

Article

Improving Leaf Photosynthetic Performance of Apple through a Novel Root-Zone Irrigation in the Loess Plateau

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Abstract: As freshwater becomes an increasingly scarce and expensive natural resource, novel water-saving irrigation methods for dwarfing apple orchards are needed in the Loess Plateau. However, studies are lacking on the effects of novel root-zone irrigation technology on leaf-level photosynthesis, which directly determines the yield and survival ability of trees. In this study, the leaf gas characteristics and water status of five-year-old dwarfing apple trees in the Loess Plateau of China were monitored during the 2016–2017 growing seasons under water storage pit irrigation (WSPI) and surface irrigation (SI) treatments. Under WSPI, the leaf water potential (Ψ_m), net photosynthesis rate (P_n), stomatal conductance (g_s), and chlorophyll content (Chl) were significantly higher than those under SI (by 12.21–28.36%), while non-photochemical quenching, superoxide dismutase, and sucrose were lower. Compared with SI, WSPI improved dwarfing apple yield by 25.4% and 26.7% in 2016 and 2017, respectively. WSPI increased the photosystem II (PSII) activity by increasing the chlorophyll fluorescence features (the potential quantum yield of PSII (F_v/F_m), actual quantum yield (Φ_{II}), photochemical quenching (q_P), and electron transport rate (ETR)). Principal component analysis showed that Ψ_m , Chl, the chlorophyll fluorescence features (q_P , Φ_{II} , and ETR), P_n , and g_s could represent the leaf photosynthetic difference between WSPI and SI treatments. Results indicated that apple under WSPI could improve its yield through enhancing leaf photosynthetic performance, and water storage pit irrigation is an effective root-zone irrigation method for apple orchards on the Loess Plateau.

Keywords: water storage pit irrigation; soil water distribution; leaf gas exchange; carbon assimilation; dwarfing apple



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1. Introduction

In the main apple regions, there has been an increasing tendency to grow dwarfing apples using high-density planting [1,2], as this cultivation pattern both increases fruit yield and decreases labor costs. Nonetheless, these dwarfing apple orchards in the Loess Plateau of China are characterized by poor irrigation water quality and small and discontinuous land use [3], which has led to high costs associated with equipment and difficulty in operating drip- and micro-irrigation systems in small orchards. For these reasons, surface irrigation (SI), such as border irrigation and flood irrigation, is still the most conventional method [4]. However, the use of SI results in substantial water loss due to evaporation from the soil surface and soil erosion risk increase due to soil compaction [5], which means that it is necessary to develop new water-saving irrigation techniques to improve both water-use efficiency and yields in these small-holding orchards.

Water storage pit irrigation (WSPI) is a novel root-zone irrigation technology, which has been tested in rainfed dwarfing apple orchards on the Loess Plateau [6,7]. WSPI allows irrigation water or rainfall runoff to directly infiltrate into the subsurface soil through special emitters (water storage pits) dug around half of the radius of a tree canopy [8]. To promote the radial movement of soil moisture and to avoid the possibility of deep percolation caused by pit irrigation, each storage pit has an impermeable bottom and an infiltrating pit wall [9].

These strategies combine the advantages of both pit irrigation [10], which is inexpensive and can easily be employed by an orchardist; and subsurface irrigation, which increases the soil water available to the deep root zone [11]. Although WSPI has been applied to improve apple yield and water-use efficiency [12,13], most research has concentrated on the plant–soil interface and the underlying mechanism, such as root growth and soil water distribution [7,14], while little is known about the leaf physiological characteristics under WSPI.

Leaf photosynthesis is the fundamental physiological process that a plant uses for growth and development, and is the ultimate basis of yield formation [15,16]. Irrigation would affect the leaf photosynthetic efficiency of the apple by influencing the soil–plant–atmosphere continuum (SPAC) water potential and root distribution [17–19]. Numerous measurements have been performed to determine the leaf photosynthetic performance for varied apple rootstocks under different irrigation levels and irrigation frequencies [20–23], and most have focused on drip irrigation [24] and partial root-zone drying irrigation [25]. However, few studies have investigated these variations in the dwarfing apple under nonuniform soil water content and root distributions. More studies on the effects of WSPI on leaf photosynthetic characteristics are needed to further clarify the carbon accumulation strategy and formulate reasonable irrigation systems in dwarfing apple orchards.

We previously found that the total fine root length was increased under WSPI, as well as increasing soil water storage in deep soil compared to SI [14,26]. In addition, we found that the depth of root water uptake for apple trees changed from 20 cm to 100 cm under WSPI, while SI mainly used 0–40 cm soil depth of soil water [27]. Deep-rooted plants would influence leaf water status and stomatal regulation [28,29], and then might lead to differences in the photosynthetic rate of apple trees. However, there are few reports on the leaf photosynthetic response to WSPI. Previous studies have shown that the leaf gas exchange characteristics in young or mature apple trees varied for WSPI at different pit depths (20 cm, 40 cm, and 60 cm) and soil water content limits, but it could not indicate whether there was a difference between WSPI and SI. To provide further insight into the relationship between net photosynthetic rate variation and leaf water status under WSPI compared to SI, a two-year field study (2016–2017) was conducted in a dwarfing apple orchard in the Loess Plateau of China. Leaf water potential, soil water content, and stomatal and nonstomatal parameters were measured to determine the differences in carbon accumulation between WSPI and SI. It was hypothesized that WSPI would improve leaf photosynthesis, resulting in an increased apple yield as compared to SI. This study aims to contribute to the scientific basis for the accelerated generalization and adaptation of irrigation theory and technologies under WSPI in the Loess Plateau.

2. Materials and Methods

2.1. Study Site

Experiments were conducted in the dwarfing apple interstock orchard at the Shanxi Academy of Agricultural Sciences, located in Taigu County, Jinzhong City, in the Shanxi Province (37°23' N, 112°32' E, 781.9 m elevation). The site has a semiarid climate with a mean annual air temperature of approximately 9.8 °C and a mean annual rainfall of 460 mm. The soil texture of 0–100 cm depth is mainly silt loam; the average soil bulk density is 1.47 g cm⁻³. The soil moisture at the field capacity is 0.30 cm³ cm⁻³, and at the saturated water content is 50%. Air temperature, rainfall, and reference evapotranspiration (ET₀, calculated using the Hargreaves equation) over the course of the experiment from April to October in 2016 and 2017 are shown in Figure 1. Table 1 is the main meteorological factors in the experiment zone.

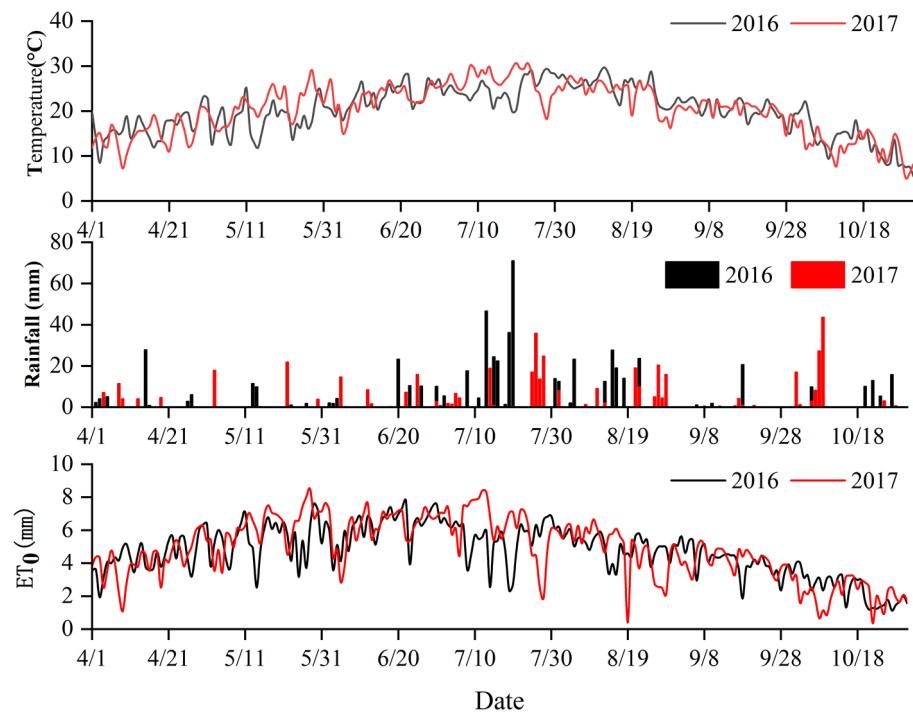


Figure 1. Air temperature, rainfall, and reference evapotranspiration (ET_0) in the growing seasons of apple in 2016 and 2017.

Table 1. Monthly average solar radiation (R_s), mean relative humidity (RH), mean atmosphere temperature (T_a), mean vapor pressure deficit (VPD), total precipitation (P), total evapotranspiration (ET_0), and irrigation amount (I) in 2016 and 2017.

Year	Month	R_s ($W\ m^{-1}$)	RH (%)	T_a ($^{\circ}C$)	P (mm)	VPD (kPa)	ET_0 (mm)	I (mm)
2016	April	239.55	55.52	16.26	48.6	0.79	130.78	45
	May	234.67	59.85	18.17	24.0	0.81	168.79	
	June	265.69	73.35	23.19	76.0	0.71	187.17	45
	July	233.92	87.91	25.05	262.8	0.35	170.89	
	August	240.54	87.64	25.24	134.2	0.35	155.29	
	September	224.33	82.37	20.18	24.2	0.39	123.81	45
	October	155.75	83.39	12.95	58.5	0.21	74.19	
2017	April	237.13	52.63	14.69	31	0.80	124.57	45
	May	258.58	49.91	21.25	43.6	1.18	191.65	45
	June	241.54	72.87	23.07	51.6	0.72	187.47	45
	July	228.24	79.65	26.75	125.4	0.67	194.26	45
	August	214.65	88.24	23.91	95	0.33	151.73	45
	September	199.51	81.01	20.29	6.6	0.42	125.15	
	October	106.32	91.23	12.08	104.2	0.12	65.83	

2.2. Experimental Design

Five-year-old ‘Red Fuji’ (*Malus domestica* Borkh.) apple trees were scion-grafted onto an SH interstock and crabapple (*Malus robusta* Rehd) rootstock, then transplanted with a row spacing of 4 m and a plant spacing of 2 m, considering future tree growth and diameter. In April 2016, two irrigation treatments, WSPI (pit diameter 30cm and depth 40 cm, Figure 2) and SI (traditional surface border irrigation, equipped with PE pipe), were randomly applied across the middle of the experimental site ($100 \times 12\ m^2$), with six replicate trees per treatment. The irrigation date and amount were the same for both treatments. According to local rainfall and farmer’s irrigation habits [27], irrigation was

performed on 21 April, 1 June, and 6 September in 2016 and on 19 April, 17 May, 19 June, 19 July, and 16 August in 2017, respectively (Table 1). The total irrigation amount was 135 mm and 225 mm in 2016 and 2017, respectively, which accounted for 61% and 64% of the calculated crop evapotranspiration (ET_c). The crop coefficient (K_c) for apple trees and ET_c were calculated according to the methodology described by Jaouhari et al. [30]. Each year, the trees were fertilized with 600 kg ha⁻¹ of urea, 150 kg ha⁻¹ of superphosphate and 100 kg ha⁻¹ of potassium sulphate [13]. Other management options such as pruning branches and weeding were kept constant throughout the experiment.

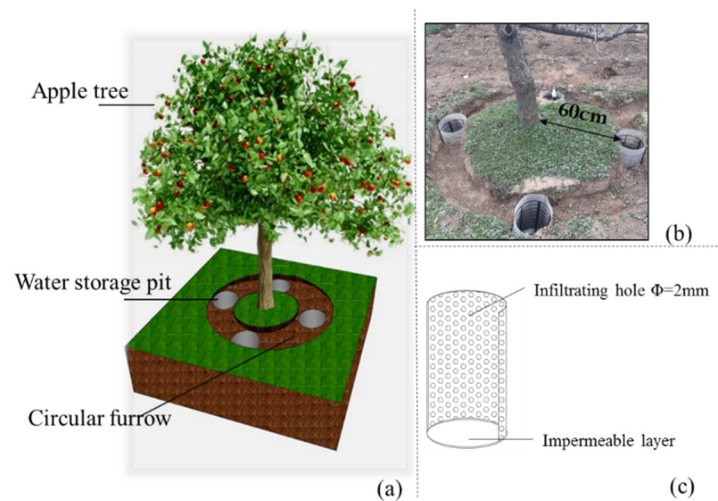


Figure 2. Diagram of water storage pit irrigation (WSPI) systems. (a) Field three-dimensional schematic. (b) Actual location of water storage pit arrangement, and the pit edge was 60 cm from the trunk. (c) Water storage pit.

2.3. Data Collection

2.3.1. Gas Exchange Parameters, Leaf Water Potential, and Soil Water Content

For one sunny day of each month in 2016 and 2017, during the growth stage of the apple trees, three fully expanded and sunlight-exposed leaves in the middle of new shoots were sampled randomly for gas exchange parameters from each tree (three randomly selected trees, $n = 9$). Between 9:00 and 11:00 am, the net photosynthesis rate (P_n), stomatal conductance (g_s), intercellular CO₂ concentration (C_i), and transpiration rate (Tr) were measured using a LI-COR 6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) with a 6400-02B LED source. Water-use efficiency (WUE) was calculated as P_n divided by Tr. The photosynthetic photon flux was set at 1500 μmol m⁻² s⁻¹, leaf temperature was 25 ± 2 °C, and relative humidity was 65 ± 5%.

On the same dates, predawn leaf water potential (Ψ_{PL}) and midday leaf water potential (Ψ_M) were measured using a chilled-mirror dewpoint potentiometer (WP4C, Decagon Devices, Inc., Pullman, WA, USA). After measuring P_n, the same leaves were sampled between 11:30 am and 1:00 pm, detached without the veins, and stored in a sealed chamber. The chamber temperature was set as to 25 °C, on fast mode. Weekly, both before and after irrigation, soil water content was measured using a time-domain reflectometry with intelligent microelements (TRIME, IMKO, Ettlingen, Germany). The measurements were performed in 20 cm increments at depths of 0–100 cm. The sites of water determination were installed at a distance of 50 cm from each tree trunk under the SI treatment and through the center of two adjacent pits under the WSPI treatment.

2.3.2. Leaf Chlorophyll Content and Chlorophyll Fluorescence Features

For each month of 2017, leaf chlorophyll (Chl) was measured according to Porra et al. [31]. Undamaged and fully expanded leaves were sampled between 9:00 am and 11:00 pm. A total of 0.2 g of fresh leaf mass was homogenized, filtered, and diluted with

80% acetone. The absorbance of the extract was measured at 645 and 664 nm using a spectrophotometer. Chlorophyll content was calculated according to the following formula:

$$\text{Chla} = 12.72 \times D_{663} \times 2.59 \times D_{645} \quad (1)$$

$$\text{Chlb} = 22.88 \times D_{645} \times 4.67 \times D_{663} \quad (2)$$

$$\text{Chl} = \text{Chla} + \text{Chlb} \quad (3)$$

During the same period, mature leaves from the upper part of three trees per treatment were selected to measure chlorophyll fluorescence. Maximum (F_m) and minimum (F_0) fluorescence yields of dark-adapted leaves were recorded in the predawn hours (between 5:00 and 6:00 am), using a LI-COR 6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) with an integrated leaf chamber fluorimeter (Li-6400-40, LI-COR Inc.). The maximum light-adapted fluorescence (F_m') and steady-state light-adapted fluorescence (F_s') were assessed diurnally. Minimal fluorescence under light conditions (F_0') was calculated using the following:

$$F_0' = F_0 / (F_v / F_m + F_0 / F_m') \quad (4)$$

The potential quantum yield of PSII (F_v / F_m) was estimated, where F_v is the maximum variable fluorescence ($F_v = F_m - F_0$). Other data-derived parameters included the actual quantum yield, $\Phi_{II} = (F_m' - F_s) / F_m'$; photochemical quenching, $q_P = (F_m' - F_s) / (F_m' - F_0')$; non-photochemical quenching, $NPQ = (F_m - F_m') / F_m'$, and electron transport rate, $ETR = \Phi_{II} \times 0.5 \times \text{PPFD} \times 0.85$.

2.3.3. Antioxidant Enzyme and Soluble Sugar Content

Simultaneously, 15 undamaged leaves from each tree were collected at midday from both WSPI and SI treatments (three replicates for each treatment). The midrib was removed from the leaves, before they were immediately frozen in liquid nitrogen, lyophilized, and kept at -80°C until analysis. For sucrose and fructose assays, 0.5 g of leaf dry mass was ground to a fine powder with a mortar and pestle, and extraction was performed three times with 10 mL 80% ethanol in a water bath at 80°C for 10 min, followed by centrifugation at $4000 \times g$. The pooled supernatants were extracted, combined, and allowed to evaporate until dry at 100°C , at which point 5 mL of distilled water was added and the solution was filtered ($0.45 \mu\text{m}$). The content of sucrose and fructose was analyzed using resorcinol colorimetric assay [32].

To measure the amount of the antioxidant enzyme, superoxide dismutase (SOD), 5 g frozen leaf samples were ground to a fine powder in liquid nitrogen and homogenized on ice in 50 mM phosphate buffer (pH 7). The homogenate was centrifuged at 4°C and $12,000 \times g$ for 30 min, to obtain the enzyme extract as the supernatant. A 3 mL total reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM nitro blue tetrazolium (NBT), 0.1 mM EDTA, 2 μM riboflavin, and 0–200 μL enzyme extract. SOD activity was measured on the basis of its ability to inhibit the photochemical reduction in NBT in the presence of riboflavin in 560 nm light [33]. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of NBT photoreduction at 25°C .

2.4. Data Analysis

Analysis of variance (ANOVA) and correlation analysis were performed using the software package SPSS (IBM SPSS Statistics 26, SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) test was used to compare means, and differences were considered significant at a level of $p < 0.05$. Principal component analysis (PCA) was used to analyze the best linear combination of variables (including leaf water potential, gas exchange parameters, chlorophyll content, chlorophyll fluorescence features, SOD, sucrose

and fructose, total 17 traits in 2017, and $n = 204$), which would account for the greatest total variance in the data.

3. Results

3.1. Soil Water Content and Leaf Water Potential

In terms of the 0–100 cm profile, the peak area of soil moisture content under SI was concentrated in 40 cm, while the high-value area under WSPI was basically located at 60–80 cm (Figure 3). In 2016 and 2017, the mean soil moisture content of 0–20 cm under SI was 21.2% and 20.29%, which were 1.12 and 1.13 times higher than those under WSPI, respectively. However, WSPI significantly increased the water content of the 40–80 cm soil layer ($p = 0.0221$) and kept the soil water content at approximately 23% (76% of field water capacity). Compared with SI, the soil moisture content of 40–80 cm under WSPI increased by 1.09 and 1.14 times in 2016 and 2017, respectively.

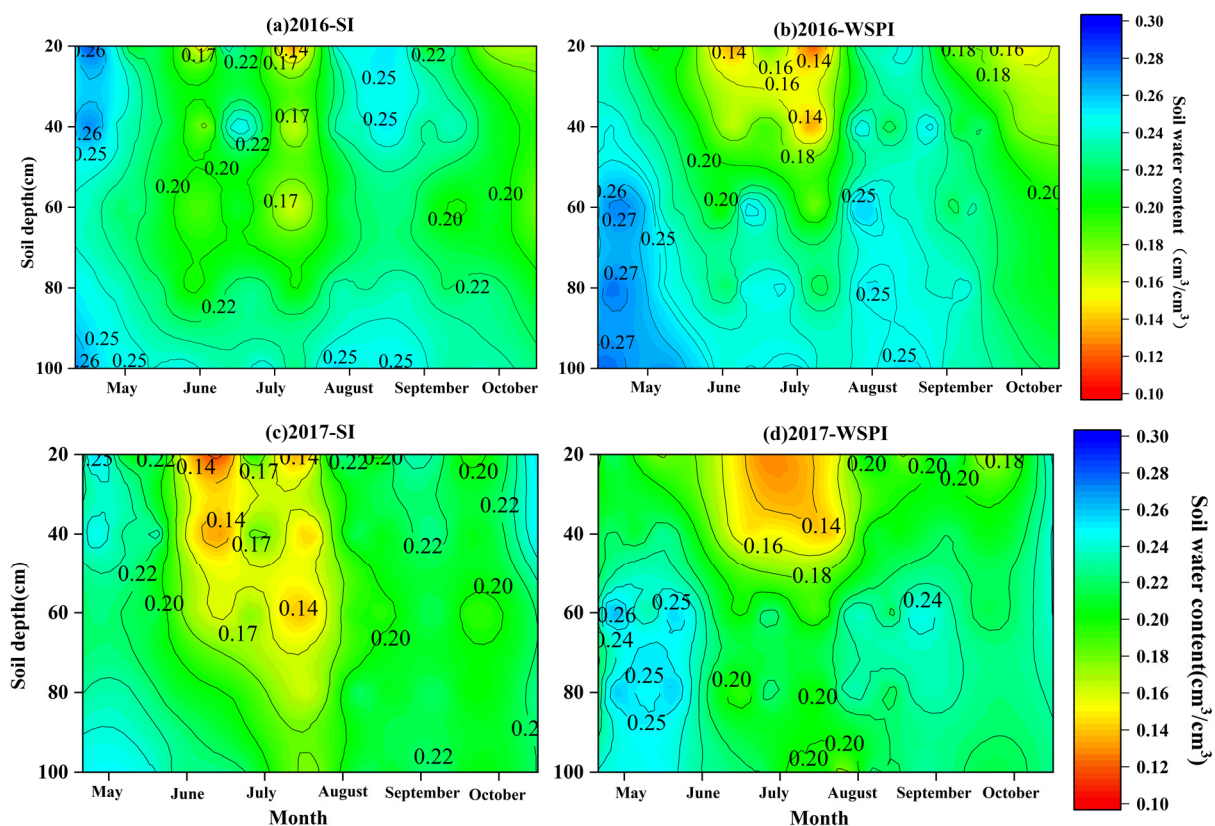


Figure 3. Seasonal variations of soil water content under surface irrigation (a,c) and water storage pit irrigation (b,d) in 2016 and 2017. WSPI means water storage pit irrigation, and SI means surface irrigation. Color changes indicate the magnitude of soil moisture content.

The seasonal variations in midday leaf water potential (Ψ_m) under different treatments during the period 2016–2017 are shown in Figure 4. There was a significant difference in Ψ_m between WSPI and SI treatment for both years ($p < 0.05$), except for July of 2016, for bud development and flowering (April and May) and September of 2017. With the same amount of irrigation, the mean value of Ψ_m from the WSPI treatment was 12.4 and 11.6% higher in 2016 and 2017, respectively, than that from SI.

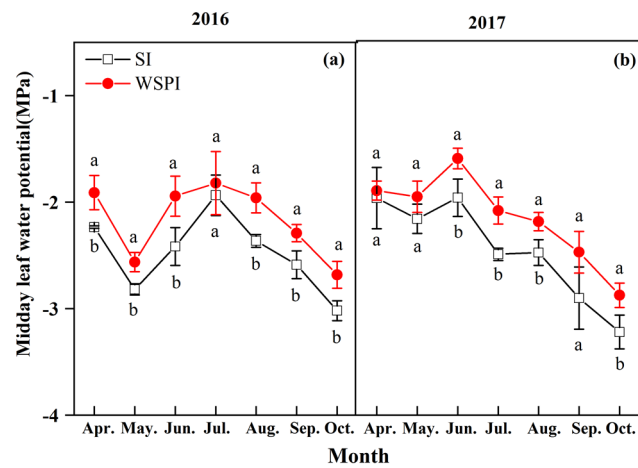


Figure 4. Seasonal course of midday leaf water potential under water storage pit irrigation (WSPI red line) and surface irrigation (SI, black line) in 2016 (a) and 2017 (b). Bars indicate standard deviation of the mean ($n = 3$); the same letters in figure indicate nonsignificant differences between the treatments, whereas different letters show significant differences ($p < 0.05$).

3.2. Gas Exchange Characteristics

The most significant differences between treatment effects were found during the hottest time of each growing season, usually from July to August (Figure 5). The WSPI treatment exhibited significantly higher Pn than the SI treatment in both years, and the single peak in August was 16.1% and 16.8% greater in 2016 and 2017, respectively (Figure 5a,e). The g_s , T_r , and C_i for WSPI were higher than those of SI, and showed a more significant difference in 2016. The mean value of g_s , T_r , and C_i for WSPI was greater than that of SI by 28%, 8.7%, and 2.7%, respectively. Significant linear correlations ($p < 0.05$) were found between leaf gas exchange and predawn leaf water potential (Ψ_{PL}) during the growing season in both years (Figure 6), as a higher Ψ_{PL} was accompanied by increased A, g_s , and T_r . However, the relationship between W_{ue} and Ψ_{PL} was not significant.

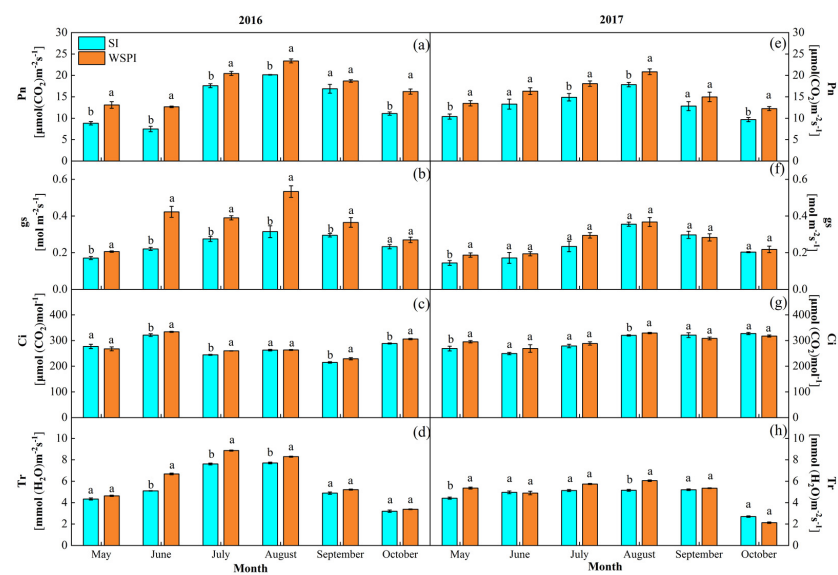


Figure 5. Net photosynthesis rate (Pn), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (T_r) of apple leaves under water storage pit irrigation (WSPI) and surface irrigation (SI) in 2016 (a–d) and 2017 (e–h). Bars indicate standard errors of the mean ($n = 9$); the same letters in figure indicate nonsignificant differences between the treatments, whereas different letters show significant differences ($p < 0.05$).

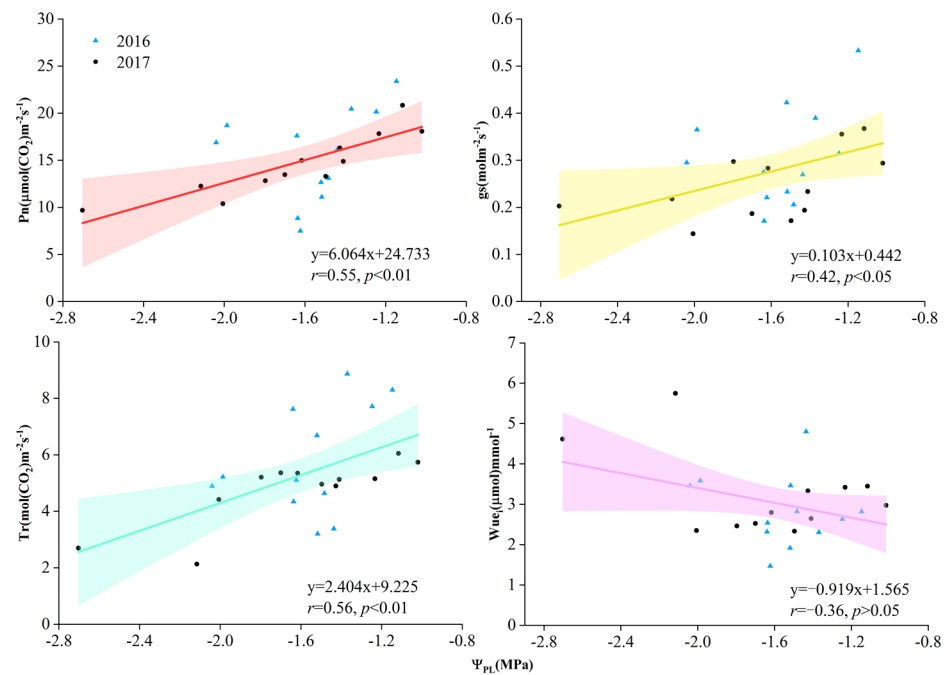


Figure 6. Relationship between leaf gas exchange and predawn stem water potential (Ψ_{PL}) during the growing season in 2016 and 2017. r indicates correlation coefficients between each pair of tested parameters. P_n means net photosynthesis rate, g_s means stomatal conductance, T_r means transpiration rate, and W_{ue} means water-use efficiency.

3.3. Chlorophyll Content and Fluorescence Features of Leaves

The analysis of the Chl content of leaves in early, mid-, and at the end of August allowed us to study the physiological changes under different treatments (Figure 7). Compared with SI, WSPI treatment increased the content of Chla, Chlb, and total chlorophyll in August of 2017 (Figure 7f–h), as those values were higher by an average of 12.2 ($p = 0.001$), 25.1 ($p = 0.049$), and 16.8% ($p = 0.014$), respectively. The WSPI treatment exhibited a higher F_v/F_m than the SI treatment ($p = 0.019$), as well as a higher qP ($p = 0.008$), Φ_{II} ($p = 0.008$), and ETR ($p = 0.002$) (Figure 7a,b,d,e). There was no significant difference in the F_v/F_m between WSPI and SI treatments in mid-August (17 August), as both treatments were irrigated (16 August) and not affected by water stress. However, the qP , Φ_{II} , and ETR of leaves in the WSPI treatment were significantly higher at the mid and final measurement points of August than those of the SI. The values of F_v/F_m , qP , Φ_{II} , and ETR decreased during August without considering irrigation treatments, but the magnitude of the decrease in SI was greater than that in WSPI. There was no significant difference in NPQ at the early and mid-points of August between treatments, but at the final point in August, the NPQ of leaves for SI was higher than that of the WSPI treatment.

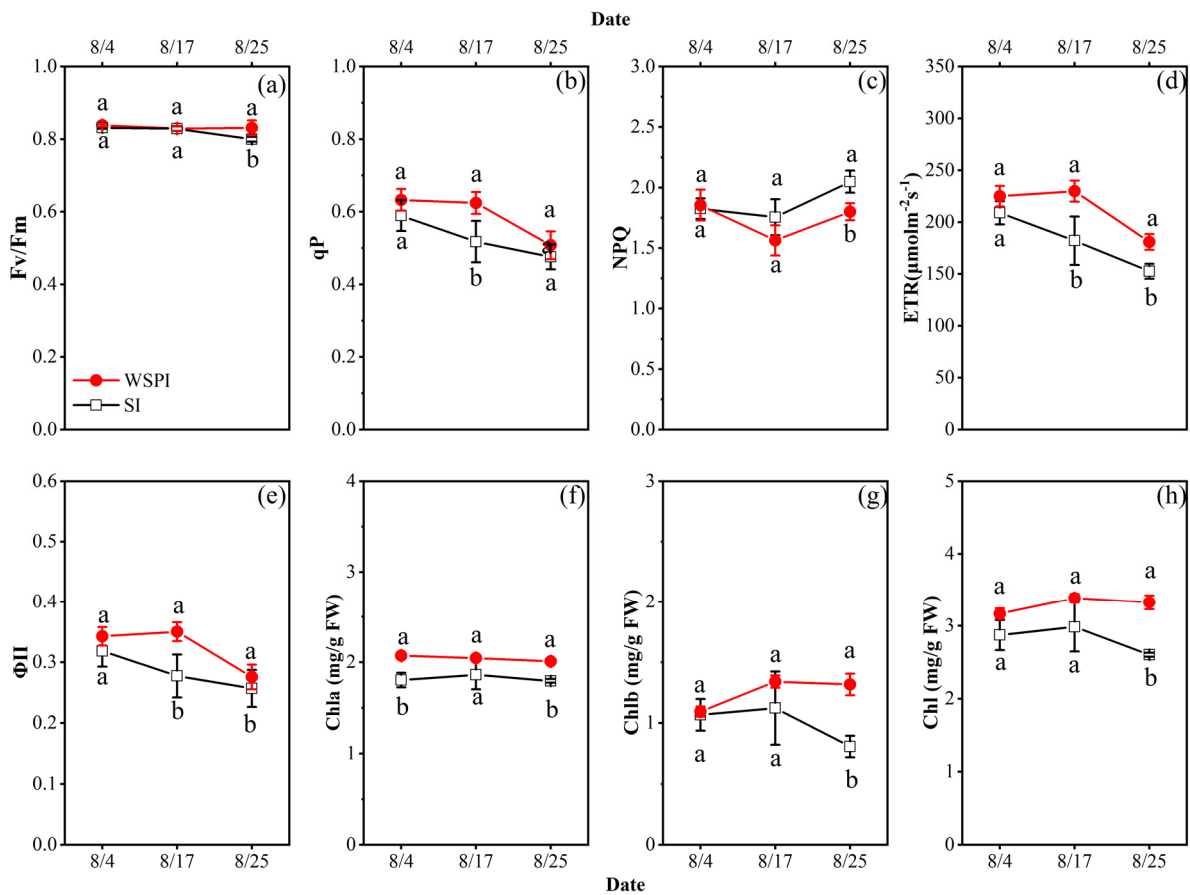


Figure 7. The potential quantum yield of PSII (F_v/F_m , shown in **a**), photochemical quenching (q_P , **b**), non-photochemical quenching (NPQ, **c**), actual quantum yield (Φ_{II} , **d**), (e) electron transport rate (ETR, **e**), and chlorophyll content (**f**–**h**) of apple leaves under water storage pit irrigation (WSPI) and surface irrigation (SI) during the August of 2017. The Bars indicate standard errors of the mean ($n = 3$ for chlorophyll content parameters, and $n = 9$ for chlorophyll fluorescence parameters); the same letters in figure indicate nonsignificant differences between the treatments, whereas different letters show significant differences ($p < 0.05$).

3.4. Antioxidant Enzyme, Soluble Sugar, and Yield

To explore whether WSPI could delay leaf senescence and reduce the extent of stress, we measured the antioxidant enzyme activity and the content of soluble sugar in August and October of 2017 (the second year after the implementation of WSPI). In October, the leaves entered senescence, showing higher SOD activity than in August (Table 2). Compared with SI, WSPI significantly decreased the activity of SOD in both August and October, by 36.5% and 29.7%, respectively. There was no significant difference in fructose content between WSPI and SI; however, WSPI significantly decreased the sucrose content in August. Compared with SI, WSPI improved dwarfing apple yield by 25.4% and 26.7% in 2016 and 2017, respectively.

Table 2. Effects of water storage pit irrigation (WSPI) and surface irrigation (SI) on superoxide dismutase (SOD), sucrose, fructose, yield, and single fruit weight in 2016 and 2017. Different letters show significant differences ($p < 0.05$).

	SOD (U/g FW)		Sucrose (mg/g DW)		Fructose (mg/g DW)		Yield (kg/tree)		Single Fruit Weight (g)	
	August	October	August	October	August	October	2016	2017	2016	2017
SI	150.24 ^a	175.67 ^a	11.34 ^a	11.41 ^a	0.78 ^a	0.23 ^a	10.28 ^b	11.47 ^b	183.72 ^b	170.64 ^b
WSPI	95.39 ^b	123.56 ^b	6.98 ^b	11.71 ^a	0.77 ^a	0.27 ^a	12.89 ^a	14.53 ^a	234.39 ^a	213.03 ^a

3.5. Principal Component Analysis (PCA)

The 17 measured parameters across the different irrigation treatments were analyzed using PCA, and the results are summarized in Table 3. Based on the factor loadings of these traits, the three principal components (PCs) could represent the effects of different irrigation treatments on leaf photosynthetic performance, which explained 86.7% of the total variance. Principle component 1 (PC1) was characterized by positive scores for Ψ_m , qP, ETR, and Φ_{II} , while PC2 was characterized by positive scores for Pn, gs, and Chl. NPQ was characterized by positive scores for PC3. The leaf water status, Chl fluorescence features, leaf stomata status, and chlorophyll content were sensitive indicators in the dwarfing apple response to WSPI.

Table 3. Eigen vectors and eigen values of the first three principal components for different irrigation treatments. The numbers in bold indicate values of factor loading > 0.8.

Traits	PC1	PC2	PC3
Ψ_m	0.969	0.055	0.029
Pn	0.405	0.885	−0.080
gs	−0.354	0.869	0.212
Ci	−0.708	0.476	0.313
Tr	0.574	0.629	0.415
WUE	−0.849	0.038	0.291
Fv/Fm	0.714	0.559	−0.123
qP	0.966	0.065	0.159
NPQ	−0.131	−0.295	0.882
ETR	0.942	0.225	−0.109
Φ_{II}	0.941	0.226	−0.108
Chla	−0.597	0.722	−0.303
Chlb	−0.498	0.782	−0.148
Chl	−0.554	0.803	−0.223
SOD	0.053	−0.706	−0.298
Sucrose	−0.770	−0.169	0.178
Fructose	0.685	0.220	0.452
Eigen value	7.991	5.023	1.731
Cumulative%	47.01	76.56	86.74

4. Discussion

Maintaining a relatively high Ψ_m was crucial for leaf photosynthesis [34,35]. Our observations were comparable to the literature values of -0.7 – -4.0 MPa in apple [36–39], and based on the results of Sircelj et al. [40], apple trees would experience severe stress when Ψ_m was -3 MPa or lower. In this study, Ψ_m did not decrease to this stress level for WSPI treatment, but it did for SI. This result was due to the following factors: (1) Soil water is an important factor that limits the plant water status. It has been demonstrated that Ψ_m is high when soil water is maintained at a high level due to irrigation [25,41]. In this study, the mean amount of SWC in a 0–100 cm soil profile under WSPI was 1.2 times that under SI treatment. (2) The availability of soil water in deeper soil profiles is indispensable for improving the leaf water content of plants and yield [42]. In this study, more irrigation water was stored at a 40–80 cm soil depth. Previous research at the same site has also shown that compared to SI, WSPI could significantly improve the soil water content of the root zone in the medium soil layer (40–150 cm) [14] and reduce surface evaporation. (3) Deeper plant roots have the ability to reduce the effect of drought stress in the Loess Plateau of China [43]. Then, the development of deep roots could have enabled the shoots to maintain a higher water status [44]. Compared with SI, the fine root length density for WSPI was improved by 10.32% at soil depths from 40 to 100 cm [14], and the average stem flow of apple trees under WSPI was 1.18–1.68 times higher from May to October [45].

The leaf stomatal strategy is regulated by the leaf water status [46], and the gs was directly related to the Pn [47]. To the best of our knowledge, this was the first investigation of the differences between stomatal regulation and photosynthetic capacity between WSPI

and SI. We found significant linear correlations between P_n , g_s , and Ψ_{PL} (the value of correlation coefficients were from 0.42 to 0.56, $p < 0.01$), which indicated that the variations in P_n for different irrigation treatments were mainly due to stomatal regulation caused by soil moisture and leaf water status [48]. In this study, the P_n , g_s , and T_r of leaves for WSPI were greater than those for SI. This observation indicated that stomatal limitation was the major limiting factor in photosynthesis [49]. However, the C_i value for WSPI and SI did not necessarily decrease with g_s ($r = 0.01$, $p > 0.05$). Based on the physiological mechanism of plant photosynthesis [50], C_i would increase when the g_s value increases, while an increase in photosynthetic capacity would consume CO_2 , thus reducing the correlation between C_i and g_s [51,52]. These relationships imply that there are also nonstomatal factors (such as Chl content, chlorophyll fluorescence, and enzymatic activity) affecting the photosynthesis of apple under different irrigation methods [53].

Chlorophyll is the key pigment for photosynthesis [54]. In general, healthy leaves have greater chlorophyll content and a higher photosynthetic rate. In this study, WSPI improved the Chl content of leaves during the growth stage (August) when compared to SI. The Chl content was increased by Ψ_m , and improved soil nutrients [55]. Li et al. [56] found that WSPI decreased the cumulative NH_3 volatilization compared with SI. The significant increase in chlorophyll content could effectively increase the light capture and conversion efficiency [57,58]. Beyond these parameters, the increase in F_v/F_m , qP , Φ_{II} , and ETR under the WSPI treatment indicated that the PSII activity of leaves was improved compared with SI. When subjected to water or temperature stress, the plant would maintain normal photosynthesis through the xanthophyll cycle [53,59,60]. In this study, the rise in NPQ for SI treatment suggested that apple trees under SI went through increasing energy dissipation to protect the photosynthetic system. Furthermore, the rate of chlorophyll degradation for WSPI slowed down when compared to SI (Figure 7h), in effect extending the functional period of effective photosynthetic time.

Under soil water stress or during a period of senescence, oxidative stress was induced by increasing the production of reactive oxygen species (ROS) [61]. SOD, the first step in the system that scavenges excess ROS, plays a central role in protecting cells against photodamage to PSII [62]. The activity of SOD under WSPI was significantly lower than that of SI due to the lower oxidative stress. In this study, the activity of SOD was increased in October when compared to August for both treatments. This increase in activity was an adaptation response to water stress, high light levels, and the lower temperatures in autumn [63]. However, this study found no significant difference in sucrose and fructose levels under WSPI and SI, with the exception of sucrose in August. Unlike other plants, sorbitol is the main soluble sugar of apple leaves and accounted for 60–70% of the photosynthesis in leaves [64]. The content of sucrose and fructose was measured in this study, considering that sorbitol is converted to fructose by sorbitol dehydrogenase, and more than 80% of the total carbon flux via fructose [65]. Further studies are needed to investigate the impacts of WSPI on sorbitol levels in apple leaves.

Under the same total amount of irrigation, WSPI increased the apple yield when compared with SI (Table 2). This is consistent with the results of Zhao et al. [14], in that they also found that the average yield for WSPI with 40 cm of pit depth was 1.17 times higher than that of surface irrigation. Li et al. [10] showed that apple yield for pit irrigation using less total water was not significantly lower than yields using furrow irrigation. The PCA analysis of leaf photosynthetic performance showed that WSPI improved apple yield by affecting the leaf water status, chlorophyll content and fluorescence features, and leaf stomata status. However, other biochemical indices are also important for interpreting the holistic photosynthesis mechanism, such as root growth, leaf area, and trunk diameter [66,67].

5. Conclusions

Based on our results, water storage pit irrigation could improve the leaf photosynthetic performance of dwarfing apple compared with SI treatment, via increasing the soil water content at the middle soil depth, leaf water potential, stomatal conductance, chlorophyll

content, and PSII activity. The accumulation of SOD and sucrose was significantly reduced under WSPI than that under SI. These findings contribute to gaining a more in-depth understanding of water-saving mechanisms when using water storage pit irrigation.

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References

1. Kviklys, D.; Samupliene, G. Relationships among the rootstock, crop load, and sugar hormone signaling of apple tree, and their effects on biennial bearing. *Front. Plant Sci.* **2020**, *11*, 1213. [[CrossRef](#)]
2. Zhao, D.; Yuan, J.; Xu, K.; Cheng, C.; Li, H. Selection of morphological, physiological and biochemical indices: Evaluating dwarfing apple interstocks in cold climate zones. *N. Z. J. Crop Hortic. Sci.* **2016**, *44*, 291–311. [[CrossRef](#)]
3. Song, X.; Gao, X.; Zhao, X.; Wu, P.; Dyck, M. Spatial distribution of soil moisture and fine roots in rain-fed apple orchards employing a rainwater collection and infiltration (rwc) system on the loess plateau of china. *Agric. Water Manag.* **2017**, *184*, 170–177. [[CrossRef](#)]
4. Zheng, L.; Ma, J.; Sun, X.; Guo, X.; Cheng, Q.; Shi, X. Estimating the root water uptake of surface-irrigated apples using water stable isotopes and the hydrus-1D model. *Water* **2018**, *10*, 1624. [[CrossRef](#)]
5. Gao, X.; Zhao, X.; Wu, P.; Yang, M.; Ye, M.; Tian, L.; Zou, Y.; Wu, Y.; Zhang, F.; Siddique, K. The economic-environmental trade-off of growing apple trees in the drylands of China: A conceptual framework for sustainable intensification. *J. Clean. Prod.* **2021**, *296*, 126497. [[CrossRef](#)]
6. Guo, X.; Lei, T.; Sun, X.; Ma, J.; Zheng, L.; Zhang, S.; He, Q. Modelling soil water dynamics and root water uptake for apple trees under water storage pit irrigation. *Int. J. Agric. Biol. Eng.* **2019**, *12*, 126–134. [[CrossRef](#)]
7. Meng, W.; Sun, X.; Ma, J.; Guo, X.; Lei, T.; Li, R. Measurement and simulation of the water storage pit irrigation trees evapotranspiration in the Loess Plateau. *Agric. Water Manag.* **2020**, *226*, 105804. [[CrossRef](#)]
8. Sun, X.; Ma, J.; Guo, X. Water storage pit irrigation technique. In Proceedings of the International Conference on Effective Utilization of Agricultural Soil & Water Resources and Protection of Environment and the 4th Annual Academic Conference of Chinese Society of Agricultural Soil and Water Engineering, Nanjing, China, 16–18 August 2006; Hohai University Press: Nanjing, China, 2007; pp. 211–214.
9. Sun, X. Effect of water storage pit irrigation on soil and water conservation. In Proceedings of the 12th ISCO Conference, Beijing, China, 26–31 May 2002; Tsinghua University Press: Beijing, China, 2002; Volume 2, pp. 367–369.
10. Li, T.; Zhang, J. Effect of pit irrigation on soil water content, vigor, and water use efficiency within vineyards in extremely arid regions. *Sci. Hortic.* **2017**, *218*, 30–37. [[CrossRef](#)]
11. Nazari, E.; Besharat, S.; Zeinalzadeh, K.; Mohammadi, A. Measurement and simulation of the water flow and root uptake in soil under subsurface drip irrigation of apple tree. *Agric. Water Manag.* **2021**, *255*, 106972. [[CrossRef](#)]
12. Zhang, B.; Hu, Y.; Hill, R.; Wu, S.; Song, X. Combined effects of biomaterial amendments and rainwater harvesting on soil moisture, structure and apple roots in a rainfed apple orchard on the Loess Plateau, China. *Agric. Water Manag.* **2021**, *248*, 106776. [[CrossRef](#)]
13. Cheng, Q.; Ma, J.; Ren, R.; Zheng, L.; Guo, X.; Sun, X. Effects of fertilization management under WSPI on soil nitrogen distribution and nitrogen absorption in apple orchard in Loess Plateau. *Agronomy* **2020**, *10*, 1386. [[CrossRef](#)]
14. Zhao, Y.; Ma, J.; Sun, X.; Guo, X. Spatial distribution of soil moisture and fine roots of apple trees under water storage pit irrigation. *J. Irrig. Drain. Eng.* **2014**, *140*, 333–340. [[CrossRef](#)]
15. Ashraf, M.; Harris, P. Photosynthesis under stressful environments: An overview. *Photosynthetica* **2013**, *51*, 163–190. [[CrossRef](#)]
16. Chen, Z.; Tao, X.; Khan, A.; Tan, D.; Luo, H. Biomass accumulation, photosynthetic traits and root development of cotton as affected by irrigation and nitrogen-fertilization. *Front. Plant Sci.* **2018**, *9*, 173. [[CrossRef](#)]
17. Kucukyumuk, C.; Kacal, E.; Ertek, A.; Ozturk, G.; Kurttas, Y.S.K. Pomological and vegetative changes during transition from flood irrigation to drip irrigation: Starkrimson Delicious apple variety. *Sci. Hortic.* **2012**, *136*, 17–23. [[CrossRef](#)]
18. Ghafari, H.; Hassanpour, H.; Jafari, M.; Besharat, S. Effect of partial root zone irrigation on physiology, water use efficiency, fruit yield, phenolic compounds and antioxidant capacity of apple. *Arch. Agron. Soil Sci.* **2020**, *67*, 1521–1538. [[CrossRef](#)]

19. Zhang, S.; Zhang, G.; Gu, S.; Xia, J.; Zhao, J. Critical responses of photosynthetic efficiency of goldspur apple tree to soil water variation in semiarid loess hilly area. *Photosynthetica* **2010**, *48*, 589–595. [[CrossRef](#)]
20. Sircelj, H.; Tausz, M.; Grill, D.; Batič, F. Detecting different levels of drought stress in apple trees (*Malus domestica*, Borkh.) with selected biochemical and physiological parameters. *Sci. Hortic.* **2007**, *113*, 362–369. [[CrossRef](#)]
21. Fujisawa, H.; Moriya, Y. Influence of rootstock and soil moisture on leaf photosynthesis of apple trees on JM1, JM7, JM8, and M.9 rootstocks. *Acta Hortic.* **2012**, *932*, 441–446. [[CrossRef](#)]
22. Bolat, I.; Dikilitas, M.; Ercisli, S.; Ikinici, A.; Tonkaz, T. The effect of water stress on some morphological, physiological, and biochemical characteristics and bud success on apple and quince rootstocks. *Sci. World J.* **2014**, *2014*, 769732. [[CrossRef](#)]
23. Tworkoski, T.; Fazio, G.; Glenn, D. Apple rootstock resistance to drought. *Sci. Hortic.* **2016**, *204*, 70–78. [[CrossRef](#)]
24. Bhusal, N.; Han, S.; Yoon, T. Impact of drought stress on photosynthetic response, leaf water potential, and stem sap flow in two cultivars of bi-leader apple trees (*Malus × domestica* Borkh.). *Sci. Hortic.* **2019**, *246*, 535–543. [[CrossRef](#)]
25. Zegbe, J.; Behboudian, M.; Clothier, B. Reduced irrigation maintains photosynthesis, growth, yield, and fruit quality in ‘pacific rose’ apple. *J. Sustain. Agric.* **2007**, *30*, 125–136. [[CrossRef](#)]
26. Guo, X.; Sun, X.; Ma, J.; Lei, T.; Zheng, L. Effects of water storage pit irrigation on root distribution and root distribution function of apple trees. *Fresen. Environ. Bull.* **2019**, *28*, 326–338.
27. Zheng, L.; Ma, J.; Sun, X.; Guo, X.; Li, Y.; Ren, R.; Cheng, Q. Effective root growth zone of apple tree under water storage pit irrigation using stable isotope methodology. *Arch. Agron. Soil Sci.* **2019**, *65*, 1521–1535. [[CrossRef](#)]
28. Rouhi, V.; Samson, R.; Lemeur, R.; Van Damme, P. Photosynthetic gas exchange characteristics in three different almond species during drought stress and subsequent recovery. *Environ. Exp. Bot.* **2007**, *59*, 117–129. [[CrossRef](#)]
29. Glenn, D. Canopy gas exchange and water use efficiency of ‘Empire’ apple in response to particle Film, irrigation, and microclimatic factors. *J. Am. Soc. Hortic. Sci.* **2010**, *135*, 25–32. [[CrossRef](#)]
30. Jaouhari, N.; Abouabdillah, A.; Bouabid, R.; Bouriou, M.; Aleya, L.; Chaoui, M. Assessment of sustainable deficit irrigation in a moroccan apple orchard as a climate change adaptation strategy. *Sci. Total Environ.* **2018**, *642*, 574–581. [[CrossRef](#)]
31. Porra, R.; Thompson, W.; Kriedmann, P. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta.* **1989**, *975*, 384–394. [[CrossRef](#)]
32. Tian, J.; Hu, Y.; Gan, X.; Zhang, Y.; Hu, X.; Gou, L.; Luo, H.; Zhang, W. Effects of increased night temperature on cellulose synthesis and the activity of sucrose metabolism enzymes in cotton fiber. *J. Integr. Agric.* **2013**, *12*, 979–988. [[CrossRef](#)]
33. Dhindsa, R.; Plumb-Dhindsa, P.; Thorpe, T. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **1981**, *32*, 93–101. [[CrossRef](#)]
34. Williams, L.; Araujo, F. Correlations among predawn leaf, midday leaf, and midday stem water potential and their correlations with other measures of soil and plant water status in vitis vinifera. *J. Am. Soc. Hortic. Sci.* **2002**, *127*, 448–454. [[CrossRef](#)]
35. Zhou, B.; Sun, J.; Liu, S.; Jin, W.; Zhang, Q.; Wei, Q. Dwarfing apple rootstock responses to elevated temperatures: A study on plant physiological features and transcription level of related genes. *J. Integr. Agric.* **2016**, *15*, 1025–1033. [[CrossRef](#)]
36. Atkinson, C.; Policarpo, M.; Webster, A.; Kingswell, G. Drought tolerance of clonal malus determined from measurements of stomatal conductance and leaf water potential. *Tree Physiol.* **2000**, *20*, 557–563. [[CrossRef](#)] [[PubMed](#)]
37. Naor, A.; Gal, Y.; Peres, M. The inherent variability of water stress indicators in apple, nectarine and pear orchards, and the validity of a leaf-selection procedure for water potential measurements. *Irrig. Sci.* **2006**, *24*, 129–135. [[CrossRef](#)]
38. O’Connell, M.; Goodwin, I. Responses of ‘pink lady’ apple to deficit irrigation and partial rootzone drying: Physiology, growth, yield, and fruit quality. *Aust. J. Agric. Res.* **2007**, *58*, 1068–1076. [[CrossRef](#)]
39. Eyles, A.; Bound, S.; Oliver, G.; Corkrey, R.; Hardie, M.; Green, S.; Close, D. Impact of biochar amendment on the growth, physiology and fruit of a young commercial apple orchard. *Trees* **2015**, *29*, 1817–1826. [[CrossRef](#)]
40. Sircelj, H.; Tausz, M.; Grill, D.; Batic, F. Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. *J. Plant Physiol.* **2005**, *162*, 1308–1318. [[CrossRef](#)]
41. Egea, G.; Dodd, I.; Gonzálezreal, M.; Domingo, R.; Baille, A. Partial rootzone drying improves almond tree leaf-level water use efficiency and afternoon water status compared with regulated deficit irrigation. *Funct. Plant Biol.* **2011**, *38*, 372–385. [[CrossRef](#)]
42. Guo, Z.; Yu, Z.; Wang, D.; Shi, Y.; Zhang, Y. Photosynthesis and winter wheat yield responses to supplemental irrigation based on measurement of water content in various soil layers. *Field Crop. Res.* **2014**, *166*, 102–111. [[CrossRef](#)]
43. Li, H.; Si, B.; Ma, X.; Wu, P. Deep soil water extraction by apple sequesters organic carbon via root biomass rather than altering soil organic carbon content. *Sci. Total Environ.* **2019**, *670*, 662–671. [[CrossRef](#)] [[PubMed](#)]
44. Black, M.; Patterson, K.; Minchin, P.; Gould, K.; Clearwater, M. Hydraulic responses of whole vines and individual roots of kiwifruit (*actinidia chinensis*) following root severance. *Tree Physiol.* **2011**, *31*, 508. [[CrossRef](#)] [[PubMed](#)]
45. Zhang, Y.; Sun, X.; Ma, J.; Guo, X.; An, J.; Li, R. Daily variation of stem flow rate of apple tree and influence factors under water storage pit irrigation. *Water Saving Irri.* **2017**, *5*, 11–15.
46. Solari, L.; Johnson, S.; Dejong, T. Relationship of water status to vegetative growth and leaf gas exchange of peach (*prunus persica*) trees on different rootstocks. *Tree Physiol.* **2006**, *26*, 1333–1341. [[CrossRef](#)]
47. Sun, X.; Yan, H.; Ma, P.; Liu, B.; Zou, Y.; Liang, D.; Ma, F.; Li, P. Responses of young ‘pink lady’ apple to alternate deficit irrigation following long-term drought: Growth, photosynthetic capacity, water-use efficiency, and sap flow. *Photosynthetica* **2012**, *50*, 501–507. [[CrossRef](#)]

48. Ma, X.; Jacoby, P.; Sanguinet, K. Improving net photosynthetic rate and rooting depth of grapevines through a novel irrigation strategy in a semi-arid climate. *Front. Plant Sci.* **2020**, *11*, 575303. [[CrossRef](#)]
49. Hamed, S.; Lefi, E.; Chaieb, M. Physiological responses of pistacia vera, l. versus pistacia atlantica desf. to water stress conditions under arid bioclimate in tunisia. *Sci. Hortic.* **2016**, *203*, 224–230. [[CrossRef](#)]
50. Farquhar, G.; Ehleringer, J.; Hubick, K. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Phys.* **1989**, *40*, 503–537. [[CrossRef](#)]
51. Garcia-Sanchez, F.; Syvertsen, J.; Gimeno, V.; Botia, P.; Perez-Perez, J. Responses to flooding and drought stress by two citrus rootstock seedlings with different water-use efficiency. *Physiol. Plant.* **2007**, *130*, 532–542. [[CrossRef](#)]
52. Yuan, J.; Xu, M.; Duan, W.; Fan, P.; Li, S. Effects of whole-root and half-root water stress on gas exchange and chlorophyll fluorescence parameters in apple trees. *J. Am. Soc. Hortic. Sci.* **2013**, *138*, 395–402. [[CrossRef](#)]
53. Wang, Z.; Li, G.; Sun, H.; Ma, L.; Guo, Y.; Zhao, Z.; Gao, H.; Mei, L. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. *Biol. Open* **2018**, *7*, bio035279. [[CrossRef](#)] [[PubMed](#)]
54. Sayyad-Amin, P.; Jahansooz, M.; Borzouei, A.; Ajili, F. Changes in photosynthetic pigments and chlorophyll-a fluorescence attributes of sweet-forage and grain sorghum cultivars under salt stress. *J. Biol. Phys.* **2016**, *42*, 601–620. [[CrossRef](#)] [[PubMed](#)]
55. Afonso, S.; Ribeiro, C.; Bacelar, E.; Ferreira, H.; Oliveira, I.; Silva, A.; Goncalves, B. Influence of training system on physiological performance, biochemical composition and antioxidant parameters in apple tree (*Malus domestica* Borkh.). *Sci. Hortic.* **2017**, *225*, 394–398. [[CrossRef](#)]
56. Li, J.; Chen, P.; Sun, X.; Liu, P. Effects of fertigation strategies on water and nitrogen distribution under water storage pit irrigation for orchards. *Int. J. Agric. Biol. Eng.* **2018**, *11*, 165–171. [[CrossRef](#)]
57. Baker, N. Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* **2008**, *59*, 89. [[CrossRef](#)]
58. Urban, L.; Aarouf, J.; Bidel, L. Assessing the effects of water deficit on photosynthesis using parameters derived from measurements of leaf gas exchange and of chlorophyll a fluorescence. *Front. Plant Sci.* **2017**, *8*, 2068. [[CrossRef](#)]
59. Ballester, C.; Zarco-Tejada, P.; Nicolas, E.; Alarcon, J.; Fereres, E.; Intrigliolo, D.; Gonzalez-Dugo, V. Evaluating the performance of xanthophyll, chlorophyll and structure-sensitive spectral indices to detect water stress in five fruit tree species. *Precis. Agric.* **2018**, *19*, 178–193. [[CrossRef](#)]
60. Petridis, A.; Therios, I.; Samouris, G.; Koundouras, S.; Giannakoula, A. Effect of water deficit on leaf phenolic composition, gas exchange, oxidative damage and antioxidant activity of four greek olive (*olea europaea* L.) cultivars. *Plant Physiol. Biochem.* **2012**, *60*, 1–11. [[CrossRef](#)]
61. Abid, G.; M'hamdi, M.; Mingeot, D.; Aouida, M.; Aroua, I.; Muhovski, Y.; Khaled, S.; Souissi, F.; Mannai, K.; Jebara, M. Effect of drought stress on chlorophyll fluorescence, antioxidant enzyme activities and gene expression patterns in faba bean (*Vicia faba* L.). *Arch. Agron. Soil Sci.* **2017**, *63*, 536–552. [[CrossRef](#)]
62. Silva, E.; Ferreira-Silva, S.; Fontenele, A.; Ribeiro, R.; Viégas, R.; Silveira, J. Photosynthetic changes and protective mechanisms against oxidative damage subjected to isolated and combined drought and heat stresses in jatropha curcas plants. *J. Plant Physiol.* **2010**, *167*, 1157–1164. [[CrossRef](#)]
63. Pina, J.; Moraes, R. Gas exchange, antioxidants and foliar injuries in saplings of a tropical woody species exposed to ozone. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 685–691. [[CrossRef](#)] [[PubMed](#)]
64. Li, M.; Feng, F.; Cheng, L. Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. *PLoS ONE* **2012**, *7*, e33055. [[CrossRef](#)]
65. Samuoliene, G.; Viškelienė, A.; Sirtautas, R.; Kviklys, D. Relationships between apple tree rootstock, crop-load, plant nutritional status and yield. *Sci. Hortic.* **2016**, *211*, 167–173. [[CrossRef](#)]
66. Hernandez-Santana, V.; Fernández, J.; Cuevas, M.; Perez-Martin, A.; Diaz-Espejo, A. Photosynthetic limitations by water deficit: Effect on fruit and olive oil yield, leaf area and trunk diameter and its potential use to control vegetative growth of super-highdensity olive orchards. *Agric. Water Manag.* **2017**, *184*, 9–18. [[CrossRef](#)]
67. Yang, Q.; Zhang, F.; Li, F. Effect of different drip irrigation methods and fertilization on growth, physiology and water use of young apple tree. *Sci. Hortic.* **2011**, *129*, 119–126. [[CrossRef](#)]