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Stable Soil Moisture Alleviates Water Stress and Improves Morphogenesis of Tomato Seedlings

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Abstract: Previous studies on soil water–plant relations have mostly focused on the soil water content (SWC), while the effect of soil moisture stability on plant growth has received surprisingly little attention. Potted tomato seedlings were used to examine the effect of stable soil moisture (SM) and fluctuating soil moisture (FM) on plant growth, development, and water use efficiency (WUE) in this study. The results showed that (i) soil moisture stability significantly affected the growth and development, photosynthetic characteristics, morphological traits, root morphology, and water physiological characteristics of seedling tomatoes, with SM being more conducive for most of these indices. (ii) SM improved the leaf WUE by reducing the content of abscisic acid in plants, regulating plant osmotic substances, maintaining a high gas exchange rate, and promoting plant morphology. (iii) SM could avoid water stress on tomato seedlings; even if the SWC of SM was equal to or lower than the SWC of FM, water stress would not occur under SM, whereas it would occur under FM. Overall, compared with FM, SM promoted beneficial plant morphology, maintained a high gas exchange rate, and did not induce water stress for tomato seedlings—ultimately improving WUE. This effect was more effective under low-SWC conditions than under high-SWC conditions. These findings provide a new perspective and theoretical basis for soil water–plant relations and indicate that SM has great potential in promoting plant growth and improving WUE.

Keywords: fluctuating soil moisture; soil water–plant relations; water use efficiency; morphogenesis; drought stress; *Solanum lycopersicum* L.

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1. Introduction

Soil moisture is intimately associated with plant growth and development, and it is always vital to achieving high crop yields or the production of specific agronomic commodities [1,2]. Numerous studies have been conducted on soil water–plant relations, with soil moisture primarily represented as soil water content (SWC) [3–5]. Recently, a newly innovated irrigation technique, named the pressure potential difference-crop initiate drawing water device or negative pressure irrigation (NPI), has been widely employed in experimental investigations and partially applied in agricultural production [6–10]. Yang [11] found that NPI improved WUE and yield of *Brassica chinensis* L. Zhang [12] showed that the activities of antioxidant enzymes and concentrations of osmotic adjustment substances in maize decreased under NPI, resulting in a substantial increase in maize dry matter accumulation and yield. A notable characteristic of NPI is its stable soil moisture (SM). Whether or not the significant advantage of NPI comes from soil moisture stability, several studies have demonstrated that plants benefit from SM [13,14].

The tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.) is one of the most widely cultivated vegetable species in the world [15], but its cultivation requires a great amount of water. The tomato seedling stage is critical for stem–leaf differentiation,

which is directly related to the yield and quality of mature tomatoes [16]. It has been demonstrated that proper water management improves the tomato photosynthetic rate, root morphology, and tomato quality while decreasing lipid peroxidation [17]. The decrease in SWC showed a negative effect on the water status of tomato leaves and roots, but it significantly improved the leaf and whole plant WUE [18]. Additionally, plants react to stress by accumulating osmolytes, such as proteins, proline, and soluble sugars [19]. An increase in the malondialdehyde content is a common response of stressed plants [20,21]. Furthermore, studies have shown that the endogenous abscisic acid (ABA) level plays a crucial role in modulating the leaf gas exchange, WUE, and quality of tomato plants [22]. Plant roots can sense changes in soil moisture, which in turn induces the ABA signaling system and the closure of stomata, thus regulating plant growth to improve WUE [22–24]. However, most previous studies were conducted under conditions of fluctuating soil moisture (FM) due to intermittent water supply [25–27], and the soil was frequently in a state of alternating drying and wetting. Excessive fluctuations and instability in soil moisture cause plants to experience drought and flood stress, which could lead to plant tissue damage and decreased root hydraulic conductivity [28] and possibly increase the incidence of pests [20,29]. Meanwhile, the physiological mechanism by which SM affects WUE is still unclear. Experiments performed on tomatoes reported that SM with continuous irrigation showed greater yield and lower water consumption in tomato production [30]. Several studies on maize revealed that SM could improve crop WUE by inhibiting root growth and alleviating water stress [8,13]. However, our understanding of the relationships between soil moisture stability and crops is still insufficient, especially with regard to the underlying mechanism of the effect of soil moisture stability on crop growth, development, and water utilization.

Our goal was to identify the differences between SM and FM in terms of tomato seedling growth, development, and water use. Compared to FM, we hypothesized the following: 1. SM could improve the plant morphogenesis of tomato seedlings, and 2. SM could improve tomato leaf WUE by regulating the ABA and osmotic substance contents of plants. To evaluate these hypotheses, we conducted a three-week-long water experiment with tomato seedlings, with FM as the control, and examined the effects of SM on the growth, development, and physiological responses of seedlings. The anticipated outcome of this study was to reveal the physiological mechanism underlying the influence of soil moisture stability on tomato seedling WUE and to deepen our understanding of soil water–tomato relationships.

2. Materials and Methods

2.1. Experimental Site and Plant Conditions

A pot experiment was conducted in a rain shelter located at the Chinese Academy of Agricultural Sciences (39.6° N, 116.2° E) in Beijing, China from August to September 2020. The study site was maintained in a typical warm-temperate, semi-humid continental climate, with hot and rainy summers and cold and dry winters. The annual mean temperature was 10–12 °C, and the annual frost-free period was 180–220 days [6]. Daily changes in temperature, humidity, and evaporation from a standard reference water surface during the test period are shown in Figure S1.

The soil texture was loam with a field capacity (FC) of 35% (*v*/*v*); total nitrogen content of 0.08%; total phosphorus content of 0.06%; alkali hydrolyzed nitrogen content of 81 $mg \cdot kg^{-1}$; organic matter content of 13.33 $g \cdot kg^{-1}$; available phosphorus of 14.81 $mg \cdot kg^{-1}$; available potassium of 125.25 $mg \cdot kg^{-1}$; and pH (soil: water, 1:5) of 8.32. Plastic pots—with a length, width, and height of 42 cm, 26 cm, and 25 cm, respectively—were used for growing tomato plants. Each pot was filled with 26.0 kg of air-dried soil, and the soil bulk density was 1.4 $g \cdot cm^{-3}$. The fertilizer amount per treatment was the same (0.11 g N, 0.05 g P_2O_5, and 0.15 g $K_2O \cdot kg^{-1}$ soil), and the fertilizer and soil were fully mixed. After filling the pots with soil, all pots were irrigated to 100% FC.

Tomato (*Solanum lycopersicum* L. Cv. Provence) seedlings were used as the test material in the experiment. Tomato seeds of uniform size were germinated on moistened filter paper, and then germinated seeds were sown in a growth medium consisting of vermiculite and perlite in nursery trays and cultured in a growth chamber, where the environmental conditions were maintained at 28/19 ± 1 °C day/night, 70 ± 5% relative humidity, and 12 h photoperiods (PAR 300 μmol m^{-2} s^{-1}). At the four-leaf and one-heart stages, the seedlings were transplanted into plastic pots on 2 August 2020, with two plants per pot. Then, the seedlings were rested for 10 days while the SWC was maintained at 80–90% of FC through uniform watering. Subsequently, a three-week water treatment was carried out.

2.2. Device Used to Maintain Stable Soil Moisture

The device used to maintain SM was designed by the Chinese Academy of Agricultural Sciences (Patent Nos. ZL201110093923.2 and ZL201310554433.7, China) (Figure 1) and consisted of a negative pressure controller, a water supply bucket (inner radius: 13.1 cm), and an irrigator (porous ceramic pipe). This device can continuously and stably supply water to plants and has been used on several species [6,8–10].

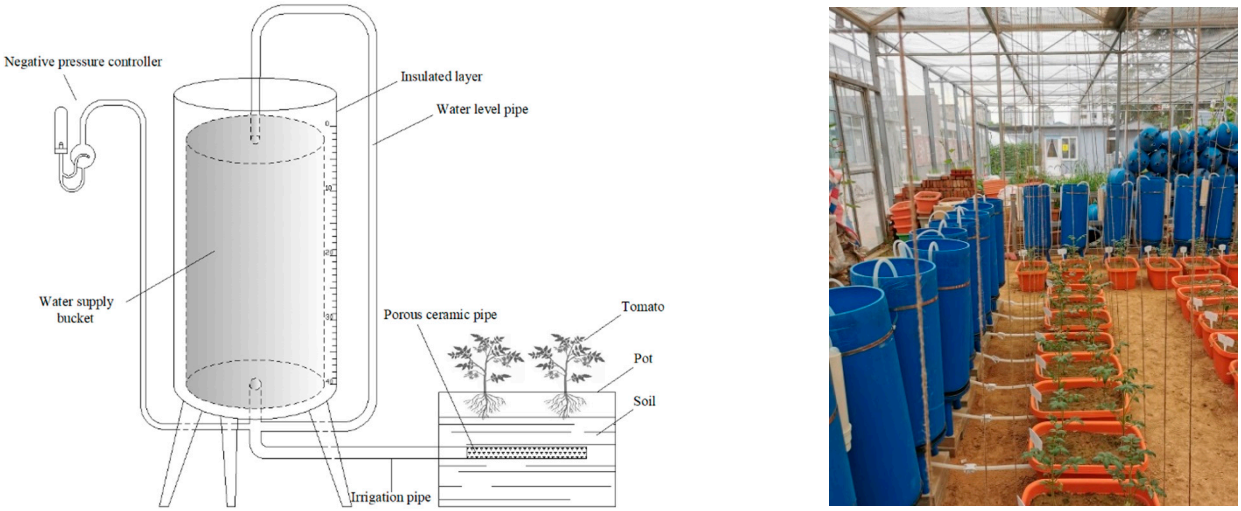

Figure 1. The schematic diagram and physical photo of the negative pressure irrigation device.

2.3. Experimental Design

The experimental factors included soil moisture stability (stable soil moisture vs fluctuating soil moisture, abbreviated as SM vs FM) and water content (low water content vs high water content, abbreviated as W1 vs W2). A completely randomized pot experiment was conducted in this study, and there were a total of four water treatments—namely SMW1, SMW2, FMW1, and FMW2—and each treatment had four replicates. The SM, which was set with the NPI system, had two water treatments of −10 kPa (SMW1) and −5 kPa (SMW2) based on previous experimental results [6,9,31]. The FM was set up through watering, and FMW1 and FMW2 had upper and lower irrigation limits of 75–55% FC and 85–65% FC, respectively. During the experiment, at 16:00 every day, four points were evenly taken around each pot to measure SWC (v/v) using a high-precision TRIME-PICO32 sensor (TRIME-PICO-IPH-TDR, IMKO, Germany), and the average value was recorded. The daily irrigation amount for SMW1 and SMW2 was calculated by multiplying the water level difference (Figure 1) by the cross-sectional area of the water supply bucket at 16:00 every day, and the cumulative irrigation amount was obtained by adding the daily irrigation amounts. When the SWC approached or dropped below the lower limits of FMW1 and FMW2, irrigation was triggered to reach the upper limits and the irrigation amount was calculated according to the measured SWC, the set upper irrigation limits, and the soil volume in the pot [31].

2.4. Sampling and Measurements

2.4.1. Fluctuation Coefficient of the SWC

The fluctuation coefficient (δ) was calculated as follows [31]:

$$\delta = \frac{1}{n-1}\sum \frac{2|\theta_i - \theta_{i-1}|}{\theta_i + \theta_{i-1}} \quad (1)$$

where δ is the fluctuation coefficient, θ_i is the observed SWC (%) at the i-th moment, θ_{i-1} is the observed SWC (%) at the i-th previous moment, and n is the number of SWC observations. The magnitude of δ reflects the soil moisture stability, and the smaller the value is, the more stable the soil moisture.

2.4.2. Sampling and Measurements of Tomato Shoots

The plants were harvested on two occasions. Four pots of tomato plants were sampled at the onset of the water treatment to measure the initial biomass. The remaining pots of tomato plants were harvested after three weeks of water treatment, and different organs (stems and leaves) of one of the tomato plants in each pot were weighed to determine the fresh weight (FW, g·plant^{-1}). Next, the materials were placed in an oven, dried at 105 °C for 30 min, and then dried at 75 °C, to a constant weight, for the determination of the dry weight (DW, g·plant^{-1}) [32]. The latest, fully expanded leaf of another tomato plant was collected to determine the leaf relative water content (LRWC, %) and leaf relative electrical conductivity (LREC, %) values [33]. The third fully expanded leaf was collected, wrapped in tinfoil, placed in liquid nitrogen, brought back to the laboratory, and stored at −80 °C to determine physiological indicators (see Section 2.4.6 for details).

2.4.3. Sampling and Measurements of Tomato Roots

The roots sampled from pots were carefully rinsed with water and wiped dry with absorbent paper. After determining the FW, one of the roots was scanned (EPSON V850 Pro scanner, Suwa, Japan), and the total root length, total root surface area, total root volume, and average root diameter were analyzed using WinRHIZO Pro software (Regent Instruments, Quebec, Canada) [34,35]. Then, the root DW was determined using the drying method [32]. The specific root length was calculated as the total root length divided by the root DW [36]. Another plant root was wrapped in tinfoil, placed in liquid nitrogen, brought back to the laboratory, and stored at −80 °C to determine its physiological indicators (see Section 2.4.6 for details).

2.4.4. Morphological Indicators

The tomato plant height, stem diameter, leaf number, leaf length, leaf width, and leaf area were measured every 7 days, beginning after treatment. The leaf length and width were obtained for the third fully expanded tomato leaf [37], and the leaf area was calculated as leaf length * leaf width * 0.64 [38].

2.4.5. Leaf Photosynthetic Pigments and Gas Exchange

The content of the tomato leaf photosynthetic pigments was determined using the alcohol extraction-spectrophotometer method (UV-2550, Shimadzu, Kyoto, Japan) [39,40].

On sunny and windless days, the third fully expanded leaf of the tomato plants was used to obtain gas exchange parameters, using a portable photosynthesis system (Li-6400XT, LI-COR, Lincoln, NE, USA), at 8:30–11:30 am. The light intensity was set to 1500 μmol·m^{-2}·s^{-1}, the flow rate was set to 500 μmol·s^{-1}, and an open gas circuit was used to measure the net photosynthetic rate (Photo, μmol CO_2 m^{-2}·s^{-1}), stomatal conductance (Cond, mol H_2O m^{-2}·s^{-1}), intercellular CO_2 concentration (Ci, μmol CO_2 mol^{-1}), and transpiration rate (Trmmol, mmol H_2O m^{-2}·s^{-1}). The intrinsic WUE of the leaves (WUE_{int}, μmol CO_2 mol^{-1} H_2O) was calculated according to the formula:

WUE_{int} = Photo/Cond. The instantaneous WUE (WUE_{ins}, μmol CO_2 $mmol^{-1}$ H_2O) value was calculated according to the formula: WUE_{ins} = Photo/Trmmol [30].

2.4.6. Physiological Indicators

Soluble sugar content was determined using the anthrone colorimetric method [8]. First, 0.3 g of the tomato sample (root, stem, and leaf) was mixed with 10 mL of distilled water in a dry 10 mL graduated test tube. Then, it was put into a boiling water bath for 30 min and then filtered into a 50 mL volumetric flask. Next, 0.5 mL of the sample solution, 1.5 mL of distilled water, 0.5 mL of anthrone ethyl acetate, and 5 mL of concentrated sulfuric acid were mixed in a 20 mL graduated test tube, and thoroughly mixed through vortexing. The solution was immediately transferred into a boiling water bath for 1 min, and absorbance was measured at 630 nm after natural cooling. The sugar content of the extract was calculated according to Zhang [8].

Soluble protein content was determined using the Coomassie Brilliant Blue method [8]. First, 0.5 g of the tomato sample (root, stem, and leaf) was mixed with 2 mL of distilled water and transferred into a 50 mL volumetric flask, which was then filled up with distilled water. After resting it for 30 min, the solution was filtered to obtain a clear liquid as the extract. Then, 1 mL of the extract and 5 mL of Coomassie Brilliant Blue reagent were added into a test tube and thoroughly mixed. After resting the solution for 2 min, absorbance was measured at 595 nm.

Free proline content was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Kete Biotechnology, Suzhou, China) [17,41]. First, 0.1 g of the tomato sample (root, stem, and leaf) was weighed, 1 mL of extracting solution was added, and it homogenized under ice bath conditions. After transferring them to a 1.5 mL centrifuge tube, they were put into a water bath for 30 min at 90 °C. Then, the mixed sample was centrifuged for 10 min at 12,000 rpm, the supernatant was collected, and the absorbance was measured at 520 nm.

Malondialdehyde content was measured using an ELISA kit [17,41]. First. 0.1 g of the tomato sample (root, stem, and leaf) was weighed, 1 mL of sulfosalicylic acid extracting solution was added, and it homogenized under ice bath conditions. After transferring them to a 1.5 mL centrifuge tube, they were put into a water bath for 30 min at 90 °C. Then, the mixed sample was centrifuged for 10 min at 12,000 rpm. After resting it for 5 min, the collected supernatant and absorbance were measured at 532 nm and 600 nm, respectively.

ABA content was measured using an ELISA kit [17,41]. First, 0.1 g of the tomato sample (root, stem, and leaf) was ground into a powder with liquid nitrogen, rinsed with 0.05 mol L^{-1} Tris-HCl (pH 7.4) in a 5 mL centrifuge tube, and made into a 10% homogenizing solution. Secondly, the mixed sample was centrifuged at 4 °C for 15 min at 3000 rpm and the supernatant was collected. The contents of ABA were assayed using an ABA ELISA kit according to the manufacturer's instructions.

After washing, the roots were wiped dry and stored at 4 °C for later use. Root activity was measured using the triphenyltetrazolium chloride reduction method and 0.06 g of fresh root [8]. The root samples were mixed with reagents in a centrifuge tube and kept in the dark at 37 °C for 3 h. Then, the sample was centrifuged for 10 min at 10,000 rpm, the supernatant was collected, and absorbance was measured at 460 nm.

2.5. Statistical Analysis

The data were processed using Microsoft Excel 2010 (Microsoft Crop, Redmond, WA, USA) and analyzed using SAS 9.0 (SAS Institute, Cary, NC, USA) to conduct one-way analysis of variance (ANOVA) tests, followed by Duncan's multiple-range tests at $p < 0.05$. All of the results were expressed as mean ± standard deviation (SD). The figures were plotted using Origin Pro 2021 software (OriginLab Corporation, Northampton, MA, USA). Principal component analysis (PCA) was used to determine the relations between soil water and plant parameters. To further investigate the relationships between morphological indicators, physiological indices, and WUE, a partial least squares path model (PLS-PM)

was constructed using the "plspm" package in R language (4.1.1) [42]. The quality of the PLS-PM was evaluated by examining the goodness-of-fit index, in which a value >0.7 indicates the acceptable overall prediction performance of the model [43].

3. Results

3.1. Soil Moisture Parameters

After water control, the SWC of all treatments showed an undifferentiated reduction trend for 8 to 10 days, at which point the SWC of FMW1 and FMW2 began to fluctuate (Figure 2). Clearly, the subsequent difference in plant performance did not result from this period, but rather from the subsequent period of fluctuation (from the eighth day after treatment). Therefore, soil moisture parameters for the entire test period and the fluctuation period were calculated (Table 1). During the fluctuation period, the δ values for SMW1, SMW2, FMW1, and FMW2 were 0.02, 0.01, 0.07, and 0.10, respectively. The mean SWC of FMW1 was 22.8% (65% FC) and was always greater than that of SMW1, for which the mean SWC was 19.9% (57% FC). The mean SWC of FMW2 was 26.1% (74% FC) and was always greater than or equal to that of SMW2, for which the mean SWC was 24.1% (69% FC). Throughout the whole test period, the mean SWC values for SMW1, SMW2, FMW1, and FMW2 were 22.6% (65% FC), 25.5% (73% FC), 24.0% (68% FC), and 26.6% (76% FC), respectively, and the δ values were 0.03, 0.02, 0.08, and 0.11, respectively. In summary, the SWC of SMW1 and SMW2 changed little, and could be categorized as SM, while the SWC of FMW1 and FMW2 changed very much, exhibiting a "sawtooth" shape, and could be categorized as FM; the mean SWC of SMW2 and FMW1 could be considered equivalent.

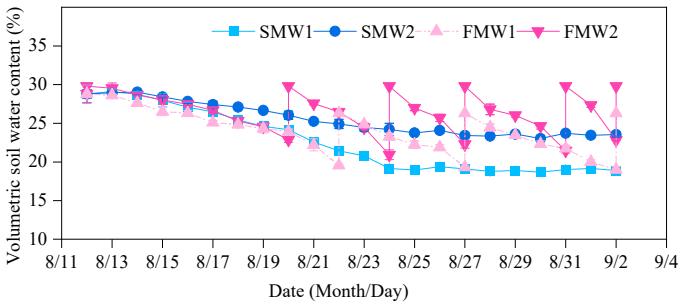

Figure 2. Changes in volumetric soil water content. Values are the mean ± SD (n = 3).

Table 1. Soil moisture parameters under different treatments.

Treatment	Test Period			Fluctuation Period		
	The Range of SWC (%)	Mean SWC (%)	δ	The Range of SWC (%)	Mean SWC (%)	δ
SMW1	18.7~29.1	22.6 (65% FC)	0.03	18.7~24.2	19.9 (57% FC)	0.02
SMW2	23.0~29.0	25.5 (73% FC)	0.02	23.0~26.0	24.1 (69% FC)	0.01
FMW1	19.0~28.8	24.0 (68% FC)	0.08	19.0~26.3	22.8 (65% FC)	0.07
FMW2	20.9~29.8	26.6 (76% FC)	0.11	20.9~29.8	26.1 (74% FC)	0.10

Note: (1) For a period of time after treatment, the soil water content (SWC) was in a downward trend without an obvious difference, and the difference in plant performance was due to the fluctuation period (from the eighth day after treatment), therefore, the soil moisture parameters were calculated for both the entire test period and the fluctuation period, respectively. (2) In terms of mean SWC, the difference between SMW2 and FMW1 was only 1.5 and 1.3% during the entire period and the fluctuation period, which was significantly less than the difference between the two treatments and other treatments. Therefore, the mean SWC of SMW2 and FMW1 can be considered equivalent.

3.2. Biomass and Morphological Traits

Regardless of SM or FM, the FW of the roots, leaves, and total plants all significantly increased with increasing SWC, while the DW of various organs showed an increasing trend with increasing SWC (Figure 3), thus indicating that a high SWC was more beneficial for biomass accumulation. The biomass of various organs and total plants (except the DW

of stems) was significantly higher in SMW2 than in FMW1, indicating that SM promoted biomass accumulation, compared to FM, under similar SWC conditions. The mean SWC of FMW1 was higher than that of SMW1, but its biomass was lower; similarly, the mean SWC of FMW2 was higher than that of SMW2, but its biomass was lower. These results indicated that soil moisture stability had a greater effect on tomato biomass than SWC.

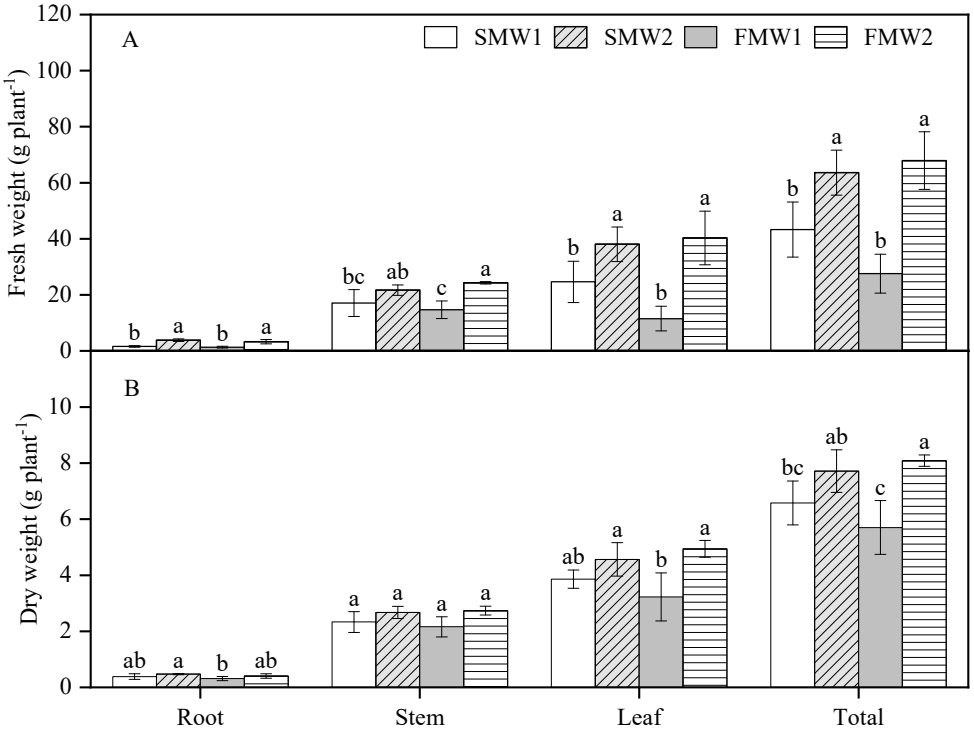

Figure 3. Effects of different treatments on the fresh weight (FW) (**A**) and dry weight (DW) (**B**) of tomato organs on the 21st day after treatment. Values are the mean ± SD (n = 3). Duncan's multiple-range test was used to test for differences among treatments at the $p < 0.05$ level. Different lowercase letters above the columns indicate significant differences among treatments within the same organ.

The differences between treatments on tomato morphological traits gradually appeared with the prolongation of water control (Figure 4). After three weeks of treatment, whether under SM or FM, the tomato plant height, stem diameter, leaf number, leaf length, leaf width, and area were significantly higher in W2 than in W1. SMW1 significantly increased tomato plant height, stem diameter, leaf number, and length compared with FMW1. SMW2 significantly increased tomato stem diameter compared with FMW2, but other morphological traits showed no significant difference. Further, SMW2 significantly increased tomato plant height, stem diameter, leaf number, leaf length, leaf width, and area compared with FMW1.

3.3. LRWC, LREC, and Photosynthetic Pigments

The LRWC increased in both SM and FM with increasing SWC, but SM was more advantageous in maintaining a higher LRWC. SMW2 increased the LRWC by 6.98% compared to SMW1. FMW2 increased the LRWC by 13.79% compared to FMW1. The LRWC of SMW1 was 10.94% higher than that of FMW1, and the LRWC of SMW2 was 4.29% higher than that of FMW2. The LRWC of SMW2 was 18.68% higher than that of FMW1. Tomato plants under low-SWC conditions exhibited a higher LREC than those under high-SWC conditions in both the SM and FM conditions, but SM was more advantageous in maintaining a lower LREC. The LREC in SMW1 decreased by 7.76% compared with FMW1, and the LREC in SMW2 decreased by 17.55% compared with FMW1. However, there were no significant differences in the LREC between SMW1 and FMW1, SMW2, and FMW2 (Table 2).

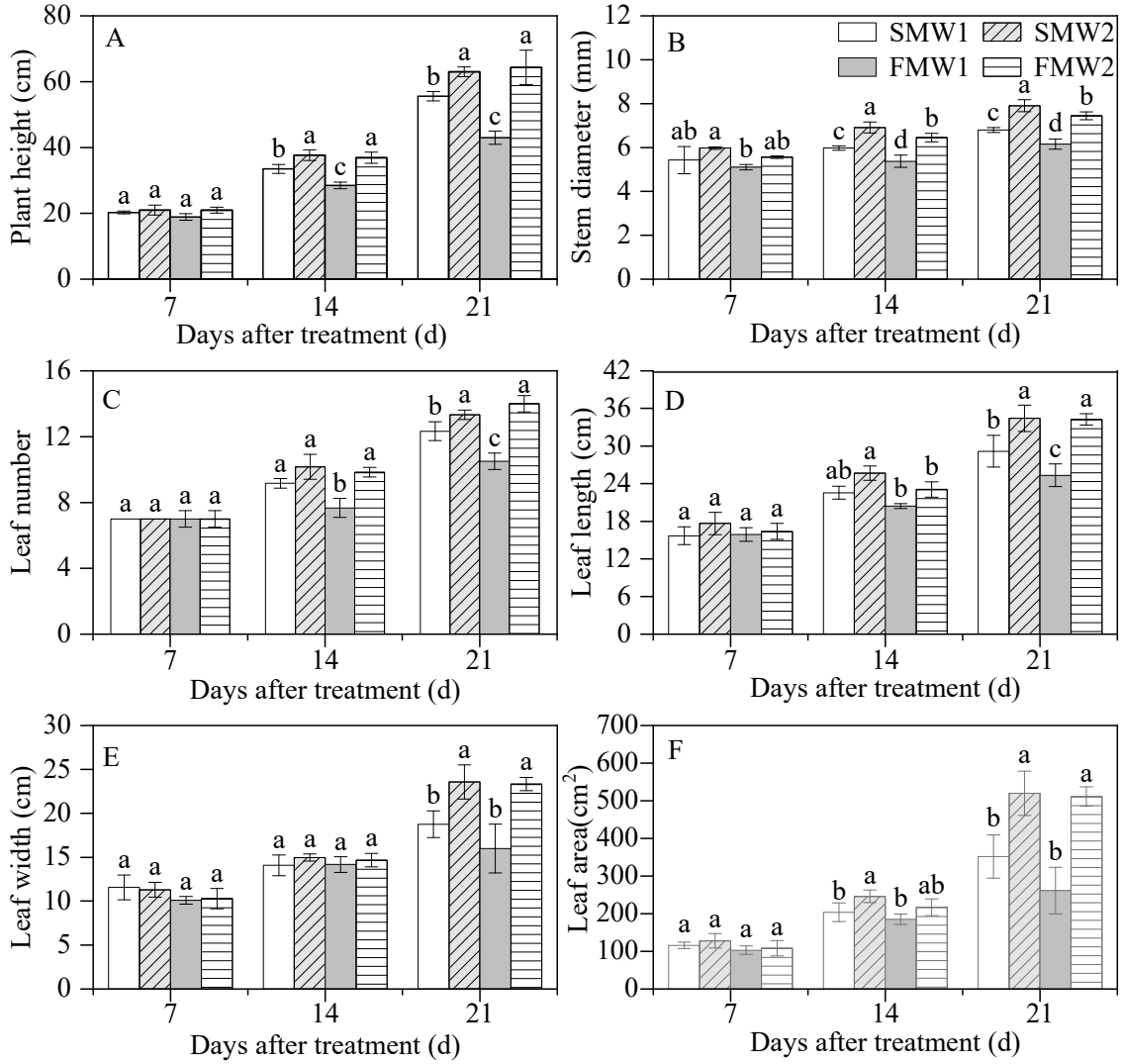

Figure 4. Effects of different treatments on tomato plant height (**A**), stem diameter (**B**), leaf number (**C**), leaf length (**D**), leaf width (**E**), and leaf area (**F**). Values are the mean ± SD (n = 3). Duncan's multiple-range test was used to test for differences among treatments at the $p < 0.05$ level. Different lowercase letters above the columns indicate significant differences among treatments within the same day.

Table 2. Relative water content (LRWC), relative electrical conductivity (LREC), chlorophyll a content, chlorophyll b content, carotenoids content, and chlorophyll a + b content of tomato leaves under different treatments.

Treatment	LRWC (%)	LREC (%)	Chlorophyll a (μg·cm^{-2})	Chlorophyll b (μg·cm^{-2})	Carotenoids (μg·cm^{-2})	Chlorophyll a + b (μg·cm^{-2})
SMW1	69.42 ± 1.15 b	17.06 ± 0.53 ab	17.70 ± 1.33 b	6.10 ± 1.28 a	1.30 ± 0.15 b	23.80 ± 0.31 b
SMW2	74.27 ± 0.94 a	15.25 ± 1.22 b	20.77 ± 1.35 a	6.97 ± 1.20 a	2.39 ± 0.47 a	27.73 ± 0.67 a
FMW1	62.58 ± 2.06 c	18.50 ± 0.64 a	17.36 ± 1.73 b	5.05 ± 1.06 a	1.37 ± 0.45 b	22.41 ± 2.68 b
FMW2	71.21 ± 0.21 b	15.19 ± 1.39 b	19.18 ± 0.54 ab	4.90 ± 0.30 a	2.62 ± 0.11 a	24.08 ± 0.52 b

Note: Values are the mean ± SD (n = 3). Duncan's multiple-range test was used to test for differences among treatments at the $p < 0.05$ level. Different lowercase letters in the same column indicate significant differences among treatments.

Tomato plants under high-SWC conditions showed higher chlorophyll a, carotenoids, and chlorophyll a + b than those under low-SWC conditions. SM increased the plants'

photosynthetic pigment contents compared to FM. The chlorophyll a, carotenoids, and chlorophyll a + b in SMW2 increased by 17.34, 84.27, and 16.53%, respectively, compared with SMW1. The chlorophyll a, carotenoids, and chlorophyll a + b in FMW2 increased by 10.47, 90.54, and 7.45%, respectively, compared with FMW1. The photosynthetic pigment contents of SMW2 were significantly higher than those of FMW1. The chlorophyll a, chlorophyll b, carotenoids, and chlorophyll a + b in SMW2 increased by 19.64, 37.90, 74.05, and 23.76%, respectively, compared with FMW1 (Table 2).

3.4. Root Morphology, Root Activity, and Root/Shoot Ratio

Tomato plants under high-SWC conditions exhibited higher root length, surface area, volume, and root activity than those under low-SWC conditions in both the SM and FM conditions. SM increased tomato root activity compared to FM. SMW1 increased the tomato root surface area by 53.80% compared to FMW1, and FMW1 decreased the tomato root length, surface area, and volume by 65.20, 57.33, and 61.73%, respectively, compared to SMW2. A significant difference in specific root length was only found between FMW1 and FMW2, wherein FMW1 had a 52.07% reduction compared to FMW2. The root activity of FMW2 was significantly lower than that of SMW2 but significantly higher than that of SMW1, while the root activity of SMW1 was significantly higher than that of FMW1. The root activity of SMW1, SMW2, and FMW2 increased by 14.88, 97.79, and 39.71%, respectively, compared with FMW1 (Table 3). However, there were no significant differences in the average root diameter and root/shoot ratio between SMW1, SMW2, FMW1, and FMW2.

Table 3. Root length, surface area, volume, average root diameter, specific root length, root activity, and root/shoot ratio of tomato plants under different treatments.

Treatment	Root Length (cm)	Root Surface Area (cm^2)	Root Volume (cm^3)	Average Root Diameter (mm)	Specific Root Length ($m \cdot g^{-1}$)	Root Activity ($\mu g \cdot h^{-1} \cdot g^{-1}$)	Root/Shoot Ratio ($g \cdot g^{-1}$)
SMW1	442.59 ± 119.45 b	39.99 ± 8.50 b	0.56 ± 0.23 bc	0.67 ± 0.12 a	12.71 ± 7.35 ab	81.63 ± 8.07 c	0.06 ± 0.01 a
SMW2	729.79 ± 101.54 a	60.93 ± 6.19 a	0.96± 0.23 a	0.86 ± 0.18 a	15.37 ± 1.50 ab	140.53 ± 3.35 a	0.07 ± 0.00 a
FMW1	254.00 ± 59.31 b	26.00 ± 4.93 c	0.37± 0.05 c	0.65 ± 0.13 a	8.18 ± 2.21 b	71.05 ± 1.18 d	0.06 ± 0.02 a
FMW2	690.71 ± 125.55 a	55.55 ± 6.28 a	0.92± 0.19 ab	0.81 ± 0.22 a	17.07 ± 1.93 a	99.27 ± 6.76 b	0.05 ± 0.01 a

Note: Values are the mean ± SD (n = 3). Duncan's multiple-range test was used to test for differences among treatments at the $p < 0.05$ level. Different lowercase letters in the same column indicate significant differences among treatments.

3.5. Leaf Gas Exchange and Leaf WUE

With increasing SWC in both the SM and FM conditions, the Photo, Cond, and Trmmol of the tomato plants increased, while the WUE_{int} and WUE_{ins} decreased (Figure 5). SM increased the tomato Photo, Cond, Trmmol, and WUE_{ins} compared to FM. On the 7th, 14th, and 21st days after water treatment, comparing SMW1 to FMW1, the Photo increased by 16.20, 8.08, and 26.73%, respectively (Figure 5A); the Cond improved by 70.33, 21.88, and 26.46%, respectively (Figure 5B); the Trmmol increased by 13.79, 2.75, and 18.97%, respectively; the WUE_{int} decreased by 31.82, 12.07, and −0.68%, respectively (Figure 5E), and the WUE_{ins} increased by 2.24, 5.17, and 6.56%, respectively (Figure 5F). On the 7th, 14th, and 21st days after water treatment, comparing SMW2 to FMW2, the Photo increased by 15.74, 9.26, and 12.19%, respectively (Figure 5A); the Cond improved by 4.86, 25.37, and 15.05%, respectively (Figure 5B); the Trmmol increased by 14.65, 2.87, and 1.97%, respectively; the WUE_{int} increased by 11.52, −12.85, and −2.53%, respectively (Figure 5E); and the WUE_{ins} increased by 0.90, 6.25, and 9.89%, respectively (Figure 5F).

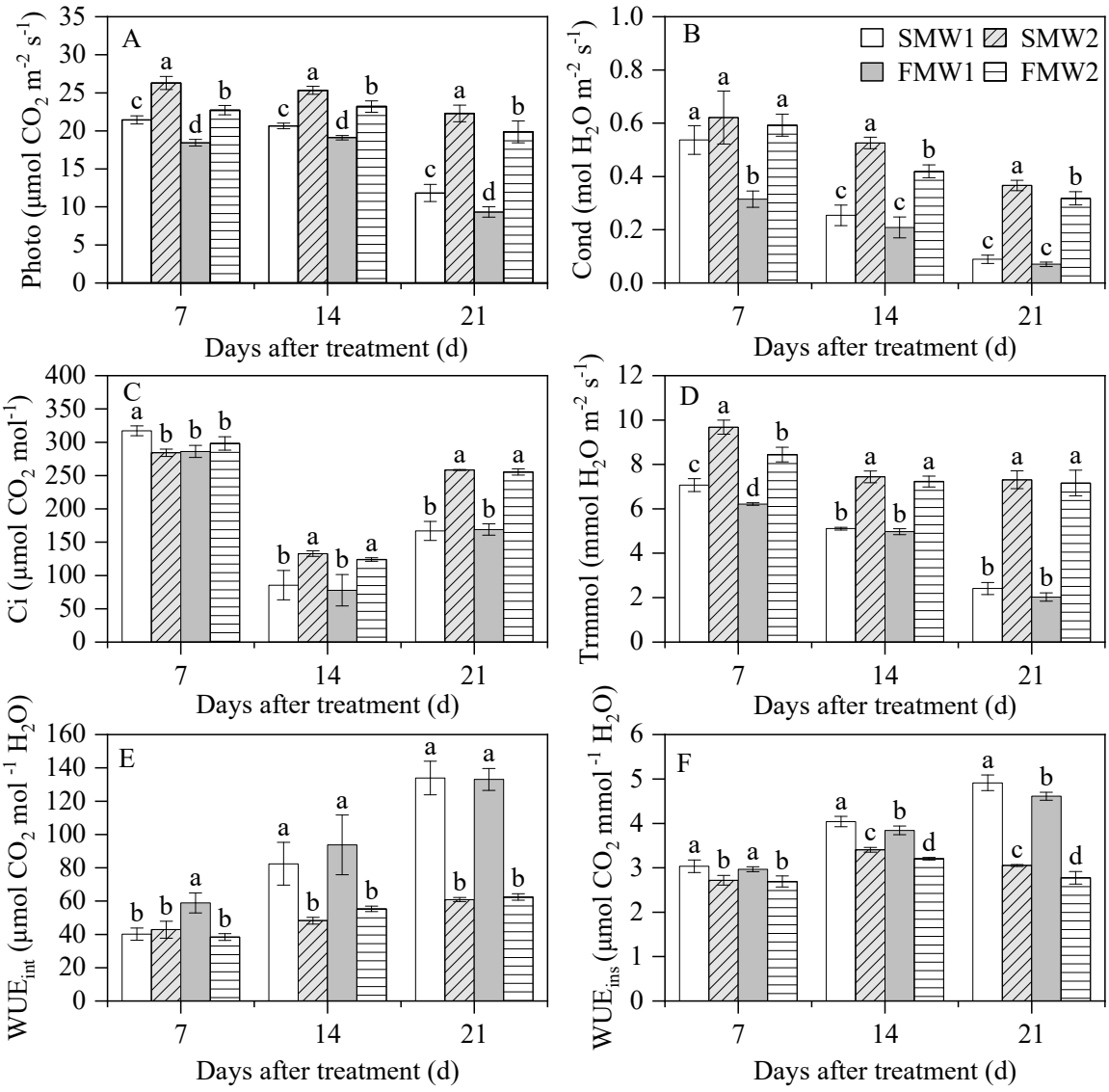

Figure 5. Effects of different treatments on the net photosynthetic rate (Photo) (**A**), stomatal conductance (Cond) (**B**), intercellular CO_2 concentration (Ci) (**C**), transpiration rate (Trmmol) (**D**), intrinsic water use efficiency (WUE_{int}) (**E**), and instantaneous water use efficiency (WUE_{ins}) (**F**) of tomato plants. Values are the mean ± SD (n = 3). Duncan's multiple-range test was used to test for differences among treatments at the $p < 0.05$ level. Different lowercase letters above the columns indicate significant differences among treatments within the same day.

3.6. Osmotic Substances, Malondialdehyde and ABA

There were no significant differences in proline content in the tomato roots, stems, or leaves (Figure 6A). The free proline in the roots, stems, and leaves decreased in both the SM and FM conditions with increasing SWC, and tomato plants accumulated more free proline under FM than under SM. The free proline in roots, stems, and leaves was significantly higher in FMW1 than in SMW1, SMW2, and FMW2, and was significantly higher in FMW2 than in SMW2. Compared with SMW1, FMW1 significantly increased the free proline content in the roots, stems, and leaves of tomato plants by 6.82, 9.32, and 6.89%, respectively. Compared with SMW2, FMW2 significantly increased the free proline content in the roots, stems, and leaves of tomato plants by 9.54, 12.76, and 5.29%, respectively.

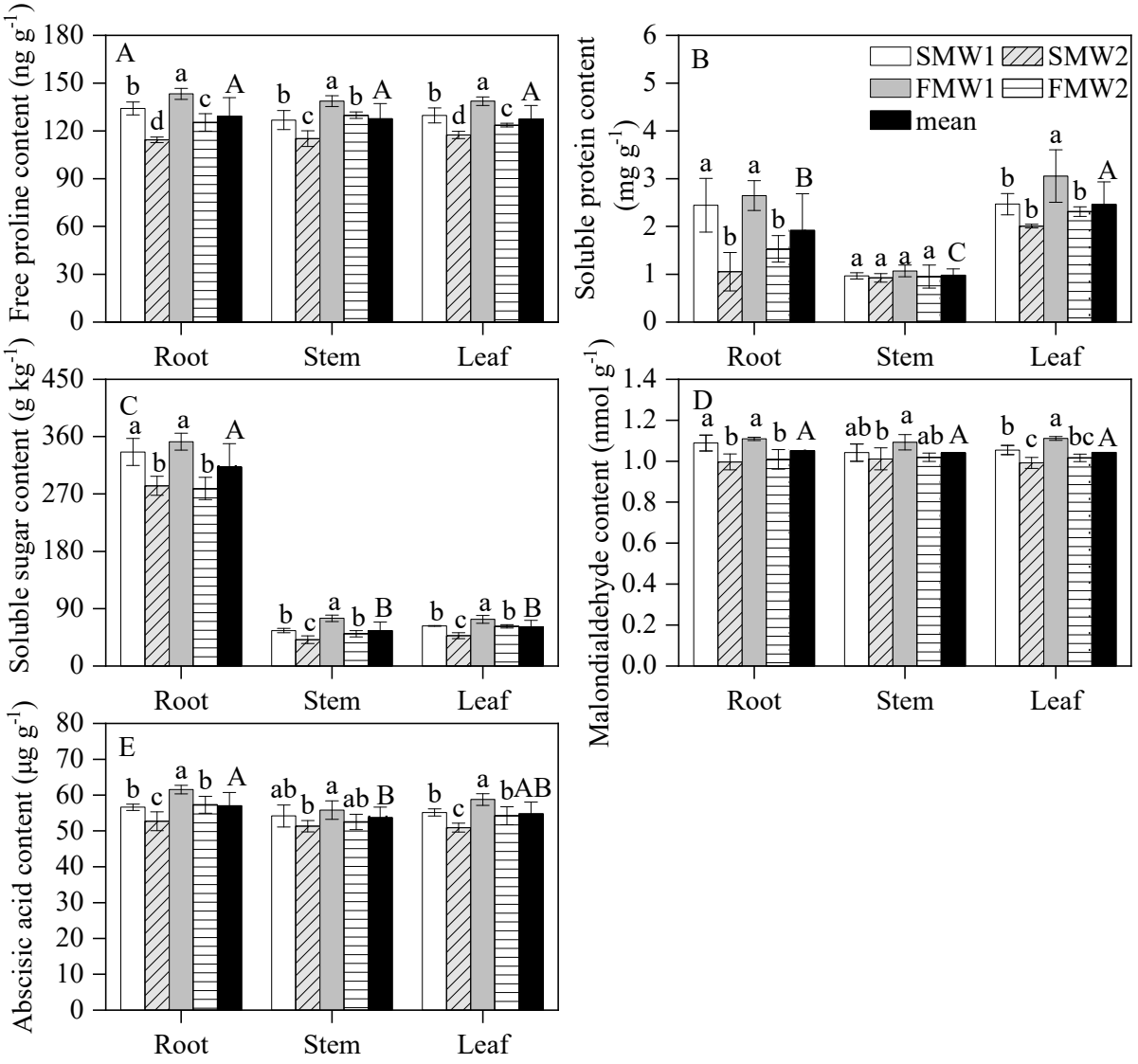

Figure 6. Effects of different treatments on osmotic substances (**A**–**C**), malondialdehyde (**D**), and abscisic acid (ABA) (**E**) contents in the tomato roots, stems, and leaves on the 21st day after treatment. Values are the mean ± SD (n = 3). Duncan's multiple-range test was used to test for differences among treatments or organs at the $p < 0.05$ level. Different lowercase letters above the columns indicate significant differences among treatments within the same organ, and different capital letters on the mean value of all treatments in each organ indicate significant differences among organs.

The soluble protein content significantly varied across different organs, with the content order of leaves > roots > stems (Figure 6B). Regardless of being in the SM or FM condition, the soluble protein content in all organs decreased with increasing SWC. Compared with FM, SM tended to reduce the soluble protein content in tomato plants. The plant stems' soluble protein content was relatively low, and there were no significant differences among treatments, while the soluble protein content of leaves in FMW1 was significantly higher than among those within SMW1, SMW2, and FMW2. Compared with FMW1, SMW1 significantly decreased the soluble protein content in the leaves of tomato plants by 19.25%. The soluble protein content of the roots was not significantly different between FMW1 and SMW1, while it was significantly higher in FMW1 than in SMW2 and FMW2.

The soluble sugar content in roots was significantly higher than those in stems and leaves (Figure 6C). The soluble sugar content in roots, stems, and leaves was significantly

reduced in both the SM and FM conditions with increasing SWC, and tomato plants accumulated more soluble sugar in their stems and leaves under FM than under SM. The soluble sugar content in stems and leaves was significantly higher (16.51–34.68%) in FMW1 than in SMW1, and it was significantly higher (23.13–30.72%) in FMW2 than in SMW2.

There were no significant differences in the content of malondialdehyde across the various organs, and the highest value was in FMW1 (Figure 6D). The malondialdehyde in each organ decreased with increasing SWC in both the SM and FM conditions. Compared with FM, SM tended to reduce the malondialdehyde content in tomato plants. The malondialdehyde in all organs was slightly lower in SMW1 than in FMW1, but the difference was significant only in the leaves. The malondialdehyde content in all organs was slightly lower in SMW2 than in FMW2, with no significant differences shown.

The ABA content varied across the different organs, in the order of roots > leaves > stems (Figure 6E). The root ABA and leaf ABA content significantly increased with increasing SWC in both the SM and FM conditions. The SM and FM conditions significantly differed, and the ABA in the roots and leaves in the FM condition was higher than those in the SM condition. The ABA content in roots and leaves was significantly higher in FMW1 than in SMW1, SMW2, and FMW2 and was significantly higher in FMW2 than in SMW2. Compared with SMW1, FMW1 significantly increased the ABA content in the roots and leaves of tomato plants by 8.71 and 6.51%, respectively. Compared with SMW2, FMW2 significantly increased the ABA content in the roots and leaves of tomato plants by 8.77 and 6.50%, respectively.

3.7. The Relations between Soil Water and Plant Parameters

The PCA, based on the measured soil–plant parameters at 21 days after treatment, revealed that the treatment observations separated into distinct clusters (Figure 7). Six principal components were extracted from the data ($\lambda > 1$), and the eigenvalues (λ) of principal component 1 (PC1) and principal component 2 (PC2) were 32.72 and 4.43, respectively, which explained 68.2 and 9.2% of the total variation, respectively (Table S1). The low-SWC conditions (FMW1 and SMW1) and high-SWC conditions (FMW2 and SMW2) were separated by PC1, where W1 treatments were generally clustered more to the left and W2 treatments more to the right on the plot. SM (SMW1 and SMW2) and FM (FMW1 and FMW2) were separated by PC2, where FM treatments were generally clustered more toward the upper area and SM treatments more toward the lower area on the plot. Vectors, such as ABA, malondialdehyde, proline, soluble sugar, and soluble protein, positively contributed to the clustering of the FMW1 treatments, whereas the SMW1 and FMW2 treatments seemed to not be influenced much by the variables. The SMW2 treatments were mainly clustered according to LRWC, root activity, chlorophyll a + b, and chlorophyll b.

3.8. The Influence Path of Changing Soil Moisture on Water Use Efficiency

PLS-PM can aggregate multiple observed variables into a latent variable and reveal the linear relationship between latent variables [43,44]. In our study, PLS-PM was used to integrate soil moisture fluctuant parameters, SWC, root activity, ABA, osmoregulation, LRWC, malondialdehyde, LREC, root morphology, photosynthetic characteristics, morphological traits, biomass, and leaf WUE (Figure 8); the goodness-of-fit index of this model was 0.89, and this index being > 0.7 indicated that the model was acceptable [43]. Soil moisture fluctuant parameters showed a significant, positive effect on ABA in tomato plants, with a path coefficient of 0.95, and exhibited a significant, negative effect on root activity, with a path coefficient of −0.91. SWC showed significant negative effects on ABA, osmoregulation, malondialdehyde, and LREC in tomato plants, with path coefficients of −0.80, −0.47, and −0.33, respectively, and exhibited a significant, positive effect on root activity, with a path coefficient of 1.00. Our PLS-PM showed that soil moisture fluctuant parameters regulated the biomass via two paths: one path followed "Soil moisture fluctuant parameters → ABA→ morphological traits → biomass", and the other path followed "Soil moisture fluctuant parameters → ABA → osmoregulation → LRWC → malondialdehyde,

LREC (→root morphology) → morphological traits → biomass". There were two paths for regulating leaf WUE: one was "Soil moisture fluctuant parameters → ABA → osmoregulation → photosynthetic characteristics → leaf WUE", and the other was "Soil moisture fluctuant parameters → ABA → osmoregulation → LRWC → malondialdehyde, LREC → photosynthetic characteristics → leaf WUE".

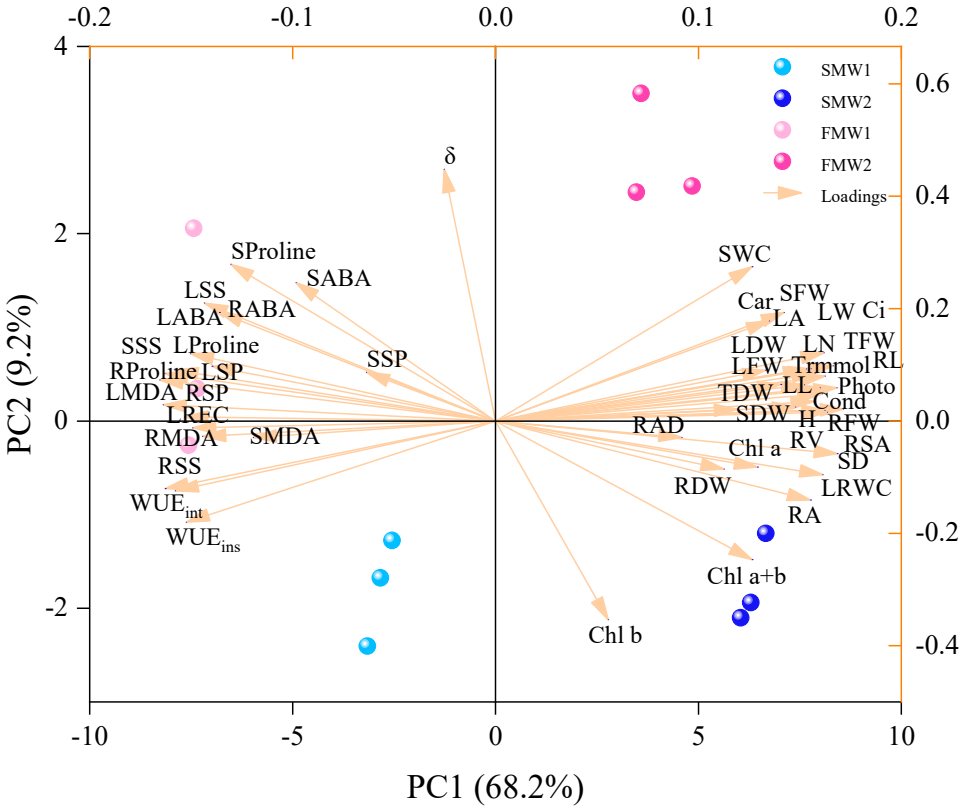

Figure 7. Biplot of the principal component analysis (PCA) of tomato indexes under different treatments. The data used in the PCA were the data from 21 days after treatment. PCn indicated the extracted principal component. SWC, soil water content; δ, fluctuation coefficient; SFW, stem fresh weight; LFW, leaf fresh weight; RFW, root fresh weight; TFW, total fresh weight; SDW, stem dry weight; LDW, leaf dry weight; RDW, root dry weight; TDW, total dry weight; H, plant height; SD, stem diameter; LN, leaf number; LL, leaf length; LW, leaf width; LA, leaf area; RL, root length; RSA, root surface area; RV, root volume; RAD, average root diameter; RA, root activity; LRWC, leaf relative water content; LREC, leaf relative electrical conductivity; Chl a, chlorophyll a content; Chl b, chlorophyll b content; Car, carotenoids content; Chl a + b, chlorophyll a and chlorophyll b contents; Photo, net photosynthetic rate; Cond, stomatal conductance; Ci, intercellular CO_2 concentration; Trmmol, transpiration rate; WUE_{int}, intrinsic water use efficiency; WUE_{ins}, instantaneous water use efficiency; SProline, stem proline content; LProline, leaf proline content; RProline, root proline content; SSP, stem soluble protein content; LSP, leaf soluble protein content; RSP, root soluble protein content; SSS, stem soluble sugar content; LSS, leaf soluble sugar content; RSS, root soluble sugar content; SMDA, stem malondialdehyde content; LMDA, leaf malondialdehyde content; RMDA, root malondialdehyde content; SABA, stem abscisic acid content; LABA, leaf abscisic acid content; RABA, root abscisic acid content.

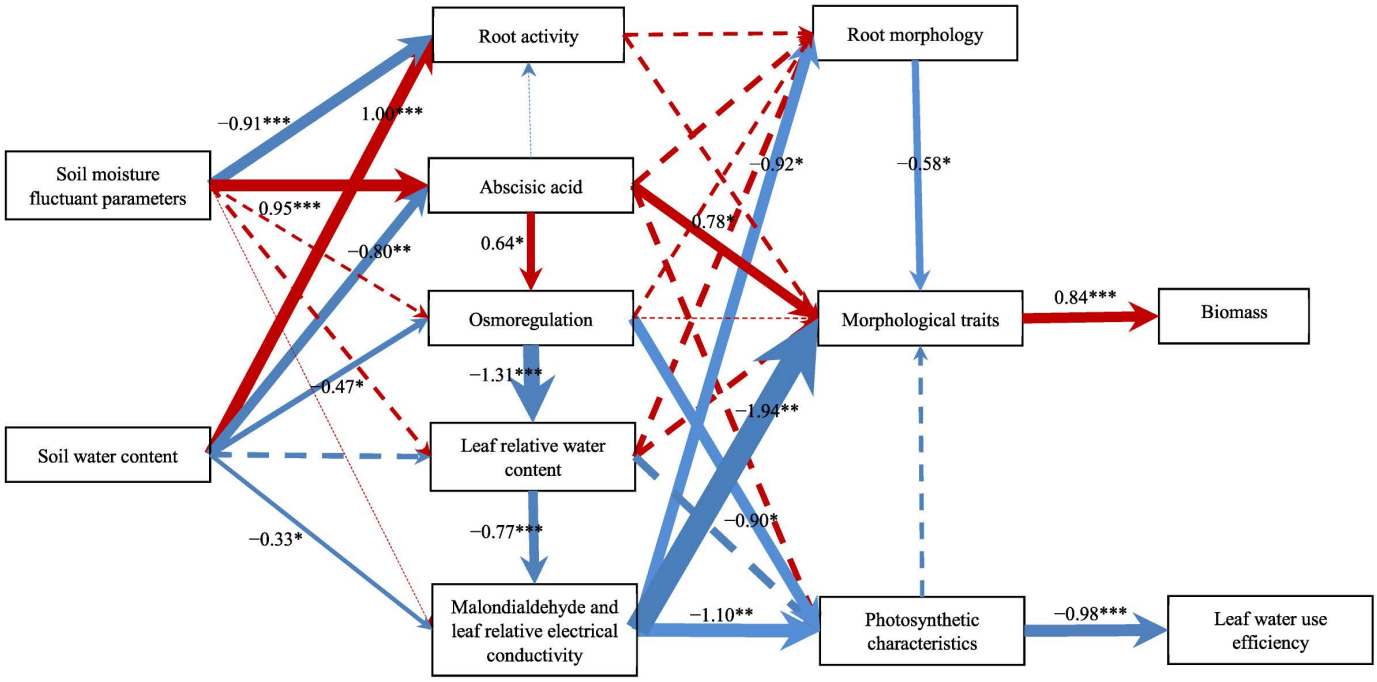

Figure 8. Partial least squares path model (PLS-PM). The path coefficients are represented by the width of the arrows. The red color indicates a positive effect, the blue color indicates a negative effect, the solid arrows indicate significant effects (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$), and the dashed arrows indicate nonsignificant path coefficients ($p > 0.05$). A goodness-of-fit (GOF) statistical evaluation model was used, and the GOF of this model was 0.89. The data used in the PLS-PM were the data from 21 days after treatment.

4. Discussion

4.1. Stable Soil Moisture Improves Plant Morphogenesis of Tomato Seedlings

Morphological indicators, such as plant height, stem diameter, and the number of leaves, are important dynamic biomarkers in the process of crop growth and development, and they are closely related to crop biomass and yield [45]. Previous studies have only centered on the effects of SWC on plant growth, development, and water use [3–5]. Our results, highlight the importance of soil moisture stability during plant growth. We found that SM (versus FM) under low-SWC conditions, significantly improved tomato plant height, stem diameter, leaf number, leaf length, root surface area, and root activity; and SM under high-SWC conditions significantly improved tomato stem diameter and root activity (Figure 4, Table 3). The results supported our first hypothesis that SM could improve the plant morphogenesis of tomato seedlings, thereby laying a good foundation for the later growth and fruit formation of tomato plants [16]. This is similar to a study on maize [31], which found that SM encouraged the formation of crop morphological traits, and our results further showed that the promotion of plant morphogenesis, in SM compared with FM, was subjected to SWC.

4.2. Stable Soil Moisture Alleviates Soil Water Stress

Drought stress has been found to interfere with crop maintenance of cellular stress, resulting in a reduction in the LRWC in maize, and the severity of its effects has increased over time [46]. Liu [23] found that two parameters, the LRWC and leaf water potential, significantly decreased only under severe soil water stress. We found that the tomato LRWC was significantly lower in low-SWC conditions than in high-SWC conditions; therefore, we infer from this result that, regardless of SM or FM, the lower SWC always caused tomato seedlings to experience water stress. Additionally, the tomato LRWC in FM was significantly lower than that in SM under similar SWC conditions, suggesting that regardless of

low or high SWC levels, FM always caused tomato seedlings to experience water stress (Table 2).

Osmoregulation, through the over-accumulation of free proline, soluble protein, and soluble sugar, is a defense mechanism employed by plants to enhance their drought stress tolerance [47–49]. Plants under water stress accumulate free proline and soluble sugars to regulate their osmotic potential, thus improving their growth characteristics and tolerance to water deficit [50]. Sánchez [51] showed that plants accumulated more proline and lower water content during turgor loss. Previous studies have shown that 60–80% FC is optimal for the growth of the majority of greenhouse crops [14]. Experiments performed on tomato plants have reported that 72–80% FC was suitable for plant growth, whereas 54–60% FC indicated a water shortage [17]. According to previous studies, the low water content (W1: 65–68% FC) and high water content (W2: 73–76% FC) used in the present study during the test period would not cause water stress in tomato plants [14,17]. However, our results showed that, compared to SM, FM caused tomato seedlings to suffer from water stress, especially in W1 treatment (Figure 6), indicating that the fluctuation of soil water led to water stress in tomato plants, and tomato plants seemed to be more sensitive to this change under low-SWC conditions.

According to previous studies, the absence of water stress in maize under SM reduced the content of free proline, soluble proteins, and soluble sugars in leaves and roots, compared to the alternation of wetting and drying treatments, which promoted leaf growth and thereby increased maize yields [8,12]. Similarly, Niu [13] found that SM decreased the accumulation of osmotic regulating substances and membrane lipid peroxidation products in maize compared to manual irrigation treatments. We found that tomato plants grown in FM accumulated more proline, soluble proteins, and soluble sugars than those grown in SM (Figure 6A–C), indicating that SM could prevent or alleviate plant water stress caused by the instability of soil moisture, thereby reducing the accumulation of osmotic substances and improving plant performance under similar SWC conditions.

Furthermore, data supported the idea that different organs of tomato plants showed different osmotic responses to soil moisture. Unlike soluble protein and soluble sugar, the proline in all organs was significantly lower in SM than in FM (Figure 6A–C), which suggested that proline might be the most sensitive osmotic substance in tomato plants in response to soil moisture fluctuations [52]. Additionally, the PCA plot showed that there were positive correlations between proline, soluble protein, and soluble sugar, and these vectors strongly contributed to the clustering of the low-SWC treatments (Figure 7), which was similar to the findings of Hessini [48] and Ferchichi [49].

Water stress could lead to the overproduction of malondialdehyde, which causes cell membrane damage and, ultimately, plant cell death. An increase in the malondialdehyde content is a common response of stressed plants [20,21]. We found that malondialdehyde levels were considerably lower in SMW2 tomato roots, stems, and leaves compared to those in FMW1 (Figure 6D), which suggested that SM decreased malondialdehyde levels compared to FM under similar SWC circumstances.

Previous studies [22,53,54] have shown that ABA is an important long-range signal of plants in response to changes in soil moisture and plays a crucial role in regulating stomata and a plant's response to water stress. High endogenous ABA levels improved the osmotic stress resistance of the tomato plants through osmotic and hydraulic regulation, and the plants usually presented higher leaf gas exchange when endogenous ABA levels were low. In our study, tomato plants grown in FM with a high ABA content (Figure 6E) accumulated a relatively large amount of osmotic substances (Figure 6A–C), and the tomato plants grown in SM with a low ABA content (Figure 6E) exhibited better photosynthetic performance (Figure 5); these results suggested that FM induced more ABA and osmotic substances than SM, which also supported our second hypothesis that SM improved the WUE by regulating the ABA and osmotic substance contents in plants.

4.3. Stable Soil Moisture Improves WUE of Tomato

Stomatal closure and reduced biochemical photosynthetic capacity are normal plant responses to water deficits [55]. According to Verma [56] and Ortega-Farias [57], water deficits significantly reduce the leaf gas exchange of plants. Similarly, we found that the Photo, Cond, and Trmmol of tomato plants significantly decreased with decreasing SWC, regardless of being in the SM or FM condition (Figure 5), thus indicating that tomato plants were sensitive to the SWC level and that even a small reduction in water content could affect tomato growth. Wang reported that stable water conditions increased the Photo of lettuce and maize [31]. Importantly, it has been found that, under NPI, cellular components, such as chloroplasts, plastids, and thylakoids—which are directly related to maize photosynthesis—significantly changed, and dry matter accumulation increased with increasing maize Photo [12]. Consistent with previous studies [12,31], our results showed that SM could significantly increase tomato Photo compared with FM. In addition, we found that SM, versus FM, resulted in an increase in the WUE_{ins} of tomato leaves due to nonlinear increases in Trmmol and Photo (Figure 5), which was consistent with previous findings on continuous irrigation and conventional irrigation [30]. Moreover, the PCA plot showed a positive correlation between the leaf WUE and ABA, and it showed a negative correlation between the leaf WUE and Cond (Figure 7), consistent with the results reported by Sun [30].

According to our PLS-PM (Figure 8), soil moisture stability affected tomato photosynthetic characteristics by affecting ABA and osmoregulation, which in turn affected the leaf WUE, solidifying our second hypothesis that SM improved WUE by regulating ABA and osmotic substance contents in plants. Notably, the total impact value of soil moisture stability on the tomato leaf WUE was 0.47. In addition, PLS-PM showed that the direct impact path between ABA and photosynthetic characteristics was not significant. Despite the fact that ABA was an important signal of the tomato response to soil moisture change, ABA alone was not sufficient, in our study, to regulate photosynthetic characteristics because ABA was easily affected by the interaction of electrical signals and other factors [54]. Although we outlined different regulatory pathways of the plants' responses to changing soil moisture, this was only a possible interpretation, and these pathways may or may not exist; thus, this needs to be verified by future studies.

5. Conclusions

This study showed that (i) soil moisture stability significantly influenced the growth and development, photosynthetic characteristics, physiological response, morphological traits, and root morphology of tomato plants, with SM being more conducive for most of these indices. (ii) SM improved the leaf WUE by regulating the abscisic acid content in plants, reducing plant osmotic substances, maintaining a high gas exchange rate, and improving plant morphology. (iii) SM could alleviate water stress on tomato seedlings; even if the SWC of SM was equal to or lower than the lower SWC of FM, water stress would not occur under SM, whereas it would occur under FM. Overall, our study showed that SM could offset the negative effects of insufficient soil moisture compared with FM and help tomato plants alleviate water stress. SM has great potential in promoting plant growth and WUE.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030391/s1, Figure S1. Daily changes in temperature, humidity, and the evaporation of the surface water during the test period. Table S1. Eigenvalues and variances of principal component analysis.

Author Contributions: Conceptualization, G.L. and H.L.; data curation, R.Z.; investigation, A.X.; writing—original draft, G.L.; writing—review & editing, H.L., R.Z., P.J.D., A.X. and L.N. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

Conflicts of Interest: The authors declare no competing interests.

Nomenclature

Abbreviation	Full Name
ABA	Abscisic acid
Ci	Intercellular CO_2 concentration
Cond	Stomatal conductance
DW	Dry weight
FC	Field capacity
FM	Fluctuating soil moisture
FW	Fresh weight
LREC	Leaf relative electrical conductivity
LRWC	Leaf relative water content
NPI	Negative pressure irrigation
PCA	Principal component analysis
Photo	Net photosynthetic rate
PLS-PM	Partial least squares path
SM	Stable soil moisture
SWC	Soil water content
Trmmol	Transpiration rate
WUE	Water use efficiency
WUE_{ins}	Instantaneous water use efficiency
WUE_{int}	Intrinsic water use efficiency
δ	Fluctuation coefficient

References

1. Farooq, M.; Hussain, M.; Ul-Allah, S.; Siddique, K.H.M. Physiological and Agronomic Approaches for Improving Water-Use Efficiency in Crop Plants. *Agric. Water Manag.* **2019**, *219*, 95–108. [CrossRef]
2. Mega, R.; Abe, F.; Kim, J.-S.; Tsuboi, Y.; Tanaka, K.; Kobayashi, H.; Sakata, Y.; Hanada, K.; Tsujimoto, H.; Kikuchi, J.; et al. Tuning Water-Use Efficiency and Drought Tolerance in Wheat Using Abscisic Acid Receptors. *Nat. Plants* **2019**, *5*, 153–159. [CrossRef]
3. Islam, M.R.; Hu, Y.; Mao, S.; Mao, J.; Eneji, A.E.; Xue, X. Effectiveness of a Water-Saving Super-Absorbent Polymer in Soil Water Conservation for Corn (*Zea mays* L.) Based on Eco-Physiological Parameters. *J. Sci. Food Agric.* **2011**, *91*, 1998–2005. [CrossRef]
4. Khapte, P.S.; Kumar, P.; Burman, U.; Kumar, P. Deficit Irrigation in Tomato: Agronomical and Physio-Biochemical Implications. *Sci. Hortic.* **2019**, *248*, 256–264. [CrossRef]
5. Nangare, D.D.; Singh, Y.; Kumar, P.S.; Minhas, P.S. Growth, Fruit Yield and Quality of Tomato (*Lycopersicon esculentum* Mill.) as Affected by Deficit Irrigation Regulated on Phenological Basis. *Agric. Water Manag.* **2016**, *171*, 73–79. [CrossRef]
6. Yang, P.; Bian, Y.; Long, H.; Drohan, P.J. Comparison of Emitters of Ceramic Tube and Polyvinyl Formal under Negative Pressure Irrigation on Soil Water Use Efficiency and Nutrient Uptake of Crown Daisy. *Agric. Water Manag.* **2020**, *228*, 105830. [CrossRef]
7. Zhang, Z.; Liu, S.; Jia, S.; Du, F.; Qi, H.; Li, J.; Song, X.; Zhao, N.; Nie, L.; Fan, F. Precise Soil Water Control Using a Negative Pressure Irrigation System to Improve the Water Productivity of Greenhouse Watermelon. *Agric. Water Manag.* **2021**, *258*, 107144. [CrossRef]
8. Zhang, J.; Ji, J.; Wang, P.; Long, H.; Wu, X. Molecular Mechanism of Negative Pressure Irrigation Inhibiting Root Growth and Improving Water Use Efficiency in Maize. *Plant Soil* **2022**, *472*, 127–143. [CrossRef]
9. Li, S.; Tan, D.; Wu, X.; Degré, A.; Long, H.; Zhang, S.; Lu, J.; Gao, L.; Zheng, F.; Liu, X.; et al. Negative Pressure Irrigation Increases Vegetable Water Productivity and Nitrogen Use Efficiency by Improving Soil Water and NO3—N Distributions. *Agric. Water Manag.* **2021**, *251*, 106853. [CrossRef]
10. Gao, X.; Zhang, S.; Song, Y.; Huaiyu, L. Negative Pressure Irrigation System Reduces Soil Nitrogen Loss for Lettuce during Greenhouse Production. *Agronomy* **2021**, *11*, 2380. [CrossRef]
11. Yang, P.; Drohan, P.J.; Long, H.; Bian, Y.; Bryant, R.B. Negative Pressure Irrigation on Water Use Efficiency, Yield and Quality of *Brassica chinensis* L. *J. Sci. Food Agric.* **2021**, *102*, 1508–1513. [CrossRef] [PubMed]
12. Zhang, J.; Wang, P.; Ji, J.; Long, H.; Wu, X. Transcriptome Analysis Reveals the Molecular Mechanism of Yield Increases in Maize under Stable Soil Water Supply. *PLoS ONE* **2021**, *16*, e0257756. [CrossRef] [PubMed]
13. Niu, L.; Wang, Z.; Zhu, G.; Yu, K.; Li, G.; Long, H. Stable Soil Moisture Improves the Water Use Efficiency of Maize by Alleviating Short-Term Soil Water Stress. *Front. Plant Sci.* **2022**, *13*, 833041. [CrossRef] [PubMed]

14. Yang, P.; Bai, J.; Yang, M.; Ma, E.; Yan, M.; Long, H.; Liu, J.; Li, L. Negative Pressure Irrigation for Greenhouse Crops in China: A Review. *Agric. Water Manag.* **2022**, *264*, 107497. [CrossRef]
15. Li, F.; Li, Y.; Li, S.; Wu, G.; Niu, X.; Shen, A. Green Light Promotes Healing and Root Regeneration in Double-Root-Cutting Grafted Tomato Seedlings. *Sci. Hortic.* **2021**, *289*, 110503. [CrossRef]
16. Zhou, T.; Wu, Z.; Wang, Y.; Su, X.; Qin, C.; Huo, H.; Jiang, F. Modelling Seedling Development Using Thermal Effectiveness and Photosynthetically Active Radiation. *J. Integr. Agric.* **2019**, *18*, 2521–2533. [CrossRef]
17. Qu, Z.; Qi, X.; Liu, Y.; Liu, K.; Li, C. Interactive Effect of Irrigation and Polymer-Coated Potassium Chloride on Tomato Production in a Greenhouse. *Agric. Water Manag.* **2020**, *235*, 106149. [CrossRef]
18. Pazzagli, P.T.; Weiner, J.; Liu, F. Effects of CO_2 Elevation and Irrigation Regimes on Leaf Gas Exchange, Plant Water Relations, and Water Use Efficiency of Two Tomato Cultivars. *Agric. Water Manag.* **2016**, *169*, 26–33. [CrossRef]
19. Wahab, A.; Abdi, G.; Saleem, M.H.; Ali, B.; Ullah, S.; Shah, W.; Mumtaz, S.; Yasin, G.; Muresan, C.C.; Marc, R.A. Plants' Physio-Biochemical and Phyto-Hormonal Responses to Alleviate the Adverse Effects of Drought Stress: A Comprehensive Review. *Plants* **2022**, *11*, 1620. [CrossRef]
20. Abbasi, S.; Sadeghi, A.; Safaie, N. Streptomyces Alleviate Drought Stress in Tomato Plants and Modulate the Expression of Transcription Factors ERF1 and WRKY70 Genes. *Sci. Hortic.* **2020**, *265*, 109206. [CrossRef]
21. Jan, A.U.; Hadi, F.; Ditta, A.; Suleman, M.; Ullah, M. Zinc-Induced Anti-Oxidative Defense and Osmotic Adjustments to Enhance Drought Stress Tolerance in Sunflower (*Helianthus annuus* L.). *Environ. Exp. Bot.* **2022**, *193*, 104682. [CrossRef]
22. Yang, X.; Bornø, M.L.; Wei, Z.; Liu, F. Combined Effect of Partial Root Drying and Elevated Atmospheric CO_2 on the Physiology and Fruit Quality of Two Genotypes of Tomato Plants with Contrasting Endogenous ABA Levels. *Agric. Water Manag.* **2021**, *254*, 106987. [CrossRef]
23. Liu, F.; Jensen, C.R.; Shahanzari, A.; Andersen, M.N.; Jacobsen, S.-E. ABA Regulated Stomatal Control and Photosynthetic Water Use Efficiency of Potato (*Solanum tuberosum* L.) during Progressive Soil Drying. *Plant Sci.* **2005**, *168*, 831–836. [CrossRef]
24. Pérez-Pérez, J.G.; Navarro, J.M.; Robles, J.M.; Dodd, I.C. Prolonged Drying Cycles Stimulate ABA Accumulation in Citrus Macrophylla Seedlings Exposed to Partial Rootzone Drying. *Agric. Water Manag.* **2018**, *210*, 271–278. [CrossRef]
25. Mehmood, F.; Wang, G.; Gao, Y.; Liang, Y.; Chen, J.; Si, Z.; Ramatshaba, T.S.; Zain, M.; Shafeeq-ur-rahman; Duan, A. Nitrous Oxide Emission from Winter Wheat Field as Responded to Irrigation Scheduling and Irrigation Methods in the North China Plain. *Agric. Water Manag.* **2019**, *222*, 367–374. [CrossRef]
26. Siakou, M.; Bruggeman, A.; Eliades, M.; Zoumides, C.; Djuma, H.; Kyriacou, M.C.; Emmanouilidou, M.G.; Spyros, A.; Manolopoulou, E.; Moriana, A. Effects of Deficit Irrigation on 'Koroneiki' Olive Tree Growth, Physiology and Olive Oil Quality at Different Harvest Dates. *Agric. Water Manag.* **2021**, *258*, 107200. [CrossRef]
27. Mazarei, R.; Soltani Mohammadi, A.; Ebrahimian, H.; Naseri, A.A. Temporal Variability of Infiltration and Roughness Coefficients and Furrow Irrigation Performance under Different Inflow Rates. *Agric. Water Manag.* **2021**, *245*, 106465. [CrossRef]
28. North, G.B.; Nobel, P.S. Changes in Hydraulic Conductivity and Anatomy Caused by Drying and Rewetting Roots of Agave Deserti (Agavaceae). *Am. J. Bot.* **1991**, *78*, 906–915. [CrossRef]
29. Salah, A.; Nwafor, C.C.; Han, Y.; Liu, L.; Rashid, M.; Batool, M.; El-Badri, A.M.; Cao, C.; Zhan, M. Spermidine and Brassinosteroid Regulate Root Anatomical Structure, Photosynthetic Traits and Antioxidant Defense Systems to Alleviate Waterlogging Stress in Maize Seedlings. *S. Afr. J. Bot.* **2022**, *144*, 389–402. [CrossRef]
30. Sun, Q.; Wang, Y.; Chen, G.; Yang, H.; Du, T. Water Use Efficiency Was Improved at Leaf and Yield Levels of Tomato Plants by Continuous Irrigation Using Semipermeable Membrane. *Agric. Water Manag.* **2018**, *203*, 430–437. [CrossRef]
31. Wang, Z. *Effects of Soil Moisture Temporal Variation on Growth, Physiology and Water Use Efficiency of Maize and Lettuces*; Chinese Academy of Agricultural Sciences: Beijing, China, 2020.
32. Li, G.; Bai, Y.; Wang, L.; Lu, Y.; Zhang, J.; Zhang, Y. Effects of Fertilizer under Different Dripline Spacings on Summer Maize in Northern China. *Sci. Rep.* **2021**, *11*, 18922. [CrossRef]
33. Challabathula, D.; Analin, B.; Mohanan, A.; Bakka, K. Differential Modulation of Photosynthesis, ROS and Antioxidant Enzyme Activities in Stress-Sensitive and -Tolerant Rice Cultivars during Salinity and Drought upon Restriction of COX and AOX Pathways of Mitochondrial Oxidative Electron Transport. *J. Plant Physiol.* **2022**, *268*, 153583. [CrossRef]
34. Altaf, M.A.; Shahid, R.; Ren, M.-X.; Altaf, M.M.; Khan, L.U.; Shahid, S.; Jahan, M.S. Melatonin Alleviates Salt Damage in Tomato Seedling: A Root Architecture System, Photosynthetic Capacity, Ion Homeostasis, and Antioxidant Enzymes Analysis. *Sci. Hortic.* **2021**, *285*, 110145. [CrossRef]
35. Jabborova, D.; Ma, H.; Bellingrath-Kimura, S.D.; Wirth, S. Impacts of Biochar on Basil (Ocimum Basilicum) Growth, Root Morphological Traits, Plant Biochemical and Physiological Properties and Soil Enzymatic Activities. *Sci. Hortic.* **2021**, *290*, 110518. [CrossRef]
36. Wang, B.; Li, P.-P.; Huang, C.-H.; Liu, G.-B.; Yang, Y.-F. Effects of Root Morphological Traits on Soil Detachment for Ten Herbaceous Species in the Loess Plateau. *Sci. Total Environ.* **2021**, *754*, 142304. [CrossRef]
37. Shu, L.-Z.; Liu, R.; Min, W.; Wang, Y.; Hong-mei, Y.; Zhu, P.; Zhu, J. Regulation of Soil Water Threshold on Tomato Plant Growth and Fruit Quality under Alternate Partial Root-Zone Drip Irrigation. *Agric. Water Manag.* **2020**, *238*, 106200. [CrossRef]
38. Gong, X.; Qiu, R.; Sun, J.; Ge, J.; Li, Y.; Wang, S. Evapotranspiration and Crop Coefficient of Tomato Grown in a Solar Greenhouse under Full and Deficit Irrigation. *Agric. Water Manag.* **2020**, *235*, 106154. [CrossRef]

39. Wellburn, A.R. The Spectral Determination of Chlorophylls a and b, as Well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *J. Plant Physiol.* **1994**, *144*, 307–313. [CrossRef]
40. Liang, Y.; Kang, C.; Kaiser, E.; Kuang, Y.; Yang, Q.; Li, T. Red/Blue Light Ratios Induce Morphology and Physiology Alterations Differently in Cucumber and Tomato. *Sci. Hortic.* **2021**, *281*, 109995. [CrossRef]
41. Guo, L.; Bornø, M.L.; Niu, W.; Liu, F. Biochar Amendment Improves Shoot Biomass of Tomato Seedlings and Sustains Water Relations and Leaf Gas Exchange Rates under Different Irrigation and Nitrogen Regimes. *Agric. Water Manag.* **2021**, *245*, 106580. [CrossRef]
42. Sanchez, G.; Trinchera, L.; Russolollo, G. Plspm: Tools for Partial Least Squares Path Modeling (PLS-PM). *R Packag. Version 0.4.9*. 2015. Available online: https://rdrr.io/cran/plspm/ (accessed on 12 October 2022).
43. Chen, J.; Song, D.; Luan, H.; Liu, D.; Wang, X.; Sun, J.; Gonzalo, M. Living and Dead Microorganisms in Mediating Soil Carbon Stocks under Long-Term Fertilization in a Rice-Wheat Rotation Study Site and Soil Sampling. *Front. Microbiol.* **2022**, *13*, 854216. [CrossRef] [PubMed]
44. Zhou, G.; Gao, S.; Lu, Y.; Liao, Y.; Nie, J.; Cao, W. Co-Incorporation of Green Manure and Rice Straw Improves Rice Production, Soil Chemical, Biochemical and Microbiological Properties in a Typical Paddy Field in Southern China. *Soil Tillage Res.* **2020**, *197*, 104499. [CrossRef]
45. Jiang, T.; Liu, J.; Gao, Y.; Sun, Z.; Chen, S.; Yao, N.; Ma, H.; Feng, H.; Yu, Q.; He, J. Simulation of Plant Height of Winter Wheat under Soil Water Stress Using Modified Growth Functions. *Agric. Water Manag.* **2020**, *232*, 106066. [CrossRef]
46. Li, Z.; Su, X.; Chen, Y.; Fan, X.; He, L.; Guo, J.; Wang, Y.; Yang, Q. Melatonin Improves Drought Resistance in Maize Seedlings by Enhancing the Antioxidant System and Regulating Abscisic Acid Metabolism to Maintain Stomatal Opening under PEG-Induced Drought. *J. Plant Biol.* **2021**, *64*, 299–312. [CrossRef]
47. Wu, S.; Hu, C.; Tan, Q.; Nie, Z.; Sun, X. Effects of Molybdenum on Water Utilization, Antioxidative Defense System and Osmotic-Adjustment Ability in Winter Wheat (Triticum Aestivum) under Drought Stress. *Plant Physiol. Biochem.* **2014**, *83*, 365–374. [CrossRef]
48. Hessini, K.; Martínez, J.P.; Gandour, M.; Albouchi, A.; Soltani, A.; Abdelly, C. Effect of Water Stress on Growth, Osmotic Adjustment, Cell Wall Elasticity and Water-Use Efficiency in Spartina Alterniflora. *Environ. Exp. Bot.* **2009**, *67*, 312–319. [CrossRef]
49. Ferchichi, S.; Hessini, K.; Dell'Aversana, E.; D'Amelia, L.; Woodrow, P.; Ciarmiello, L.; Fuggi, A.; Carillo, P. Hordeum Vulgare and Hordeum Maritimum Respond to Extended Salinity Stress Displaying Different Temporal Accumulation Pattern of Metabolites. *Funct. Plant Biol.* **2018**, *45*, 1096–1109. [CrossRef]
50. Yooyongwech, S.; Samphumphuang, T.; Tisarum, R.; Theerawitaya, C.; Cha-um, S. Arbuscular Mycorrhizal Fungi (AMF) Improved Water Deficit Tolerance in Two Different Sweet Potato Genotypes Involves Osmotic Adjustments via Soluble Sugar and Free Proline. *Sci. Hortic.* **2016**, *198*, 107–117. [CrossRef]
51. Sánchez, F.J.; Manzanares, M.; de Andres, E.F.; Tenorio, J.L.; Ayerbe, L. Turgor Maintenance, Osmotic Adjustment and Soluble Sugar and Proline Accumulation in 49 Pea Cultivars in Response to Water Stress. *Field Crop. Res.* **1998**, *59*, 225–235. [CrossRef]
52. Ghaffari, H.; Tadayon, M.R.; Bahador, M.; Razmjoo, J. Investigation of the Proline Role in Controlling Traits Related to Sugar and Root Yield of Sugar Beet under Water Deficit Conditions. *Agric. Water Manag.* **2021**, *243*, 106448. [CrossRef]
53. Li, S.; Wang, X.; Liu, X.; Thompson, A.J.; Liu, F. Elevated CO_2 and High Endogenous ABA Level Alleviate PEG-Induced Short-Term Osmotic Stress in Tomato Plants. *Environ. Exp. Bot.* **2022**, *194*, 104763. [CrossRef]
54. da Silva, F.B.; Macedo, F.D.C.O.; Daneluzzi, G.S.; Capelin, D.; Silva, A.R.; Müller, C.; de Oliveira, R.F. Action Potential Propagation Effect on Gas Exchange of ABA-Mutant Microtomato after Re-Irrigation Stimulus. *Environ. Exp. Bot.* **2020**, *178*, 104149. [CrossRef]
55. Tezara, W.; Domínguez, T.S.T.; Loyaga, D.W.; Ortiz, R.N.; Chila, V.H.R.; Ortega, M.J.B. Photosynthetic Activity of Oil Palm (Elaeis Guineensis) and Interspecific Hybrid Genotypes (Elaeis Oleifera × Elaeis Guineensis), and Response of Hybrids to Water Deficit. *Sci. Hortic.* **2021**, *287*, 110263. [CrossRef]
56. Verma, K.K.; Song, X.-P.; Zeng, Y.; Guo, D.-J.; Singh, M.; Rajput, V.D.; Malviya, M.K.; Wei, K.-J.; Sharma, A.; Li, D.-P.; et al. Foliar Application of Silicon Boosts Growth, Photosynthetic Leaf Gas Exchange, Antioxidative Response and Resistance to Limited Water Irrigation in Sugarcane (*Saccharum officinarum* L.). *Plant Physiol. Biochem.* **2021**, *166*, 582–592. [CrossRef] [PubMed]
57. Ortega-Farias, S.; Villalobos-Soublett, E.; Riveros-Burgos, C.; Zúñiga, M.; Ahumada-Orellana, L.E. Effect of Irrigation Cut-off Strategies on Yield, Water Productivity and Gas Exchange in a Drip-Irrigated Hazelnut (*Corylus avellana* L. Cv. Tonda Di Giffoni) Orchard under Semiarid Conditions. *Agric. Water Manag.* **2020**, *238*, 106173. [CrossRef]