrogen compound biosynthesis process (65). The differentially down-regulated genes were concentrated in response to stimulus (14) and cellular response to stimulus (8). In cellular components (CCs), differentially up-regulated genes were concentrated in the cytoplasm (129), cytoplasmic part (119), and photosynthetic membrane (88). In molecular functions (MFs), differentially up-regulated genes focused on structural molecular activity (213) and the structural constituent of ribosome (72). The differentially down-regulated genes were concentrated in oxidoreductase (8). In summary, differentially expressed genes were mainly enriched in the direction of cell components.



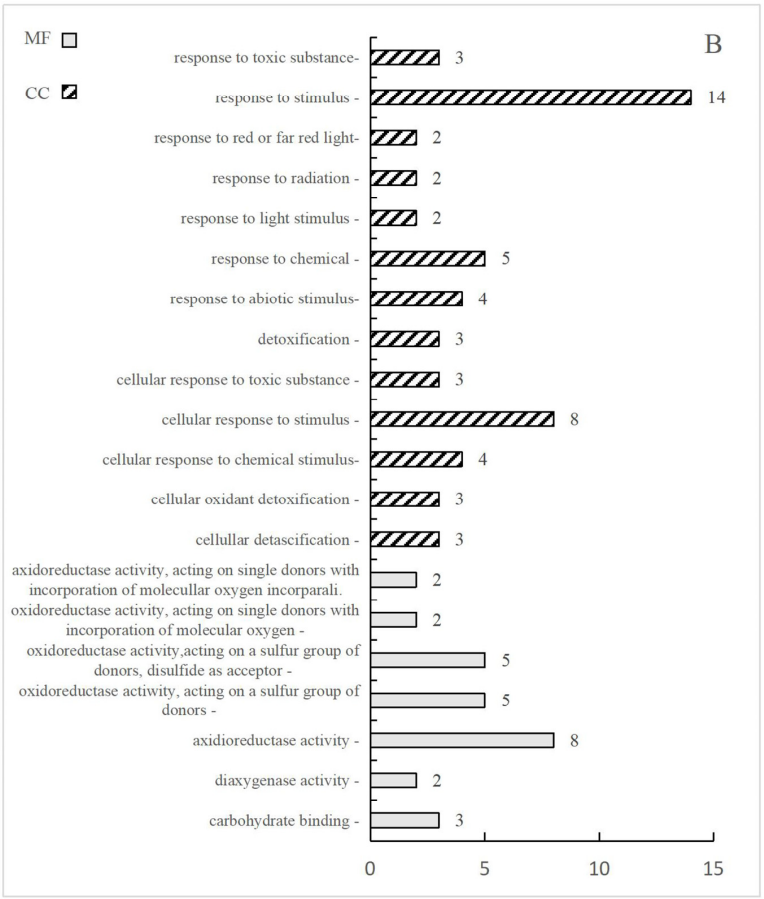


Figure 1. Histogram of GO enrichment analysis (CC: cell component; MF: Molecular function). Note: (**A**) shows the top 20 significant enrichment pathways of up-regulated genes and (**B**) shows the top 20 significant enrichment pathways of down-regulated genes.

KEGG enrichment analysis was performed on differential genes under sprayed Se treatment. The results showed that 32 metabolic pathways showed differences, and the *p*-value was lower than the specific threshold, and the error detection rate was <0.3. These metabolic pathways were mainly divided into energy metabolism (5), global and overview maps (4), amino acid metabolism (4), carbohydrate metabolism (4), the biosynthesis of other secondary metabolites (3), the metabolism of other amino acids (2) the metabolism of cofactors and vitamins (2), the metabolism of terpenoids and polyketides (2), translation (1), lipid metabolism (1), membrane transport (1), nucleotide metabolism (1), signal transduction (1), and transport and catabolism (1). The results of Table 5 show that the effect of selenium on the bolting stage of rapeseed Xiangzayou 787 was mainly focused on growth and development. Energy metabolism, amino acid metabolism, carbohydrate metabolism, the biosynthesis of other secondary metabolites, the metabolism of other amino acids, the metabolism of cofactors and vitamins, the metabolism of terpenes and polyketones, lipid metabolism, and growth and development are closely related, which indicates that spraying Se treatment during the bud stage has a significant promoting effect on the growth of Xiangzayou 787. Based on the functional annotations provided by NCBI and combined with bioinformatics analysis, six differentially expressed genes have been found (Table 6).

Table 5. KEGG enrichment pathway.

Pathway ID	Pathway	Up Number	Down Number	Level
bna00195	Photosynthesis	40	0	Energy metabolism
bna00710	Carbon fixation in photosynthetic organisms	27	0	
bna00196	Photosynthesis-antenna proteins	11	0	
bna00910	Nitrogen metabolism	12	0	
bna00920	Sulfur metabolism	0	5	
bna01100	Metabolic pathways	161	25	Global and overview maps
bna01200	Carbon metabolism	48	0	
bna01110	Biosynthesis of secondary metabolites	98	14	
bna01230	Biosynthesis of amino acids	22	0	
bna00250	Alanine, aspartate, and glutamate metabolism	10	0	Amino acid metabolism
bna00260	Glycine, serine, and threonine metabolism	11	4	
bna00360	Phenylalanine metabolism	4	0	
bna00220	Arginine biosynthesis	4	0	
bna00630	Glyoxylate and dicarboxylate metabolism	31	0	Carbohydrate metabolism
bna00030	Pentose phosphate pathway	12	0	
bna00030	Pentose and glucuronate interconversions	12	0	
bna00500	Starch and sucrose metabolism	0	3	
bna00940	Phenylpropanoid biosynthesis	17	4	Biosynthesis of other secondary metabolites
bna00941	Flavonoid biosynthesis	6	0	
bna00960	Tropane, piperidine, and pyridine alkaloid biosynthesis	0	2	
bna00460	Cyanoamino acid metabolism	9	3	Metabolism of other amino acids
bna00430	Taurine and hypotaurine metabolism	0	2	
bna00670	One carbon pool by folate	4	0	Metabolism of cofactors and vitamins
bna00860	Porphyrin and chlorophyll metabolism	6	0	
bna00906	Carotenoid biosynthesis	9	0	Metabolism of terpenoids and polyketides
bna00902	Monoterpenoid biosynthesis	2	0	
bna03010	Ribosome	66	0	Translation
bna00073	Cutin, suberine, and wax biosynthesis	6	0	Lipid metabolism
bna02010	ABC transporters	3	0	Membrane transport
bna00240	Pyrimidine metabolism	0	2	Nucleotide metabolism
bna04016	MAPK signaling pathway—plant	0	3	Signal transduction
bna04146	Peroxisome	14	0	Transport and catabolism

Table 6. RT-qPCR gene primer sequence.

Gene Name	Gene ID	Sequence (5′-3′)
Actin	Actin	F: CGTTGGTGGAGTTGCACTTG R: AGCACGTTACGGGATTGGTT
CKX5	BnaC06g36220D	F: GCCTTCCGACTTAGCCTCC R: ACCACACCGTTCCTCCCTG
BrabHLH139	BnaA03g13450D	F: ATAAGGTAACAGGGAAGGC R: CAAGATCCGAACCGAAGTC
PP2C78	BnaC09g34350D	F: GGGTGGTCGGGTTATCT R: TTACTTCCGGTTTGCTG
LOG1	BnaC04g39630D	F: AGTGGGAGAAGTGAAGGCAG R: ACGAGCAGTTGGTGAAATGA
LOC106301310	BnaC07g07830D	F: TGAAAAGATCCGAGGCTAC R: TGGGAAAATGTTCTAAATG
LOC106399213	BnaC01g06270D	F: TCACCGTTAAGGCAGAGAAG3 R: CCGCTTACGATTTCGGAGAAAC

Note: Actin as internal control.

3.2.3. RT-qPCR Verification and the Expression Pattern of Differential Genes

Six differentially related genes affecting the growth of rapeseed buds were screened (Table 6): cytokinin dehydrogenase 5 *(CKX5)*, BHLH domain-containing protein *(BrabHLH139)*, proteinphosphatase2C78 *(PP2C78)*, cytokinin riboside 5′-monophosphate phosphoribohydrolase *(LOG1)*, 30S ribosomal protein S1 *(BnaC07g07830D)*, and Chloroplast synthase subunit b′, *(BnaC01g06270D)*. The results of RT-qPCR verification were consistent with the sequencing results (Figure 2), which indicates that the sequencing results were reliable.

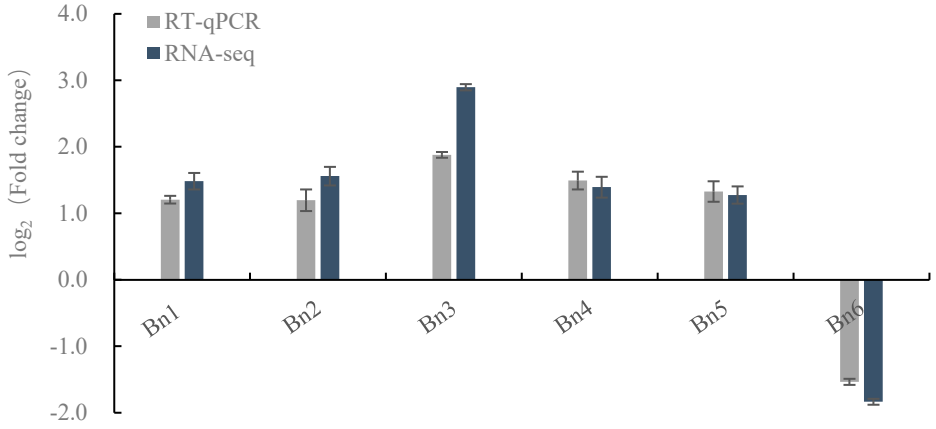


Figure 2. Expression analysis of differentially expressed genes related to growth and development of rapeseed at the bolting stage. Note: Fold change: gene expression in treatment group/CK group; *Bn1-Bn6: BnaC06g36220D (CKX5)*, *BnaC04g39630D (LOG1)*, *BnaA03g13450D (BrabHLH139)*, *BnaC07g07830D (BnaC07g07830D)*, *BnaC01g06270D (BnaC01g06270D)*, and *BnaC09g34350D (PP2C78)*.

The Expression Pattern of Differential Genes

Combined with the functional annotations provided by NCBI and previous studies [34–37], four genes, *CKX5*, *BrabHLH139*, *PP2C78*, and *LOG1*, were screened. RT-qPCR analysis was performed on the expression of the potential key differential genes in leaves, silique wall, and seeds. As shown in Figure 3, the expression levels of the four genes in leaves were significantly lower in the treatment group than in CK, and the *PP2C78* gene and *LOG1* gene increased significantly with the development of the growth stage. In flowers, the expression of the *LOG1* gene in the treatment group was significantly lower than that in CK, and gradually decreased with the progression of the growth stage. In the silique wall, the expression levels of the *CKX5* gene, *PP2C78* gene, and *LOG1* gene in the treatment group were significantly higher than those in the CK silique wall at 21 days

and 28 days after pollination, while the *BrabHLH139* gene has opposite expression. At the same time, *CKX5* gene expression decreased gradually with the progression of growth, while *BrabHLH139* gene expression increased gradually. In addition, the expressions of the *PP2C78* gene and *LOG1* gene increased with the growth of the silique wall until 28 days after pollination and then gradually decreased. The expression level of the *BrabHLH139* gene and *PP2C78* gene were significantly higher than the CK in the silique wall 35 days after pollination, while the *CKX5* gene and *LOG1* gene have the opposite results, with a higher expression level in the CK.

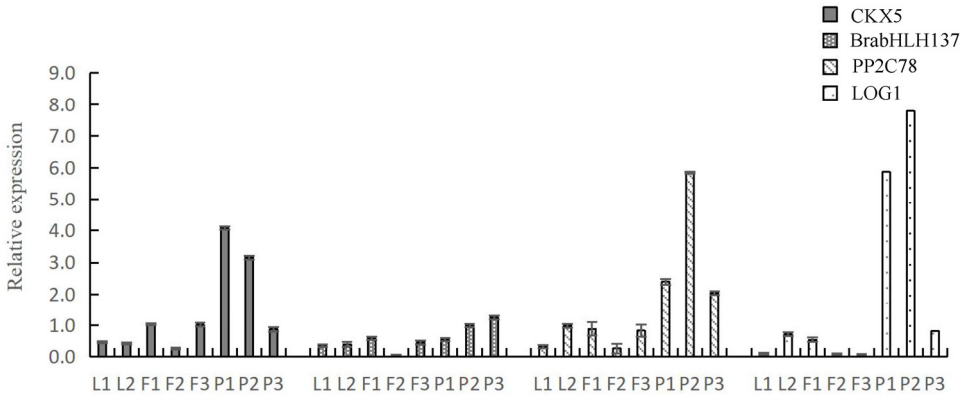


Figure 3. RT-qPCR analysis of genes. Note: The expression level of CK is 1; From left to right, *CKX5*, *BrabHLH137*, *PP2C78*, and *LOG1*; L1~L2: leaves at the bolting stage and flowering stage; F1~F3: flowers of initial flowering, full flowering, and final flowering; P1~P3: silique wall at 21 days, 28 days, and 35 days after pollination. Reference gene: BnActin; n = 3.

3.3. Correlation Analysis between Different Indicators

3.3.1. Correlation Analysis between Potential Key Genes Expression and Physiological Indexes Content

The correlation analysis between differential gene expression and physiological indexes content were conducted. Table 7 shows that the expression level of the *PP2C78* gene was significantly positively correlated with chlorophyll content and soluble sugar content in leaves. The correlation coefficients were 0.559 and 0.547, and there was also a significant negative correlation with the soluble protein content, with a correlation coefficient of −0.577.

Table 7. Correlation analysis between gene expression and physiological indexes content.

Index		Gene			
		CKX5	**BrabHLH139**	**PP2C78**	**LOG1**
Chlorophyll	Leaf	0.112	0.142	0.559 *	0.201
	Flower	0.130	0.210	0.115	0.371
	Thorn peel	0.279	−0.146	−0.175	0.194
	Seed	−0.211	0.235	−0.056	0.448
Content of soluble sugar	Leaf	0.140	0.254	0.547 *	0.320
	Flower	0.047	0.481	0.238	−0.05
	Thorn peel	−0.327	0.140	−0.134	0.041
	Seed	−0.118	−0.272	−0.239	0.151
Content of soluble protein	Leaf	−0.015	0.458	−0.577 *	0.299
	Flower	−0.144	−0.110	−0.123	−0.032
	Thorn peel	−0.216	−0.202	−0.222	0.111
	Seed	0.216	0.128	0.117	0.175

Note: * $p < 0.05$.

3.3.2. Correlation Analysis between Physiological Indexes Content and Rapeseed Yield

Correlation analysis was conducted between the content of physiological indexes and the yield of rapeseed. Table 8 showed that the chlorophyll content in flowers at full flowering stage was significantly positively correlated with the yield of rapeseed and the correlation coefficient was 0.694. At maturity stage, the content of soluble sugar was significantly correlated with the yield of rapeseed 25d and 30d after pollination.

Table 8. Correlation analysis of physiological indexes content and rapeseed yield.

Stage		Index		
		Chlorophyll	**Content of Soluble Sugar**	**Content of Soluble Protein**
Leaf	The bolting stage	0.258	−0.454	−0.220
	Flowering period	−0.104	−0.526	0.341
Flower	Early flowering period	−0.570	0.028	−0.073
	Full flowering stage	0.694 *	0.270	−0.457
	Final flowering period	0.269	−0.487	0.075
Silique wall	20d	−0.144	−0.347	0.350
	25d	0.412	−0.789 *	−0.073
	30d	0.386	0.700 *	0.092
	35d	0.406	0.662 *	−0.373
	40d	−0.273	0.606 *	0.556
Seed	20d	−0.126	−0.236	0.379
	25d	−0.434	−0.327	0.365
	30d	0.158	−0.923 **	−0.174
	35d	−0.164	0.770 *	0.331
	40d	−0.351	0.682 *	0.173

Note: * $p < 0.05$ and ** $p < 0.01$.

A correlation analysis was conducted between the expression levels of differentially expressed genes and rapeseed yield. Table 9 showed that the expression levels of the *CKX5* gene in the flowers during the full flowering period was significantly negatively correlated with rapeseed yield, with a negative correlation coefficient of −0.545. The expression levels in the flowers during the final flowering period and in the silique wall 28 days after pollination were significantly positively correlated with rapeseed yield and the correlation coefficients were 0.557 and 0.599. The expression level of the *PP2C78* gene in flowering leaves was negatively correlated with rapeseed yield and the correlation coefficient was −0.732 35 days after pollination; the expression level in the silique wall was positively correlated with rapeseed yield and the correlation coefficient was 0.60.

Table 9. Correlation analysis between gene expression and rapeseed yield.

Stage		Gene			
		CKX5	**BrabHLH139**	**PP2C78**	**LOG1**
Leaf	Budding stage	−0.036	0.035	0.021	−0.058
	Flowering period	0.179	0.331	−0.732 *	−0.177
Flower	Early flowering period	−0.258	0.391	−0.417	−0.128
	Full flowering stage	−0.545 *	−0.360	0.115	−0.253
	Final flowering period	0.557 *	0.276	0.548	−0.094
Silique wall	21d	0.398	0.109	0.426	0.379
	28d	0.599 *	0.108	0.465	0.112
	35d	0.490	0.437	0.600 *	−0.262

Note: * $p < 0.05$.

4. Discussion

In the case of an excessive application of nitrogen phosphorus potassium compound fertilizer and limited increase in rapeseed yield, the appropriate application of foliar selenium fertilizer (Se) during the growth period of rapeseed played an important role in improving the final yield traits of rapeseed [38,39]. The appropriate application of selenium fertilizer can not only increase the content of nitrogen, phosphorus, potassium, and selenium in different organs of crops, but also improve the quality and yield of crops to different degrees. Rapeseed has a large demand for mineral nutrients. In order to promote the growth of rapeseed, it is necessary to absorb and utilize a large amount of mineral elements. It is not enough to rely solely on the available nutrients in the soil, and it needs to be supplemented by the application of chemical fertilizers [40]. However, the excessive use of chemical fertilizers not only causes great pollution to farmland soil, but also increases economic costs [41]. Selenium can also enhance the disease and stress resistance of plants, and an appropriate amount of exogenous selenium has a significant effect on inhibiting the growth of rapeseed sclerotium pathogens [42]. Se can regulate plant photosynthesis and affect plant growth and development [11,43]. By spraying exogenous selenium, the chlorophyll content of foxtail millet increased, which promoted the photosynthesis of millet and was conducive to growth and development [44]. The photosynthetic rate of soybean leaves was increased after the appropriate concentration of selenium fertilizer was sprayed [45]. With the application of selenium, the transpiration rate and photosynthetic rate of rice increased, which improved the photosynthetic capacity of rice and promoted the growth and development of rice [46]. In this study, foliar Se spraying treatment resulted in higher chlorophyll, soluble sugar, and soluble protein content in rapeseed compared to the CK, proving that Se could promote the photosynthetic efficiency of rapeseed. At the same time, under Se treatment the number of green leaves on the main stem, total number of leaves on the main stem, root and stem thickness, and plant height of rapeseed were all increased compared to the CK, indicating that Se could promote the growth and development of rapeseed at the bud stage. Compared with the CK, the chlorophyll content in the leaves during the flowering period was significantly increased, while the soluble sugar content in the silique wall and seeds during the mature period was significantly increased. The content of soluble protein significantly increased during both flowering and maturity stages, then gradually decreased with the development of the growth period in the silique wall. However, the content in the seeds first decreased and then increased, reaching its lowest point 30 days after pollination. After foliar spraying with 375 g/hm^2 of nano selenium, the 1000-grain weight, spike length, plant height, and yield of foxtail millet were increased [47]. After treatment with appropriate dose of sodium selenite, the 1000-grain weight and yield of rice were increased [48]. By applying selenium fertilizer to peanuts, their growth and development were promoted, and the full fruit weight and yield per plant were both increased [49]. In addition, Zahedi et al. [26] found that the foliar application of Se had a significant additive effect on the effective number of siliques, 1000-grain weight, and biomass and harvest indexes of rapeseed plants. This study showed that under foliar fertilizer treatment, the effective number of siliques, 1000-grain weight, and yield per plant of rapeseed all increased, which was consistent with the above research results. This indicated that the application of foliar Se fertilizer during the flowering stage of rapeseed had a significant yield increase effect and could be used for the high-yield cultivation of rapeseed.

Selenium can enhance plant photosynthesis and promote the synthesis of chlorophyll. During the rapid growth period of rapeseed, there is a greater demand for energy, so photosynthesis is relatively important [50,51]. Selenium also plays an important role in energy metabolism and root vitality improvement. Compared with the control, wheat's root vitality was significantly enhanced after selenium spraying [52]. Selenium can also change the biosynthesis of metabolites and promote the accumulation of beneficial metabolites [53]. After spraying selenium, lettuce not only promoted the synthesis of chlorophyll, but also increased the content of soluble protein [54]. By foliar spraying selenium on flax, the activity

of antioxidant enzymes was enhanced at an appropriate selenium concentration [55]. After foliar spraying selenium fertilizer on cucumber, the activities of SOD, POD, and CAT were increased, the contents of soluble sugar and soluble protein were increased, and the content of MDA was decreased [56]. The soluble sugar content of foxtail millet increased after the used of exogenous selenium, which improved the quality of foxtail millet [57]. Selenium has a certain preventive and resistance effect on stress. Selenium could prevent cadmium poisoning and enhance plant resistance to heavy metals. Selenium had an antagonistic effect on the enrichment of lead, cadmium, and mercury in peach and pear fruits [58]. Selenium could also alleviate the toxic effects of herbicides and increase the content of chlorophyll and glutathione in plants, as well as the activity of antioxidant enzymes [59].

Protein phosphatase 2C (*PP2C78*) played an important role in abscisic acid (ABA) signaling and signaling processes, controlling many stress responses and developmental processes in plants [60,61]. Studies have shown that the down-regulated and antisense mediation of the protein phosphatase 2C gene could not only promote plant development, but also enhance its frost resistance [62]. This study analyzed the correlation between differential gene expression and physiological indexes and found that the *PP2C78* gene was significantly positively correlated with chlorophyll and soluble sugar content in leaves, then significantly negatively correlated with soluble proteins. Chlorophyll was the main photosynthetic pigment in plants, and participates in photosynthesis and crop growth and development. The changes in chlorophyll content were closely related to yield [62]. Soluble sugar was an important source of energy for plant growth and development, providing energy and metabolic intermediates for plant growth and development [63]. Soluble protein is an important nutrient and physiological indicator in the growth process of rapeseed, which is of great significance for physiological processes such as nutrient and organic matter accumulation in rapeseed leaves [64]. The results indicated that the expression of the *PP2C78* gene affects the growth and development of rapeseed during the nutritional growth period, and is closely related to rapeseed yield. It can be used for the early screening of foliar fertilizers and yield estimation, which helps to analyze the mechanism of yield increase and provides a reference for screening new types of foliar fertilizers. This study hopes to solve the problem of an insufficient supply of edible oil by lowering costs, increasing rapeseed production, increasing farmers' income, and expanding production. However, this study has some limitations because there is no large-scale experiment.

5. Conclusions

In this study, different foliar fertilizer treatments had a promoting effect on the growth and yield traits of three varieties, and Xiangzayou 787 had the highest yield per plant, which increased by 24.3% under sprayed Se treatment. Differentially expressed genes related to growth and development were found using absolute quantitative transcriptome sequencing, and the key gene *PP2C78* that may affect rapeseed growth was screened. The expression level of the *PP2C78* gene in the silique wall was significantly higher than that in the CK. With the development of the growth period, the expression level first increased and then decreased. Compared with the expression level at different stages, the highest level was reached in the silique wall 28 days after pollination. The *PP2C78* gene was significantly positively correlated with chlorophyll and soluble sugar content in leaves and the correlation coefficient was 0.539 and 0.547. The expression level of the *PP2C78* gene in flowering leaves was negatively correlated with rapeseed yield and the correlation coefficient was −0.732. There was a significant negative correlation with soluble protein content and the correlation coefficient was −0.577. The *PP2C78* gene may be a key functional gene affecting rapeseed yield. The results of this study indicate that the foliar spraying of appropriate concentrations of Se could not only promote the photosynthetic efficiency and growth and development of rapeseed during the bud stage, but also increase the effective number of pods, thousand-grain weight, and single plant yield of rapeseed, with significant yield increasing effects. This provides a basis for further exploring the

high-yield cultivation of rapeseed. With the development of molecular biology, this study screened the key gene *PP2C78* related to the growth and development of rapeseed during the bud stage through absolute quantitative sequencing, which could be used for the early screening of foliar fertilizers and yield estimation. Next, we will further explore the relationship between the optimal period and concentration of selenium fertilizer application, and promote the efficient utilization of selenium fertilizer in agriculture.

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