Alterations in leaf photosynthetic electron transport in Welsh onion (*Allium fistulosum* L.) under different light intensity and soil water conditions

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Abstract

Background: Welsh onions are often affected by stressful environments, such as high light and drought, during summer cultivation, which hinders their growth. To date, few studies have focused on leaf photosynthesis of Welsh onions during summer. We used carbon dioxide assimilation and OJIP transient and MR curves to analyze the photosynthetic characteristics of Welsh onions. **Results:** The results showed that strong light and drought could lead to a decrease in leaf pigment content. Simple high light stress caused a decrease in the net photosynthetic rate through stomatal limitation, while the simple drought treatment and the two stress factors combined caused a nonstomatal limitation. PSII energy distribution indicated that strong light and drought stress reduced the photochemical quantum efficiency of PSII. OJIP curve analysis showed that FO and FJ were increased, Fm was decreased, and a distinct K-phase was induced. In addition, OJIP parameters, including RC/CSO, TRO/ABS, ETO/TRO, and PIABS, were significantly reduced. MR analysis showed that strong light and drought stress blocked MR transients, leading to a gradual decrease in VPSI and VPSII-PSI. **Conclusions:** In general, the photosynthesis of Welsh onion was inhibited by high light and drought, which destroyed the receptor and donor side of PSII and reduced the electron transport capacity of PSII and PSI.

Background

In the summer in northern China, a combination of high irradiance, high temperature and water stress (i.e., drought) is very common[1]. These limiting factors usually last a long time in the summer, which seriously affects plant growth and survival. When exposed to both drought and high light, plants respond with defensive reactions that involve more efficient water use, maintain high carbon dioxide assimilation with few stomatal openings, and initiate several photoprotective measures [2, 3]. Some studies have shown that photosynthesis is affected when plants are exposed to two or more stresses in the environment [4]. Photosynthesis is the main physiological process of plants. The two photosystems [Photosystem I (PSI) and Photosystem II (PSII)] are vulnerable to damage during photosynthesis [4, 5].

Drought stress can induce stomatal closure and reduce the maximum quantum efficiency of PSII, resulting in reduced photosynthesis[6, 7]. As drought progresses, photosynthetic carbon assimilation and growth become limited. Photoinhibition occurs in plants when the amount of available light exceeds that required for photosynthesis[4, 5, 7]. PSII is the main target of photosynthetic photoinhibition [7, 8]. The photosynthetic electron transfer process is driven by light excitation from H₂O to coenzyme II (NADP⁺). After the photosynthetic pigment absorbs light energy, it concentrates the energy in the reaction center, causing charge separation and a photochemical reaction. PSII takes electrons from water, oxidizes the water, releases oxygen, and then PSI transfers the electrons to NADP⁺ and reduces the NADP⁺ to NADPH, which passes through a series of electron carriers, such as plastoquinone (PQ), plastocyanin (PC), the cytochrome b₆f complex (Cyt b₆f), and ferredoxin (Fd)[9, 10]. Any change in the sites of the electron transport chain affects photosynthetic efficiency. The existing techniques, including use of the fast chlorophyll fluorescence induction kinetics curve (OJIP curve) and the modulated 820 nm reflection curve (MR curve), can be used to study the changes in the photosynthetic electron transport chain[11]. As the name suggests, the typical OJIP curve includes phases O, J, I, P, etc., which contain a large amount of information about the far-end photochemical reactions of the PSII reaction center. Through JIP-test analysis, changes in the photosynthetic apparatus of plant material under the influence of environmental factors can be obtained. Determining the modulated reflection mode at 820 nm is an effective way to study the redox state of PSI under continuous light[12]. In fully dark-adapted plant leaves, the PSI reaction center and the P₇₀₀ primary electron donor (PC) were in a reduced state. After illumination, P₇₀₀ and PC were converted to an oxidized state. Therefore, the reduction of PSI and PC can result in a decrease in absorbance at 820 nm, while the oxidation of PSI and PC can increase the absorbance[13].

Welsh onion (*Allium fistulosum* L.), the most important seasoning vegetable in Asia, is mainly cultivated during summer, so the seedlings are susceptible to high light intensity and drought. It is necessary to evaluate the growth status of Welsh onions during summer. Surprisingly, there are no reports on the effects of the combined stresses of drought and high light on Welsh onions. In this experiment, we aimed to study the resistance of Welsh onion leaves under drought and high light conditions by examining photosynthetic parameters, focusing on the interaction between PSII and PSI using carbon dioxide assimilation, the OJIP curve and the MR curve. Our research further elucidates the photosynthetic reaction mechanism of Welsh onions under drought and high light stress and provides a theoretical basis for summer planting.

Methods

Plant material and experimental design

'Zhangqiu', a typical Chinese Welsh onion cultivar, was used as the plant material for all experiments. The Welsh onion seeds, obtained from Taian Denghai Wuyue Taishan Seed Industry Co., Ltd., were rinsed with sterilized distilled water, sown in plugs containing substrate, and grown in a greenhouse with standard irrigation and fertilization. Representative seedlings with four leaves were transferred to plastic pots (diameter 110 mm, height 140 mm; four seedlings per pot) containing 1000 g substrate composed of a sandy loam-soil: peat mixture (1: 1, v: v). The experiment was designed using a two-factor random block design. Factor A was the shading treatment and included no shading (N) and 20% shading (S). Factor B was the water stress treatment and included drought conditions (40% of field capacity, D) and a normal water supply (80% of field capacity, W). There were four treatments, ND, NW, SD, SW. In the 'N' treatment, plants were exposed to natural conditions. Plants in the 'S' treatment were maintained on a plastic tunnel covered with shade cloth with a retention capacity of 80% of sunlight. To prevent the roots of the potted seedlings

from absorbing water by capillary action from the ground, a watering basin was placed under each pot. A rainproof shelter was set up in the treatment area, the shelter was opened before rain events and was closed on sunny days. The plants were watered once daily at 09:00 using the pot weighing method to estimate soil moisture. Each treatment was replicated three times, and each replicate included twenty seedlings. In addition, HOBO Data Loggers (MicroDAQ.com, Ltd., NH, USA) were fixed around the test plants of each treatment to record the photosynthetically active radiation (PAR) and air temperature every 30 min (Fig. 1). All measurements were conducted at 10 and 20 d of treatment unless otherwise stated.

Leaf pigments

For each treatment, 0.51 cm² leaf discs were cut from functional leaves. Five discs were immersed in 25 mL tubes with 20 mL of 95% ethanol, incubated in the dark at 25°C, and then extracted for 24 h. The amount of pigment in the solution was quantified using an ultraviolet-visible spectrophotometer (UV-2450, Shimadzu, Japan) as described in Zhao[14].

Photosynthetic gas exchange measurements

Using a CIRAS-3 portable system equipped with a 1.75 cm² leaf chamber, we measured the net photosynthetic rate (Pn), stomatal conductance (Gs) and substomatal CO₂ concentrations (Ci) between 8:00 and 11:00 in the afternoon. For all the gas exchange measurements, the constant flow rate was set at 200 μmol/s, the CO₂ partial pressure was 380 μmol/mol (unless otherwise stated), and the leaf temperature was 25°C. The relative air humidity was set between 60% and 70%. The photon flux density (PFD) was provided by a mixture of red (90%) and blue (10%) LEDs in the leaf chamber. All gas exchange measurements were taken from the youngest, fully expanded leaf, and corrected for diffusive leaks between the cuvette and the surrounding atmosphere. The diurnal variation in photosynthesis was measured using a CIRAS-3 portable photosynthetic apparatus. The functional leaf assay was selected, and the measurements were taken from 7:00-17:00 every 2 h. Three leaves were chosen for each treatment as repeats.

Chlorophyll fluorescence

Using the Mini-Imaging-PAM chlorophyll fluorescence system (Heinz Walz GmbH, Effeltrich, Germany), we performed the chlorophyll fluorescence imaging in detached leaves from the four treatments. Plants used for chlorophyll fluorescence were dark adapted for 30 min before measurement. We acquired images of the effective PSII quantum yield [Y(II)], the quantum yield of regulated energy dissipation [Y(NPQ)], and the quantum yield of nonregulated energy dissipation [Y(NO)].

Measurements of chlorophyll a transient fluorescence

Chlorophyll a transient fluorescence was measured with dark-adapted leaves (in dark for 1 h) at room temperature (25°C) by a Handy PEA fluorometer (Hansatech Instruments, UK). Dark-adapted leaves were illuminated for 1 s with 3000 μmol/m²/s while chlorophyll fluorescence was induced. A typical OJIP curve resembles the different fluorescence states; the first rise from the origin is denoted as O which ascends to an intermediate state (the J step, at 2 ms) followed by a second slower rise involving a second intermediate (the I step, at 30 ms), while the P step is the maximum fluorescence measured. To compare measurements made on different samples, all values of fluorescence were standardized and analyzed with the JIP-test[9]. The JIP-test parameter used in this study and its formulae and explanation are listed in Supplementary Table 1.

MR analysis

The MR transient was measured with a Multi-Functional Plant Efficiency Analyzer (M-PEA, Hansatech Instruments, UK) as described by Strasser[12]. Before measurement, the plants were dark adapted for 20 min. MR was expressed as MR/MR₀, where MR represented the modulated reflection signal during illumination, MR₀ was the value at the onset of the actinic illumination, and MR/MR₀ represented the value of the 820 nm reflection at the onset of actinic illumination. The maximal decrease in the slope (V_{PSI}, the maximum PSI oxidation rate) and the maximal increase in the slope (V_{PSII-PSI}, the rate of PSI reduction) were derived from the MR/MR₀.

Statistical analysis

Experimental data were collected from the four treatments in a random order and using three independent replicates. The data are expressed as the mean ± standard deviation of three independent experiments. All statistical analyses were performed using DPS software. Calculations were performed using one-way ANOVA and Duncan's multiple range test. p < 0.05 was considered to be statistically significant.

Results

Leaf pigment and photosynthetic gas exchange parameters

Table 1 shows that the chlorophyll and carotenoid contents and Pn decreased significantly under drought conditions, with decreases of 19.1%, 21.1%, and 64.5% under high light, and 20.7%, 18.7%, and 55.1%, respectively, under 20% shading. Compared with natural light, shading increased the leaf chlorophyll and carotenoid contents, which increased by 4.9% and 9.4% under drought conditions and by 7.0% and 6.2%, respectively, under

normal irrigation conditions. As the processing time progressed, the effects of high light and drought on plants increased. The chlorophyll content, carotenoid content and net photosynthetic rate of SW were significantly higher than those of the other three treatments at 20 d. The chlorophyll content of SW was 49.2%, 34.3%, and 45.0% higher than that of ND, NW and SD, respectively. The carotenoid content of SW was 37.6 %, 32.3%, and 27.3% higher, and the Pn was 6.3, 0.7, 2.3 times higher than the corresponding values of the other treatments. With prolonged drought stress time, the Pn decreased significantly. At 10 d, compared with SW, the net photosynthetic rate of ND, NW and SD decreased significantly, while the Gs and Pn showed the same trend, and the Ci decreased slightly, but this difference was not significant. We hypothesized that the decrease in Pn was due to the closure of the pores, resulting in a reduction in the carbon dioxide supply. When treated for 20 d, the decrease in Pn was intensified, and the Gs trend was basically the same, while the Ci of ND and SD was higher than that of SW, which may be the reason for the decrease in Pn caused by nonstomatal limiting factors; NW showed a decrease in Gs and an increase in Ci, which may have been caused by stomatal factors.

The trends in the diurnal variation of Pn were similar under the different treatments, and all showed a “double peak” curve (Fig. 2). The highest peak appeared at approximately 11:00, and the Pn was the highest for SW. Between 11:00 and 13:00, as the PAR and temperature rose, the Pn dropped rapidly and peaked at approximately 15:00.

The quantum efficiencies of PSII

Hendrickson et al. (2004) noted that the excitation energy of PSII absorption in plant leaves was mainly used in the following three ways: Y(II), Y(NPQ), and Y(NO). Under natural light, the photosynthetic mechanism operates quickly, and the light energy absorbed by the Welsh onion leaves was mainly used for photochemical processes and heat dissipation. SW had the highest Y(II), which was 22.1%, 20.0%, and 14.1% higher than that of ND, NW, and SD, respectively. The Y(NPQ) was highest with NW and lowest with SW. The Y(NO) of ND was significantly higher than that of NW, SD, and SW by 15.0%, 13.7%, 12.9%, respectively.

OJIP curve and JIP-test

All OJIP curves showed multiphase changes as the OJIP phase increased. At the O-step, the fluorescence yield was minimal because Q_A was almost completely oxidized after dark adaptation. When exposed to saturated pulsed light, with the accumulation of Q_A^- , the fluorescence yield gradually increased. Once Q_A completely entered the reduced state, the PSII reaction center was completely closed, and the quantum yield was no longer accepted. At this time, the maximum PF intensity (F_m) was reached. The occurrence of points J and I was mainly related to the exchange of a reduced Q_A with an oxidized PQ molecule at the Q_B site and the reoxidation of PQ. After 10 d of treatment, there was a significant difference in the change in the OJIP transient. Compared with that of SW, the F_m value of ND, NW, and SD decreased while the minimum PF intensity (F_o) increased (Fig. S1-A). This trend was more pronounced at 20 d. Fig.4 -A, D shows an induction curve normalized by the fluorescence value at point P. The normalized curve did not change significantly at 10 d but did at 20 d; the fluorescence signal between the O-P step of ND and SD increased significantly, especially the J-step. In the SW treatment, the OJIP curve showed similar trends over all measured days. Fig.4 -B, E shows a subtraction process with NW as a control. Four treatments were significantly different in the J-phase. At 10 d, ΔJ under ND and SD increased to 0.02 and 0.01 compared to that of NW, while that of SW decreased to 0.02. At 20 d, ΔJ increased to 0.08 under ND and SD and decreased to approximately 0.03 under SW. First, the O-J phase normalization was performed for each treatment and then subtracted from each treatment with NW as a control (Fig.4 -C, F). At 10 d, SD had a more obvious K peak than did NW. The difference between the treatments at 20 d was more noticeable than that at 10 d. The K peak of the ND treatment was 11.6% higher than that of the NW treatment, while the difference between the SD and NW was not significant, and the K peak of SW was 15.3% smaller than that of NW.

A large amount of raw data was obtained from the OJIP curve. To better reflect the relationship between the kinetic curves and the material, the methods of Strasser based on the biofilm flow were used to calculate the energy flow and energy ratio to measure the given physical parameters [12, 16]. The variation in the interior of the sample material in a given state was used to establish a highly simplified energy flow model diagram. The data from the energy model diagram was used for the JIP-test. Compared with SW, the F_o of ND, NW, and SD increased significantly at 10 d, by 18.6%, 12.9%, and 19.2%, respectively. At 20 d, the F_o value was highest in ND and lowest in SW. The difference in F_o between ND and SD was not significant. The F_m value of SW was significantly higher than that of ND, NW, SD, by 29.6%, 16.6%, and 17.1%, respectively. The V_j reflects the degree of closure of the active reaction center at 2 ms of illumination. The V_j of ND and SD increased notably, while the difference between SW and NW was not distinct. When the donor side of the PSII (oxygen-releasing complex, OEC complex) was damaged, the chlorophyll fluorescence yield increased before the J point (approximately 300 μ s), so the relative fluorescence value W_k of this point increased as the donor of PSII. The degree of increase in W_k represents the level of damage on the donor side of PSII. ND had the highest W_k , and SW had the lowest. S_m is typically used to characterize the size of the PSII reaction center PQ pool. At 20 d, there was a significant difference in S_m between different treatments, with SW being the highest at 27.6%, 19.8%, and 26.6% higher than those of ND, NW, and SD, respectively. There were significant differences in the PI_{ABS} of the Welsh onion leaves under each treatment, in which drought stress notably decreased the PI_{ABS} , while the PI_{ABS} was increased by 20% shading.

MR curve

P_{700} can absorb light at 820 nm when the light is in the oxidation state P_{700}^+ , so the change in light absorption or reflection at 820 nm can represent the redox state of P_{700} . The 820 nm light reflection kinetic curve in this test was represented by MR/MR_0 . Under different treatment conditions, the redox state of PSI changed in the leaves of Welsh onion. The lowest point of the MR/MR_0 rapid decline phase represents the turning point of the PSI oxidation state. The fast decline phase of the synchronously measured MR/MR_0 curve changed at 10 d, while the lowest point of the reduction was increased in ND, and the MR/MR_0 drop rate V_{PSI} was significantly reduced (Fig. 5). After 20 d of treatment, the rapid drop in the MR/MR_0 of ND was significantly shifted backward and increased. In addition, the V_{PSI} of ND was notably reduced compared with that of NW, SD, and SW by 27.5%, 20.5%, and 48.9%, respectively. For the rate of the reduction of P_{700}^+ by electrons from PSII and the reduced PQ pool, the rate of increase of $V_{PSII-PSI}$ in ND was significantly reduced after 10 d of treatment. After 20 d of treatment, the rate of increase of MR/MR_0 was highest in SW, followed by NW and SD, and was the lowest in ND. Among them, the rate of increase of MR/MR_0 in SW was 34.6%, 35.5%, and 80.9% higher than that in NW, SD and ND, respectively.

Discussion

At present, few studies have examined changes in the photosynthesis of Welsh onions during the summer. In this experiment, to investigate the regulatory mechanisms of onion leaves under high light and drought conditions during summer, we measured the rate of carbon dioxide assimilation using CIRAS-3, PSII energy distribution with Imaging-PAM and the fast fluorescence induction curve and 820 nm light reflection signal using Handy PEA and M-PEA.

The chlorophyll content is the basis of photoreactions and can be used to measure the light energy absorption and utilization of leaves. Environmental stress leads to a decrease in the chlorophyll content [3, 17, 18]. The leaf pigment content of Welsh onion leaves under high light and drought conditions decreased significantly, and the decreasing trend became more serious with the treatment time. Under the combined high light and drought treatment, a significant drop in P_n indicated a decrease in photosynthesis in the leaves of Welsh onion. According to the changed direction of C_i , we speculated that the reduction in the P_n of the ND and SD treatments was mainly caused by nonstomatal limiting factors when treated for 20 d, while the P_n of NW decreased because the pores limited photosynthesis. This trend was also found in the daily variation in measurements between treatments. The decrease in $Y(II)$ under natural light and drought treatment was accompanied by an increase in $Y(NPQ)$ and a significant increase in $Y(NO)$ [19]. We hypothesized that the decrease in $Y(II)$ was mainly due to the decrease in the carbon assimilation ability of the leaves and the excessive accumulation of linear electron transport products, which suppress the linear electron transfer rate [20].

Plants must be able to grow in a changing light environment throughout their life cycle [21]. The intensity of PAR directly affects the photosynthesis of plants, but when the light energy exceeds the need of photosynthesis, a decrease in the efficiency of light energy utilization occurs, causing photoinhibition [22, 23]. Generally, PSII is more sensitive to photoinhibition under conditions of high light and drought compared with PSI [7]. Our results indicated that the fast-induced fluorescence kinetics curve was very sensitive to light intensity and drought stress [9, 24-26]. The entire photosynthetic electron transport chain of Welsh onion leaves was significantly affected under each treatment, but to a slightly different extent. Compared with SW, an increase in F_0 and a decrease in F_m were observed in ND, SD and NW, indicating a decline in active reaction centers in PSII under drought and high light conditions. Through JIP-test analysis, we obtained the same results, including the increase of ABS/RC and the reduction of RC/CS_0 and TR_0/ABS . In addition, some studies have shown that an elevated F_0 caused by drought stress reflects PSII damage associated with light-harvesting complex II (LHCII) dissociation [27]. Therefore, the dissociation of LHCII may be a cause of the decrease in PSII activity. However, moderate dissociation of LHCII and degradation of the PSII core protein can accelerate the turnover of PSII and stabilize the normal function of PSII [28].

The J-phase appears because Q_B cannot accept electrons from Q_A^- in time and thus cannot oxidize Q_A^- , resulting in the accumulation of Q_A^- . A significantly elevated J phase indicates that electron transport beyond Q_A^- was limited [29]. We normalized the O-P segment and observed that the ND, SD, and NW treatments showed different degrees of increase in the J-phase compared with SW. V_J reflects the closure level of the active reaction center when illuminated for 2 ms, M_o is generally considered to be the rate at which Q_A is reduced during O-J, and S_m reflects the size of the receptor side PQ pool in the PSII reaction center [25, 26]. V_J and M_o increased under natural light, while S_m decreased. These results indicated that the Welsh onion leaves under natural light received too much light energy, which led to the limitation of Q_A^- transport in PSII, and the PQ was reduced by the capacity of the receptor library. The drop in ET_0/TR_0 can also verify this result.

The appearance of the K-phase is caused by the inhibition of the water cleavage system and the inhibition of the receptor side portion before Q_A [30]. In this inhibition process, mainly the donor side (OEC) of PSII is damaged, so the increase in K-phase fluorescence is usually used to characterize the damage to the donor side of PSII or OEC [31]. At 20 d, the significant increase in the K-phase of ND indicated that the PSII donor side may be damaged. OEC participates in the photooxidation of water during the photoreaction of photosynthesis [31]. We hypothesize that the water oxidation of Welsh onions under high light and drought treatment was impaired. ND, SD, and NW all had significant K phases compared with that of SW, and F_K , V_K , and W_K all increased (Table S2). An increase in F_K caused a negative change in the L-band, indicating the detachment of LHCII from the reaction center, which again validates the results of the previous paragraph (the dissociation of LHCII may be a cause of the reduced PSII activity, Fig. S1-B, D). The strong uncoupling of the PSII donor side (OEC) can cause a significant increase in V_K [8, 32]. The increase in V_K indicates that the

manganese clusters of the oxygen-releasing complex may be the cause of the PSII photoinhibition. W_K can also be used to reflect the extent of damage to the OEC. According to the calculation formula for W_K and previous studies, W_K should be used as an indicator to reflect the relative damage of the PSII donor and receptor sides. Binding to the PSII receptor side was also compromised, which may have also caused an increase in W_K [33, 34].

PI_{ABS} is a performance index based on absorbed light energy, which can reflect the parameters of plant comprehensive photosynthetic performance and is sensitive to stress, especially drought stress[26]. The PI_{ABS} was significantly reduced by ND, especially after 20 d of treatment. A decrease in the PI_{ABS} value suggested decreased PSII activity and ultimately a diminished rate of electron transport through PQ to P_{700}^+ .

The MR/MR_0 is reduced from MR_0 to the minimum level, reflecting the PSI oxidation process, and then increases to the maximum level indicating that the PSI is in the reduction process[12, 26]. After 20 d of treatment, MR delivery was significantly affected, with the PSI oxidation process shortening and the PSI reduction process was suppressed, as evidenced by the significant decreases in V_{PSI} and $V_{PSII-PSI}$. However, we need to pay attention to the fact that PSI photoinhibition poses a major threat to the entire photosynthetic mechanism compared to PSII photoinhibition because it is difficult to recover the process and feedback suppression of PSII[21, 35, 36]. The ϕR_0 decreased significantly with the ND, SD, and NW treatments (Table S2, reduction efficiency of PSI terminal electron acceptor), indicating that electron transfer between photosystems was suppressed, and the difference between treatments was not significant at 10 d, which may be related to linear electron flow. The effect was not significant in the early treatment stage but was strongly suppressed in the later stages of treatment. Surprisingly, the SW treatment significantly reduced the PSI terminal electron acceptor-reduced electron flow RE_0/RC (Table S2) at 10 d, while the difference between the treatments was eliminated at 20 d. We hypothesize that under the conditions of donor side restriction, the stability of PSI terminal electron acceptor reduction is likely to be due to the presence of circular electron transport around PSI[12].

Conclusions

High light and drought treatment can lead to a decrease in the leaf pigment content and net photosynthetic rate during summer. By measuring the QJIP and MR curves, we observed a large amount of environmental stress-induced changes in the photosynthetic electron transport chain. High light and drought stress damaged multiple sites in the electron transport chain. The dual stresses deactivated the PSII reaction center, hindered electron transfer between Q_A and Q_B , caused OEC damage, reduced connectivity between independent PSII units, and inhibited electron transfer between optical systems, ultimately leading to the imbalance between PSII and PSI.

Abbreviations

Ci, substomatal CO_2 concentrations; Cyt b_6f , the cytochrome b_6f complex; Fd, ferredoxin; F_m , the maximum PF intensity; F_o , the minimum PF intensity; Gs, stomatal conductance; LHCII, the light-harvesting complex II, MR, modulated 820 nm reflection; OEC complex, oxygen-releasing complex; PAR, photosynthetically active radiation; PC, plastocyanin; PF, prompt chlorophyll a fluorescence; PFD, photon flux density; Pn, net photosynthetic rate; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; P680, PSII reaction center; P700, PSI reaction center; Y(NPQ), the quantum yield of regulated energy dissipation; Y(NO), the quantum yield of nonregulated energy dissipation; Y(II), the effective PSII quantum yield; V_{PSI} , maximum slope decrease of MR/MR_0 ; $V_{PSII-PSI}$, maximum slope increase of MR/MR_0 .

Declarations

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

KX and XNL designed the research. XNL and SG performed the experiments. XNL and YL analyzed the data. XNL wrote the manuscript, and KX revised the intellectual content of this manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zi-ying H, Jun-ju Z, LI-LI Z, Wei W, Jian-jun C: **Climate dry-wet and drought evolution characteristics of different dry-wet areas in northern China.** *Acta Ecologica Sinica* 2018, **38**(06):1908-1919.
2. Gilbert ME, Zwieniecki MA, Holbrook NM: **Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought.** *Journal of Experimental Botany* 2011, **62**(8):2875-2887.
3. Gilbert ME, Medina V: **Drought Adaptation Mechanisms Should Guide Experimental Design.** *Trends in Plant Science* 2016, **21**(8):639-647.
4. Niyogi KK: **Photoprotection revisited: genetic and molecular approaches.** *Annual Review of Plant Physiology and Plant Molecular Biology* 1999, **50**(1):333-359.
5. S P Long, S Humphries a, Falkowski PG: **Photoinhibition of Photosynthesis in Nature.** *Annual Review of Plant Physiology and Plant Molecular Biology* 1994, **45**(1):633-662.
6. Lawlor DW, Cornic G: **Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants.** *Plant, Cell & Environment* 2002, **25**(2):275-294.
7. Yang XQ, Zhang QS, Zhang D, Sheng ZT: **Light intensity dependent photosynthetic electron transport in eelgrass (Zostera marina L.).** *Plant Physiology and Biochemistry* 2017, **113**:168-176.
8. Shen J-R, Terashima I, Katoh S: **Cause for Dark, Chilling-Induced Inactivation of Photosynthetic Oxygen-Evolving System in Cucumber Leaves.** *Plant Physiology* 1990, **93**(4):1354-1357.
9. Strasser R, Srivastava A, Tsimilli-Michael M: **The fluorescence transient as a tool to characterize and screen photosynthetic samples;** 2000.
10. Gollan Peter J, Lima-Melo Y, Tiwari A, Tikkanen M, Aro E-M: **Interaction between photosynthetic electron transport and chloroplast sinks triggers protection and signalling important for plant productivity.** *Philosophical Transactions of the Royal Society B: Biological Sciences* 2017, **372**(1730):20160390.
11. Walker BJ, Busch FA, Drier SM, Kromdijk J, Lawson T: **Survey of Tools for Measuring In Vivo Photosynthesis.** In: *Photosynthesis: Methods and Protocols*. Edited by Covshoff S. New York, NY: Springer New York; 2018: 3-24.

12. Strasser RJ, Tsimilli-Michael M, Qiang S, Goltsev V: **Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*.** *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 2010, **1797**(6):1313-1326.
13. Schansker G, Srivastava A, Govindjee, Strasser RJ: **Characterization of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves.** *Functional Plant Biology* 2003, **30**(7):785-796.
14. Zhao S, Shi GA, Dong XC: **Plant Physiology Experiment Report.** Beijing: Chinese Agriculture Science & Technology Press; 2002.
15. Hendrickson L, Förster B, Furbank RT, Chow WS: **Processes contributing to photoprotection of grapevine leaves illuminated at low temperature.** *Physiologia Plantarum* 2004, **121**(2):272-281.
16. Straaer BJ: **Measuring fast fluorescence transients to address environmental questionsL;the JIP-test.** *Photosynthesis:from Light to Biosphere* 1995:977-980.
17. Larkunthod P, Nounjan N, Siangliw JL, Toojinda T, Sanitchon J, Jongdee B, Theerakulpisut P: **Physiological responses under drought stress of improved drought-tolerant rice lines and their parents.** *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 2018, **46**(2):679-687.
18. Rapacz M, Wójcik-Jagła M, Fiust A, Kalaji HM, Kościelniak J: **Genome-Wide Associations of Chlorophyll Fluorescence OJIP Transient Parameters Connected With Soil Drought Response in Barley.** *Frontiers in plant science* 2019, **10**:78-78.
19. G H Krause, Weis E: **Chlorophyll Fluorescence and Photosynthesis: The Basics.** *Annual Review of Plant Physiology and Plant Molecular Biology* 1991, **42**(1):313-349.
20. Durand M, Brendel O, Buré C, Le Thiec D: **Altered stomatal dynamics induced by changes in irradiance and vapour-pressure deficit under drought: impacts on the whole-plant transpiration efficiency of poplar genotypes.** *New Phytologist* 2019, **222**(4):1789-1802.
21. Li P, Ma F: **Different effects of light irradiation on the photosynthetic electron transport chain during apple tree leaf dehydration.** *Plant Physiology and Biochemistry* 2012, **55**:16-22.
22. Yong-zheng Z, Hai-dong L, Xiu L, Jing X, Kun X: **Effects of Light Intensity and Water Stress on Leaf Photoysnthetic Chatacteristics of Ginger.** *Acta Horticulturae Sinica* 2013, **40**(11):2255-2262.
23. Mathur S, Jajoo A: **Alterations in photochemical efficiency of photosystem II in wheat plant on hot summer day.** *Physiology and Molecular Biology of Plants* 2014, **20**(4):527-531.
24. Stirbet A, Govindjee: **On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient.** *Journal of Photochemistry and Photobiology B: Biology* 2011, **104**(1):236-257.
25. Kalaji HM, Račková L, Paganová V, Swoczyna T, Rusinowski S, Sitko K: **Can chlorophyll-a fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill?** *Environmental and Experimental Botany* 2018, **152**:149-157.
26. Liu J, Guo YY, Bai YW, Camberato JJ, Xue JQ, Zhang RH: **Effects of drought stress on the photosynthesis in Maize.** *Russian Journal of Plant Physiology* 2018, **65**(6):849-856.
27. Briantais J-M, Dacosta J, Goulas Y, Ducruet J-M, Moya I: **Heat stress induces in leaves an increase of the minimum level of chlorophyll fluorescence, Fo: A time-resolved analysis.** *Photosynthesis Research* 1996, **48**(1):189-196.
28. Oukarroum A, Madidi SE, Schansker G, Strasser RJ: **Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering.** *Environmental and Experimental Botany* 2007, **60**(3):438-446.
29. Li P, Cheng L, Gao H, Jiang C, Peng T: **Heterogeneous behavior of PSII in soybean (*Glycine max*) leaves with identical PSII photochemistry efficiency under different high temperature treatments.** *Journal of Plant Physiology* 2009, **166**(15):1607-1615.
30. Wang Y, Zhang B, Jiang D, Chen G: **Silicon improves photosynthetic performance by optimizing thylakoid membrane protein components in rice under drought stress.** *Environmental and Experimental Botany* 2019, **158**:117-124.
31. Yan K, Chen P, Shao H, Shao C, Zhao S, Brestic M: **Dissection of Photosynthetic Electron Transport Process in Sweet Sorghum under Heat Stress.** *PLOS ONE* 2013, **8**(5):e62100.
32. Kaniuga Z, Ząbek J, Sochanowicz B: **Photosynthetic apparatus in chilling-sensitive plants : III. Contribution of loosely bound manganese to the mechanism of reversible inactivation of hill reaction activity following cold and dark storage and illumination of Leaves.** *Planta* 1978, **144**(1):49-56.
33. Strasser BJ: **Donor side capacity of Photosystem II probed by chlorophyll a fluorescence transients.** *Photosynthesis Research* 1997, **52**(2):147-155.
34. Jin L-Q, Che X-K, Zhang Z-S, Gao H-Y: **The relationship between the changes in Wk and different damage degree of PSII donor side and acceptor side under high temperature with high light in Cucumber.** *Plant Physiology Journal* 2015, **51**(6):969-976.
35. Sonoike K: **Photoinhibition of photosystem I.** *Physiologia Plantarum* 2011, **142**(1):56-64.
36. Salvatori E, Fusaro L, Strasser RJ, Bussotti F, Manes F: **Effects of acute O3 stress on PSII and PSI photochemistry of sensitive and resistant snap bean genotypes (*Phaseolus vulgaris* L.), probed by prompt chlorophyll "a" fluorescence and 820 nm modulated reflectance.** *Plant Physiology and Biochemistry* 2015, **97**:368-377.

Tables

Table 1 Effects of different light intensity and drought treatments on leaf pigment and gas exchange parameters, including the net photosynthetic rate (Pn), intercellular CO₂ concentration (Ci) and stomatal conductance (Gs). The data are from three replicated experiments (n=3) and represent means± SD. Different letters indicate significant differences between different treatments.

Days after treatment	Treatment	Chlorophylls (µg/cm ²)	Carotenoids	Pn (µmol/m ² /s)	Ci (µmol/mol)	Gs (mmol/mol)
(µg/cm ²)						
10 d	ND	31.94±0.51d	4.45±0.13b	3.63±0.06d	280.33±2.31b	64.00±5.29d
	NW	39.49±0.76b	5.64±0.18a	10.23±0.12b	289.00±7.81ab	198.33±8.62b
	SD	33.52±0.67c	4.87±0.23b	6.47±0.12c	328.67±40.25a	135.67±11.59c
	SW	42.25±0.27a	5.99±0.04a	14.40±0.01a	266.67±3.51b	217.33±15.04a
20 d	ND	28.31±0.09d	4.17±0.12c	1.87±0.38d	304.67±9.87ab	49.67±11.93c
	NW	31.45±0.43b	4.32±0.12bc	8.13±0.21b	248.67±4.93c	115.67±2.52b
	SD	29.13±0.16c	4.51±0.12b	4.13±0.12c	321.33±12.06a	124.67±2.52b
	SW	42.23±0.59a	5.74±0.04a	13.6±0.17a	300.67±2.08b	413.67±14.47a

Table 2 Effects of different light intensity and drought treatments on the JIP-test parameters. The data are from three replicated experiments (n=3) and represent means± SD. Different letters indicate significant differences between different treatments.

Days after treatment	Treatment	F _O	F _m	V _J	W _K	S _m	M _O	ABS/RC	RC/CS	TR _O /ABS	ET _O /TR _O	PI _{ABS}
10 d	ND	541±36	2240±241	0.41±0.02	0.32±0.06	33.52±0.99	0.96±0.45	2.49±0.15	206±106	0.76±0.01	0.59±0.02	1.83±0.13
	NW	aA 515±8	cB 2639±50	aA 0.39±0.01	aA 0.31±0.01	aA 27.68±1.28	aA 0.89±0.05	aA 2.37±0.08	aA 182±15	dD 0.8±0.01	bA 0.61±0.01	cC 2.77±0.09
	SD	aA 543±7	abA 2533±59	abA 0.4±0.01	aA 0.33±0.04	bAB 25.58±2.37	aA 0.95±0.14	aAB 2.52±0.22	aA 181±30	bB 0.79±0.01	abA 0.6±0.01	bB 2.21±0.16
	SW	aA 456±5	bAB 2841±43	aA 0.37±0.01	aA 0.25±0.01	bB 27.17±0.68	aA 0.71±0.04	aA 1.91±0.04	aA 200±14	cC 0.84±0.01	bA 0.63±0.01	cC 4.65±0.21
20 d		bB	aA	bA	aA	bAB	aA	bB	aA	aA	aA	aA
	ND	586±12	2106±103	0.46±0.01	0.4±0.02	24.27±0.68	1.17±0.17	2.49±0.15	168±31	0.72±0.01	0.53±0.01	1.04±0.05
	NW	aA 525±14	cB 2342±83	aA 0.38±0.01	aA 0.31±0.01	bB 25.86±1.41	aA 0.88±0.11	aA 2.37±0.08	aA 178±27	dD 0.78±0.01	bB 0.62±0.01	cC 2.28±0.04
	SD	bB 569±7	bB 2332±52	bB 0.45±0.04	bcAB 0.35±0.01	bAB 24.47±0.86	bcAB 1.06±0.05	aAB 2.52±0.22	aA 186±11	bB 0.76±0.01	aA 0.54±0.04	bB 1.45±0.16
	SW	aA 452±20	bB 2729±127	aA 0.35±0.01	abAB 0.26±0.05	bB 30.98±3.48	abAB 0.7±0.13	aA 1.91±0.04	aA 197±50	cC 0.83±0.01	bB 0.65±0.01	cBC 4.69±0.56
		cC	aA	bB	cB	aA	cB	bB	aA	aA	aA	aA

Table 3 Parameters derived from the modulated 820 nm reflection (MR/MR_O) curve of the different treatments on the 10th and 20th d of treatment. V_{PSI} was the maximum decrease in the slope of MR/MR_O, and V_{PSII-PSI} was the maximum increase in the slope of MR/MR_O. The data are from three replicated experiments (n=3) and represent means± SD. Different letters indicate significant differences between different treatments.

Days after treatment	Treatment	V _{PSI} ×10 ³	V _{PSII-PSI} ×10 ³
10 d	ND	3.47±0.22cC	5.16±0.38bB
	NW	4.61±0.09bB	7.80±0.49aA
	SD	4.34±0.06bB	6.90±0.66aA
	SW	5.10±0.26aA	7.86±0.57aA
20 d	ND	2.70±0.03cB	4.59±0.40cC
	NW	3.72±0.02bB	6.16±0.33bB
	SD	3.39±0.12bB	6.12±0.08bB
	SW	5.27±0.57aA	8.30±0.08aA

Figures

Figure 1

Photosynthetically active radiation (PAR) and air temperature during the experimental period. PAR (A) and air temperature (B) in the no shading (closed circles) and 20% shading (open circles) treatments. Each point represents the highest PAR or average air temperature of each day.

Figure 2

The diurnal variation in the net photosynthetic rate, PAR and air temperature on the 20th d. Average half-hourly PAR (A) and air temperature (B) within the 20th day in the no shading (closed circles) and 20% shading (open circles) treatments. (C) The diurnal variation in the net photosynthetic rate (Pn) on the 20th day.

Figure 3

Effects of different light intensity and drought treatments on the quantum efficiencies of PSII. The quantum efficiencies of PSII (A) and associated images (B) on the 20th d of treatment. The data are from three replicated experiments (n=3). Different letters indicate significant differences between different treatments.

Figure 4

Effects of different light intensity and drought treatments on the OJIP transients. Normalized transients at the 10th (A) and 20th d (D), expressed as $V_t = [(F_t - F_0)/(F_m - F_0)]$. The ΔV_t curves at the 10th (B) and 20th d (E) expressed as $\Delta V_t = [(F_t - F_0)/(F_m - F_0)]_T - [(F_t - F_0)/(F_m - F_0)]_{NW}$. (C) Normalized curves between FO and FJ, and their difference in kinetics (K-band) at the 10th (C) and 20th d (F) expressed as $\Delta W_{OJ} = [(F_t - F_0)/(F_J - F_0)]_T - [(F_t - F_0)/(F_J - F_0)]_{NW}$. The data are from three replicated experiments (n=3).

Figure 5

Effects of different light intensity and drought treatments on the MR curves. Kinetics of modulated light reflection at 820 nm (MR/MRO) in dark-adapted leaves based on different treatments at the 10th (A) and 20th d (B) of treatment. All data are normalized to the initial measured value of the signal. The data are presented in relative units. The data are from three replicated experiments (n=3).

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