horticulturae

MDPI

Article

Integrative Phytohormone and Transcriptome Analyses Reveal the Inhibitory Mechanism of Ethylene on Potato Tuber Sprouting at Room Temperature

Meixue Zhang [1], Wenxiao Jiao [1], Qingmin Chen [2], Maorun Fu [1] and Cong Han [1,*]

[1] College of Food Science and Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, China; zhangmeixue1216@163.com (M.Z.); jiaowx9857@qlu.edu.cn (W.J.); skyfmr@126.com (M.F.)
[2] College of Food Science and Engineering, Shandong Agricultural and Engineering University, Jinan 250100, China; coconut508@163.com
* Correspondence: hancong6559@126.com; Tel./Fax: +86-531-89631195

Abstract: As a commercial potato sprout suppressant, ethylene (Eth) is usually used under a low temperature for long-term storage of potato tubers. However, in many cases, potato tubers are usually transferred from a refrigeration house and sold at room temperature. In the present research, Eth's inhibitory effects on tuber sprouting at room temperature were investigated. The potential molecular mechanisms of Eth-induced sprout suppression were revealed by phytohormone and transcriptome analyses. Results showed that exogenous Eth significantly suppressed sprout growth in potato tubers during two weeks of storage. The endogenous plant hormone levels of abscisic acid (ABA) and auxin (Aux) were markedly reduced by Eth treatment. Transcriptomic analysis revealed that more transcriptional changes occurred in the early stage of sprouting. The differentially expressed genes (DEGs) assigned to the pathways of plant hormone signal transduction, photosynthesis, starch and sucrose metabolism and phenylpropanoid biosynthesis, which were closely associated with sprouting, were extensively down-regulated by Eth treatment. In addition, the heat map of six hormone signal transduction pathways showed that the expression of most DEGs annotated into the Aux signal transduction pathway was suppressed by Eth treatment, while the expression of many DEGs annotated as *ETR* (Eth receptor) and *ERF1/2* (Eth-responsive transcription factor 1 and 2) in the Eth signal transduction pathway was enhanced by Eth treatment. Taken together, our results indicated that Eth-induced sprout inhibition might be closely related to the suppression of internal Aux production and signal transduction and the activation of Eth signal transduction.

Keywords: potato; sprouting; RNA-seq; auxin; signal transduction

Citation: Zhang, M.; Jiao, W.; Chen, Q.; Fu, M.; Han, C. Integrative Phytohormone and Transcriptome Analyses Reveal the Inhibitory Mechanism of Ethylene on Potato Tuber Sprouting at Room Temperature. *Horticulturae* **2024**, *10*, 286. https://doi.org/10.3390/horticulturae10030286

Academic Editor: Aisheng Xiong

Received: 31 January 2024
Revised: 11 March 2024
Accepted: 14 March 2024
Published: 17 March 2024

1. Introduction

Potatoes (*Solanum tuberosum* L.) are an excellent staple food for human consumption and occupy the fourth largest food crops after maize, rice, and wheat, on account of its high worldwide production [1]. In order to meet the demand of year-round supply, lots of freshly harvested potatoes endure long-term storage with strict storage conditions to maintain consistent postharvest quality. However, after endodormancy is released, the occurrence of sprouting is a major threat to the storability of potatoes. Sprouting not only damages tuber appearance but also adversely affects the nutritional and processing features of potatoes including the accelerated weight loss [2], the remobilization of starch and proteins [3], and the generation of toxic steroid glycoalkaloids [4]. Therefore, control of tuber sprouting is crucial for the potato industry.

Chlorpropham (CIPC) is a common suppressant applied to inhibit potato sprouting and extend storage life [5]. Although the commercial utilization of CIPC was ratified by European Annex I under Directive EC/91/414 with a maximum residue level of 10 mg kg^{-1}

and came into force in April 2007, the security of CIPC has always been a concern [6]. More research had reported that natural plant extracts such as peppermint, coriander, citronella and eucalyptus essential oils can be used as alternative management options for controlling sprout growth as well as preventing phytopathogenic damage of potato tubers [7,8]. However, due to their volatility and high cost, their usage is facing restrictions. It has also been demonstrated that sprouting can be inhibited by physical methods, e.g., low temperature, gamma irradiation or UV-C irradiation, but each of these methods has its limitations. Long-time low temperature usually results in undesirable tissue sweetening [9], gamma irradiation has detrimental effects on potato quality [10], and the sprout inhibition effect of UV-C irradiation varies upon the cultivar [6].

In comparison with these sprout suppressants, continuous Eth supplementation is established as an effective strategy for sprout inhibition, with the benefits of being relatively inexpensive and having no chemical residue concerns. In 2003, Eth gas was approved as a commercial potato sprout suppressant by the Chemical Residue Directorate in the United Kingdom [11]. Despite its positive effect on sprout inhibition, Eth can also induce the accumulation of reducing sugars. To alleviate this negative effect, the use of Eth is constantly being optimized, like filling Eth gas in a time increment gradient or using 1-MCP prior to Eth treatment [12,13]. In previous studies, the inhibitory effects of Eth on tuber sprouting were mainly evaluated during long-term storage at a low temperature. However, in many cases, potato tubers are usually transferred from a refrigeration house and sold at room temperature. There is a paucity of information on the impact of Eth on tuber sprouting as well as its potential mechanisms at room temperature.

Eth-induced sprout inhibition in stored potatoes is a complicated process that involves many metabolic pathways. Apart from increasing carbohydrate metabolism, Eth also leads to an obvious reduction in the concentration of phenolics [14]. As a classical phytohormone, the linkage between Eth and other phytohormones was also studied. For example, ABA is thought to be directly involved in the maintenance of tuber dormancy [15], and conflicting data have been published concerning the interaction of Eth and ABA. Foukaraki et al. [16] observed that the content of ABA increased with the use of Eth, while Tosettia et al. [17] found that Eth treatment could lead to the catabolism of ABA in potato. Besides ABA, the information about the relationship between Eth and other phytohormones in sprout inhibition still remains poor. The objectives of this manuscript were to investigate the effect of Eth on potato tuber sprouting at room temperature and to explore mechanisms of Eth-induced sprout inhibition from the perspective of endogenous plant hormone biosynthesis and signal transduction. The sprout inhibition effect by Eth, the changes in endogenous hormone levels and the transcriptional changes in related metabolic pathways were therefore investigated.

2. Materials and Methods

2.1. Plant Material and Treatment

Short-term dormancy potato cultivar 'Favourita' was harvested in late May 2021 in Jisuo Town, Tengzhou, Shandong, China (35°0′ 32.39′ N, 117°0′ 13.05′ E). The dormancy period of 'Favourita' was about 2–3 months at 20 °C and the dormancy period was longer at a low temperature. Prior to long-term storage, all the potato tubers underwent a 14-day wound healing period at 20 °C and 95% relative humidity (RH) in the dark. After wound healing, the tubers of similar size (weighing approximately 180–200 g and 60–70 mm in diameter) with no sprouting, no rot and no mechanical damage were selected and stored in a local refrigeration house at 1–3 °C for three months to pass the period of dormancy. After storage, the chosen tubers were transported to our laboratory within 3 h and conditioned overnight at room temperature. The room temperature was controlled by the central air conditioning and set to 20 °C.

The tubers were roughly washed with tap water and air-dried. Before treatment, 60 tubers were taken out, sampled and used as control. The sampling method of the control was same as the treatments below. Other samples were randomly separated into

two groups marked A and B. All of the chosen samples were placed into unsealed plastic containers, and each container contained 20 individuals with 6 containers per group. Tubers in group A were treated with exogenous Eth using commercial solid Eth-releasing agents. The agents were purchased form Shandong Nutrition Source Food Technology Co., Ltd., Dezhou, China, with a patent number of CN101715810A. For each package, the weight was 2.5 g and effective ingredients content was 20%. According to previous research in our laboratory [18], the utilization number for each container was determined as two. After dipping in water, two packages of agents were put into the container. Meanwhile, the container was enveloped with a transparent polyethylene plastic bag to form a closed environment, and the measured highest concentration of exogenous Eth was 117.9 μL L^{-1}. The changes in exogenous Eth level during storage were presented in Supplementary Figure S1. Tubers in group B were treated similarly but without Eth. All the containers were placed at room temperature for 2 weeks. The sprouting of potato tubers was observed and recorded at day 7 and day 14. Each time, 3 containers from each group were opened and all the tubers were withdrawn. After removing the sprouts, the tissues below the bud eye with a size of 1 cm diameter and 5 mm height were taken out by a hole puncher. And then, the tissues were frozen in liquid nitrogen and stored at −80 °C for phytohormone and transcriptome analyses.

2.2. Sprouting during Storage

Sprouting rate of all tubers was assessed at day 7 and day 14. Three plastic bags of tubers were randomly taken out from per treatment at each sampling time. At each tuber eye, the recognition of sprouting was defined as a length of bud exceeding 2 mm [18]. The sprouting rate was calculated as the following equation: SR = n/N*100%, where 'n' was the number of sprouted potatoes and 'N' was the total number of the potatoes.

Sprouting index of all tubers was measured at day 7 and day 14. According to Dai et al. [19], the levels of sprouting were defined as follows: level 0, no sprouting was observed; level 1, the percentage of the sprouted eyes in all eyes was higher than 0 but less than 25%; level 2, the percentage of the sprouted eyes in all eyes was between 25% and 50%; level 3, the percentage of the sprouted eyes in all eyes was over 50%. The sprouting index was calculated upon the following equation: SI = $\Sigma(S \times n)/(3 \times N)$, where 'S' was the sprouting level, 'n' was the number of the potatoes in this level, and 'N' was the total number of potatoes in each polyethylene plastic bag.

The sprout length and sprout weight of all tubers were evaluated at day 7 and day 14. The buds were cut off from each tuber eye using a biological scalpel and a tweezer. Then, the sprout length was measured by a vernier caliper, and the average length of the longest sprout from each tuber was recorded [20]. All buds in each plastic bag were gathered together and weighed by a 1/10,000 analytical balance.

2.3. Determination of Plant Hormones

2.3.1. Standard Curve

A total of twenty kinds of plant endogenous hormones and one kind of Eth precursor were analyzed in potato tissues, including trans-zeatin (tZ), cis-zeatin (cZ), cis-zeatin riboside (czR), trans-zeatin riboside (tzR), isopentenyladenine (iP), isopentenyladenosine (iPR), indole-3-acetic acid (IAA), ABA, gibberellin A1 (GA1), gibberellin A3 (GA3), gibberellin A4 (GA4), gibberellin A7 (GA7), 1-Aminocyclopropanecarboxylic acid (ACC), salicylic acid (SA), jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), cis-12-oxo-phytodienoic acid (cis-OPDA), brassinolide (BL), castasterone (CS) and typhasterol (TY). The standard working solutions were prepared with different concentration gradients by diluting plant hormone standards in 50% (v/v) methanol (Merck, 144282), and the calibration curves were obtained by the isotope internal standard method [21].

2.3.2. Metabolites Extraction

According to Le et al. [21], the frozen tissues obtained in the first experiment were ground into powder under liquid nitrogen using a grinding miller (A11 basic, IKA®-Werke GMBH & CO. KG, Staufen, Germany), and approximately 100 mg of each sample was weighed and put into 2 mL centrifuge tubes. Subsequently, approximately 50 µL of internal standard solution and 1 mL of 50% (v/v) methanol containing 0.2% formic acid were added as extraction solvent. The samples were shaken for 2 min using a vortex mixer, followed by extraction for 12 h at 4 °C in the dark. After centrifugation at 14,000× g for 10 min at 4 °C, 800 µL of the supernatant was taken out and dried by a nitrogen evaporator. Thereafter, the remaining samples were redissolved in 200 µL of 50% (v/v) methanol and centrifugated again at 14,000× g for 10 min at 4 °C. The supernatant was directly injected into the chromatographic column and analyzed by Liquid Chromatograph-Mass Spectrometry (LC-MS).

2.3.3. LC-MS Analysis

According to Le et al. [21], the 5500 QTRAP triple quadrupole mass spectrometry (AB SCIEX, MA, USA) coupled to the 1290 Infinity LC ultra-high performance liquid chromatography system (Agilent, CA, USA) was used. ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm, Waters, MA, USA) was used for analysis. The gradient solvent systems were A (0.04% of formic acid in water) and B (0.04% of formic acid in acetonitrile). The mobile phase gradient conditions were performed as follows: 0–10 min, 2–98% B; 10–11.1 min, 98–2% B; 11.1–13 min, 2% B. The injection volume was 4 µL at a flow rate of 400 µL min^{-1} and the temperature of autosampler was 4 °C. The column temperature was 45 °C.

The MS analysis was performed in the negative ionization mode. The conditions of 5500 QTRAP ESI were as follows: source temperature, 500 °C; ion source gas 1, 45 psi; ion source gas 2, 45 psi; curtain gas, 30 psi; ion spray voltage floating, −4500 V. All detections were in multiple-reaction monitoring (MRM) mode. The chromatographic peak area and retention time were obtained using Multiquant software. According to the standard curve, the content of plant hormones in samples was calculated.

2.4. RNA Sequencing (RNA-Seq)

Frozen tissues acquired in the first experiment were utilized for RNA-seq. Total RNA was extracted using a Plant Total RNA Extraction Kit (TaKaRa 9769, Shanghai, China). The purity of RNA was appraised by the Nanodrop 2000 (Thermo Scientific, MA, USA) and the length of RNA segment was detected by the Agilent 2100 bioanalyzer (Agilent, CA, USA). After completing the detection, the qualified RNA was then transferred to Shanghai Applied Protein Technology Co., Ltd. (Shanghai, China) to construct and sequence the cDNA libraries. The libraries were sequenced using Illumina Novaseq 6000 instrument. To obtain clean reads, the generated raw reads were filtered by removing the impure sequences (adaptor reads and low-quality reads which contained more than 60% low-quality bases or the N ratio was greater than 5% reads).

The clean reads were mapped to a reference genome of potato (https://dec2021-plants.ensembl.org/Solanum_tuberosum/Info/Index (accessed on 13 March 2024)) using HISAT2 software (v 2.2.1). The screening criteria of DEGs were $|\log_2{}^{\text{fold change}}| > 1$ and Padj (adjusted p-value) < 0.05. The KEGG orthology (KO) database (http://www.genome.jp/kegg/) (accessed on 27 January 2022) was used for pathway mapping. The KEGG pathway enrichment analysis was carried out to annotate and classify the identified DEGs [22]. p values < 0.05 through Fisher's exact test were considered to be significant when identifying enhanced KEGG pathways, thereby identifying metabolic and signal transduction pathways that were significantly affected. Heat mapping of plant hormone signal transduction-related DEGs was performed using R software (v 4.0.5).

The raw data of RNA-seq have been deposited in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra (accessed on 13 March 2024)) under accession numbers SRX23891566–SRX23891580.

2.5. Quantitative Real-Time PCR (qRT-PCR)

According to Zhen et al. [23], the total RNA was extracted from frozen potato tissues using a Plant Total RNA Extraction Kit (TaKaRa: 9769), and then the cDNA was synthesized using a RT-PCR Kit (TaKaRa: RR036A), in accordance with manufacturer's instructions. Ten genes related with Aux signal transduction pathway were screened, including *PGSC0003DMG400008504*, *PGSC0003DMG400027712*, *PGSC0003DMG400006108*, *PGSC0003DMG400003771*, *PGSC0003DMG400012261*, *PGSC0003DMG400025856*, *PGSC0003DMG400024997*, *PGSC0003DMG400025017*, *PGSC0003DMG400003773* and *PGSC0003DMG400001655*. The details of their primer sequences are listed in Supplementary Table S1. The relative expression levels of selected genes were analyzed by qRT-PCR using SYBR® *Premix Ex* Taq™ (TaKaRa: RR420A). According to Neubauer et al. [24], the reaction procedure consisted of 95 °C pre-denaturation for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 34 s. The amplification was carried out on the Applied Biosystems 7500 Real-Time PCR System (Waltham, MA, USA). The relative gene expression was calculated based on $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical Analysis

Sprouting assessment, RNA-seq and qRT-PCR analysis were all performed with three biological replicates, while LC-MS analysis was performed with five biological replicates. SPSS software (V 23, IBM CO., NY, USA) and one-way analysis of variance (ANOVA) were used for statistical analysis. The experiment results were considered as mean ± standard deviation (SD). The significant differences were calculated by Fisher's least significant differences (LSD) tests with a confidence level of $p < 0.05$.

3. Results

3.1. Effects of Exogenous Eth Treatment on Potato Tuber Sprouting

The difference in sprouting statues between the Eth-treated and control potato tubers is clearly seen in the photograph during two weeks of storage (Figure 1a). The sprouts of control tubers arose in the first week and grew rapidly in the second week. However, the application of exogenous Eth effectively inhibited sprout growth. As shown in Figure 1b, after one week of storage, the sprouting rate of control tubers reached 100%, while in the Eth-treated tubers, this value was no more than 10%. Meanwhile, an obvious reduction in the sprouting index was observed in Eth-treated tubers during two weeks of storage, as compared with the control (Figure 1c). In addition, the sprout length and sprout weight of all tubers were measured. On the 7th day, the average length of the longest sprout in control and Eth-treated tubers was 3.6 mm and 1.1 mm, respectively (Figure 1d). On the 14th day, the former was 10.2 mm and the latter was 3.5 mm. Likewise, the sprout weight of the Eth-treated tubers was much lower than that of the control during the entire storage time (Figure 1e). The maximum sprout weight was observed in the control group after two weeks of storage, which was 13.4 times higher than that of the Eth group.

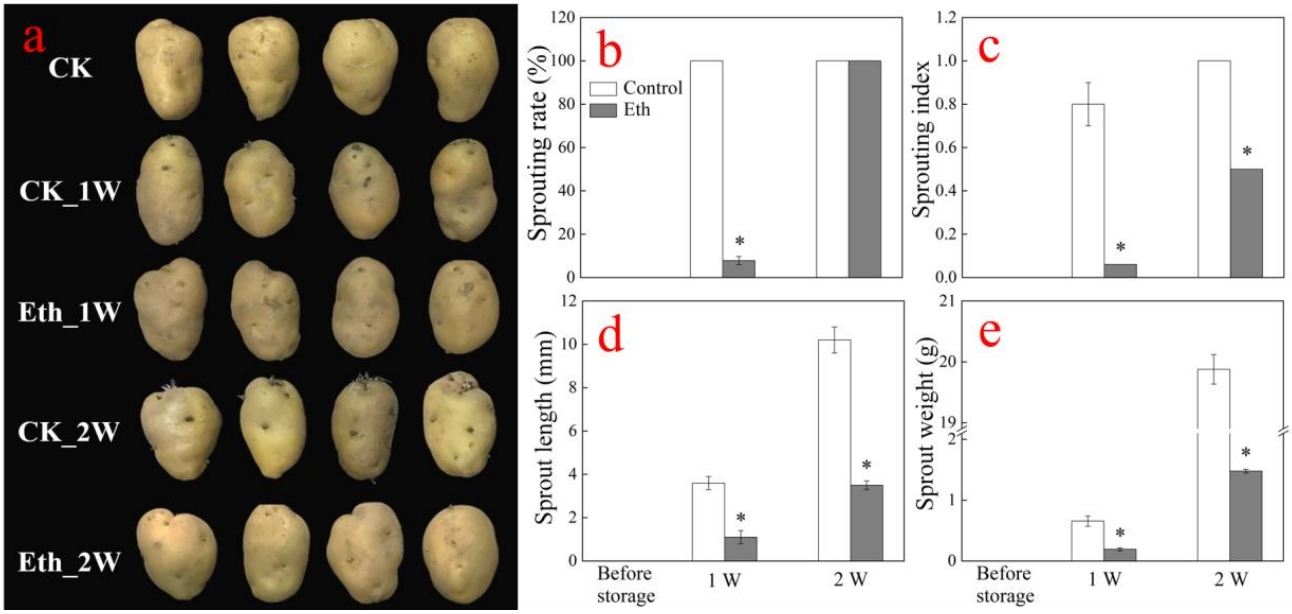

Figure 1. Effect of exogenous Eth on sprouting of potato during two weeks storage. (**a**) Appearance. (**b**) Sprouting rate. (**c**) Sprouting index. (**d**) Sprout length. (**e**) Sprout weight. Each value is a mean ± SD of triplicate assays. Asterisk represented significant difference ($p < 0.05$) between control and Eth treatments.

3.2. Exogenous Eth Treatment Affects the Levels of Endogenous Hormones and Their Related Metabolites

As shown in Table 1, five kinds of cytokinins including tZ, czR, tzR, iP and iPR were detected in the potato tubers. Generally, the concentrations of these substances in the control tubers increased dramatically in the first week and were maintained at relatively high levels in the second week. Eth treatment enhanced the concentration of iPR in the first week, and elevated the concentrations of most substances in the second week except czR. The IAA content in the control tubers increased sharply during storage and exhibited a 970% increase after two weeks of storage, with respect to the initial value. The application of exogenous Eth pronouncedly suppressed the upward trend of IAA content during the entire storage period. The IAA content in the Eth-treated tubers decreased by 41.2% after one week of storage and 35.0% after two weeks of storage, as compared with the control. Furthermore, the ABA content in the control tubers increased slightly in the first week and remained stable in the second week. The ABA content was evidently reduced in the Eth-treated tubers, which was lower than its initial value at each time point. The ACC content remained relatively stable in both the control and Eth-treated tubers during storage, and no significant difference ($p > 0.05$) was observed between the two groups. As for SA, although Eth treatment reduced its content in the first week, a higher level appeared in the second week in all tubers. In addition, three JA-related metabolites including JA, JA-Ile and cis-OPDA were detected in the potato tubers. Among them, cis-OPDA was detected in all tubers, while JA and JA-Ile were only detected in the control after one and two weeks of storage. The contents of JA and JA-Ile showed an upward pattern with prolonged storage time, whereas the cis-OPDA content increased initially but decreased thereafter. It appeared that Eth treatment delayed the peak time of cis-OPDA content in sprouted potato.

Table 1. Effect of exogenous Eth on levels of endogenous hormones and their related metabolites in potato tuber (ng/g FW) *.

Types	Metabolites	Mass Info	Retention Time (min)	CK	CK_1W	Eth_1W	CK_2W	Eth_2W
Cytokinin	tZ	220.0/136.0	2.031	16.6 ± 4.1^{B}	43.4 ± 15.2^{Aa}	48.2 ± 12.8^{a}	45.1 ± 11.3^{Ay}	75.8 ± 12.7^{x}
	czR	352.0/220.0	2.592	15.8 ± 1.3^{B}	19.4 ± 3.6^{ABa}	17.5 ± 1.9^{a}	22.9 ± 3.9^{Ax}	21.0 ± 2.6^{x}
	tzR	352.0/220.0	2.608	1.4 ± 0.1^{B}	19.9 ± 3.1^{Aa}	25.5 ± 5.3^{a}	20.2 ± 2.0^{Ay}	50.1 ± 11.8^{x}
	iP	204.0/136.1	3.872	0.05 ± 0.01^{C}	0.17 ± 0.04^{Aa}	0.14 ± 0.02^{a}	0.09 ± 0.01^{By}	0.18 ± 0.04^{x}
	iPR	336.0/204.1	5.331	7.3 ± 1.2^{C}	14.5 ± 1.1^{Ab}	28.8 ± 4.7^{a}	11.0 ± 0.2^{By}	23.2 ± 4.9^{x}
Auxin	IAA	176.0/130.0	5.834	26.7 ± 9.0^{C}	132 ± 25.5^{Ba}	77.6 ± 11.6^{b}	286 ± 15.9^{Ax}	186 ± 72.9^{y}
Abscisic acid	ABA	263.1/153.0	7.045	81.9 ± 4.6^{B}	96.9 ± 6.1^{Aa}	61.0 ± 3.2^{b}	105 ± 13.3^{Ax}	63.5 ± 6.3^{y}
Ethylene	ACC	101.9/56.1	0.608	29.1 ± 2.7^{B}	33.0 ± 4.1^{ABa}	33.9 ± 1.1^{a}	34.5 ± 3.6^{Ax}	35.4 ± 1.5^{x}
Salicylic acid	SA	137.0/93.0	5.474	27.1 ± 4.6^{BC}	33.8 ± 5.3^{Ba}	23.6 ± 5.8^{b}	64.2 ± 13.5^{Ax}	59.2 ± 28.9^{x}
Jasmonic acid	JA	209.1/59.1	7.743	nd	0.64 ± 0.17^{Ba}	nd	2.19 ± 1.14^{Ax}	nd
	JA-ILe	322.0/130.0	8.691	nd	0.02 ± 0.01^{Ba}	nd	0.10 ± 0.03^{Ax}	nd
	cis-OPDA	291.1/165.0	10.071	404 ± 44.0^{B}	537 ± 65.0^{Aa}	446 ± 54.5^{b}	325 ± 20.8^{Cy}	507 ± 97.5^{x}

* Data are expressed as the mean ± SD (n = 5). Values in the same storage temperature with different letters were significantly different at $p < 0.05$. Capital letters represent significant difference among control samples, lowercase letters marked a and b represent significant difference among control and Eth-treated samples in the first week, and x and y represent significant difference among control and Eth-treated samples in the second week. nd = not detected.

3.3. Transcriptomic Analysis Predicted DEGs in Plant Hormone Signal Transcription in Response to Eth Treatment

To investigate the changes in plant hormone signal transcription-related genes in response to Eth treatment, the transcriptome profiles of the control and Eth-treated tubers were analyzed. CK represented the potato tubers before storage, which was used as control; CK_1W and Eth_1W represented the potato tubers of the control and Eth-treated tubers after one week of storage, respectively; CK_2W and Eth_2W represented the potato tubers of control and Eth-treated tubers after two weeks of storage, respectively. As shown in Table 2, after removing the low-quality reads, a total of 839,353,478 clean reads were obtained, with approximately 50–60 million reads per sample. For each sample, the unique matching ratio was higher than 86% and the value of Q30 exceeded 92%. Overall, the basic information herein indicated the high quality of RNA-Seq data.

Table 2. Summary of RNA-seq data generated from potato.

Samples	Total Clean Reads	Total Mapped Reads	Unique Mapped Reads	Q30 (%)	GC Content (%)
CK_1	49117490	46554246	44539512 (90.68%)	95.60	42.96
CK_2	60085322	56922492	54362940 (90.48%)	95.34	42.96
CK_3	48721916	46245828	44193020 (90.7%)	95.45	43.01
CK_1W-1	54690870	51614444	47759724 (87.33%)	95.56	42.48
CK_1W-2	52915110	50062774	46359266 (87.61%)	95.25	42.62
CK_1W-3	50413250	47688552	44281584 (87.84%)	95.39	42.47
Eth_1W-1	64327208	60276068	55524598 (86.32%)	93.36	42.30
Eth_1W-2	55367714	51993362	48167102 (86.99%)	93.23	42.52
Eth_1W-3	58716980	55194672	51091124 (87.01%)	93.09	42.56
CK_2W-1	57957378	54643194	50825122 (87.69%)	93.21	42.79
CK_2W-2	49102098	46318434	43340896 (88.27%)	93.28	42.74
CK_2W-3	54091952	51011764	47733536 (88.25%)	93.16	42.65
Eth_2W-1	61568930	57767596	54604050 (88.69%)	93.24	43.00
Eth_2W-2	60278752	56639274	53698588 (89.08%)	93.15	42.98
Eth_2W-3	61998508	57898058	54490272 (87.89%)	92.93	43.23

The DEGs screened between two groups are shown in Figure 2. Here, four comparison groups were analyzed, including CK_1W vs. CK, Eth_1W vs. CK, CK_2W vs. CK_1W and Eth _2W vs. Eth _1W. For each comparison group, the sample in the back was used as control. In total, 4949 (3293 up-regulated and 1656 down-regulated), 4737 (2751 up-regulated and 1986 down-regulated), 531 (338 up-regulated and 193 down-regulated), and 815 (311 up-regulated and 504 down-regulated) DEGs were observed, respectively. To further characterize the potential functions of DEGs, KEGG pathway enrichment analysis

was carried out for all DEGs. The top 20 items of enriched KEGG pathways between CK_1W and CK are listed in Table 3. Specifically, the results of Eth_1W vs. CK in the same items are also included. According to the KEGG annotation, most DEGs were classified into "metabolic pathways (ko01100)" and "biosynthesis of secondary metabolites (ko01110)". Meanwhile, "plant hormone signal transduction (ko04075)", "biosynthesis of secondary metabolites (ko01110)", "phenylpropanoid biosynthesis (ko00940)", and "biosynthesis of amino acids (ko01230)" were also main classifications. In the comparison of CK_1W vs. CK, an extensive up-regulation of DEGs was observed in most of the enriched KEGG pathways. The application of Eth treatment distinctly reduced the number of up-regulated DEGs in many enriched KEGG pathways, like "metabolic pathways (ko01100)", "biosynthesis of secondary metabolites (ko01110)", "photosynthesis—antenna proteins (ko00196)", "starch and sucrose metabolism (ko00500)", "plant hormone signal transduction (ko04075)", and "phenylpropanoid biosynthesis (ko00940)".

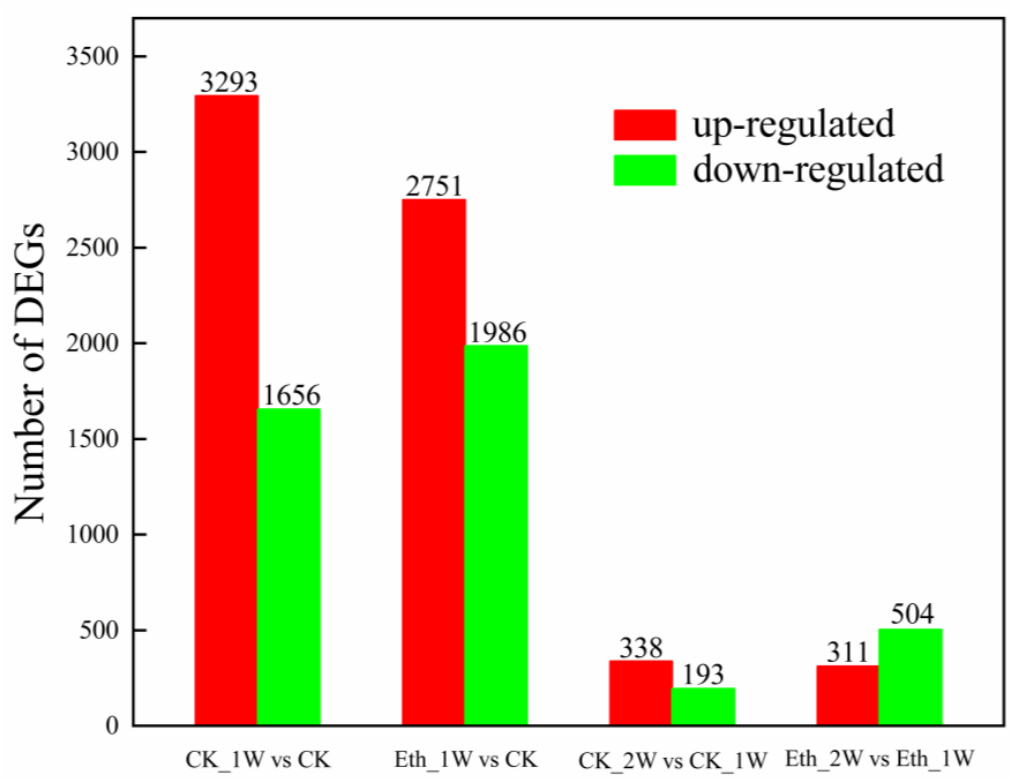

Figure 2. Counts of DEGs identified from potato under Eth treatment.

To visualize the changes in the DEGs involved in plant hormone signal transduction of potato tubers, the expression patterns of DEGs in all comparison groups were functionally annotated into six hormone signal transduction pathways and presented in a heatmap (Figure 3). It revealed that a total of 40 DEGs were identified in the Aux signal transduction pathway, encoding proteins of *AUX1* (Aux influx carrier), *TIR1* (transport inhibitor response 1), *AUX/IAA* (Aux responsive protein IAA), *ARF* (Aux response factor), *GH3* (Aux responsive GH3 gene family), and *SAUR* (SAUR family protein). Most of these genes were up-regulated in control tubers after one week of storage. Treatment with exogenous Eth markedly and widely suppressed the expression of these genes during the whole sprouting process. There were 11 and 7 DEGs annotated into cytokinin and brassinosteroid signal transduction pathways, respectively. In general, the expression of these genes at each time point was down-regulated by Eth treatment. With regard to the ABA signal transduction pathway, no visual changes were observed between the control and Eth groups. In the gibberellin signal transduction pathway, three DEGs annotated as *DELLA* were identified and two of them were down-regulated by Eth treatment during storage. In addition, 19 DEGs

were found in the Eth signal transduction pathway. Among them, the expression levels of six genes encoding *ETR* and six genes encoding *ERF1/2* were significantly higher in Eth-treated tubers, as compared with the control.

Table 3. Statistics of DEGs in the top 20 enriched KEGG pathways based on CK_1W vs. CK (the results of Eth_1W vs. CK in the same items were also included).

KEGG Pathways	CK_1W vs. CK		Eth_1W vs. CK		Background Genes
	Up	Down	Up	Down	
Metabolic pathways	374	126	293	146	2227
Biosynthesis of secondary metabolites	236	72	190	91	1292
Photosynthesis—antenna proteins	30	0	24	0	34
Starch and sucrose metabolism	54	19	40	18	254
Photosynthesis	31	0	21	2	87
Porphyrin and chlorophyll metabolism	18	3	14	4	48
Zeatin biosynthesis	18	4	15	7	52
Plant hormone signal transduction	60	19	44	19	326
Phenylalanine metabolism	20	4	12	8	62
Phenylpropanoid biosynthesis	56	4	37	8	245
Biosynthesis of amino acids	49	12	39	13	251
Glyoxylate and dicarboxylate metabolism	14	12	8	10	81
Cyanoamino acid metabolism	16	3	10	0	52
Steroid biosynthesis	1	2	1	2	40
Butanoate metabolism	9	3	9	4	28
Carbon metabolism	38	26	33	24	290
2-Oxocarboxylic acid metabolism	14	7	12	7	68
Cysteine and methionine metabolism	24	6	20	5	118
Glycolysis/Gluconeogenesis	24	9	20	10	135
Pentose phosphate pathway	14	4	15	6	61

3.4. Verification of DEGs by qRT-PCR Analysis

To verify the reliability of the experimental data derived from RNA-seq, 10 DEGs annotated as *AUX1*, *TIR1*, *AUX/IAA*, *ARF*, *GH3*, and *SAUR* in the Aux signal transduction pathway were selected, and the expression level of each DEG was detected by qRT-PCR (Figure 4). It was observed that two genes of *PGSC0003DMG400008504* and *PGSC0003DMG400027712* were up-regulated by Eth treatment after one week of storage, while other genes were down-regulated by Eth treatment in most of the detecting points. Specifically, the inhibition effect of Eth was more evident for the expressions of *PGSC0003DMG400006108* (annotated as *AUX/IAA*) and *PGSC0003DMG400003773* (annotated as *SAUR*), which was approximately 7 and 10 times lower than that of the control, respectively. In general, the changes in the expression profiles of these genes obtained from qRT-PCR were in accordance with their variation trend observed in the heatmap. In addition, a correlation analysis between qRT-PCR and RNA-seq was performed by calculating $\log_2^{\text{fold change}}$. As shown in Figure 5, a significant correlation coefficient of 0.84 was observed between qRT-PCR and RNA-seq, which reflected the reliability of RNA-seq results.

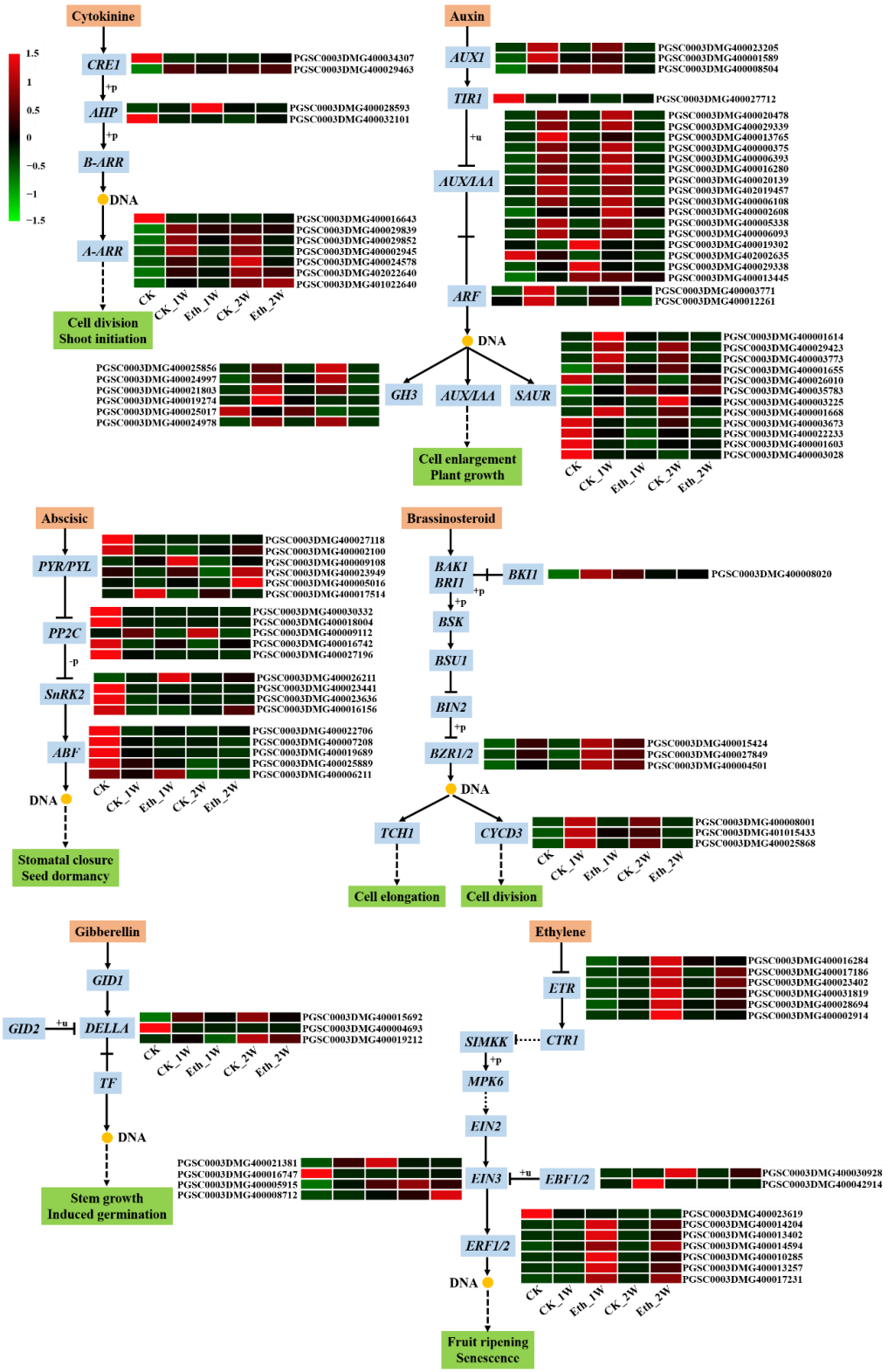

Figure 3. Expression profiles of DEGs associated with different plant hormones signal transduction, including cytokinin, Aux, ABA, brassinosteroid, gibberellin and Eth. The rows in the heat map represent screened DEGs, and the columns indicate different samples (CK, CK_1W, Eth_1W, CK_2W and Eth_2W). The color gradient, ranging from green through black to red, represents low, middle, and high values of the FPKM value. The denser the red color, the more expression is up-regulated, while the denser the green color, the more expression is down-regulated.

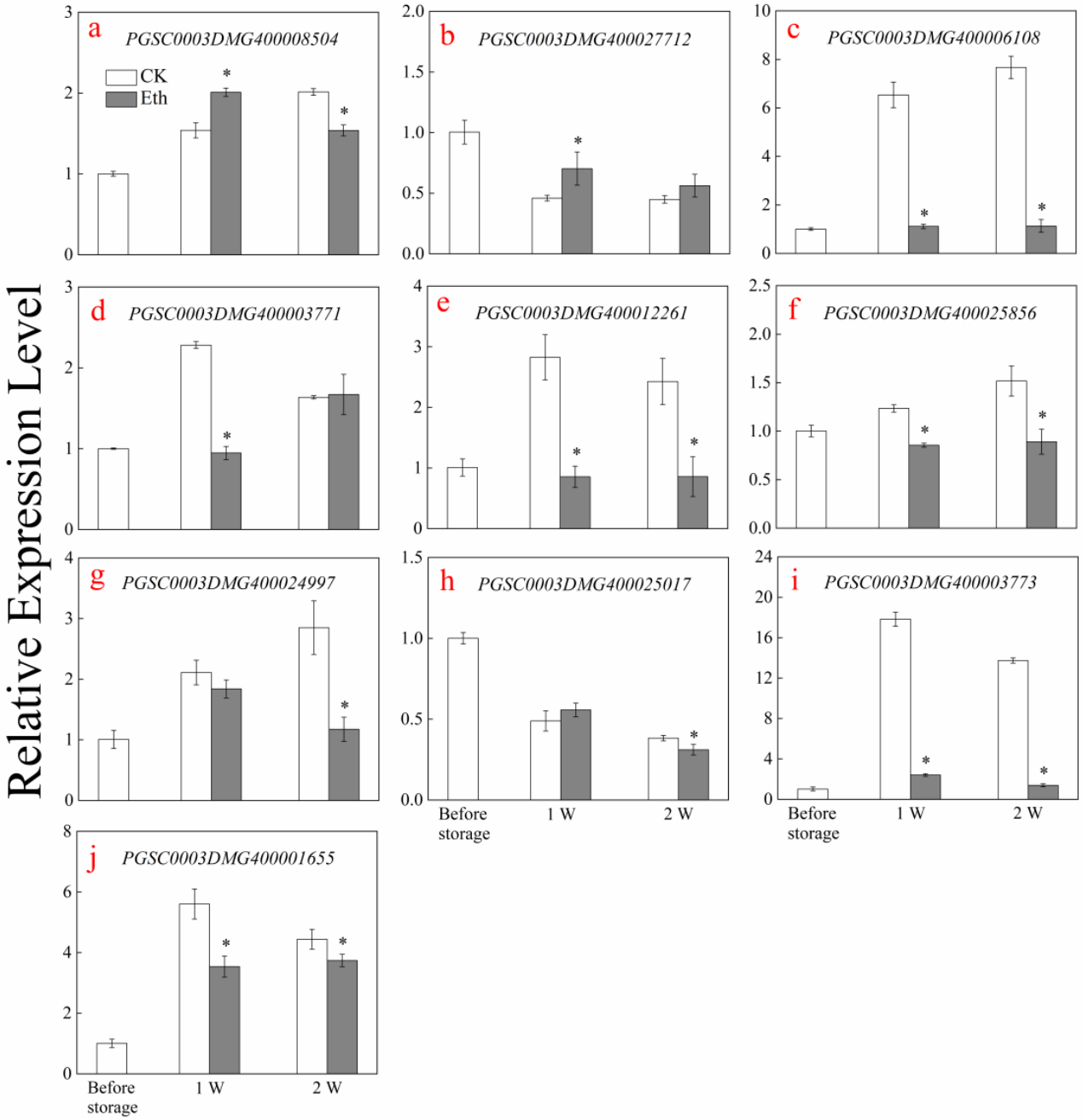

Figure 4. Expression of 10 DEGs involved in encoding the proteins of *AUX1*, *TIR1*, *AUX/IAA*, *ARF*, *GH3* and *SAUR* in the Aux signal transduction pathway. (**a**) *PGSC0003DMG400008504* (annotated as *AUX1*). (**b**) *PGSC0003DMG400027712* (annotated as *TIR1*). (**c**) *PGSC0003DMG400006108* (annotated as *AUX/IAA*). (**d**) *PGSC0003DMG400003771* (annotated as *ARF*). (**e**) *PGSC0003DMG400012261* (annotated as *ARF*). (**f**) *PGSC0003DMG400025856* (annotated as *GH3*). (**g**) *PGSC0003DMG400024997* (annotated as *GH3*). (**h**) *PGSC0003DMG400025017* (annotated as *GH3*). (**i**) *PGSC0003DMG400003773* (annotated as *SAUR*). (**j**) *PGSC0003DMG400001655* (annotated as *SAUR*). Asterisk represented significant difference ($p < 0.05$) between control and Eth treatments.

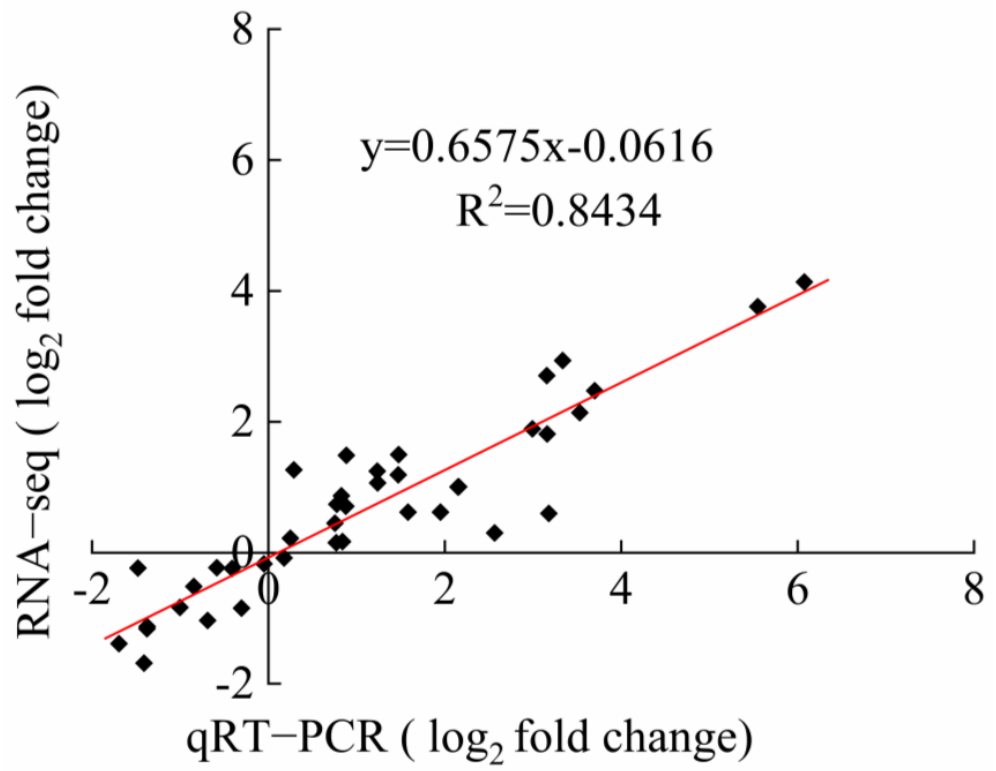

Figure 5. Correlation of fold change analyzed by RNA-seq (*x* axis) and qRT-PCR (*y* axis). The values of RNA-Seq and qRT-PCR were derived from Supplementary Table S2 and Figure 4.

4. Discussion

Sprouting is the main problem in postharvest storage of potato tubers, since it affects tuber appearance, reduces nutritional and processing quality, and causes unpredictable economic losses in the supply chain [25]. CIPC, as the most effective anti-sprouting agent, has been approved for commercial application in many countries, with different residual limits [26]. However, regarding the potential/possible safety and environmental concerns related with CIPC, in June 2019, the use of CIPC was prohibited in the European Union [17]. With restrictions coming into force, finding a viable CIPC alternative is imperative for controlling tuber sprouting. Exogenous Eth has been demonstrated as an environmentally acceptable sprout suppressant for extending potato tubers' storage life, although the use of Eth is partly constrained by the propensity to induce the accumulation of reducing sugars. In the current research, the application of exogenous Eth was implemented using solid Eth-releasing agents and the sprout inhibition effect of Eth for potato tubers was evaluated at room temperature. Our results revealed that the sprout growth in potato tubers was inhibited by exogenous Eth during two weeks of storage (Figure 1), suggesting the great inhibition effect of Eth for potato tuber sprouting at room temperature. This observation was in agreement with the earlier publications that exogenous Eth treatment inhibited potato tuber sprouting under low storage temperature [13,27]. In addition, it was worth noting that the inhibitory effect of Eth was dramatically weakened in the second week. This result might be attributed to the low concentration of Eth in the last few days of storage (Supplementary Figure S1).

It is widely believed that potato tuber sprouting is primarily regulated by plant hormones. ABA, Eth, Aux, cytokinins, and gibberellins have been implicated in the processes of tuber development [28]. ABA and Eth are mainly linked with the onset and maintenance of tuber dormancy, whereas cytokinins and gibberellins are related with dormancy termination and tuber sprout growth [2,29]. With regard to Aux, it plays an essential role in cell expansion, division, and differentiation and is involved in early phases of dormancy termination and sprouting [30,31]. In this study, the changes in

endogenous hormone levels were examined to illustrate their differences in response to Eth treatment (Table 1). It turned out that Eth treatment had no influence on the Eth precursor of ACC, but caused an evident decrease in ABA content. Usually, the content of ABA in tissue is high during tuber dormancy and declines to a certain threshold before the induction of tuber sprouting [32]. The depletion of ABA presented herein was similar to previous studies that showed that short-term exposure to Eth easily led to the catabolism of ABA [17]. Moreover, the levels of two kinds of cytokinins (tzR and iPR) in potato tubers increased by Eth treatment during two weeks of storage, while no HPLC-derived data were observed with respect to gibberellins, which might be attributed to their low concentrations. Usually, the endogenous cytokinin content is low during tuber dormancy but rises coincident with the onset of sprouting [33]. As interpreted by Turnbull and Hanke [34], the increase in cytokinin content is the principal factor resulting in the release of dormancy, but probably does not control the subsequent sprout growth. In addition, Eth treatment strongly inhibited the increase in IAA content during the whole storage period. This result implied that the decreased IAA content in Eth-treated tubers might be an important reason for sprouting inhibition.

To further understand the potential molecular mechanism of how Eth inhibits tuber sprouting, whole transcriptome sequencing of control and Eth-treated tubers was conducted. Transcriptomic analysis showed that the number of DEGs for CK_1W vs. CK comparison (3293 up-regulated and 1656 down-regulated) was approximately 10 times higher than that of the CK_2W vs. CK_1W comparison (338 up-regulated and 193 down-regulated), indicated that more transcriptional changes occurred in the early stage of sprouting (Figure 2). The application of Eth reduced the number of up-regulated genes and increased the number of down-regulated genes, which indicated that Eth treatment could retard the metabolic processes during tuber sprouting. To better characterize the biological meanings of these DEGs, KEGG enrichment was performed (Table 3). According to the annotation results, genes involved in metabolic pathways for carbohydrate metabolism (e.g., "starch and sucrose metabolism (ko00500)"), phytohormone metabolism (e.g., "plant hormone signal transduction (ko04075)"), and secondary metabolism (e.g., "phenylpropanoid biosynthesis (ko00940)") have been reported as being closely involved in the tuber dormancy release process [35]. Within the first week of storage, over 20% of genes in the pathway of "phenylpropanoid biosynthesis (ko00940)" were up-regulated in the control tubers, while this trend was suppressed markedly by Eth treatment, which matched with the results of a decreased total phenol concentration in a previous study [14]. Meanwhile, the number of up-regulated genes related to starch and sucrose metabolism was reduced drastically by Eth treatment. This result might indicate that the initial mobilization of deposited starch in sprouted tubers was restrained after Eth treatment. Interestingly, many genes involved in the pathways of "photosynthesis" and "photosynthesis—antenna proteins" were up-regulated in the comparison of CK_1W vs. CK, while a smaller number of genes were observed in the comparison of Eth_1W vs. CK. This observation presented the potential physiological role of photosynthesis in tuber sprouting, since it involved the generation of carbohydrates, ATP synthesis and energy conversion [36]. Additionally, plenty of genes in the pathway of "plant hormone signal transduction (ko04075)" were altered by Eth treatment, which might play a vital role in controlling tuber sprouting.

The heat map in Figure 3 presents that many DEGs encoding *ETR* and *ERF1/2* in the Eth signal transduction pathway were evidently up-regulated by Eth treatment, as compared with expression in control tubers. *ETR* is one of the five membrane-bound receptors and acts as an Eth-responsive negative regulator [37]. The up-regulation of this negative regulator might be explained as an increase in the Eth sensitivity of Eth-treated tissues or the adaptation to prolonged high Eth exposure [38]. *ERFs*, the transcription factors that are located operationally downstream of the Eth signaling pathway, have been shown to play an important role in regulating plant growth and development [39]. The higher expression levels of *ERF*-related genes in Eth-treated tubers implied that *ERF* might be the key factor in signal transduction contributing to Eth-induced sprout suppression.

In addition to that, the expression of genes regulating cell enlargement and plant growth (*AUX1*, *AUX/IAA*, *ARF*, *GH3*, and *SAUR*) in the Aux signal transduction pathway was mostly down-regulated by Eth treatment, as compared with expression in control tubers. In this study, combining the physiological and transcriptional results of Aux, we hypothesized that one possible mechanism of ethylene-inhibited potato tuber sprouting might be through repressing the biosynthesis of internal Aux and the expression levels of related genes in the Aux signal transduction pathway, thereby weakening the effect of Aux. Furthermore, Eth treatment suppressed the expressions of six *A-ARR* (two-component response regulator ARR-A family) genes in the cytokinin signal transduction pathway and three *CYCD3* (cyclin D3) genes in the brassinosteroid signal transduction pathway, respectively, subsequently controlling the cell division and shoot initiation. In addition, compared with other plant hormones, the signal transduction processes of ABA and gibberellin were less affected by Eth treatment, indicating their limited effects on Eth-induced sprout suppression.

5. Conclusions

In the present study, the application of exogenous Eth significantly suppressed sprout growth of potato tubers at room temperature during two weeks of storage. According to the levels of endogenous hormones and their related metabolites examined, Eth treatment markedly reduced the concentrations of ABA and Aux, while it had no impact on the Eth precursor of ACC. Moreover, transcriptomic analysis revealed that many DEGs related to plant hormone signal transduction, photosynthesis, starch and sucrose metabolism and phenylpropanoid biosynthesis were down-regulated by Eth treatment. By analyzing the DEGs associated with plant hormone signal transduction, it was shown that Eth supplementation affected several different components of the Eth, Aux, cytokinin, and brassinosteroid pathways. Particularly, the genes regulating cell enlargement and plant growth in the Aux signal transduction pathway were extensively down-regulated by Eth treatment. Taken together, both the physiological and transcriptional results herein highlighted that Eth-induced sprout inhibition might be closely related to the suppression of internal Aux production and signal transduction and the activation of Eth signal transduction. Our findings provide valuable information for the application of Eth in controlling potato tuber sprouting at room temperature, and will be useful to help elucidate the transcriptional regulation of Eth-induced sprout inhibition in potato tubers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10030286/s1, Figure S1: The changes of exogenous Eth concentration during two weeks of storage; Table S1: Primers of the 10 genes in Aux signal transduction pathway and one internal control gene in potato; Table S2: FPKM values of 10 DEGs associated with Aux signal transduction pathway.

Author Contributions: Conceptualization and methodology, C.H.; formal analysis M.Z. and W.J.; investigation, M.Z., W.J. and Q.C.; data curation, M.Z. and Q.C.; writing—original draft, M.Z. and W.J.; writing—review and editing, M.F. and C.H.; funding acquisition, M.F. and C.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Talent Research Project of Qilu University of Technology (2023RCKY235), the Shandong Provincial Science and Technology of Innovation Ability Enhancement Project of Small and Medium Enterprises (2023TSGC0153), the Agricultural Industrial Technology System of Shandong Province (SDAIT-05) and the Science and Technology Project of Dafeng District, Yancheng City (DFJH20230 *).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

Eth, Ethylene; Aux, Auxin; ETR, Eth receptor; CIPC, Chlorpropham; tZ, trans-zeatin; czR, *cis*-zeatin riboside; iP, isopen-tenyladenine; IAA, indole-3-acetic acid; GA3, gibberellin A3; GA7, gibberellin A7; SA, salicylic acid; JA-Ile, jasmonoyl-isoleucine; BL, brassinolide; TY, typhasterol; RNA-seq, RNA Sequencing; KO, KEGG orthology; SD, standard deviation; AUX1, Aux influx carrier; ARF, Aux response factor; SAUR, SAUR family protein; CYCD3, cyclin D3; ABA, abscisic acid; DEGs, differentially expressed genes; ERF1/2, Eth-responsive transcription factor 1 and 2; RH, relative humidity; cZ, *cis*-zeatin; tzR, trans-zeatin riboside; iPR, isopentenyladenosine; GA1, gibberellin A1; GA4, gibberellin A4; ACC, 1-Aminocyclopropanecarboxylic acid; JA, jasmonic acid; *cis*-OPDA, *cis*-12-oxo-phytodienoic acid; CS, castasterone; LC-MS, Liquid Chromatograph-Mass Spectrometry; MRM, multiple-reaction monitoring; ANOVA, one-way analysis of variance; LSD, least significant differences; TIR1, transport inhibitor response 1; AUX/IAA, Aux responsive protein IAA; GH3, Aux responsive GH3 gene family; A-ARR, two-component response regulator ARR-A family.

References

1. Ezekiel, R.; Singh, N.; Sharma, S.; Kaur, A. Beneficial phytochemicals in potato—A review. *Food Res. Int.* **2013**, *50*, 487–496. [CrossRef]
2. Sonnewald, S.; Sonnewald, U. Regulation of potato tuber sprouting. *Planta* **2014**, *239*, 27–38. [CrossRef]
3. Sonnewald, U. Control of potato tuber sprouting. *Trends Plant Sci.* **2001**, *6*, 333–335. [CrossRef] [PubMed]
4. Sancer, O.; Şahin, U.; Ates, M.; Yünlü, S. Evaluation of genotoxic and apoptotic effects of sprouted potato. *Potato Res.* **2022**, *65*, 903–913. [CrossRef]
5. Huang, Z.; Tian, S.L.; Ge, X.; Zhang, J.; Li, S.Q.; Li, M.; Cheng, J.X.; Zheng, H.W. Complexation of chlorpropham with hydroxypropyl-β-cyclodextrinand its application in potato sprout inhibition. *Carbohyd. Polym.* **2014**, *107*, 241–246. [CrossRef] [PubMed]
6. Cools, K.; Alamar, M.C.; Terry, L.A. Controlling sprouting in potato tubers using ultraviolet-C irradiance. *Postharvest Biol. Technol.* **2014**, *98*, 106–114. [CrossRef]
7. Gómez-Castillo, D.; Cruz, E.; Iguaz, A.; Arroqui, C.; Vírseda, P. Effects of essential oils on sprout suppression and quality of potato cultivars. *Postharvest Biol. Technol.* **2013**, *82*, 15–21. [CrossRef]
8. Jia, B.; Xu, L.X.; Guan, W.Q.; Lin, Q.; Brennan, C.; Yan, R.X.; Zhao, H. Effect of citronella essential oil fumigation on sprout suppression and quality of potato tubers during storage. *Food Chem.* **2019**, *284*, 254–258. [CrossRef]
9. Orawetz, T.; Malinova, I.; Orzechowski, S.; Fettke, J. Reduction of the plastidial phosphorylase in potato (*Solanum tuberosum* L.) reveals impact on storage starch structure during growth at low temperature. *Plant Physiol. Biochem.* **2016**, *100*, 141–149. [CrossRef] [PubMed]
10. Ezekiel, R.; Singh, B. Effect of cooking and processing on CIPC residue concentrations in potatoes and processed potato products. *Potato Res.* **2007**, *50*, 175–184. [CrossRef]
11. Alamar, M.C.; Tosetti, R.; Landahl, S.; Bermejo, A.; Terry, L.A. Assuring potato tuber quality during storage: A future perspective. *Front. Plant Sci.* **2017**, *8*, 2034. [CrossRef]
12. Daniels-Lake, B.J.; Prange, R.K.; Kalt, W.; Walsh, J.R. Methods to minimize the effect of ethylene sprout inhibitor on potato fry colour. *Potato Res.* **2007**, *49*, 303–326. [CrossRef]
13. Foukaraki, S.G.; Cools, K.; Chope, G.A.; Terry, L.A. Impact of ethylene and 1-MCP on sprouting and sugar accumulation in stored potatoes. *Postharvest Biol. Technol.* **2016**, *114*, 95–103. [CrossRef]
14. Dako, E.; Jankowski, C.K.; Gnimassou, Y.M.; Lebeau, D. Study of inhibition of germination of potato by ethylene. *Heliyon* **2021**, *7*, e06175. [CrossRef]
15. Suttle, J.C.; Hultstrand, J. Role of endogenous abscisic acid in potato microtuber dormancy. *Plant Physiol.* **1994**, *105*, 891–896. [CrossRef] [PubMed]
16. Foukaraki, S.G.; Cools, K.; Terry, L.A. Differential effect of ethylene supplementation and inhibition on abscisic acid metabolism of potato (*Solanum tuberosum* L.) tubers during storage. *Postharvest Biol. Technol.* **2016**, *112*, 87–94. [CrossRef]
17. Tosettia, R.; Waters, A.; Chopec, G.A.; Cools, K.; Alamar, M.C.; McWilliamc, S.; Thompsona, A.J.; Terry, L.A. New insights into the effects of ethylene on ABA catabolism, sweetening and dormancy in stored potato tubers. *Postharvest Biol. Technol.* **2021**, *173*, 11420. [CrossRef] [PubMed]
18. Zhao, S.; Chen, Q.M.; Fu, M.F.; Yang, X.Y.; Qu, Q.L.; Dai, H.F. The inhibition of exogenous ethylene generated by solid ethylene-releasing agents on sprouting of potato tubers in relation to carbohydrate metabolism. *Qual. Assur. Saf. Crop.* **2015**, *7*, 423–429. [CrossRef]

19. Dai, H.F.; Fu, M.F.; Yang, X.Y.; Chen, Q.M. Ethylene inhibited sprouting of potato tubers by influencing the carbohydrate metabolism pathway. *J. Food Sci. Technol.* **2016**, *53*, 3166–3174. [CrossRef] [PubMed]
20. Visse-Mansiaux, M.; Tallant, M.; Brostaux, Y.; Delaplace, P.; Vanderschuren, H.; Dupuis, B. Assessment of pre-and post-harvest anti-sprouting treatments to replace CIPC for potato storage. *Postharvest Biol. Technol.* **2021**, *178*, 111540. [CrossRef]
21. Le, V.N.; Nguyen, Q.T.; Nguyen, N.T.; Le, T.G.; Janda, T.; Szalai, G.; Yu-Kui, R.U.I. Simultaneous determination of plant endogenous hormones in green mustard by liquid chromatography–tandem mass spectrometry. *Chin. J. Anal. Chem.* **2021**, *49*, 111–117. [CrossRef]
22. Fan, W.J.; Wang, Y.Q.; Zhang, L.; Fang, Y.J.; Yan, M.X.; Yuan, L.; Yang, J.; Wang, H.X. Sweet potato ADP-glucose pyrophosphorylase small subunit affects vegetative growth, starch content and storage root yield. *Plant Physiol. Biochem.* **2023**, *200*, 107796. [CrossRef]
23. Zhen, W.N.; Tu, Y.; Lin, Z.H.; Xu, X.X.; Fu, M.R.; Han, C. Comparative transcriptome analysis reveals the molecular mechanism of UV-B irradiation in promoting the accumulation of phenolic compounds in wounded carrot. *Horticulturae* **2022**, *8*, 896. [CrossRef]
24. Neubauer, J.D.; Lulai, E.C.; Thompson, A.L.; Suttle, J.C.; Bolton, M.D.; Campbell, L.G. Molecular and cytological aspects of native periderm maturation in potato tubers. *J. Plant. Physiol.* **2013**, *170*, 413–423. [CrossRef]
25. Kleinkopf, G.E.; Oberg, N.A.; Olsen, N.L. Sprout inhibition in storage: Current status, new chemistries and natural compounds. *Am. J. Potato Res.* **2003**, *80*, 317–327. [CrossRef]
26. Visse-Mansiaux, M.; Soyeurt, H.; Herrera, J.M.; Torche, J.M.; Vanderschuren, H.; Dupuis, B. Prediction of potato sprouting during storage. *Field Crop. Res.* **2022**, *278*, 108396. [CrossRef]
27. Daniels-Lake, B.J.; Prange, R.K.; Nowak, J.; Asiedu, S.K.; Walsh, J.R. Sprout development and processing quality changes in potato tubers stored under ethylene: 1. Effects of ethylene concentration. *Am. J. Potato Res.* **2005**, *82*, 389–397. [CrossRef]
28. Liu, B.L.; Zhang, N.; Wen, Y.K.; Si, H.J.; Wang, D. Identification of differentially expressed genes in potato associated with tuber dormancy release. *Mol. Biol. Rep.* **2012**, *39*, 11277–11287. [CrossRef] [PubMed]
29. Suttle, J.C. Involvement of endogenous gibberellins in potato tuber dormancy and early sprout growth: A critical assessment. *J. Plant Physiol.* **2004**, *161*, 157–164. [CrossRef] [PubMed]
30. Rosquete, M.R.; Barbez, E.; Kleine-Vehn, J. Cellular auxin homeostasis: Gatekeeping is housekeeping. *Mol. Plant* **2012**, *5*, 772–786. [CrossRef] [PubMed]
31. Sorce, C.; Lombardi, L.; Giorgetti, L.; Parisi, B.; Ranalli, P.; Lorenzi, R. Indoleacetic acid concentration and metabolism changes during bud development in tubers of two potato (*Solanum tuberosum*) cultivars. *J. Plant Physiol.* **2009**, *166*, 1023–1033. [CrossRef] [PubMed]
32. Muthoni, J.; Kabira, J.; Shimelis, H.; Melis, R. Regulation of potato tuber dormancy: A review. *Aust. J. Crop. Sci.* **2014**, *8*, 754–759.
33. Suttle, J.C.; Huckle, L.L.; Lu, S.; Knauber, D.C. Potato tuber cytokininoxidase/dehydrogenase genes: Biochemical properties, activity, and expression during tuber dormancy progression. *J. Plant. Physiol.* **2014**, *171*, 448–457. [CrossRef] [PubMed]
34. Turnbull, C.G.N.; Hanke, D.E. The control of bud dormancy in potato tubers. *Planta* **1985**, *165*, 359–365. [CrossRef] [PubMed]
35. Liu, B.L.; Zhang, N.; Wen, Y.K.; Jin, X.; Yang, J.W.; Si, H.J.; Wang, D. Transcriptomic changes during tuber dormancy release process revealed by RNA sequencing in potato. *J. Biotechnol.* **2015**, *198*, 17–30. [CrossRef] [PubMed]
36. Wang, H.B.; Zhang, M.L.; Song, Y.X.; Li, H.; Huang, H.; Shao, M.W.; Liu, Y.; Kang, Z.H. Carbon dots promote the growth and photosynthesis of mung bean sprouts. *Carbon* **2018**, *136*, 94–102. [CrossRef]
37. Chen, Y.F.; Etheridge, N.; Schaller, G.E. Ethylene signal transduction. *Ann. Bot.* **2005**, *95*, 901–915. [CrossRef]
38. Alamara, M.C.; Anastasiadia, M.; Lopez-Cobollob, R.; Bennettb, M.H.; Thompsona, A.J.; Turnbullb, C.G.N.; Mohareba, F.; Terrya, L.A. Transcriptome and phytohormone changes associated with ethylene induced onion bulb dormancy. *Postharvest Biol. Technol.* **2020**, *168*, 111267. [CrossRef]
39. Nakano, T.; Suzuki, K.; Fujimura, T.; Shinshi, H. Genome-wide analysis of the *ERF* gene family in Arabidopsis and rice. *Plant Physiol.* **2006**, *140*, 411–432. [CrossRef] [PubMed]