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*Article*

# Effects of Different Water Treatments on Physiological Characteristics of Film Free Cotton in Southern Xinjiang

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**Abstract:** In order to explore the effects of different irrigation methods on the physiological characteristics of film free cotton in southern Xinjiang, the following experiments were carried out: (1) Different irrigation amounts test: 300, 375, 450, 525 and 600 mm (represented by W1, W2, W3, W4 and W5) and a control (450 mm for film free cotton, represented by WCK) were set; (2) Drip irrigation frequency test: drip irrigation 12 times, 10 times, 8 times and 6 times during the growth period (expressed as P12, P10, P8 and P6), chlorophyll fluorescence, leaf area index, SPAD, single leaf photosynthesis, stress enzyme activities and MDA content were observed. The results showed that: compared with film cotton, the average leaf green fluorescence parameters of film free cotton under suitable water treatment (W3 and W4) decreased by 9.03%~50.44%, the photosynthetic indexes (Pn: net photosynthetic rate, Gs: stomatal conductance, Tr: transpiration rate, Ci: intercellular $CO_2$ concentration) decreased by 4.64%-12.93%, the activities of protective enzymes (SOD: superoxide dismutase, POD: peroxidase) decreased by 3.36%-3.58%, the SPAD value decreased by 5.55%, and the MDA content increased by 3.17%, indicating that film free cotton reduced the physiological function of cotton leaves, and the yield decreased by 42.07%. With the increase of irrigation water and irrigation frequency, the initial fluorescence ($F_0$) of leaves in each period of film free cotton showed a downward trend, and the maximum fluorescence ($F_m$), variable fluorescence ($F_v$), maximum photochemical efficiency ($F_v/F_m$), potential photochemical activity of PS Ⅱ ($F_v/F_0$), electron transfer of PS Ⅱ ($F_m/F_0$), and photosynthetic performance index (PIABS) showed an upward trend. In all water treatments, W3 and P12 had the highest SPAD value, photosynthetic index and protective enzyme activity, and the lowest MDA content, which was significantly different from other treatments except W4 and P10. The yield order of different treatments was W3>W4>W5>W2>W1, and the difference between W3 and W4 was not significant, but significant with W2 and W1; The irrigation frequency test was P12>P10>P8>P6, and there was no significant difference between P12 and P10. Conclusion: no film mulching has a certain impact on the physiological function of cotton leaves. When the irrigation amount is 450-525 mm and irrigation times is 10-12 times, it is beneficial to play the physiological function of film-free cotton leaves, which can provide reference for water management in production practice.

**Keywords:** Southern Xinjiang; film-free cotton; moisture treatment; physiological characteristics

## 1. Introduction

Xinjiang is the largest cotton planting area in China, with a planting area of 2.4479 million hectares and a total output of 5.686 million tons in 2024, accounting for 86.25% and 62.25% of the country, respectively. Southern Xinjiang is rich in light and heat resources, which is very suitable for

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cotton production, and its planting area accounts for more than 65% of the whole Xinjiang. In production, people generally use plastic film mulching cultivation, and the planting area of plastic film mulching is more than 98%. Plastic film mulching cultivation has become a key measure for high and stable yield of cotton in southern Xinjiang [1]. Although plastic film mulching can significantly optimize soil ecological conditions, promote cotton growth and increase yield, there are also major problems, mainly reflected in the difficulty of residual film recovery, high manual recovery cost, difficult application, and low mechanical recovery rate, resulting in the accumulation of residual film in the field year by year, resulting in pollution and serious damage to soil ecology. Therefore, the implementation of film free cultivation is the fundamental way to solve white pollution [11]. At present, people have cultivated new cotton varieties suitable for film free cultivation, laying the foundation for film free cultivation[3]. However, the problems of slow emergence, late development, low yield and low water and fertilizer utilization efficiency in film free cultivation of cotton seriously restrict the application of film free cultivation. Compared with cotton with film mulching, cotton without film mulching has obvious differences in moisture, nutrient ecology and cotton growth physiology. How to carry out reasonable water and fertilizer management, promote the effective growth of cotton, stimulate the adverse physiological activity of cotton yield formation, and improve the growth performance of cotton is the key to the formulation of cotton planting technology without film mulching. Water stress has an important impact on the growth physiology of cotton. It changes the antioxidant enzyme activity [4,5] and chlorophyll fluorescence kinetic parameters in cotton, reduces the photosynthetic performance of leaves, and has adverse effects on the synthesis and transportation of yield substances. This experiment investigated the effects of varying irrigation amounts and frequencies on the physiological characteristics of film-free cotton, revealed the physiological mechanism of corresponding moisture conditions of film-free cotton, and provided a theoretical basis for the formulation of irrigation schedule for the efficient growth of film free cotton.

## 2. Materials and Methods

### *2.1. Overview and Management of Test Area*

The experiment was conducted in the net-house of the Agricultural Experiment Station of Tarim University, situated on the northwest edge of the Tarim Basin (40°33′N, 81°16′E, elevation 1012.2 m). This location represents a typical extremely arid desert region, characterized by an average annual temperature of 11.2℃, an accumulated temperature of ≥10℃ reaching 4760℃, an average annual precipitation of 53.6 mm, an average annual evaporation of 1988.4 mm, and an average annual relative humidity consistently below 55%. It belongs to a typical inland climate in the warm temperate zone. The soil in the experimental field is composed of sandy loam soil, with a soil bulk density of 1.39 $g·cm^{-3}$ in the 0-40 cm soil layer. The field water holding capacity stands at 23.8% (by weight), the wilting coefficient is 10.7%, the groundwater level is approximately 8.0 m, the content of soil organic matter is 10.25 $g·kg^{-1}$., total nitrogen is 0.09%, alkali hydrolyzed nitrogen is 49.27 $mg·kg^{-1}$, available phosphorus is 32.01 $mg·kg^{-1}$, and available potassium is 214.10 $mg·kg^{-1}$.

The test was conducted in 2022 and 2023.The plot area was 4.5 m × 10 m=45 $m^2$, and each area was separated by impermeable plate (PVC polyester plate) with an isolation depth of 80 cm to prevent leakage. The soil moisture was stored by 180 mm pressure alkali irrigation in spring before sowing, and 600 $kg·hm^{-2}$ of nitrogen, phosphorus and potassium ternary compound fertilizer (NPK 19-17-6) was evenly applied and turned 25 cm deep after a week. 'Zhongmian 619' (Cotton Research Institute of the Chinese Academy of Agricultural Sciences) was selected as the test material. On April 5 (2022) and April 11 (2023), the cotton was sown in the mode of no film deepening on-demand (sowing depth of 3.5 cm) and (66+10) × 10 cm mechanized cotton with shallow burying in drip irrigation belt. The model of one film, four rows and two tubes was adopted, and the theoretical number of plants was 239200 $plants·hm^{-2}$. The drip irrigation belt was placed in the middle of the narrow row and buried shallowly for 3 cm. In order to control weeds, 2250$ml·hm^{-2}$ of 33% pendimethalin EC (produced by Wuhan jiyesheng Chemical Co., Ltd.) was sprayed before sowing, and 300$kg·hm^{-2}$ of water was mixed. In order to maintain the weed control effect, 3-5cm of soil was

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mixed after spraying. During the growth period, urea 600 kg·hm$^{-2}$, high phosphorus ternary compound water-soluble fertilizer (NPK 10-30-10+TE) 300 kg·hm$^{-2}$ and high potassium ternary compound water-soluble fertilizer (NPK 12-8-30+TE) 225 kg·hm$^{-2}$ were applied with water drops. The top was topped on July 15, and the chemical control was carried out three times at the full bud stage, the first flowering stage and the full flowering stage. The dosage of 98% mepiquat wettable powder was 22.5 g·hm$^{-2}$, 37.5 g·hm$^{-2}$ and 120 g·hm$^{-2}$, respectively. The irrigation water volume is recorded by the water meter in the community.

*2.2. Test Materials and Design*

Set up 2 tests. 1) Different drip irrigation water volume experiments. Based on the production practice of drip irrigation quantity and drip irrigation time, after emergence, increase or decrease the irrigation amount according to the recommended irrigation amount in production practice, and five irrigation quotas were set: 300 mm, 375 mm, 450 mm, 525 mm and 600 mm, with 450 mm of irrigation under the film as the control, and named them as W1, W2, W3, W4, W5 and WCK respectively. There were six treatments in the experiment, repeated three times, a total of 18 cells. Drip irrigation was conducted 9 times at the pregnant bud stage, the early bud stage, the full bud stage, the initial flowering stage, the full flowering stage, the late flowering stage, the early boll stage, full boll stage and the initial boll opening stage according to the proportion of 0.1:0.1:0.12:0.12:0.14:0.12:0.1:0.1. See **Table 1** for specific allocation.

**Table 1.** Experimental setup for different irrigation amounts (mm).

| Treatment | Pregnant bud stage (5-18) | Early bud stage (5-26) | Full budding period (6-14) | Early flowering stage (6-28) | Full blooming period (7-10) | Late blooming stage (7-22) | Early boll stage (8-5) | Full boll stage (8-18) | Early boll opening stage (8-28) | Irrigation quota |
|---|---|---|---|---|---|---|---|---|---|---|
| W1 | 30 | 30 | 30 | 36 | 36 | 42 | 36 | 30 | 30 | 300 |
| W2 | 37.5 | 37.5 | 37.5 | 45 | 45 | 52.5 | 45 | 37.5 | 37.5 | 375 |
| W3 | 45 | 45 | 45 | 54 | 54 | 63 | 54 | 45 | 45 | 450 |
| W4 | 52.5 | 52.5 | 52.5 | 63 | 63 | 73.5 | 63 | 52.5 | 52.5 | 525 |
| W5 | 60 | 60 | 60 | 72 | 72 | 84 | 72 | 60 | 60 | 600 |
| WCK | 45 | 45 | 45 | 54 | 54 | 63 | 54 | 45 | 45 | 450 |

Note: the number in brackets in the header is the date, M-D. The following table is the same.

2) Experiment of different drip irrigation frequency. After emergence, four drip irrigation frequencies were set: 12 times, 10 times, 8 times and 6 times, represented by p12, P10, P8 and p6. The drip irrigation quota is 450 mm. The experiment consisted of four treatments, repeated three times for a total of 12 cells. According to the law of cotton water demand with more in the middle stage and less in the early and late stages, the water distribution in each growth period was carried out. The distribution of water in each growth period is shown in **Table 2**.

**Table 2.** Experimental setup for different irrigation frequencies (mm).

| Treatment | Early bud stage (6-10) | Full budding period (6-17) | Full budding period (6-20) | Early flowering stage (6-24) | Early flowering stage (6-27) | Early flowering stage (7-1) | Early flowering stage (7-7) | Full flowering period (7-11) | Full flowering Period (7-13) |
|---|---|---|---|---|---|---|---|---|---|
| P12 | 27 | 27 | 0 | 36 | 0 | 36 | 45 | 0 | 45 |
| P10 | 36 | 0 | 45 | 0 | 0 | 45 | 0 | 54 | 0 |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| P8 | 0 | 45 | 0 | 0 | 54 | 0 | 54 | 0 | 63 |
| P6 | 0 | 63 | 0 | 0 | 0 | 72 | 0 | 81 | 0 |
| P12 | 45 | 0 | 45 | 36 | 36 | 0 | 36 | 36 | 450 |
| P10 | 54 | 0 | 54 | 45 | 45 | 0 | 36 | 36 | 450 |
| P8 | 0 | 63 | 0 | 63 | 0 | 54 | 0 | 54 | 450 |
| P6 | 81 | 0 | 0 | 81 | 0 | 0 | 72 | 0 | 450 |

*2.3. Determination Content and Method*

Single leaf photosynthetic index: photosynthetic ecological indexes such as net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intercellular $CO_2$ concentration (Ci) of the largest functional leaf (the top four leaves before topping, and the top largest leaf after topping) were measured by Li-6400xt photosynthetic analyzer at 12:00 on a sunny day.

Chlorophyll fluorescence characteristics: During the main growth period of cotton, clear and cloudless weather is selected to measure the main stem leaves of cotton. Before measurement, the leaves are dark treated for 30 minutes, and then the Yaxin-1105 chlorophyll fluorescence analyzer is used to measure the rapid chlorophyll fluorescence induction kinetic parameters of leaves under regular dark adaptation, including initial fluorescence ($F_0$), maximum fluorescence ($F_m$), variable fluorescence ($F_V$), potential photochemical activity of PSII ($F_V/F_0$), electron transfer of PSII ($F_m/F_0$), maximum photochemical efficiency (Fv/Fm), and photosynthetic performance index based on light absorption (PIABS).

Group Leaf Area Index (LAI): Cut 2 consecutive plants from each plot at different stages, pick all green leaves, lay them flat on white paper with no overlap facing down, place the label board (black cardboard 10cm × 10cm) and plot number in a corner of the white paper, take a frontal photo with more than 8 million pixels (based on complete framing), calculate the green leaf area using digital image processing technology [6], and calculate the group leaf area index (LAI) based on the total number of plants.

Chlorophyll value (SPAD): 10 cotton plants were randomly selected from each plot, and the relative chlorophyll value of the largest functional leaf on the main stem was measured using a 502 SPAD meter, and the average value was taken.

Functional leaf stress biochemical substances: Using an ELISA kit and a double antibody one-step sandwich enzyme-linked immunosorbent assay, the activity of stress enzymes such as peroxidase (POD) and superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in cotton functional leaves were determined.

## 3. Results and Discussion

*3.1. Effects of Different Water Treatments on Chlorophyll Fluorescence Induction Kinetic Parameters of Film Free Cotton*

Chlorophyll fluorescence is an important component of photosynthesis and can reflect changes in most photosynthetic processes [7]. As shown in **Table 3**, with the increase in irrigation water, all chlorophyll fluorescence parameters of film-free cotton showed an upward trend, except for $F_0$ which showed a downward trend. Among them, the $F_0$ of the W1 treatment was the largest, significantly higher than the W5 treatment during the bud, flower, boll, and lint stages, with increases of 25.05%, 32.67%, 12.55%, and 18.16% respectively. The $F_m$ and $F_V$ of the W5 treatment were the largest during the bud, flower, and lint stages, with increases of 14.94%, 15.45%, 30.09% and 16.32%, 20.52%, 34.71% compared to the W1 treatment. The $F_m$ and $F_V$ of the W4 treatment were the largest during the boll stage, with increases of 8.78% and 15.13% compared to the W1 treatment. The $F_V/F_m$, $F_V/F_0$, $F_m/F_0$, and PIABS of the W5 treatment were the largest during all periods, with increases of 5.48%-14.06%, 23.39%-34.92%, 10.74%-25.49%, and 55.78%-116.59% compared to the W1 treatment. It is evident that drought stress has a significant impact on the chlorophyll fluorescence parameters of film-free cotton,

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with PIABS being the most affected (the coefficient of variation CV for each treatment is 19.37%-27.10%). Appropriate higher irrigation levels (W4, W5 treatments) are beneficial to improve the photosynthetic fluorescence characteristics of film-free cotton and promote its material production capacity. Comparing the average values of each parameter of film-free cotton with the WCK treatment (mulched cotton), the $F_0$ of the WCK treatment is not significantly different from that of film-free cotton, while its $F_m$, $F_V$, $F_V/F_m$, $F_V/F_0$, $F_m/F_0$, and PIABS are increased by14.49%-20.18%, 22.13%-36.67%, 8.87%-12.25%, 24.72%-44.33%, 15.22%-36.08%, and 37.82%-132.97% compared to the average values of film-free cotton. It is clear that non-mulching has a significant impact on the leaf photosynthetic fluorescence parameters, with PIABS and $F_V/F_0$ being the most affected.

**Table 3.** Effect of irrigation amount on chlorophyll fluorescence parameters of cotton.

| Period | Process | $F_0$ | $F_m$ | $F_V$ | $F_V/F_m$ | $F_V/F_0$ | $F_m/F_0$ | PIABS |
|---|---|---|---|---|---|---|---|---|
| Bud stage | W1 | 216.33 a | 723.00 c | 514.73 d | 0.65 d | 2.18 d | 3.26 cd | 2.45 d |
| | W2 | 206.00 ab | 724.00 c | 524.41 cd | 0.67 cd | 2.32 cd | 3.18 d | 2.99 c |
| | W3 | 194.33 bc | 740.67 c | 535.64 c | 0.69 bc | 2.26 d | 3.32 cd | 3.24 c |
| | W4 | 188.00 c | 824.67 b | 535.86 c | 0.71 b | 2.55 bc | 3.49 bc | 3.38 c |
| | W5 | 173.00 d | 831.00 b | 598.73 b | 0.71 b | 2.69 b | 3.61 b | 4.38 b |
| | WCK | 198.33 bc | 910.67 a | 740.57 a | 0.77 a | 3.41 a | 4.43 a | 7.66 a |
| Blossom period | W1 | 246.33 a | 733.67 d | 545.91 d | 0.72 c | 2.52 d | 3.57 d | 5.54 c |
| | W2 | 219.00 b | 754.33 cd | 562.91 cd | 0.73 c | 2.79 c | 3.69 cd | 5.20 c |
| | W3 | 214.00 bc | 773.67 c | 580.93 cd | 0.75 bc | 2.84 c | 3.82 cd | 6.42 b |
| | W4 | 193.00 d | 792.33 c | 602.00 c | 0.74 c | 3.00 c | 3.87 c | 6.61 b |
| | W5 | 185.67 d | 847.00 b | 657.94 b | 0.78 b | 3.40 b | 4.48 b | 8.63 a |
| | WCK | 210.67 c | 937.67 a | 740.25 a | 0.81 a | 4.20 a | 5.04 a | 9.09 a |
| Boll stage | W1 | 275.00 a | 819.67 c | 568.27 d | 0.73 bc | 2.73 c | 3.52 d | 6.34 d |
| | W2 | 264.00 b | 849.33 bc | 596.90 c | 0.69 b | 2.48 c | 3.74 c | 7.21 d |
| | W3 | 256.00 bc | 868.33 bc | 609.99 c | 0.74 b | 2.89 bc | 3.83 c | 8.49 c |
| | W4 | 247.33 cd | 891.67 b | 654.23 b | 0.74 b | 2.88 bc | 3.89 c | 8.53 c |
| | W5 | 244.33 d | 855.33 bc | 643.71 b | 0.77 ab | 3.48 ab | 4.31 b | 10.55 b |
| | WCK | 258.00 b | 981.00 a | 750.63 a | 0.80 a | 4.04 a | 5.25 a | 11.79 a |
| Lint stage | W1 | 201.67 a | 585.00 d | 403.58 c | 0.64 d | 1.99 d | 2.98 d | 2.23 d |
| | W2 | 189.67 b | 612.33 cd | 423.48 c | 0.67 cd | 2.02 d | 3.03 d | 3.16 c |
| | W3 | 182.67 bc | 726.33 b | 522.13 b | 0.69 bc | 2.32 c | 3.26 c | 4.08 b |
| | W4 | 176.67 cd | 639.67 c | 426.30 c | 0.70 bc | 2.22 c | 3.22 c | 3.84 b |
| | W5 | 170.67 d | 761.00 a | 543.65 b | 0.73 ab | 2.49 b | 3.48 b | 4.83 a |
| | WCK | 183.67 bc | 792.33 a | 604.15 a | 0.76 a | 2.82 a | 3.68 a | 5.00 a |

Note: $F_0$ is the initial fluorescence intensity of the leaf; $F_m$ is the maximum fluorescence intensity; $F_V$ is the variable fluorescence intensity; $F_V/F_m$ is the maximum photochemical efficiency of PS II; $F_V/F_0$ is the potential photochemical activity of PS II; $F_m/F_0$ is the electron transport status of PS II; PIABS reflects the photosynthetic performance index based on absorbed light energy; different lowercase letters in the same column indicate significant differences between treatments ($P$<0.05), the same below.

As the frequency of irrigation increases, all fluorescence parameters except for $F_0$ show an increasing trend during various growth stages. Among them, the $F_0$ value is the highest for the P6 treatment, and it significantly differs from other treatments except for the P8 treatment during the bell stage. $F_m$, $F_V$, $F_V/F_m$, $F_V/F_0$, $F_m/F_0$, and PIABS all reach their maximum under the P12 treatment,

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which is significantly higher than the P6 treatment, with increases ranging from 8.48% to 42.80%, 13.71% to 43.98%, 11.11% to 27.66%, 28.81% to 64.29%, 15.14% to 45.27%, and 42.51% to 163.64%, respectively. This indicates that higher irrigation frequencies can improve leaf photosynthetic fluorescence parameters and enhance leaf photosynthetic function. From the perspective of the CV changes of each parameter under different treatments at various stages, PIABS has the highest variation, ranging from 15.37% to 44.75%, followed by F0, which varies from 20.55% to 25.59%, while $F_V/F_m$ has the smallest variation, ranging from 4.85% to 10.18%. This suggests that the irrigation frequency mainly affects the photosynthetic performance index and the initial fluorescence intensity, with a smaller impact on the maximum photochemical efficiency. (**Table 4**)

**Table 4.** Effect of Drip irrigation frequency on chlorophyll fluorescence parameters of cotton.

| Period | Process | $F_0$ | $F_m$ | $F_V$ | $F_V/F_m$ | $F_V/F_0$ | $F_m/F_0$ | PIABS |
|---|---|---|---|---|---|---|---|---|
| Bud stage | P12 | 192.67 c | 694.67 a | 408.29 a | 0.60 a | 1.52 a | 2.51 a | 1.16 a |
| | P10 | 271.67 b | 622.33 b | 384.32 b | 0.55 ab | 1.49 a | 2.24 b | 0.84 b |
| | P8 | 245.00 b | 622.00 b | 335.08 c | 0.52 bc | 1.21 b | 2.22 b | 0.46 c |
| | P6 | 324.00 a | 568.33 c | 321.05 c | 0.47 c | 1.18 b | 2.18 b | 0.44 c |
| Blossom period | P12 | 289.33 b | 916.33 a | 682.54 a | 0.70 a | 2.51 a | 3.50 a | 3.52 a |
| | P10 | 278.33 b | 852.00 ab | 575.75 b | 0.64 bc | 1.88 b | 3.39 a | 2.79 bc |
| | P8 | 288.67 b | 795.67 b | 549.38 b | 0.67 ab | 2.36 a | 2.71 b | 3.2 ab |
| | P6 | 439.67 a | 641.67 c | 474.07 c | 0.60 c | 1.66 c | 2.49 b | 2.47 c |
| Boll stage | P12 | 288.33 b | 993.33 a | 750.93 a | 0.80 a | 3.91 a | 4.91 a | 9.51 a |
| | P10 | 243.33 b | 990.00 a | 711.43 b | 0.74 b | 3.28 b | 4.06 b | 9.12 a |
| | P8 | 386.00 a | 920.00 b | 745.26 ab | 0.73 b | 3.06 b | 4.27 b | 6.31 b |
| | P6 | 430.67 a | 915.67 b | 660.39 c | 0.65 c | 2.38 c | 3.38 c | 4.59 c |
| Lint stage | P12 | 193.33 b | 861.67 a | 406.19 a | 0.60 a | 1.53 a | 2.53 a | 2.29 a |
| | P10 | 189.00 b | 630.67 c | 381.63 ab | 0.58 ab | 1.33 b | 2.33 b | 1.48 b |
| | P8 | 219.67 b | 679.00 b | 365.02 bc | 0.55 b | 1.13 c | 2.12 c | 1.00 c |
| | P6 | 288.00 a | 628.67 c | 340.65 c | 0.54 b | 1.03 c | 2.03 c | 0.90 c |

*3.2. Effects of Different Water Treatments on SPAD of Film Free Cotton*

From **Figure 1**, it can be seen that the SPAD value of film-free cotton leaves shows a trend of first increasing and then decreasing with the growth process, reaching its maximum at 88 days after seedling emergence (peak flowering period). In the irrigation experiment, the average SPAD value of WCK leaves was the highest, increasing by 2.86%-19.78% compared to non film cotton. The average SPAD value of cotton leaves without film treatment showed W3>W4>W2>W5>W1, with W3 treatment significantly higher than W1, W5, and W2 treatments, a decrease of 1.60 compared to WCK, but the difference was not significant. In addition, from the perspective of the variation in SPAD values during the growth period (CV) of each treatment, W3 had the smallest variation (CV=8.95%), which was not significantly different from WCK (CV=9.66%). The W1, W2, and W5 treatments showed greater variation (CV=14.56%-14.84%), indicating that appropriate irrigation can promote the improvement of leaf function in non film cotton, prevent premature aging of leaves, and that excessive or insufficient irrigation can be detrimental to leaf function. In the drip irrigation frequency test, the average SPAD value of the leaves showed P10>P8>P12>P6, with P10 being 57.09, an increase of 11.01%, 4.99%, and 5.32% compared to P6, P8, and P12, respectively. The CV values of SPAD for each treatment ranged from 11.84%-12.96%, indicating that appropriately higher irrigation times are beneficial for the development of leaf function.

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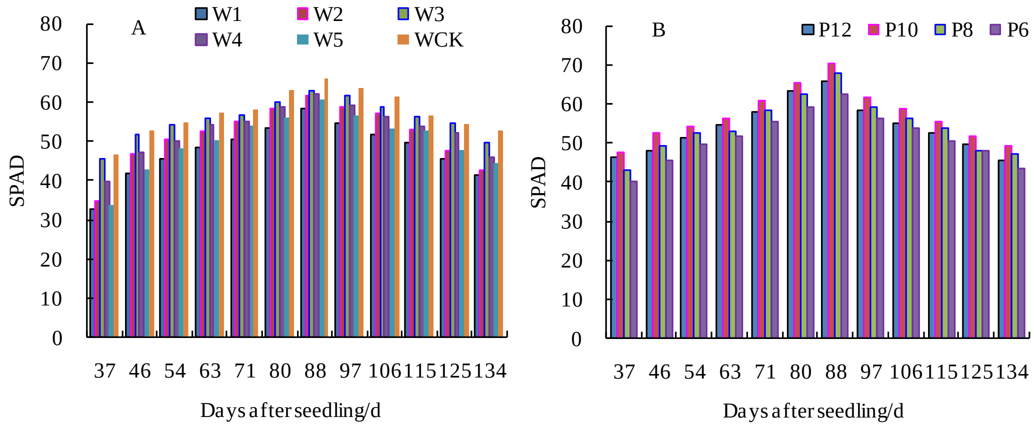


**Figure 1.** Effects of different irrigation amount (A) and irrigation frequency (B) on the relative chlorophyll content (SPAD) of cotton.

*3.3. Effects of Different Water Treatments on Leaf Area Index (LAI) of Film Free Cotton*

From **Figure 2**, it can be seen that the LAI of film-free cotton shows a trend of first increasing and then decreasing as the growth process progresses. It rapidly increases after the first flowering period, reaches its maximum during the peak flowering period, and rapidly decreases after the bolling period. In the irrigation experiment, the LAI of all treatments of non film cotton increased with the increase of irrigation water, showing W5>W4>W3>W2>W1. W5 increased by 20.52%-60.37% compared to WCK at each stage. During the boll opening period, WCK experienced a sharp decrease in LAI due to its shallow root system and the cessation of irrigation. From the average value of LAI, there is not much difference between W3 treatment and WCK, but its CV value of LAI is smaller than W1, W2, and WCK, indicating that appropriate irrigation can maintain good LAI dynamics and maintain the photosynthetic area in the later stage of the canopy. In the drip irrigation frequency experiment, the LAI of each treatment increased with the increase of drip irrigation frequency, showing a trend of P12>P10>P8>P6 at all stages. The P12 treatment increased by 18.78%-50.42% compared to the P6 treatment. Due to fewer irrigation cycles, there is significant variation in LAI during each period in P6 treatment. It can be seen that increasing irrigation frequency is beneficial for the development of canopy leaf area in non membrane cotton.

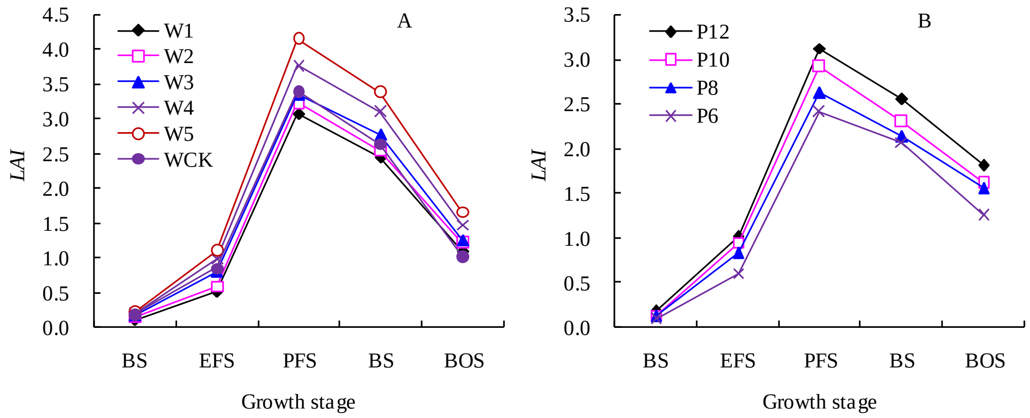


**Figure 2.** LAI dynamics of cotton population under different irrigation volume (A) and irrigation frequency (B), BS,EFS,PFS,BS and BOS respectively represent the budding stage ,early flowering stage, peak flowering stage, boll stage and boll opening stage. The same are as below.

*3.4. Effects of Different Water Treatments on Photosynthetic Characteristics of Film Free Cotton*

As shown in **Figure 3**, the net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) of film-free cotton leaves exhibited a trend of initially increasing and then

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decreasing with the progression of the growth stage, reaching their maximum during the peak flowering period. Specifically, the average values of Pn, Gs, and Tr for different irrigation treatments of film-free cotton during this period decreased by 19.30%, 9.55%, and 18.26% compared to the WCK, respectively. The intercellular $CO_2$ concentration (Ci) showed a trend of initially decreasing and then increasing with the growth stage, reaching its lowest point during the peak flowering period. The average Ci value for the film-free cotton treatments decreased by 22.87% compared to the WCK during this period. The coefficients of variation (CV) for the average values of Pn, Gs, Tr, and Ci for each film-free cotton treatment at different stages were 23.32%, 49.17%, 33.08%, and 34.51%, respectively, indicating an increase of 0.32, 10.42, 4.71, and 2.69 percentage points compared to the WCK. This suggests that the photosynthetic parameters of film-free cotton leaves are generally lower than those of film-covered cotton, and there are significant variations in their changes during the growth period. Among different irrigation treatments, the order of Pn values for film-free cotton during the flowering period was W4>W3>W2>W5>W1, with Gs values being W3>W4>W2>W5>W1, Tr values being W4>W3>W2>W5>W1, and Ci values being W3>W4>W5>W2>W1. The average values of each photosynthetic parameter showed a trend of W3>W4>W2>W5>W1. Specifically, the Pn, Gs, Tr, and Ci values for the W3 treatment reached 23.75 $\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$, 0.40 $mol \cdot m^{-2} \cdot s^{-1}$, 15.18 $mmol \cdot m^{-2} \cdot s^{-1}$, and 226.21 $\mu mol \cdot mol^{-1}$, respectively, which increased by only 3.15% to 7.21% compared to W4, a slight difference. Compared to W2, it increased by 5.56% to 19.42%, compared to W5, it increased by 6.59% to 33.16%, and compared to W1, it increased by 11.02% to 39.53%. It can be seen that the W3 and W4 irrigation treatments effectively improved the photosynthetic function of film-free cotton leaves, mainly reflected in the increase of Pn, Gs, and Tr indicators. Increasing the frequency of irrigation is beneficial to improving leaf photosynthetic parameters, with the P12 treatment having the highest photosynthetic parameters and the P6 treatment having the lowest. Among the average photosynthetic parameter values for each treatment throughout the period, the P12 treatment increased by 2.15% to 7.18% compared to the P10 treatment, increased by 1.58% to 8.78% compared to the P8 treatment, and increased by 9.04% to 15.84% compared to the P6 treatment. From the perspective of the impact of irrigation amount and frequency on leaf photosynthetic parameters, the average CV of Pn, Gs, Ci, and Tr for each irrigation treatment was 13.21%, 13.51%, 3.88%, and 8.70%, respectively, while the CV for irrigation frequency treatment was 4.97%, 6.17%, 3.64%, and 5.98%. It can be seen that the irrigation amount has a greater impact on leaf photosynthetic parameters, especially on Pn and Gs.

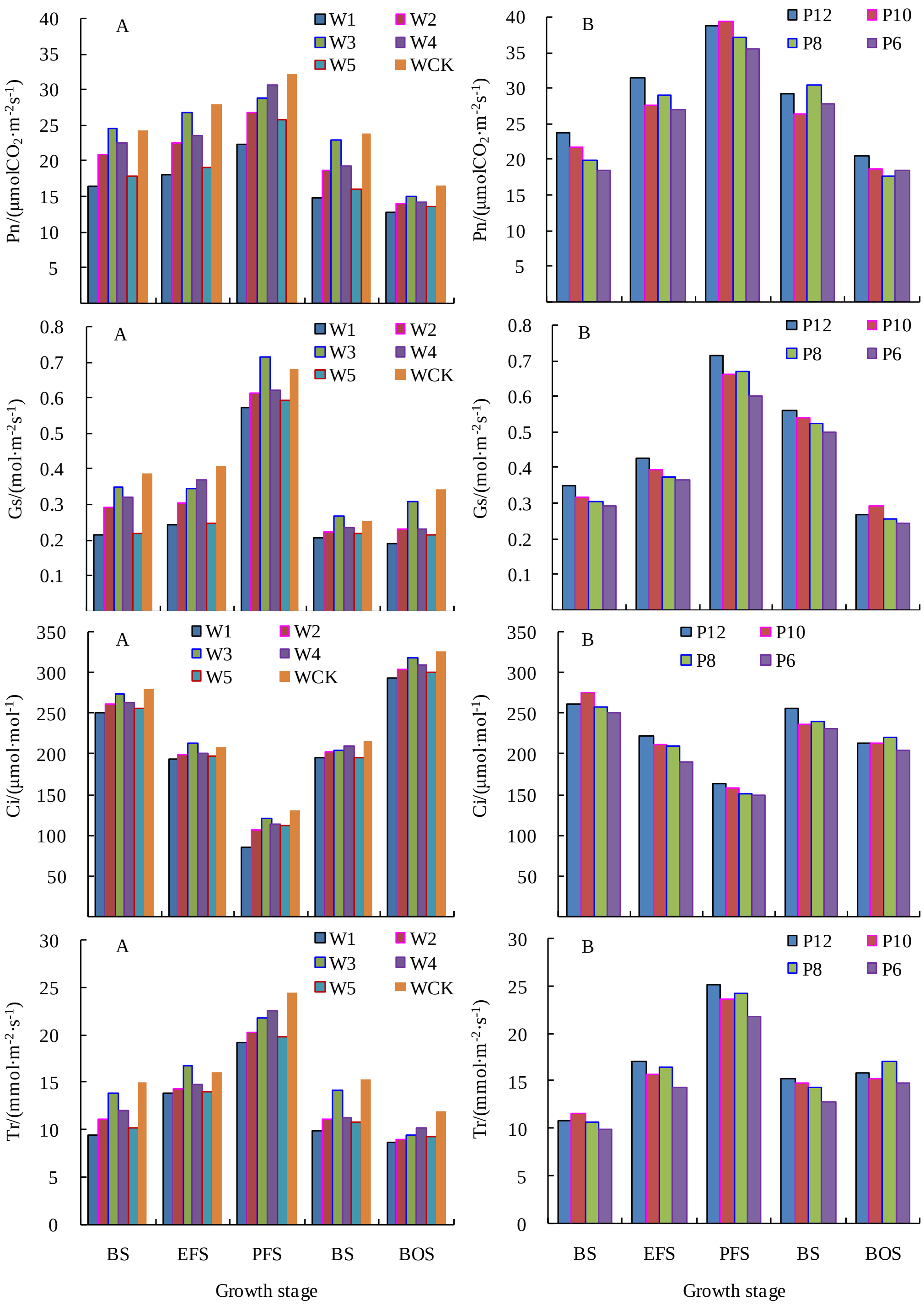


**Figure 3.** Effects of different irrigation amount (A) and irrigation frequency (B) on leaf light and parameters of film free cotton.

### *3.5. Effects of Different Water Treatments on Stress Biochemical Substances of Film Free Cotton*

#### 3.5.1. Effect on Malondialdehyde (MDA) Content

From **Figure 4**, it can be seen that the MDA content in leaves shows an upward trend with the growth process, with an average increase of 24.31% during the boll open period compared to the bud period in each treatment. There is not much difference between the initial flowering period and the peak flowering period. The MDA content in film-free cotton leaves is generally higher than that in WCK, with an average value of 4.97%-8.42% higher in each treatment and stage compared to WCK, indicating that non membrane coverage has certain adverse effects on cotton growth. The MDA content showed a trend of first decreasing and then increasing with the increase of irrigation volume

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among different irrigation volumes, manifested as W3<W4<W2<W5<W1. The MDA content of W3 treatment was 0.94 mol·gFW$^{-1}$ during the peak flowering period, which was not significantly different from W4, but decreased by 2.53%, 4.63%, and 6.57% compared to W2, W5, and W1, with an average decrease of 5.32%, 8.95%, and 9.95%, respectively; In the drip irrigation frequency experiment, the MDA content of each treatment showed a decreasing trend with the increase of drip irrigation frequency. The P6 treatment had the highest MDA content, which increased by 9.05%-13.62% compared to P12. This indicates that appropriate water treatment (W3, W4) and increasing irrigation frequency are beneficial for reducing the harm of water stress in film-free cotton.

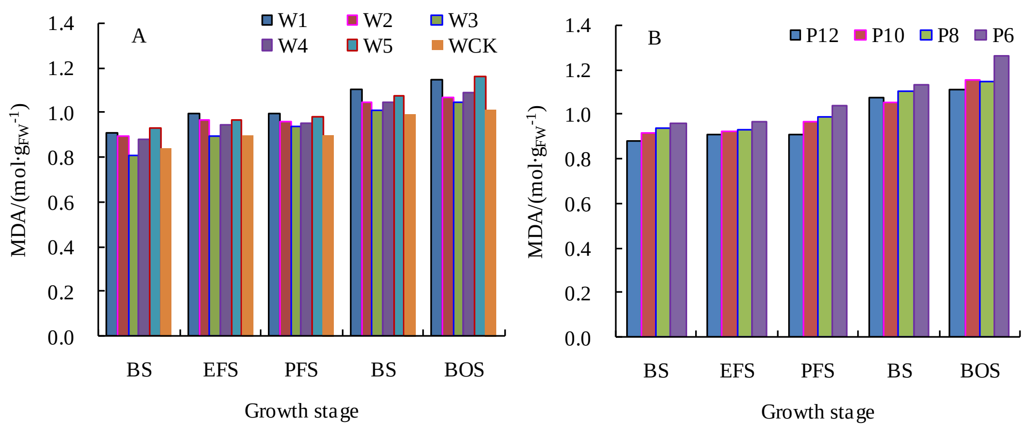


**Figure 4.** Effects of different irrigation amount (A) and irrigation frequency (B) on the MDA content of film free cotton.

3.5.2. Effect on the Activity of Stress Protective Enzymes

Superoxide dismutase (SOD) is an important antioxidant enzyme in the stress protective enzyme system, playing a crucial role in cellular antioxidant and anti-aging. Peroxidase (POD) is an enzyme that removes peroxides from the plant body and can reduce the damage of reactive oxygen species to cotton plants. According to **Figure 5**, as the growth period progresses, the activity of various protective enzymes shows a trend of first increasing and then decreasing, with the highest activity occurring during the peak flowering period to the boll stage. The SOD and POD activities of film-free cotton were lower than those of WCK, and the average values of each irrigation treatment were 6.43%-13.46% and 4.52%-12.32% lower than those of WCK. The SOD and POD activities between different irrigation treatments were W3>W4>W2>W5>W1. The average SOD and POD activities of W3 treatment were not significantly different from W4 treatment (2.08%-2.94%), but significantly different from W5 and W1 treatments, increasing by 11.23%, 22.44%, 8.35%, and 11.63%, respectively. The appropriate irrigation amount is beneficial for improving the activity of protective enzymes. Increasing the number of irrigation sessions is beneficial for improving SOD and POD activities, with the average values of P12 treatment increasing by 3.81%, 6.00%, 10.56% and 3.89%, 3.87%, 10.06% respectively compared to P10, P8, and P6 treatments.

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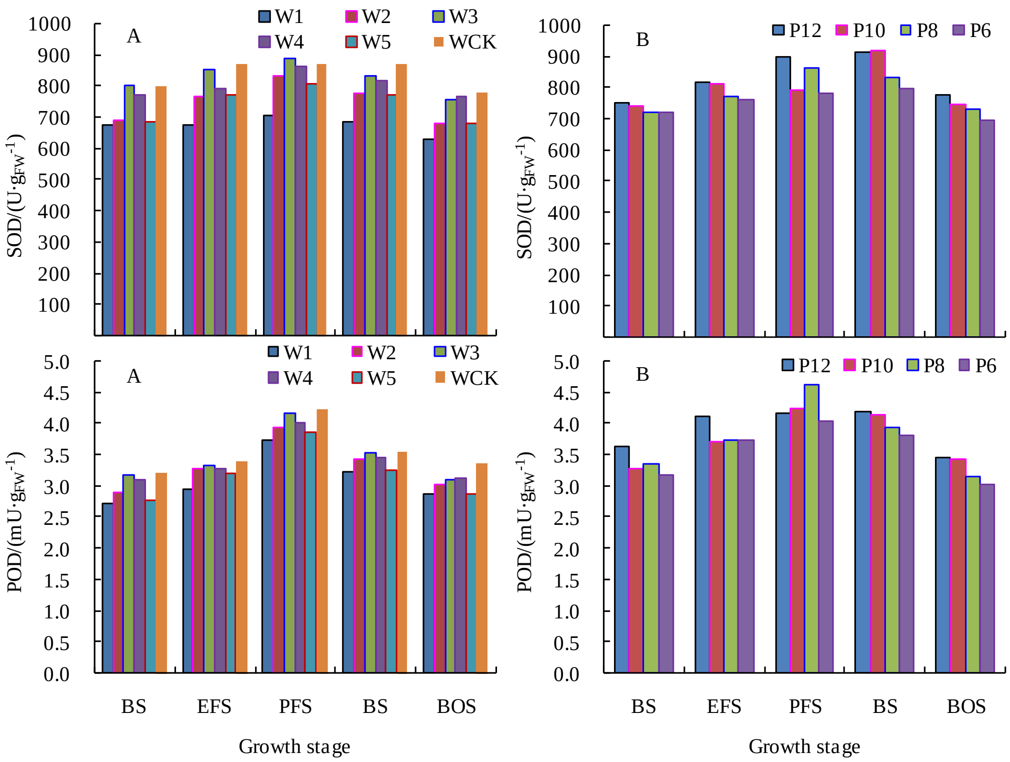


**Figure 5.** Effects of different irrigation amount (A) and irrigation frequency (B) on SOD and POD activities of film free cotton.

*3.6. Effects of Different Water Treatments on Yield of Film Free Cotton*

According to **Figure 6**, among different irrigation treatments, WCK had the highest yield, increasing by 38.5% to 130.7% compared to the no film treatment. With the increase of irrigation water, the yield of film-free cotton showed a trend of first increasing and then decreasing. The W3 treatment had the highest yield, reaching 4833.25 kg·hm$^{-2}$, which was not siFgnificantly different from the W4 treatment but significantly higher than other treatments; In different irrigation frequency treatments, the yield of film-free cotton gradually increased with the increase of irrigation frequency. The yield of P12 treatment reached 5183.71 kg·hm$^{-2}$, which was not significantly different from P10 treatment but significantly higher than P8 and P6 treatments. It can be seen that a suitable amount of irrigation and a higher frequency of irrigation are beneficial for unleashing the potential yield of film-free cotton.

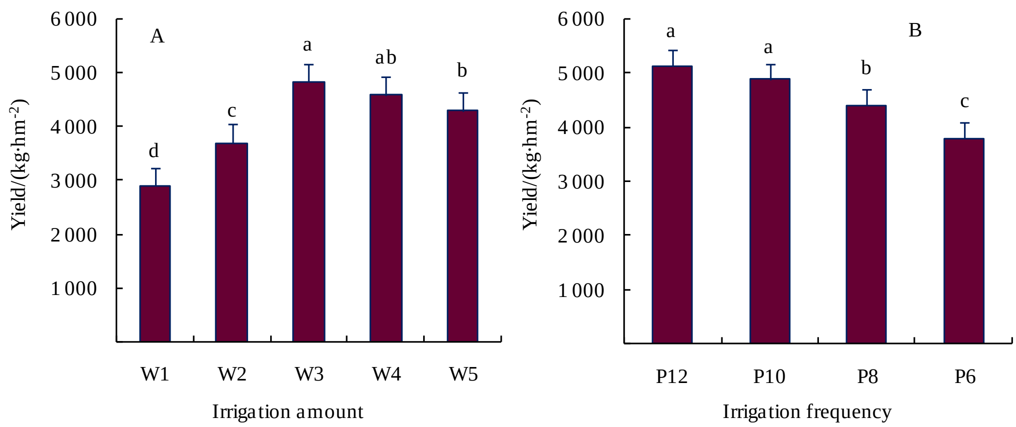


**Figure 6.** Effects of different irrigation amount (A) and irrigation frequency (B) on seed cotton yield of film free cotton. Different lowercase letters in the same column indicate significant differences between different irrigation treatments at the same time ($P$<0.05).

## 4. Discussion

Chlorophyll fluorescence is closely related to photosynthesis, reflecting the absorption, transmission, and conversion of light energy by leaves [8,9]. Fluorescence changes can reflect the situation of photosynthesis and heat dissipation [10,11] . Genty et al. 12 ]reported that drought had little effect on the chlorophyll fluorescence parameters of cotton. The results of this study indicate that water deficit has a significant impact on chlorophyll fluorescence parameters. The $F_0$ of water deficit treatment significantly increased, while the $F_m$, $F_V$, $F_V/F_m$, $F_V/F_0$, $F_m/F_0$, and PIABS indicators showed a decreasing trend with the deepening of water deficit. This is consistent with the research results of Xue et al. [13] and Dou et al. [14]. Abdulkadir et al. [15] believe that drip irrigation 7 times in film covered cotton fields in southern Xinjiang is beneficial for the development of leaf photosynthetic function. In this study, increasing the number of irrigation times can effectively reduce the degree of photoinhibition or damage to PSII complexes. The fluorescence parameters of leaves treated with P12 reached a good level, which may be related to the ecological differences in soil moisture between the film covered and non film covered plots. Maintaining a high drip irrigation frequency for non film covered cotton can effectively maintain the soil moisture content in the root zone and promote leaf activity of cotton plants [16].

The moisture condition is an important factor affecting photosynthesis. Previous studies have found that when there is a water deficit, the stomata of crop leaves close, inhibiting the absorption of $CO_2$, resulting in a decrease in photosynthesis and a decrease in light energy utilization efficiency [17,18]. The results of this study indicate that appropriate irrigation amounts (W3, W4) can effectively improve leaf photosynthetic parameters and enhance photosynthetic function, which is consistent with previous research [19]. However, excessive water irrigation is not conducive to maintaining high photosynthetic function in film-free cotton leaves. Increasing the number of irrigation cycles can promote the photosynthetic capacity of film-free cotton leaves, with P12 having the best effect, which is different from Li et al. [20] who believed that drip irrigation for 8 cycles resulted in the highest photosynthetic capacity of leaves. In addition, this experiment found that the photosynthetic parameters of cotton with film were higher than those of cotton without film, and the differences in their growth period were smaller. This may be related to the improvement of ecological conditions in the root and canopy layers of cotton under film conditions [21].

It is generally believed [22,23]that LAI increases with the increase of irrigation volume, and the time to reach its peak shifts later. When the water volume is too low, the population becomes smaller and the photosynthetic area decreases. As an antioxidant response indicator of plant membrane system stress perception, MDA content increases with the degree of water stress, while the activity of protective enzymes such as SOD and POD, which regulate the content of osmotic substances in the body, shows a decreasing trends [24,25]. In this study, both water deficit and excess water had negative effects on the LAI, MDA content, and protective enzymes of film-free cotton. The CV of LAI, MDA content, SOD activity, and POD activity changes during cotton growth in water deficit treatment (W1, W2) were 84.54%-87.61%, 9.10%-10.09%, 6.16%-8.61%, 12.15%-13.29%, respectively, which were higher than those in multi water treatment (W4, W5) (77.10%-78.17%, 8.29%-9.10%, 5.11%-7.67%, 11.23%-12.86%). This indicates that water deficit has a greater impact on the physiological functions of film-free cotton than excessive water, which is also one of the reasons why film-free cotton has higher water requirements [26]. In addition, it was found in this study that the appropriate irrigation amount treatments (W3, W4) increased LAI, SOD activity, and POD activity by 4.90%-17.64% and 21.07%-42.68%, 3.99%-4.99% and 2.40%-6.53%, 0.29%-8.66% and 2.45%-8.08%, respectively, compared to WCK during the boll and opening stages. This indicates that the population growth of film-free cotton is higher in the later stage, which may be related to the more developed root system of film-free cotton [27], and this is also one of the reasons why non membrane cotton is less prone to premature aging [28].

The test found that compared with WCK, the average value of chlorophyll fluorescence parameters in the whole period of the suitable irrigation amount treatment (W3, W4) of film-free cotton , except for $F_0$, other parameters such as $F_0$, $F_m$, $F_V$, $F_V$ / $F_m$, $F_V$ / $F_0$, $F_m/F_0$, PIABS were reduced by 20.58%, 33.21%, 10.19%, 48.22%, 36.77%, 70.68%, leaf SPAD value and Pn, Gs, Ci, Tr were reduced by 5.55%, 9.04%, 10.16%, 4.64%, 12.93%, protective enzyme activity was reduced by 3.36%-3.58%, MDA content increased by 3.16% and the yield decreased by 42.07%. It can be seen that the physiological function of the leaves of cotton without film is reduced to a certain extent compared with that with film, which may be related to the fact that the field water ecological conditions of cotton without film mulching are not as good as those with film mulching [29], resulting in a significant decline in yield. There are also studies that there is no significant difference between the yield of cotton cultivated without film and that of cotton with film [30], which may be related to the cultivation method and needs further study.

## 5. Conclusions

(1) The leaf physiological function of film-free cotton was the strongest at the flowering and bolling stage, and its function peak had a backward trend compared with film covered cotton. However, the leaf physiological function of film-free cotton was lower than that of film covered cotton, and it was greatly affected by water conditions, and the degree of influence was greater than irrigation frequency.

(2) Appropriately increasing the amount and frequency of irrigation was beneficial to improve the chlorophyll fluorescence parameters of film-free cotton leaves, which mainly showed that PIABS and $F_v/F_0$ increased significantly. The SPAD value and photosynthetic indexes (Pn, Gs, Tr, Ci) of leaves in W3 and P12 treatments were larger, and had little difference with W4 and P10 treatments. The LAI and WCK in W3 treatment had little difference, and the changes in each growth period were small. The MDA content of W3, W4 and P12 treatments were lower, the activities of SOD and POD were higher, and the yield was the largest, indicating that the irrigation amount of 450-525 mm and irrigation times of 10-12 times were beneficial to the physiological function of the leaves of film-free cotton, which could be used as a reference for water management in production practice.

**Author Contributions:** J.W. and F.S. initiated and designed this study. F.S., Z.G., B.W. and S.C. conducted experiments and collected data. F.S. and J.W. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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