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Ebb-and-Flow Subirrigation Improves Seedling Growth and Root Morphology of Tomato by Influencing Root-Softening Enzymes and Transcript Profiling of Related Genes

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Abstract: Ebb-and-flow subirrigation is a promising strategy to increase water use efficiency, avoid waterlogging or drought conditions, and promote the overall growth of vegetable crops. The aim of this study was to evaluate the seedling growth, root morphology, activities of root-softening enzymes, and transcript profiling of those enzyme-related genes in tomato under top sprinkle irrigation and ebb-and-flow subirrigation. The results showed that ebb-and-flow subirrigation could significantly improve stem diameter, root fresh weight, root dry weight, root volume, and root diameter of tomato by 9.42%, 45.92%, 44.82%, 15.61%, and 9.41%, as compared with top sprinkle irrigation, respectively. The ebb-and-flow subirrigation also maintained the root activity and photosynthetic rate of tomato seedlings. The activities of superoxidase dismutase, peroxidase, catalase, glutathione reductase, and ascorbate peroxidase of tomato roots under ebb-and-flow subirrigation were remarkably increased, while the malondialdehyde content was decreased compared with the plants grown under top sprinkle irrigation. Correlation analysis among activities of root-softening enzymes and transcriptomic profiling of their biosynthesis-related genes revealed that 10 genes might be responsible for regulation of studied enzymes. Overall, ebb-and-flow subirrigation could significantly promote the growth of tomato seedlings, so as to maintain high activity and promote the cultivation of high-quality and strong seedlings.

Keywords: peroxidase; root activity; photosynthesis; antioxidant enzymes; transcriptome

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1. Introduction

Tomato (*Solanum lycopersicum* L.), belonging to the Solanaceae family, is a greenhouse vegetable crop native to Peruvian and Mexican regions [1,2]. It is a rich source of natural antioxidants, including flavonoids, carotenoids (mainly lycopene and β -carotene), and vitamins A, B, and C [3–6]. Vegetable production in greenhouses demands high fertilizer and water inputs in order to achieve better yield and superior quality produce [7–10]. Common irrigation practices for greenhouse vegetable production include the top sprinkle irrigation system (TSI), which is not considered to be environmentally friendly as large volumes of water and fertilizers are frequently wasted and may runoff/leach, polluting surface and groundwater systems [11–13]. Besides, the seedlings around the hole plate will grow poorly due to water shortage, resulting in inconsistent seedling height [14–16].

The ebb-and-flow irrigation (EFI) system, which is an evolved form of continuous floating system, was originally designed to grow tobacco (*Nicotiana glauca* L.) plants to increase field survival and reduce transplant shock and is now being used in China, Japan, USA, and other developing countries to grow a large number of commercial vegetables [17]. It has many advantages such as root moisture optimization, water saving, and fertilizer saving as compared to top sprinkler irrigation [18–20]. Seedling trays are suspended on

metal wires ≈ 0.20 m above a concrete floor, and every 2–3 days the irrigation water is raised to the level of the container, maintained for 15–45 min and then decreased to its original level or returned to the main reservoir until the next irrigation. In this system, water or nutrient solution is transported to the plant root through the bottom of a cultivation container by the capillary action of the cultivation medium, which can effectively avoid edge or umbrella effects and improve plant uniformity [17,21,22]. Some studies in subirrigation, performed mainly with ornamental species, have demonstrated that the concentration of the nutrient solution may be reduced by up to 50% when compared to nutrient solutions for top sprinkle irrigation, with no detrimental effects on plant growth and quality [9,23]. Subirrigation systems improve the uniformity and quality of bell pepper (*Capsicum annuum* L.) and tomato (*S. lycopersicum* L.) if grown with minimal nutrient and drought stress [24–26].

Different irrigation methods cause differences in root growth [27]. However, prolonged durations of waterlogging and anaerobic respiration ultimately lead to the accumulation of aldehydes, combined with an increase in reactive oxygen species (ROS), thus eventually leading to cell death and plant senescence [28,29]. Hindered gaseous exchange can also lead to the rapid accumulation or degradation of plant hormones and further affect plant waterlogging tolerance [30,31]. When plants are under waterlogging or drought stress, the activities of protective enzymes in plants change dynamically [32,33]. In a recent study, the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and other protective enzymes in the roots of sesame seedlings increased at the initial stage of flooding and decreased significantly with the extension of water stress time [34,35].

There are few reports about the mechanism of plant root growth, development, and physiological changes under ebb-and-flow irrigation and top sprinkler irrigation. Therefore, taking tomato as material, this experiment studied the seedling growth and physiology, root morphology, activities of root softening enzymes, and transcript patterns of their metabolism-related genes in tomato under top sprinkle and ebb-and-flow irrigation systems, so as to provide a theoretical basis for the popularization and application of ebb-and-flow irrigation technology.

2. Materials and Methods

2.1. Plant Source, Experimental Design and Irrigation Treatments

A greenhouse experiment was conducted at experimental farm of Wenzhou Vocational College of Science and Technology, Wenzhou, China ($28^{\circ}05'39.5''\text{N}$ $120^{\circ}30'55.2''\text{E}$) from 15 August 2019 to 19 September 2019. Six-month-old seeds of the pure line tomato cultivar ‘Ouxiu 201’ (round shaped fruit with regular leaves) were obtained from Institute of Vegetable Science, Wenzhou Academy of Agricultural Sciences, Wenzhou, China. The initial germination and seed moisture content before sowing were 84% and 10.5%, respectively. Tomato seeds were sown in seedlings trays (540 mm $\times$ 280 mm) having 50 plugs (each having volume of 55 mL with upper and lower diameter of 48 and 18 mm, respectively). Seedling trays were purchased from Taizhou Longji Plastics Co., Ltd., Taizhou, China. Seedling plugs were filled with growing media containing peat, vermiculite, and perlite (3:1:1), which were obtained from Hangzhou Lin’an Jindalu Industrial Technology Co., Ltd., Hangzhou, China. The pH, EC, and organic matter of the growing media were 6.34, $0.87 \text{ ms}\cdot\text{cm}^{-1}$, and 95.4%, respectively. The ebb-and-flow subirrigation system (Patent No.: ZL201520333950.6) used in the experiment was developed by Wenzhou Academy of Agricultural Sciences, Wenzhou, China. It contained a nursery frame, a number of ebb-and-flow trays horizontally placed on the nursery frame, and a water or nutrient solution circulation device (Figure 1).

Figure 1. Lateral (A) and top (B) view of ebb-and-flow nursery raising system. 1-nursery frame; 2-ebb-and-flow tray; 3-water reservoir; 4-disinfection equipment; 5-inlet main pipe; 6-drainage branch pipe; 7-drainage hole; 8-filtering parts; 9-inlet branch pipe; 10-five-way connector; 11-drainage main pipe.

Tomato seedling trays were placed on ebb-and-flow irrigation (EFI) seedling raising system [36], as well as on simple beds for top sprinkle irrigation (TSI) water treatment. The top sprinkle irrigation was carried out by manually holding the watering can. There was no fertilizer applied during the experiment. Six seedling trays were used for this experiment, each being considered as a replication of different irrigation treatments, i.e., EFI and TSI. Each irrigation treatment had three repetitions with 50 plants in each repeat. The experiment was designed under a completely randomized design (CRD).

The seedlings receiving both irrigation treatments were placed in the same greenhouse under natural environmental conditions. During the experimental period, maximum and minimum temperatures were 30.85~37.95 °C and 21.43~25.48 °C, respectively. The highest relative humidity was 82.39~99.65% and the lowest was 45.11~71.67%. The maximum and minimum light intensity was 204.26~1258.53 and 1.28~37.26 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively.

2.2. Morphological Attributes

Thirty-five days after sowing (DAS), with the plants at the stage of 6 true leaves, plant height and stem diameter were determined on 10 randomly selected seedlings from each replication of each treatment. On the same date, whole plants were taken out and the fresh weight of each plant was measured using a digital weighing balance (MJ-W176P, Panasonic, Japan). Each plant was then separated into leaves, roots, and stem. The roots of tomato seedlings were washed with water to remove media and other impurities, then dried with paper. The root morphological attributes, i.e., root length, root surface area, root volume, average root diameter, and number of root hairs were analyzed by WinRhizo Pro 2012 root analysis system (STD4800, WinRHIZO, Quebec, QC, Canada). For dry weight determination, individual plant sections were oven-dried at 70 °C until constant dry mass was attained [7,37]. Root weight ratio and leaf weight ratio were calculated by dividing the dry weight of roots and leaves by the dry weight of the whole plant, respectively [37–39]. Root-to-shoot ratio was calculated by dividing the dry weight of below-ground parts by the dry weight of above-ground parts of the plant [37,38].

2.3. Physiological Attributes

At 35 DAS, before the biological harvesting of tomato seedlings, net photosynthetic rate (P_n), stomatal conductance (G_s), and transpiration rate (T_r) were recorded from the fully expanded leaves from the top of the plant canopy using the Portable Photosynthesis System (LI-6400XT, LI-COR Biosciences, Nebraska, USA). Measurements were made between 6:00 a.m. and 7:00 a.m. The chlorophyll content of the leaves was measured with a

chlorophyll SPAD meter (CCM-200 plus, Opti-Sciences, Hudson, NH, USA) according to the manufacturer's instructions and presented as SPAD values. Water use efficiency (WUE) was calculated by dividing net photosynthetic rate with transpiration rate (P_n/T_r) as described earlier [40]. The root activity was measured by the TTC method [41].

2.4. Enzymes Activity Assay

At 35 DAS, root samples (0.5 g) were collected from 10 randomly selected seedlings from each replication of each irrigation treatment and quickly frozen in liquid nitrogen. The 1 mL of 0.1 M phosphoric acid buffer (pH = 7) was added into frozen root samples, ground, and homogenized in an ice bath. The final volume of 5 mL was made by adding 0.1 M phosphoric acid buffer (pH = 7), and 2 mL was taken and centrifuged at 10,000 rpm for 10 min. The supernatant was obtained as the crude extract of enzyme solution [42] and stored at 4 °C for further analysis.

The activity of superoxidase dismutase (SOD; EC 1.15.1.1) was assayed using a xanthine-xanthine oxidase system [43]. The change in absorbance was read at 560 nm. Peroxidase (POD; EC 1.11.1.7) activity was determined using a previously described method [44]. The change in absorbance was read at 470 nm for 4 min. Catalase (CAT; EC 1.11.1.6) activity was assayed as described previously [45]. The reaction was initiated with the enzyme extract. The decrease in absorbance (due to decomposition of H_2O_2) at 240 nm was recorded for 1 min. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured as previously described by Nakano and Asada [46]. The absorbance was measured at 290 nm for 1 min. The activity of glutathione reductase (GR; EC 1.6.4.2) was measured using the method earlier described by Cakmak et al. [47]. The decrease in absorbance was recorded for 1 min at 340 nm due to NADPH oxidation.

Cellulase (C-ase; EC 3.2.1.4) activity was determined by a test kit (A138-1-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), while ACC synthase (ACS; EC 4.4.1.14) was determined by an ELISA Kit (JM-09939p2, Jiangsu Jingmei Biotechnology Co., Ltd., Taizhou, China). The malondialdehyde (MDA) content was determined with TBA reaction method [48].

2.5. RNA Extraction, Illumina Sequencing and Gene Expression

Total RNA was extracted from tomato roots using an RNase plants mini-Kit (Omega, New York, NY, USA). The nano-spectrophotometer (ND2000, Thermo Scientific, Waltham, MA, USA) was used to assess the quality and quantity of RNA. Libraries were constructed and sequenced using Illumina HiSeq (Xten's PE150, Novogene, Beijing, China). Reads containing adapters and sequences with more than 10% unknown nucleotides (N) were first removed from the data set. The filtered clean reads were compared with the genome of *S. lycopersicum* using TopHat (ver. 2.0.12) [49]. The HTSeq (ver. 0.6.1) was used to count the reads numbers mapped to each gene [50]. Gene expression levels were standardized using the fragments per kilobase million mapped fragments (FPKM). Then, FPKM of each gene was calculated based on the length of the gene and read count mapped to this gene. All analyses consisted of 3 biological and 3 technical replicates. Table S1 contains the gene IDs, FPKM values, and descriptions of all studied genes.

2.6. Statistical Analysis

Collected data were subjected to Student's *t*-test using Microsoft Excel (ver. 2016). Correlation coefficient values were determined with Pearson (*n*) method using IBM SPSS software (ver. 17.0) and visualized through a heat-map using TBtools software (ver. 0.6655) [51].

3. Results

3.1. Morphological Attributes

The plant height and stem diameter of tomato seedlings under the influence of ebb-and-flow irrigation were 12.36% and 9.42% higher than those receiving top sprinkle irrigation, respectively (Figure 2A,B). The fresh and dry weight of tomato roots was also 45.92% and

44.82% higher with ebb-and-flow irrigation as compared to top sprinkle irrigation, respectively (Figure 2C,D). Under the influence of irrigation treatments, the biomass accumulation percentage in tomato leaves was almost same as in roots, as evidenced by measured fresh and dry weight of tomato leaves. The plants irrigated with ebb-and-flow irrigation exhibited maximum leaf fresh (4.45 g) and dry weight (0.27 g) as compared with top sprinkle irrigation (Figure 2E,F). The root length, total root volume, average root diameter, and number of root hairs of tomato seedlings increased under the influence of ebb-and-flow irrigation by 62.54%, 15.61%, 93.81%, 9.41%, and 49.81%, respectively, compared with those that received overhead irrigation (TSI) (Figure 2G,I–K). However, root surface area remained unchanged with changed irrigation strategy (Figure 2H). Leaf weight ratio, root weight ratio, and root-to-shoot ratio of tomato seedlings was positively influenced by ebb-and-flow irrigation (Figure 2L–N). Overall, plants receiving ebb-and-flow irrigation exhibited 13.85%, 23.25%, and 12.74% increases in leaf weight ratio, root weight ratio, and root-to-shoot ratio compared with control, respectively.

Figure 2. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on morphological attributes of tomato seedlings. Vertical bars indicate means $\pm$ SE ($n = 3$, 10 plants per replicate). The *, ** and *** represent significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, according to Student's *t*-test. NS—not significant.

3.2. Physiological Attributes

At 35 DAS, the photosynthetic rate (P_n), stomatal conductance (G_s), and transpiration rate (T_r) of tomato seedlings were $8.813 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $0.606 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and $1.341 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under ebb-and-flow irrigation, and significantly ($p \leq 0.01$) increased by 28.38%, 21.71%, and 19.79% compared with the top sprinkle irrigation treatment, respectively (Figure 3A–C). Water use efficiency (WUE) of tomato seedlings was $7.409 \mu\text{mol}\cdot\text{mmol}^{-1}$

under the influence of ebb-and-flow irrigation, significantly ($p \leq 0.05$) lower than that of the plants receiving top sprinkle irrigation (Figure 3D).

Figure 3. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on physiological attributes of tomato seedlings. Vertical bars indicate means $\pm$ SE ($n = 3$, 10 plants per replicate). The * and ** represent significance at $p \leq 0.05$ and $p \leq 0.01$, respectively, according to Student's *t*-test.

Chlorophyll content in leaves is an important index to reflect the photosynthetic capacity of the plants. The SPAD values depicting the chlorophyll content of tomato leaves were 28.66 and 26.70 under ebb-and-flow and top sprinkler irrigation, respectively (Figure 3E). Similarly, the root activity of tomato seedlings grown under ebb-and-flow irrigation system was $78.92 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, which was 14.15% more as compared with the plants receiving top sprinkle irrigation treatment (Figure 3F).

3.3. Enzymes Activity Assay

Tomato plants grown under ebb-and-flow irrigation showed significantly ($p \leq 0.01$) increased SOD, POD, CAT, GR, APX, and cellulase activities as compared to those receiving top sprinkle irrigation (Figure 3). The SOD, POD, and CAT activities of tomato roots under ebb-and-flow irrigation were 96.75 , 705.67 , and $10.67 \text{ U} \cdot \text{g}^{-1} \text{ protein}$, respectively, which were 12.99%, 16.98%, and 18.25% higher than those plants grown under top sprinkle irrigation system (Figure 4A–C). Similarly, the plants receiving ebb-and-flow irrigation showed 46.51% and 28.18% increases in root GR and APX activities as compared with the top sprinkle irrigation treatment (Figure 4D,E). The activity of ACC synthase of tomato roots receiving ebb-and-flow irrigation was $21.676 \text{ U} \cdot \text{mg}^{-1} \text{ protein}$, which was significantly ($p \leq 0.05$) higher than that of top sprinkle irrigation treatment (Figure 4F). The cellulase activity of tomato roots under ebb-and-flow irrigation was $0.0745 \text{ U} \cdot \text{g}^{-1} \text{ protein}$, which was significantly ($p \leq 0.01$) higher than those plants grown under top sprinkle irrigation (Figure 4G). A 6.39% reduction was observed in the MDA content of tomato roots when treated with the ebb-and-flow irrigation strategy (Figure 4H).

Figure 4. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on activities of SOD (A), POD (B), CAT (C), GR (D), APX (E), ACC synthase (F) and cellulase (G), and MDA content (H) of tomato root. Vertical bars indicate means $\pm$ SE ($n = 3$). The * and ** represent significance at $p \leq 0.05$ and $p \leq 0.01$, respectively, according to Student's t -test.

3.4. Transcript Profiling of Genes Related to the Metabolism of Tomato Root Softening Enzymes

A total of five genes related to SOD metabolism were identified through transcriptome data analysis of tomato roots. The genetic expression of *SISOD1-3* was significantly ($p \leq 0.05$) up-regulated in tomato roots under the influence of ebb-and-flow irrigation as compared with top sprinkle irrigation. Meanwhile, transcript levels of *SISOD4* and *SISOD5* remained unchanged with irrigation treatments ($p \leq 0.05$) (Figure 5).

Figure 5. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on transcript profiling of SOD genes in tomato root. Vertical bars indicate means $\pm$ SE (3 biological and 3 technical replicates). The *, ** and *** represent significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, according to Student's t -test. NS—not significant.

Among 16 POD genes, the genetic expressions of *SIPOD2*, *SIPOD7*, *SIPOD8*, and *SIPOD13* were up-regulated, while *SIPOD1*, *SIPOD3*, *SIPOD5*, *SIPOD9*, *SIPOD11*, and *SIPOD16* were significantly down-regulated under the influence of ebb-and-flow irrigation as compared with top sprinkle irrigation. The transcript levels of *SIPOD4*, *SIPOD6*, *SIPOD10*, *SIPOD12*, *SIPOD14*, and *SIPOD15* remained almost the same ($p \leq 0.05$) in roots of the tomato seedlings grown under both irrigation systems (Figure 6).

Figure 6. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on transcript profiling of POD genes in tomato root. Vertical bars indicate means $\pm$ SE (3 biological and 3 technical replicates). The *, ** and *** represent significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, according to Student's *t*-test. NS—not significant.

In the case of CAT genes, the genetic expression of only one gene (*SICAT1*) was significantly ($p \leq 0.05$) up-regulated, while *SICAT4-6* showed significantly ($p \leq 0.01$) down-regulated expression under the influence of ebb-and-flow irrigation as compared with top sprinkle irrigation. The transcript levels of *SICAT2-3* remained unchanged ($p \leq 0.05$) in roots of the tomato seedlings grown under both irrigation systems (Figure 7).

There was only one gene (*SIGR1*) identified as responsible for GR metabolism through transcriptome data analysis of tomato roots. The expression level of *SIGR1* was significantly reduced ($p \leq 0.05$) under the effect of the ebb-and-flow irrigation system as compared with the top sprinkle irrigation strategy (Figure 8).

In the case of APX genes, the genetic expression of only one gene (*SIAPX4*) was significantly ($p \leq 0.05$) up-regulated, while *SIAPX1-2* showed significantly ($p \leq 0.05$) down-regulated expression under the influence of ebb-and-flow irrigation as compared with top sprinkle irrigation. The transcript level of *SIAPX3* remained non-significantly ($p \leq 0.05$) different in plants grown under both irrigation systems (Figure 9).

Figure 7. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on transcript profiling of CAT genes in tomato root. Vertical bars indicate means $\pm$ SE (3 biological and 3 technical replicates). The *, ** and *** represent significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, according to Student's *t*-test. NS—not significant.

Figure 8. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on transcript level of GR gene in tomato root. Vertical bars indicate means $\pm$ SE (3 biological and 3 technical replicates). The * represents significance at $p \leq 0.05$, according to Student's *t*-test.

Figure 9. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on transcript profiling of APX genes in tomato root. Vertical bars indicate means $\pm$ SE (3 biological and 3 technical replicates). The * and *** represent significance at $p \leq 0.05$ and $p \leq 0.001$, respectively, according to Student's *t*-test. NS—not significant.

There were seven genes identified as responsible for ACC synthase metabolism through transcriptome data analysis of tomato roots. The transcript levels of three genes (*SIACC2*, *SIACC5*, *SIACC6*) were significantly ($p \leq 0.05$) up-regulated, while two genes (*SIACC1* and *SIACC4*) showed significantly ($p \leq 0.001$) down-regulated expression under the influence of ebb-and-flow irrigation as compared with top sprinkle irrigation. The transcript levels of *SIACC3* and *SIACC7* did not statistically significantly differ between irrigation treatments (Figure 10).

Figure 10. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on transcript profiling of ACC synthase genes in tomato roots. Vertical bars indicate means $\pm$ SE (3 biological and 3 technical replicates). The *, ** and *** represent significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, according to Student's *t*-test. NS—not significant.

In total, six genes were identified as being responsible for cellulase metabolism through transcriptome data analysis of tomato roots. The transcript level of only one gene (*SICesA4*) was significantly ($p \leq 0.05$) up-regulated, and one gene (*SICesA1*) was significantly ($p \leq 0.05$) down-regulated under the influence of ebb-and-flow irrigation as

compared with top sprinkle irrigation. The transcript levels of *SlCesA2,3,5,6* remained non-significantly ($p \leq 0.05$) different in plants grown under both irrigation systems (Figure 11).

Figure 11. Effect of top sprinkle (TSI) and ebb-and-flow (EFI) irrigation on transcript profiling of cellulase metabolism-related genes in tomato roots. Vertical bars indicate means $\pm$ SE (3 biological and 3 technical replicates). The * represents significance at $p \leq 0.05$, according to Student's *t*-test. NS—not significant.

3.5. Pearson (*n*) Correlation between Root-Softening Enzymes Activities and Transcript Profiling of Their Metabolism-Related Genes

The correlation between “root-softening enzymes activities (SOD, POD, CAT, GR, APX, ACC synthase and cellulase) and MDA content” and “transcript profiling of their metabolism-related genes” in the roots of tomato seedlings was analysed (Figure 12). The SOD activity was significantly ($p \leq 0.05$) positively correlated with the transcript levels of *SISOD1*, *SIPOD7*, *SIPOD8*, *SIPOD12*, *SIPOD13*, *SIAPX1*, and *SIAPX2*. The POD activity was positively associated with *SIPOD2*, *SIPOD8*, *SIACAT1*, *SIACC2*, *SIACC5*, and *SIACC6*. Similarly, CAT activity was positively and significantly ($p \leq 0.05$) correlated with *SISOD1*, *SISOD2*, *SIPOD2*, *SIPOD7*, *SIPOD8*, *SIPOD13*, *SIACAT1*, *SIAPX1*, *SIACC2*, *SIACC5*, *SIACC6*, and *SIACC7*. The activity of GR enzyme was positively associated with the expressions of *SISOD1*, *SISOD2*, *SIPOD2*, *SIPOD7*, *SIPOD8*, *SIPOD13*, *SIACAT1*, *SIAPX1*, *SIACC5*, and *SIACC6*.

The correlation analysis showed that APX activity was positively associated with the transcript levels of *SIACAT1*, *SIACC5*, *SIACC6*, *SIACC7*, and *SlCesA4*. Interestingly, ACC synthase activity was positively and significantly correlated with the genetic expressions of *SIPOD14*, *SIPOD15*, and *SIAPX2*. Similarly, cellulase activity positively correlated with *SIPOD4*, *SIACC2*, and *SlCesA2*. The MDA content showed the opposite trend to all other root-softening enzymes. It was negatively correlated with *SISOD5* while it showed a significantly ($p \leq 0.05$) positive correlation with *SlCesA5*.

Figure 12. The heat map showing Pearson (n) correlation between root-softening enzymes activities and transcript profiling of their metabolism-related genes. Correlation coefficient values were determined with Pearson (n) method using IBM SPSS software (ver. 17.0) and visualized through a heat-map using TBtools software (ver. 0.6655). Color codes: red—higher correlation; green—lower correlation.

4. Discussion

Compared to top sprinkle irrigated plants, ebb-and-flow sub-irrigated tomatoes increased plant biomass. These responses have been ascribed to a more uniform watering [52], lower water stress, and to an improved nutrition status due to a higher nutrient retention in the growing medium [53] in sub-irrigated when compared to top sprinkle-irrigated tomato plants. Recent studies on tomato and cucumber show that under ebb-and-flow irrigation, the plant growth potential is strong, the growth period is advanced, and the bottom water supply can enhance the quality of plug seedlings [54,55]. In this experiment, it was found that compared with the traditional top sprinkle irrigation, the ebb-and-flow irrigation treatment could significantly increase the stem diameter, as well as the root dry and fresh weight of tomato seedlings, indicating the importance of the ebb-and-flow irrigation strategy. At the same time, the total root length, root volume, and average root diameter of tomato seedlings under ebb-and-flow irrigation were significantly higher than

those under traditional top sprinkler irrigation, indicating that the subirrigation method is conducive to promote the rooting of tomato seedlings.

In the process of seedling raising, water management is very important for plant growth [56]. Too much water can cause waterlogging and less water will lead to drought stress [57,58]. The cell membranes and ROS system are severely affected by stress [59]. Malondialdehyde (MDA) is the final product of peroxidation of unsaturated fatty acids in plant cell membrane [60–62]. The higher its content, the greater the degree of membrane damage [63]. The SOD, POD, and CAT are the most important antioxidant enzymes in plants [64,65]. They interact to decompose free radicals into water and oxygen [66,67]. At the same time, they cooperate with the ascorbic acid (AsA) and glutathione (GSH) cycle to regulate reactive oxygen species metabolism [67] and maintain the redox balance of plant cells [68,69]. APX takes AsA as substrate and scavenges H_2O_2 with the help of AsA-GSH cycle system located on chloroplast membrane participated by glutathione dehydrogenase reductase (DHAR) and glutathione reductase (GR) [70]. Higher contents of APX and GSH help to improve plant defense mechanism [71]. The research shows that when tomato is subjected to water stress, the antioxidant enzyme activities of plant leaves and roots will increase with the aggravation of water stress to eliminate the adverse effects of free radicals [72]. In this experiment, it was found that the activities of SOD, POD, and CAT in tomato roots sub-irrigated with ebb-and-flow irrigation were higher than those irrigated with top sprinkle irrigation, but the content of MDA decreased significantly, indicating that the tomato roots treated with EFI maintained high growth activity, which could effectively remove the accumulation of MDA and reduce the damage of membrane lipid peroxidation. At the same time, roots of tomato plants grown under the ebb-and-flow subirrigation system maintained high APX and GR activities, which was conducive to enhance the antioxidant capacity of tomato seedlings. The stem diameter and root fresh weight were correlated with SOD, POD, CAT, and GR, which also reflect that the plants grew better under ebb-and-flow subirrigation and maintained high antioxidant enzyme activity.

Chlorophyll content is an important parameter reflecting the photosynthetic capacity of crops [73,74]. Net photosynthetic rate (P_n) reflects the plant photosynthetic capacity [75] and stomatal conductance (G_s) is closely related to plant stress resistance [76]. The water content of soil or substrate is closely related to plant photosynthesis [77,78]. Under moderate water stress, the leaf stomata remain closed, resulting in a decrease of evaporation and decrease of P_n and G_s [79]. In this experiment, the P_n and G_s of tomato seedlings grown under ebb-and-flow subirrigation were higher than those under top sprinkle irrigation, indicating that tomato plants maintained high photosynthetic capacity under EFI, while TSI treatment might lead to drought due to uneven watering.

Root activity is an important indicator to reflect the strength of the plant root system [80]. It is closely related to the plant life cycle and can reflect the dynamic relationship between root growth and water supply [80,81]. Studies on maize [82] and duckweed [83] show that the root activity of plants decreases or increases first and then decreases under drought stress. In this experiment, the root activity of tomato seedlings grown under an ebb-and-flow irrigation system was significantly higher than those irrigated with top sprinkle irrigation, maintaining vigorous metabolic activity and indicating that the water supply of EFI treatment was more uniform, and the effects of drought and other uneven water changes caused by TSI treatment were reduced.

5. Conclusions

This experiment was designed to investigate the comparison between traditional top sprinkle irrigation and ebb-and-flow subirrigation systems in terms of their effects on plant growth and the root morphology of tomato. The results indicate that ebb-and-flow subirrigation is a feasible system for soilless-cultivated tomato with no negative effects on its growth. Ebb-and-flow subirrigation of tomato resulted in beneficial changes to root morphology, as evidenced by increased root length, root surface area, root volume, average root diameter, and number of root hairs. The ebb-and-flow subirrigation strategy also

enhanced the activities of root-softening enzymes, i.e., SOD, POD, CAT, GR, APX, ACC synthase, and cellulase, resulting in better plant growth. Transcriptomic analysis of tomato roots revealed that ebb-and-flow subirrigation system caused significant variation in the expression of genes related to the biosynthesis of the aforementioned enzymes. Thus, ebb-and-flow subirrigation is a promising treatment to reduce water stress and improve the overall growth of tomato plants. This research laid the basis for further elucidation of the mechanism behind the coordinated growth of above-ground and underground plant parts under the influence of different irrigation techniques.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy12020494/s1>, Table S1: Gene IDs, FPKM values and description of studied tomato genes.

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