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Study on Regulation Mechanism of Tomato Root Growth in Greenhouse under Cycle Aerated Subsurface Drip Irrigation

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Abstract: Aerobic irrigation can effectively improve the oxygen environment in the root zone, and enhance crop quality and yield. However, how aerobic irrigation regulates root growth has not been elucidated. In this study, tomato plants were irrigated with three levels of oxygen (high, medium, and low) under underground drip irrigation. The morphology, activity, transcriptome, and hormone content of tomato roots under oxygen irrigation were analyzed. We found that the aeration irrigation significantly promoted root development. Notably, in the high-aeration irrigation treatment (HAI), the total root length, total surface area, total volume, and root activity were 12.41%, 43.2%, 79.1%, and 24.15% higher than in the non-aeration irrigation treatment (CK), respectively. The transcriptome of tomato roots under aeration irrigation was determined with a total of 272 differentially expressed genes (DEGs), including 131 up-regulated and 141 down-regulated genes. The Kyoto encyclopedia of genes and genomes (KEGG) analysis revealed that the DEGs were enriched mainly in the metabolic pathways and plant hormone signal transduction. Among the plant hormone signal transduction, 50% of DEGs belonged to IAA signal-related genes and were upregulated. LC-MS analysis showed that the content of auxin hormones in the tomato roots subjected to aeration irrigation was significantly higher than that in CK. The content of Indole-3-acetic acid (IAA), Indole-3-carboxylic acid (ICA) and Indole-3-carboxaldehyde (ICAld) were 2.3, 2.14 and 1.45 times higher than those of the CK, but insignificant effects were exerted on the contents of cytokinins, salicylic acid, jasmonic acid, abscisic acid, and ethylene. Meanwhile, the key enzyme of auxin synthesis flavin monooxygenase (YUCCA) was significantly up-regulated. The aforementioned results show that aeration irrigation may promote the growth and development of roots by auxin regulation.

Keywords: cycle aerated irrigation; root development; transcriptome analysis; hormone signal transduction; auxin

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1. Introduction

In the whole life, plants were subjected to different abiotic stresses such as flood, freezing, drought and salt stress. For most crops, flood seriously affected the growth and development of plants, which made the water around the soil saturated and extrudes the oxygen in the soil, resulting in root hypoxia [1–4]. O_2 and CO_2 are the main gas components of the plant rhizosphere, and their concentrations play an important role in root metabolism and soil microbial activity [5]. Low levels or lack of oxygen in rhizosphere of plants can have a more or less detrimental effect on plant fitness, depending on species. In tomato, root asphyxia will impair metabolic functions and causes reversible or irreversible damage to plants, potentially leading to death [6,7].

The current techniques of flood irrigation and long-term underground drip irrigation often cause hypoxic stress in the crop rhizosphere, affecting adversely the root normal

growth and reducing crop yield and quality [8,9]. The irrigation technology of the application of oxygen or oxygen-containing substances, evenly mixed with water and transported to the crop root zone through the underground drip irrigation system, is called oxygenated underground drip irrigation. This irrigation method can not only improve the irrigation water use efficiency, but also effectively alleviate the problem of root zone hypoxia. Previous studies have shown that aerobic irrigation improves the rhizosphere oxygen environment, ensures normal root respiration, and promotes root activity, improving the absorption and utilization rate of water and nutrients [10–14]. Bhattara et al. (2004) [15] found that aeration irrigation increased significantly the root length and weight and the photosynthetic activity of soybean and cotton, achieving yields that were 82–96% and 14–28% higher than those of the control. Bhattara et al. (2008) [16] performed an irrigation experiment on tomato planted in heavy clay and saline soils. Their results showed that the tomato fruit yield in the heavy clay soil increased by 16%, whereas in the saline soil it increased by remarkable 38%; the water and fertilizer utilization efficiency increased by 16% and 32% under aeration irrigation. Additionally, Niu et al. (2013) [13] found that post-irrigation aeration enhanced greenhouse cucumber plant growth, yield, irrigation-use efficiency, and nutritional quality.

The root system is an important organ for normal plant growth and development. Its main function is to obtain nutrients and water from soil for aboveground growth and development. The internal factors affecting plant root development include organic nutrition [17] and endogenous hormones, while the most significant external factors are soil permeability and temperature [18], soil moisture [19], soil nutrient availability [20], soil pH value [21], etc. Plant hormones are critical to the growth and development of plant roots. Auxins, cytokinins (CTKs), ethylene, abscisic acid (ABA), and gibberellins participate in the regulation of root growth and development. As growth promoting hormones, plant auxins have been extensively studied, which are widely distributed in plant tissues and organs, such as the roots, stems, leaves, flowers, fruits, seeds, and the coleoptile. However, auxins are concentrated mainly in the parts with vigorous growth (the coleoptile, young stem tip, root tip, leaves, post-spermatogenesis ovary, and young seeds). Auxins are involved in the regulation of the growth of plant taproot, promoting the formation of lateral and adventitious roots and root hair, and inducing vascular differentiation [22–24]. Exogenous application of a low concentration of auxin was found to promote root growth, but high auxin concentrations inhibited taproot elongation [25]. The main function of cytokinins is the stimulation of plant cell division. The application of CTKs alone usually inhibits the growth of taproot, but it can promote the formation and development of the root canal in synergy with auxins [26,27]. Gibberellins regulate the primary root development of plants. The gibberellin signal was established to degrade the DELLA protein, reducing the inhibitory effect of DELLA protein on growth and stimulating the elongation of root cells [28]. The content of endogenous ethylene in plants promotes the growth and development of roots within a certain threshold range. Exceeding this threshold inhibits the formation of main and lateral roots. In addition, ethylene is needed for the growth of root hair [29,30]. ABA and jasmonic acid are important stress factors in plants. Exogenous application of ABA or jasmonic acid usually inhibits the growth and development of roots [30–32].

Tomato is a kind of crop with large water demand. And the long-term underground drip irrigation usually leads to hypoxia, which affects the growth and development of tomato roots. To solve this problem, tomato under underground drip irrigation was irrigated with oxygen in this study. We found that the aeration irrigation significantly promoted root development including total root length, total surface area, total volume, and root activity. Moreover, aeration irrigation promoted the increase of auxin content in plant root, and the transcript of the corresponding synthetic genes were also up-regulated significantly. This study was helpful to understand the regulatory mechanism of aerobic irrigation exerts on root growth from perspective of hormone.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in a solar greenhouse with 5.5 m high, 13 m wide, and 52 m long from east to west in Tai'an, Shandong, China (36°10′ N, 117°10′ E). The site was in the semi-humid monsoon climate zone with an annual average temperature of 13 °C and precipitation of 697 mm. The soil type in greenhouse was loam clay, which had the following basic properties: soil bulk density 1.32 g/cm^3, soil alkali-hydrolyzable nitrogen 136.5 mg/kg, soil available phosphorus 51.72 mg/kg, soil available potassium 168.07 mg/kg, pH = 6.5, and EC (The value of Electrical Conductivity) = 0.62 ms/cm. In this experiment, the fertilization level is N 180 kg/hm^2 - P_2O 590 kg/hm^2 - K_2O 112.5 kg/hm^2.

2.2. Experimental Design

Tomato seedlings ('Jinglu 6335') at four leaves stage were transplanted in the greenhouse on 22 September 2019, and harvested on 1 February 2020. The area of each experimental plot was 8 m^2 with length of 10 m and width of 0.8 m. Drip irrigation pipelines were located 20 cm below the soil surface with the diameter of 16 mm, the drip head flow rate of 2 L/h, the distance between drip heads of 33 cm, and the maximum working pressure of 0.2 MPa. To prevent water from seeping sideways, each ridge was separated by a waterproof plastic film to prevent interaction between treatments, which was buried 100 cm deep. Row spacing was 50 cm, with 33 cm between plants within rows. Each plot water supply pipeline is controlled separately, and equipped with precision metering water meter. Fertilization, irrigation and plant management were the same in all plots. Each treatment combination was replicated three times.

The amount of irrigation water was calculated by the following Equation (1) [33]:

$$I = A \times Kcp \times Ep/1000 \quad (1)$$

where I is amount of irrigation a single dropper each time (mL); A is the plot area (m^2) controlled by each two branch pipes. In this experiment, A = 0.8 m × 10 m = 8 m^2; Ep represents the cumulative evaporation value of the evaporation pan in the greenhouse between two irrigation intervals (mm), Ep = 20 mm in this experiment; Kcp is evaporation pan coefficient, Kcp = 1 in this experiment.

In this experiment, the cycle aeration device is used for aeration, as shown in Figure 1 (patent No.: CN103314697A). Three pressure levels of 0.05 MPa, 0.1 MPa and 0.15 MPa are set as three aeration levels: high aeration rate (HAI), medium aeration rate (MAI) and low aeration rate (LAI) with the aeration ratio 17.25% (HAI), 14.58% (MAI) and 10.79% (LAI), respectively (the aeration ratio is the ratio of air volume to liquid volume) under the temperature of irrigation water of 18 °C. In the test, the maximum pressure of drip irrigation pipe used is 0.2 MPa. The irrigation water is circulated for 15 min for aeration treatment, and then the water and gas mixture is transported to each test area through the underground drip irrigation belt. CK represents subsurface drip irrigation without aeration treatment.

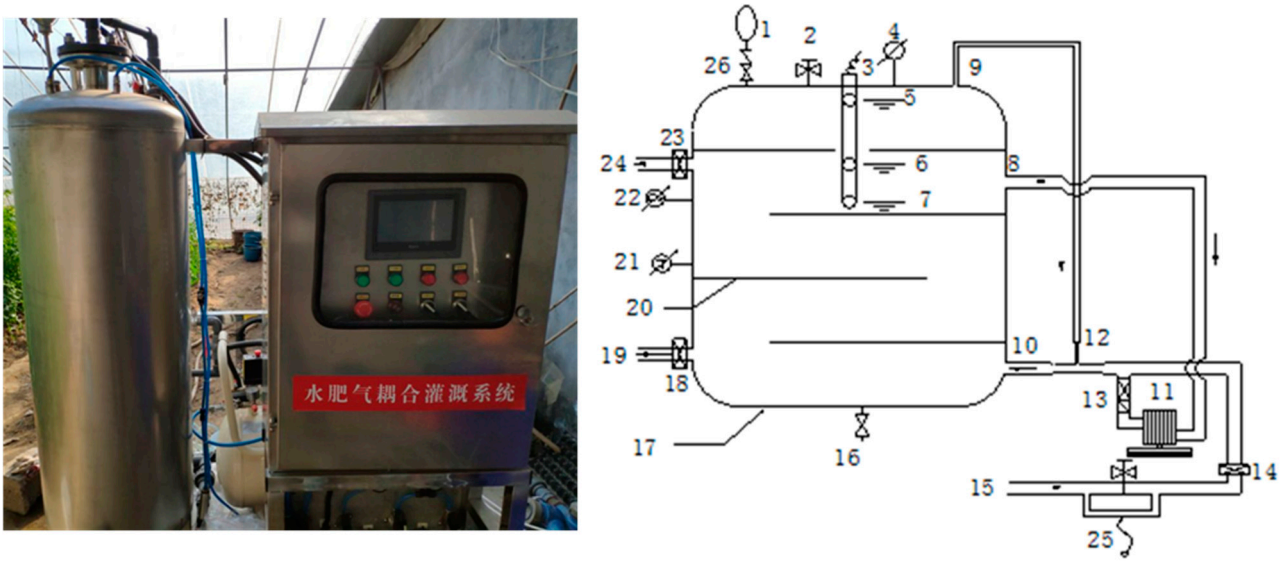

Figure 1. The cycle aeration device and schematic diagram. 1: Air compressor, 2: Pressure safety valve, 3: Water monitor, 4: Pressure control valve, 5: High water level, 6: Lower water level, 7: Reference level, 8: Internal circulation orifice, 9: Air circulation port, 10: Water inlet, 11: Booster pump, 12: Air jet generator, 13: Check valve, 14: Inlet solenoid valve, 15: Water inlet, 16: Drain outlet, 17: Pressure water tank, 18: Water outlet solenoid valve, 19: Low level water outlet, 20: Deflector, 21: Temperature transmitter, 22: Dissolved oxygen controller, 23: Water outlet solenoid valve, 24: High level water outlet, 25: Fertilizer applicator, 26: Check valve.

2.3. Experimental Method

2.3.1. Determination of Root Morphology and Root Activity Index

The total root length, root surface area, root volume and root tip number of tomato were scanned by MRS-9600TFU2L root scanner (Seiko Epson Corporation, Nagano, Japan). Root activity was measured by TTC method [34].

2.3.2. Transcriptome Sequencing

Tomato root RNA was extracted using the Omega RNA extraction kit (Omega Bio-tek, Norcross, GA, USA), followed by determination of the RNA concentration and purity with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). A sequencing library was next constructed by Wuhan Lianchuan Biological Co., Ltd. and Illumina NovaSeq sequencing was performed with a sequencing reading length of 2 × 150 bp (PE150).

2.3.3. Data Processing and Differentially Expressed Gene Analysis

Clean data were obtained using online cutadap and fptrim software (http://github.com/marcelm/cutdapt/, accessed on 20 October 2021) to remove adaptors and unqualified sequences, respectively. The clean data of different samples were mixed and assembled by Trinity software (https://trinity-software.informer.com/, accessed on 20 October 2021), and the transcripts were clustered by TGICL software (https://sourceforge.net/projects/tgic/files/?source=navbar, accessed on 20 October 2021) to remove redundancy and were normalized to obtain unigenes. The FPKM expression and read number of the unigene were calculated by Bowtie2 and Express Online software (http://bowtie-bio.sourceforge.net/bowtie2/, accessed on 20 October 2021). The screening of differentially expressed genes were Fold Change ≥ 2 and p-value software cutadap and fptrim were used gene was used by Blast2GO software (https://blast2go.com/, accessed on 20 October 2021).

2.3.4. Real Time PCR Analysis

The first strand of cDNA was synthesized by the first strand of cDNA synthesis kit (Takara, Beijing, China). The primer design is shown in Table S1. Real-time fluorescence quantitative PCR was performed on a CFX96 PCR cycler (Bio-Rad Laboratories, Hercules, CA, USA) using SYBR Premix Ex Taq II kit (Takara, Beijing, China). The relative expression was calculated by the $2^{-\triangle\triangle ct}$ method with the tomato actin gene as the reference [35].

2.3.5. Hormone Analysis

Tomato roots under cycle aeration irrigation and control were harvested. The hormones were extracted and quantified by MetWare (http://www.metware.cn/, accessed on 20 October 2021) based on a Liquid Chromatograph Mass Spectrometer (LC-MS) platform (MetWare Company, Wuhan, China). Three biological replicates of each assay were performed.

3. Results

3.1. Effects of Aerated Irrigation on Tomato Root System in Greenhouse

Low (LAI), medium (MAI), and high (HAI) aeration drip irrigation were performed under subsurface drip irrigation with non-aeration irrigation as control (CK). The root development was observed at the fruit expansion period. The root was better developed under irrigation treatment with different levels of aeration than that in the CK treatment (Figure 2). The dry weight of root under LAI, MAI and HAI treatments were 1.25, 1.78 and 2.26 times higher than in the CK treatment (Table 1).

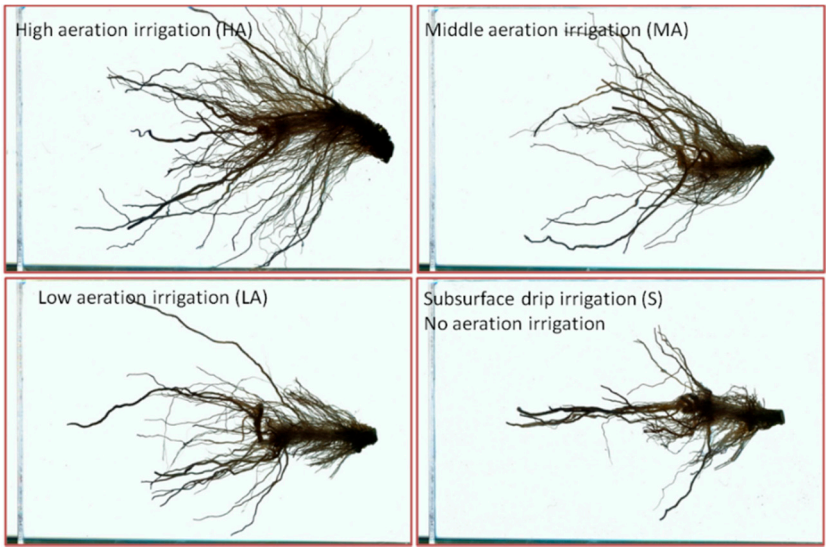

Figure 2. The growth of greenhouse tomato roots under cycle aerated subsurface drip irrigation.

Table 1. Effects of cycle aerated subsurface drip irrigation on root dry matter accumulation.

Factor	Root Dry Matter Accumulation (g/Plant)
Aeration rate	**
HAI	3.7 ± 0.68 a
MAI	2.92 ± 0.18 a
LAI	2.05 ± 0.19 b
CK	1.64 ± 0.12 b

Note: Factor level means not followed by the same letter are significantly different at the 0.05 level. ANOVA F-value for main and interaction effects were not significant or significant at <0.05 and <0.01 level (**).

The total root length under aeration irrigation treatment was significantly larger than those of the control. The total tomato root length increased with the rise in the dose of aeration, from 2274.86 cm under CK to 4645.07 cm under HAI (Table 2). The HAI treatment

had the highest root length, which was 104.19% higher than that of the CK treatment. The root length with a diameter of 0–5 mm under the aeration irrigation treatment was significantly higher than that under the CK treatment and the root length increased with the elevation of the aeration volume. The root length with a diameter of >5 mm of aerated irrigation treatment was significantly higher than that of CK treatment but LAI treatment, with 89.93% increase in HAI treatment and 37.40% increase in MAI treatment.

Table 2. Treatment main effects on total root length and length distribution by root diameter for greenhouse tomato on cycle aerated subsurface drip irrigation.

Factor	Root Length (cm/plant)			
	Total Surface Length	By Root Diameter (D) in cm		
		$0 < D \leq 2$	$2 < D \leq 5$	$5 < D$
Aeration rate	**	*	**	**
HAI	4645.07 ± 352.4 a	1699.19 ± 49.51 a	2518.05 ± 267.6 a	591.41 ± 122.0 a
MAI	3527.64 ± 78.8 b	1284.22 ± 161.0 b	1652.0 ± 328.0 b	427.83 ± 55.90 ab
LAI	3279.92 ± 257.7 b	1580.66 ± 87.17 a	1178.78 ± 191.5 bc	253.81 ± 36.24 b
CK	2274.86 ± 147.5 c	1296.13 ± 111.50 b	667.35 ± 58.06 c	311.38 ± 87.58 b

Note: Factor level means not followed by the same letter are significantly different at the 0.05 level. ANOVA F-value for main and interaction effects were not significant or significant at <0.05 (*) and <0.01 level (**).

The total root surface area length under aeration irrigation treatment was significantly larger than those of the control (Table 3). In the diameter range of 0–2 mm, the root surface area of aerated irrigation treatment was significantly higher than that of CK treatment with 48.89% increase in HAI treatment, 28.60% increase in MAI treatment and 16.78% increase in LAI treatment. In the diameter range of 2–5 mm or >5 mm, the root surface area of aerated irrigation treatment was also significantly higher than that of CK treatment, with the biggest increase of 64.68% and 165.35% in HAI treatment, respectively.

Table 3. Treatment main effects on total root surface area and surface area distribution by root diameter forgreenhouse tomato on cycle aerated subsurface drip irrigation.

Factor	Root Surface Aera (cm^2/plant)			
	Total Surface Aera	By Root Diameter (D) in cm		
		$0 < D \leq 2$	$2 < D \leq 5$	$5 < D$
Aeration rate	**	*	**	**
HAI	4278.46 ± 69.47 a	617.19 ± 68.49 a	2340.67 ± 114.30 a	1320.61 ± 39.09 a
MAI	3481.81 ± 142.0 b	533.10 ± 34.10 ab	1819.63 ± 108.0 b	1112.4 ± 59.60 b
LAI	3079.32 ± 68.33 c	484.08 ± 61.81 ab	1651.09 ± 45.02 b	944.15 ± 30.91 c
CK	2366.90 ± 161.60 d	414.53 ± 61.84 b	1421.35 ± 48.73 c	497.68 ± 123.0 d

Note: Factor level means not followed by the same letter are significantly different at the 0.05 level. ANOVA F-value for main and interaction effects were not significant or significant at <0.05 (*) and <0.01 level (**).

3.2. Effect of Aerated Irrigation on Tomato Root Activity in Greenhouse

The aeration treatment had significant effects on tomato root activity. At the same fertilization level, the root activity under the HAI treatment was the highest, followed by MAI and LAI treatments, which was 24.51%, 12.41%, and 5.55% higher than that of CK respectively (Table 4).

Table 4. Effects of aeration on root activity of tomato.

Treatment	Root Acivity ($mg.g^{-1}.h^{-1}$)
Aeration Rate	*
HAI	1.713 ± 0.08 a
MAI	1.623 ± 0.05 a
LAI	1.523 ± 0.07 ab
CK	1.375 ± 0.09 b

Note: Data are means ± SE. Values followed by different letters in the same column meant significant differences at 0.05 levels. Single asterisk means $p < 0.05$.

3.3. Effect of Aerated Irrigation on the Overground Organ Development of Tomato in Greenhouse

The aeration treatment had significant effects on the overground organ development of tomato. At the same fertilization level, the plant height, stem diameter, Chlorophyll content, dry matter (stem, leaves, fruit) under the aeration irrigation were significantly higher than that of CK respectively, with the highest was HAI treatment, followed by MAI and LAI treatments (Tables S2–S4).

3.4. Transcriptome Analysis of Tomato Roots under Aeration Irrigation

To explore the regulatory mechanism of aeration irrigation on tomato root development, tomato roots subjected to aeration irrigation were sequenced using the Illumina HiSeq2500 platform (Illumina Inc., San Diego, CA, USA). A total of 39.01 G clean bases (with an average size of 6.50 Gb) were obtained. The Q20 value of each sample was higher than 98.01%, the Q30 value was more than 94.07%, and the GC content was 42% (Table 5), indicating that the sequencing results were feasible and could be used for subsequent splicing and gene annotation.

Table 5. The transcriptome sequencing dataset and quality check.

Sample	Raw Reads	Clean Reads	Clean Base (G)	Q20 (%)	Q30 (%)	GC Content (%)
HAI1	42,547,436	40,922,608	6.14	98.07	94.28	42.36
HAI2	41,329,814	39,998,504	6.0	98.08	94.23	42.07
HAI3	43,711,800	41,211,314	6.18	98.01	94.07	41.98
CK1	50,831,674	48,398,154	7.26	98.19	94.56	42.09
CK2	50,378,580	48,594,630	7.29	98.11	94.31	42.12
CK3	42,408,998	40,913,598	6.14	98.09	94.3	42.07

The obtained unigenes were compared by Gene ontology (GO), the Kyoto encyclopedia of genes and genomes (KEGG), Pfam, Swiss-Prot, eggnog, and non-redundant (NR) databases for function annotation. A total of 26,118 unigenes were annotated, with an annotation rate of 100%, of which 75.7% were annotated in the tomato genome (Figure 3A).

Based on the fold change (FC), the differentially expressed genes of tomato roots under aeration irrigation were screened, and FC ≥ 2 and p-value ≤ 0.05 were set. Compared with the control, 272 differentially expressed genes were obtained, including 131 up-regulated genes and 141 down-regulated genes (Figure 3B).

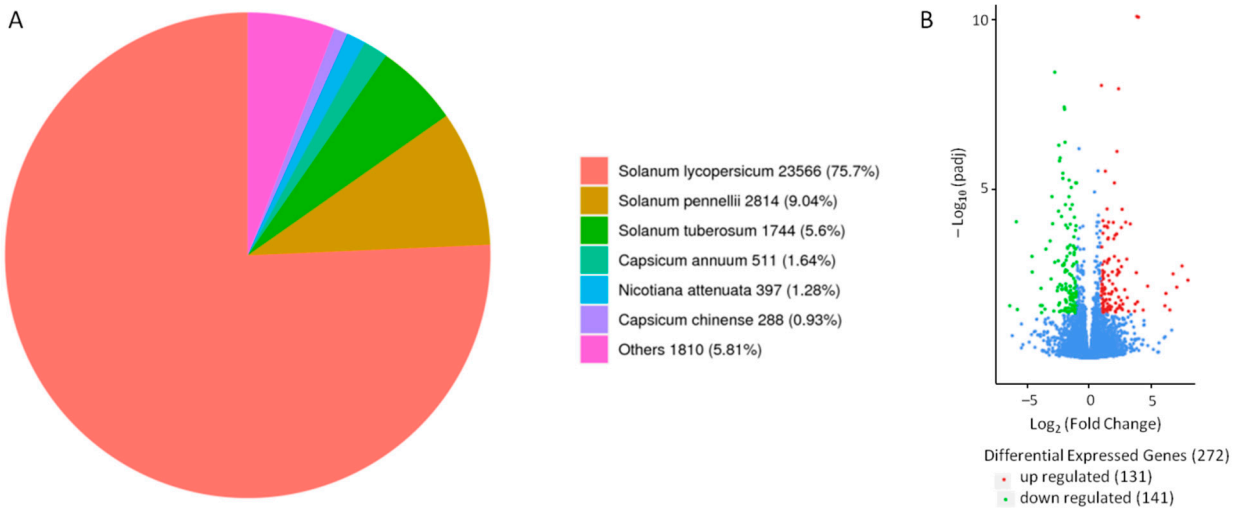

Figure 3. Functional annotation statistics of the unigenes (**A**) and the expression pattern of DEGs (**B**) in each sample based on FPKM values and numbers of DEGs of tomato root under aeration irrigation.

KEGG analysis showed that the difference expressed genes were mainly enriched in "metabolic pathway", "secondary metabolic biosynthesis pathway", "plant hormone signal transduction pathway", "pathogen interaction", "endoplasmic reticulum protein treatment", and other metabolic pathways (Figure 4). The metabolic pathways contained 47 differentially expressed genes, 31 were upregulated and 16 were downregulated, involving those of the glycolysis pathway, tricarboxylic acid cycle pathway, pentose phosphate pathway, glycogen synthesis pathway, gluconeogenesis pathway, fatty acid synthesis pathway, etc. (Table S5). The secondary biological metabolic pathways contained 27 differentially expressed genes, 12 of which were upregulated and 15 downregulated, involving genes related to the synthesis of hormones, alkaloids, toxins, and other substances (Figure 5A). Among them, there were six hormone synthesis-related genes including five auxin synthesis-related genes (YUCCA family) that were all upregulated, and one ABA synthesis key enzyme gene ZEP that was downregulated. These results indicated that hormones were involved in the regulation of aeration irrigation on root growth.

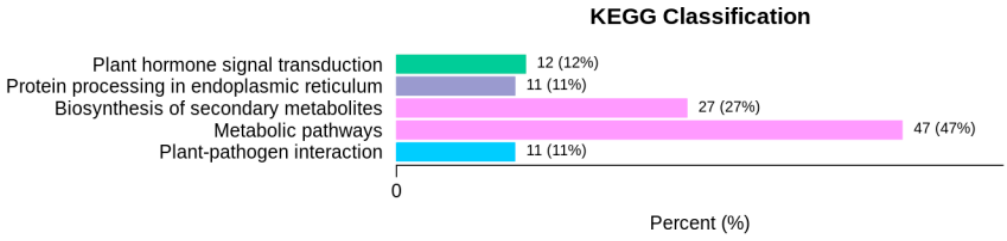

Figure 4. KEGG classification of tomato root differential gene under aeration irrigation.

Twelve differentially expressed genes were enriched in the plant hormone signal transduction pathway (Figure 5B), including six indoleacetic acid (IAA) signal-related genes, accounting for 50% of the total genes and were upregulated. In addition, two ABA signal-related genes, three ethylene signal-related genes, and one gibberellin signal-related gene were down-regulated. These DEGs were validated by quantitative expression using qPCR (Figure 6). We speculated that IAA may play an important role in the positive influence exerted by aeration irrigation (Table 5).

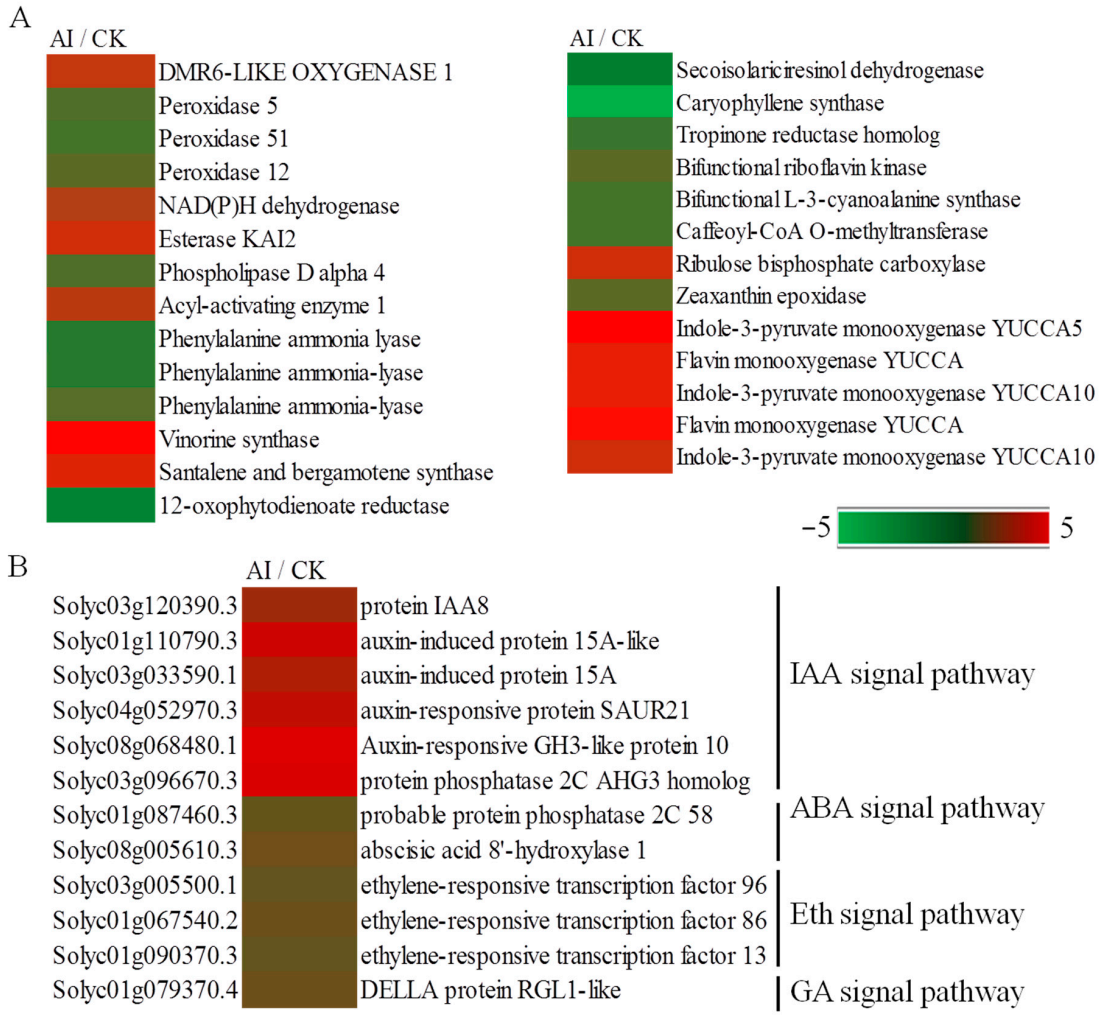

Figure 5. DEGs analysis of tomato root secondary metabolism pathway (**A**) and hormone signal transduction (**B**) under aeration irrigation.

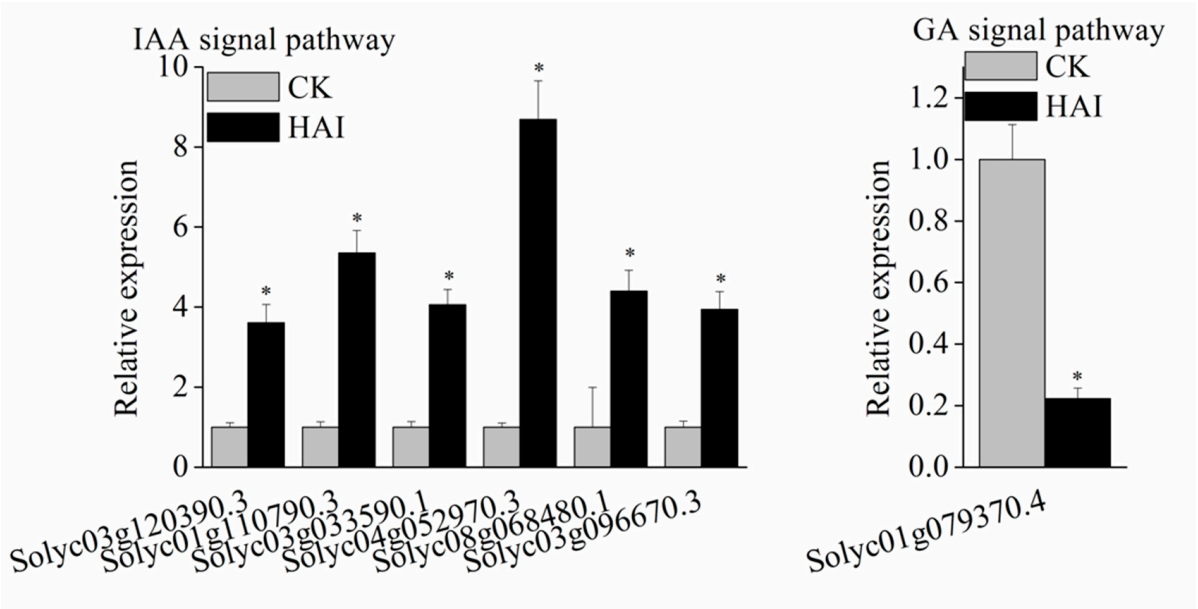

Figure 6. *Cont.*

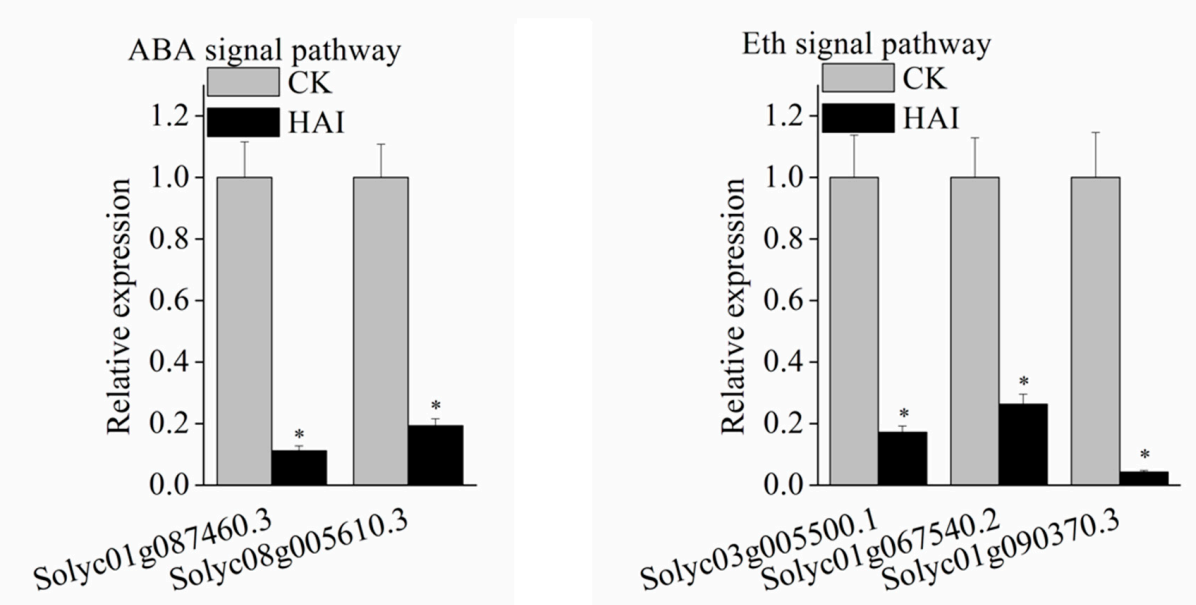

Figure 6. Validation of DEGs quantitative expression in hormone signal transduction. Data is mean of three biological replicates with ±SD. * Shows significant difference at $p < 0.05$.

3.5. Analysis of Hormone Content in Tomato Roots under Aeration Irrigation

The contents of endogenous hormones in tomato roots were determined by liquid chromatography-mass spectrometry (LC-MS). The results are shown in Figure 7. The contents of auxin hormones in tomato roots under aeration irrigation were significantly higher than those in the control (non-aeration). The contents of IAA, ICA, and ICAld were 2.3, 2.14, and 1.45 times higher than those in the control, respectively (Figure 7A). However, aeration irrigation had little effect on the contents of CTKs, salicylic acid, jasmonic acid, ABA, and ACC, with no significant difference compared with the control (Figure 7B–F). Meanwhile, the expression of key enzyme genes of hormone synthesis was detected. As can be seen in Figure 8, the key enzyme of auxin synthesis flavin monooxygenase (YUCCA) was significantly upregulated. Under aeration irrigation, the expression of YUCCA5, YUCCA2, YUCCA10, YUCCA2.1, and YUCCA10.1 genes was 18.25, 9.83, 8.25, 10.56, and 3.78 times higher than that of the control, respectively. Under aeration irrigation, the expression of CTK synthesis enzyme gene IPT (isopentenyl transfer) and salicylic acid synthesis enzyme gene ICS (isochorismate synthase) was up-regulated. The expression of ABA synthesis enzyme gene NCED (9-cis-epoxycarotenoid dioxygenase, zeaxanthin epoxidase), ZEP (zeaxanthin epoxidase) gene, ethylene synthesis enzyme gene ACS (1-aminocyclopropane-1-carboxylate synthase), and ACO (1-aminocyclopropane-1-carboxylate oxidase) was down-regulated, and the jasmonic acid synthesis enzyme gene AOS (allene oxide synthase) and AOC (allene oxide cyclase) gene were down-regulated; however, the expression levels of the aforementioned synthesis enzyme genes were not significantly lower except for the ZEP gene.

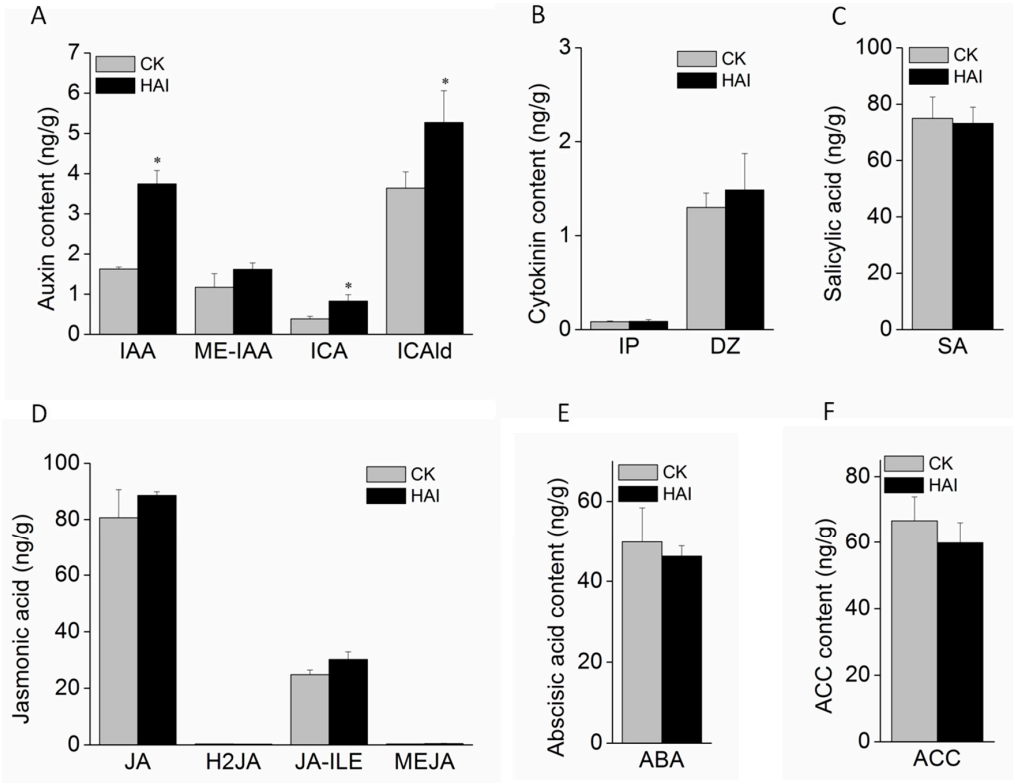

Figure 7. Determination of endogenous hormone content in tomato root under aeration irrigation using GC-MS. Data is mean of three biological replicates with ±SD. * Shows significant difference at $p < 0.05$.

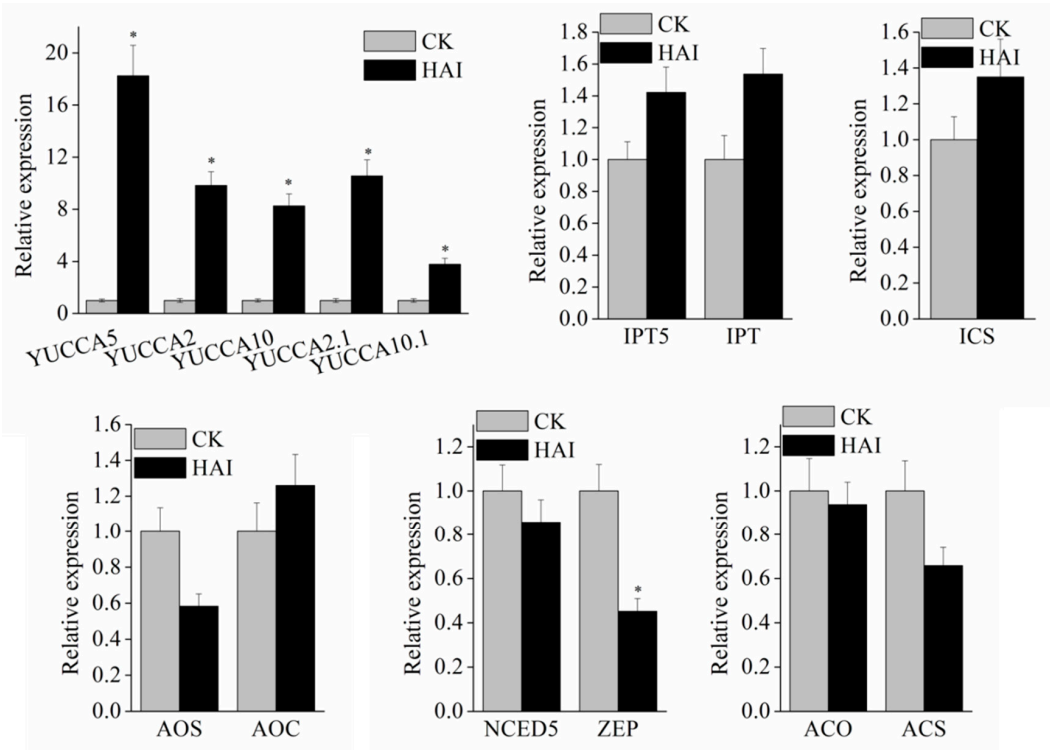

Figure 8. Expression analysis of hormone synthesis related genes in tomato roots under aeration irrigation. * Shows significant difference at $p < 0.05$.

4. Discussion

The content of rhizosphere soil gas directly affects the growth and development of crop plants, and soil hypoxia inhibits the absorption of nutrients and water, resulting in the reduction of crop yield [36]. Tomato is a crop sensitive to soil oxygen content. Hence, the aeration in the root zone is conducive to to an increase in the oxygen content of the root zone soil, enabling the physiological response of tomato roots. In this study, aeration irrigation had significant effects on the total root length, total surface area, and total volume of tomato plants, which was consistent with previous findings [14,37] that the aeration treatment in the crop root zone significantly increased the root length and volume. And we also found that high aeration treatment can significantly improve fine root growth and thus increase the fine root root surface area and root volume, suggesting the improvement of oxygen environment in root zone is conductive to the growth of roots. The aeration treatment of the root zone soil improves root activity. In this study, we found that the best aeration treatment to improve root activity was the high-aeration treatment, which is consistent with previous research results that root zone aeration treatment can significantly improve plant root activity [38]. The reason for this effect may be that aeration treatment improves the oxygen content of root zone soil. Plant roots secrete and release strong oxidizing sites into the rhizosphere soil in the root zone soil environment with good oxygen content. Furthermore, the sufficient oxygen content in the root zone soil suppresses nitrification and vulcanization reaction in root zone soil, which is conducive to enhancing root activity [39].

To explore the molecular mechanism of aeration irrigation promoting root development, we conducted transcriptome analysis of roots of tomato plants grown under aeration irrigation. KEGG analysis showed that the differentially expressed genes were mainly enriched in the "metabolic pathway" and "secondary metabolic biosynthesis pathway". Root metabolic capacity was reflected in the root activity, and its strength is reflected in root growth, absorption of water and nutrient elements or synthetic transportation of important organic matter. The metabolic pathway contained 47 differentially expressed genes, 65.9% of which were upregulated. The secondary biological metabolic pathway contained 27 differentially expressed genes, 44% of which were upregulated. These results are consistent with the enhancement of root viability, indicating that aeration irrigation promoted metabolic pathway and secondary metabolic synthesis pathway, and improved root viability.

Moreover, we found that differentially expressed genes were enriched in the hormone signal transduction pathways, of which IAA signal transduction genes accounted for 50%, including those of IAA8, auxin responsive protein SAUR21, auxin responsive GH3 like protein 10, protein phosphatase 2C AHG3, auxin induced protein 15A, and these genes were all upregulated. Auxin signal transduction is conducted by transcriptional regulation genes. Auxin early-response genes are divided into three categories: the Aux/IAA, GH3, and SAUR (small auxin up RNA) gene families [40]. The Aux/IAA protein is an early auxin responsive protein that participates in auxin signaling pathway by interacting with the ARF protein as a transcription inhibitor [41]. There are 29 Aux/IAA family members in Arabidopsis [42], and 25 Aux/IAA genes were identified in tomato [43]. Arabidopsis mutants crane-1 and crane-2 (IAA18) had suppressed lateral root formation and response to auxins [44]. The gene Aux/IAA19 regulates lateral root formation jointly with the transcription activator *NPH4/ARF7* [45]. The *OsIAA11* function acquisition mutant had seriously hindered occurrence of lateral root primordia and reduced number of lateral roots, but no effect was observed on the development of adventitious roots [46]. The SAUR gene family encodes a class of highly unstable transcripts; its mRNA half-life is very short and widely exists in various plants [47]. There are 99 SAUR family members in tomato [48]. Overexpression of *SAUR41* in Arabidopsis plants significantly increased taproot length and vigorous lateral root development [49]. The GH3 gene family encodes a class of acyl amide synthetases, which can promote the binding of auxins and amino acids, is responsible for the feedback regulation of auxins, and is involved in the control of the relative stability of

intracellular auxin content [50]. Therefore, we speculated that auxins are involved in the regulation of the effects of aeration irrigation on root development.

The hormone content of tomato roots under aeration irrigation was measured. The content of auxin hormones in tomato roots under aeration irrigation was significantly higher than that of the control (non-aeration). Although the CTK content was also upregulated, this change was not significant. Aeration exerted minor influence on the contents of other hormones, such as salicylic acid, jasmonic acid, ABA, and ACC. Auxins play an important role in plant root development, regulating the development of main lateral roots and adventitious roots in Arabidopsis, tomato, rice, grape, and other species. Therefore, we consider that IAA is critically involved in the regulation of root development under aeration irrigation. In addition, five up-regulated YUCCA genes and one downregulated ZEP gene were found in the secondary metabolic pathways. Meanwhile, the expression of key enzyme genes of hormone synthesis in tomato roots under aeration irrigation was assessed. Five key auxin synthesis YUCCA genes were significantly upregulated, whereas no significant differences were established in the other key enzymes, except for the ABA synthesis enzyme ZEP. The YUCCA gene encodes flavin monooxygenase-like enzyme (FMO), which catalyzes the conversion of tryptamine to hydroxytryptamine. This is a rate limiting enzyme in the tryptophan IAA synthesis pathway. There are 17 YUCCA family members in tomato and 18 in rice. Rice YUC1 gene regulates auxin synthesis and promotes crown root development [51]. Maize GmYUC2a was revealed to increase plant auxin content and to promote root hair growth and root diameter [52]. The above results show that aeration irrigation can promote root growth and development by regulating auxin synthesis.

5. Conclusions

In the present study, we investigated root morphological development, endogenous hormone content, and molecular regulation mechanism of tomato plants under aerated irrigation. Aerated irrigation significantly promoted the root development and length, total surface area, total volume, total diameter, and root activity, which were significantly higher than those in the control group. Transcriptome sequencing revealed that the differentially expressed genes were enriched mainly in the auxin signal transduction pathways, except for the metabolic and secondary metabolic pathways. Meanwhile, the content of auxin hormones and the upregulated expression of genes related to auxin synthesis were significantly increased under aerated irrigation. These results indicate that aerated irrigation can promote root growth and development by auxin regulation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12112609/s1, Table S1: Sequence of the primers in the experiment for qPCR. Table S2: Impacts of cycle aerated subsurface drip irrigation on plant height and stem diameter in tomato. Table S3: Effects of cycle aerated subsurface drip irrigation on photosynthetic pigment content of leaves. Table S4: Impacts of cycle aeration subsurface drip irrigation on plant dry matter in tomato. Table S5: DEGs analysis of tomato root metabolic pathway under aeration irrigation.

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