

Article

Changes in Photosynthetic Characteristics of *Paeonia suffruticosa* under High Temperature Stress

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Abstract: This study explored the changes in the photosynthetic characteristics of the Fengdan peony under high-temperature stress to provide a reference for understanding the tolerance of peony plants under heat stress. In this study, the effects of high-temperature stress (40 °C) on the photosynthetic characteristics of the Fengdan leaves were studied. At 25 °C, the net photosynthetic rate (P_n), stomatal conductance (G_s), and transpiration rate (Tr) of the leaves decreased gradually with the increase in heat stress time, and intercellular CO₂ concentration (C_i) decreased first and then increased. High-temperature stress reduced the light energy absorption (ABS/RC) and capture (TR_O/RC), light energy for electron transport (ET_O/RC), and heat dissipation (DI_O/RC) per unit leaf area. The maximum photochemical efficiency (F_v/F_m), leaf photochemical performance index (PI_{abs}), the probability that captured excitons can transfer electrons to other electron acceptors in the electron transport chain beyond Q_A (Ψ_O), and the quantum yield for electron transport (φE_o), decreased gradually. The results showed that high temperatures damaged the photosynthetic capacity of the peony leaves and destroyed the photosynthetic apparatus of leaves. This study provides a reference for understanding the photosynthetic characteristics and tolerance of peony plants under heat stress.

Keywords: tree peony; high temperature stress; photosynthetic characteristics; chlorophyll fluorescence induction kinetics; JIP-test



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1. Introduction

Tree peony (*Paeonia suffruticosa* Andr.), a deciduous shrub in the Genus Paeoniaceae, is known as “the king of flowers” due to its large, magnificent flowers. This peony is known for its appearance, color, and fragrance. In recent years, it has been widely used in landscaping, garden beautification, and ornamental potted plants. Peonies prefer a dry, cool environment; however, the summer in south China is rainy and hot. Under humid, hot conditions, the peony plant becomes severely stressed: the stem, leaf, and root system will develop poorly, and forced dormancy can occur, which has led to incompatibility and southward migration of the peony [1,2]. Therefore, in order to expand the cultivation of peonies, studying the peony's tolerance to high-temperature stress could assist in screening for high-temperature-tolerant peony varieties and meeting the market demand.

In recent years, global climate change characterized by temperature rise has become the focus of the world. China is a sensitive and significantly affected area of global climate change. From 1951 to 2017, the warming rate in China reached 0.24 °C per decade, significantly higher than the global average in the same period [3]. According to global Climate

Extremes in 2016, high temperatures exceeding 40 degrees Celsius have occurred in more than 90 countries, and the frequency will continue to increase [4]. Long-term, high temperatures affect the growth and development of plants, and the damage of high-temperature stress is manifested in the reduction of their photosynthetic capacity and photosynthetic rate [5], resulting in changes in the structure and function of their photosynthetic organs. The photosystem (PSI and PSII), photosynthetic pigments, CO₂ reduction pathway, and electron transport chain are important components of photosynthesis, and damage to any part affects the whole photosynthesis [6]. High-temperature stress can easily cause damage to PSII [7]. Light energy is easily absorbed by plants and can exceed the amount that can be utilized by CO₂ assimilation, resulting in an accumulation of reactive oxygen species (ROS) and subsequent damage to PSII [8–11]. Therefore, studying the structure and function of PSII can broaden our understanding of the effects of high-temperature stress on plants. The rapid chlorophyll fluorescence induction kinetic curve can reflect the original photochemical reaction of PSII as well as the changes in the structure and state of the photosynthetic apparatus [12]. In recent years, chlorophyll fluorescence technology has been widely used in the study of plant physiology and ecology. Based on the theory of energy flow in biofilms, previous research established an analysis method (JIP-test) for the rapid chlorophyll fluorescence kinetic curve [13], and it has also been used in high-temperature stress studies [14–16]. The typical kinetic curve of rapid chlorophyll fluorescence induction presents OJIP sites, the OJIP curve deforms into an OKJIP curve under high-temperature treatments, and it then reflects the degree of damage to the OEC [17–19].

At present, the research on high-temperature stress in peonies has focused on the effect of high temperatures on the photosynthetic characteristics; the physiological, biochemical, and molecular mechanisms of the peony leaves; and the effect of exogenous additives on the high-temperature resistance of peonies. Studies have found that high-temperature stress can damage the photosynthetic machinery of the peony leaves and affect PSII function and physiological characteristics [20,21]. Zhu et al. [22] used isotope-labeling, relative quantifications, and absolute quantifications (iTRAQ) combined with mass spectrometry identification and the corresponding biological analysis to discover the response pattern of the Jiangnan peony under high-temperature stress and further discovered possible heat-resistant target proteins. Previous studies have shown that Fengdan, as an important variety in the Jiangnan region, has a certain ability to withstand high-temperature stress, and there are few reports on the changes in photosynthetic characteristics of peonies under high-temperature stress, the change in chlorophyll fluorescence characteristics, and the study of the effect of high-temperature stress on the photosynthesis of peonies by analyzing the changes of photosynthetic parameters. Persistent high temperatures can cause serious damage to the photosynthetic mechanism of the peony leaves, which may damage the oxygen release complex as well as the acceptor and donor sites of PSII and reduce electron-transport capacity. Therefore, the objective of this study was to analyze the effects of different high temperature conditions and durations on peony photosynthetic characteristics and chlorophyll fluorescence characteristics via JIP-testing, and to provide reference for understanding the tolerance of peony under high temperature stress.

2. Materials and Methods

2.1. Experimental Materials

In November 2020, Fengdan was planted in a plastic flowerpot with an upper diameter of 28 cm, a lower diameter of 19 cm, and a height of 23 cm. The matrix was composed of garden soil/sand/perlite (mass ratio 5:3:2), normal water, and fertilizer management. In June 2021, healthy, 4-year-old peony seedlings with similar growth and size as well as no pests or diseases were selected and treated at 25 °C for control and 40 °C for 7 days with 10 plants in each treatment. During the experiment, the air humidity in the incubator was set at 70%, and the light intensity was set to 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; the ratio of day/night cycles was 14 h/10 h.

2.2. Determination Items and Methods

2.2.1. Measurement of Photosynthetic Gas Exchange Parameters

Photosynthetic parameters of the peony leaves during a period of zero-to-seven days under high-temperature stress were measured in June 2021. The healthy functional leaves (a pair of leaves under the terminal bud) with similar growth in the middle and upper parts of the plant were selected and using a Li-6400 portable photosynthetic apparatus (Li-Cor6400XT PSC-4817, Lincoln, NE, USA), the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO₂ concentration (Ci), and transpiration rate (Tr) of the peony leaves were measured every morning (09:00–11:00 h on a sunny day) and repeated in triplicate. Leaves were tagged for the next measurement.

2.2.2. Determination of the Kinetic Curve of Rapid Chlorophyll Fluorescence Induction

The Handy Plant Efficiency Analyzer (PEA, Hansatech, Norfolk, UK) was used to determine the rapid chlorophyll fluorescence induction kinetics (OJIP) and related parameters of the peony leaves from 09:00–11:00 h daily from day zero to seven-under high-temperature stress. Before the measurement, the same leaves were clamped with dark adaptation clips for 30 min, and the dark reaction was measured. There were three plants and three leaves per plant for each measurement. After the first measurement, the leaves were labeled for continuous measurement. The terms and formulas used in the analysis of the rapid chlorophyll fluorescence induction kinetics curve (OJIP) [23] are shown in Table 1.

Table 1. Formulae and terms used in the analysis of the OJIP fluorescence induction dynamics curve.

Formula and Terms	Illustrations
Data extracted from the recorded fluorescence transient OJIP	
F_t	Fluorescence at time t after onset of actinic illumination
$F_{50 \mu s}$ or $F_{20 \mu s}$	Minimal reliable recorded fluorescence, at 50 μs or 20 μs
$F_{300 \mu s}$	Fluorescence intensity at 300 μs
$F_j \equiv F_{2ms}$	Fluorescence intensity at the J-step (2 ms) of OJIP
$F_I \equiv F_{30ms}$	Fluorescence intensity at the I-step (30 ms) of OJIP
F_P	Maximal recorded fluorescence intensity, at the peak P of OJIP
t_{FM}	Time (in ms) to reach the maximal fluorescence intensity F_M
Fluorescence parameters derived from the extracted data	
$F_0 \cong F_{50\mu s}$ or $\cong F_{20\mu s}$	Minimal fluorescence (all PSII RCs are assumed to be open)
$F_M (=F_P)$	Maximal fluorescence, when all PSII RCs are closed (equal to F_P when the actinic light intensity is above 500 μmol photons $m^{-2} s^{-1}$ and provided that all RCs are active as Q_A reducing)
$F_v \equiv F_t - F_0$	Variable fluorescence at time t
$F_V \equiv F_M - F_0$	Maximal variable fluorescence
F_V/F_M	Maximal quantum efficiency of PSII
V_j	J point is relatively variable fluorescence intensity
$V_t \equiv F_v/F_V \equiv (F_t - F_0)/(F_M - F_0)$	Relative variable fluorescence at time t
$\Delta V_t = (F_t - F_0)/(F_M - F_0) - V_t$ (control)	Relative variable fluorescence
$M_0 \equiv [(\Delta F/\Delta t)_0]/(F_M - F_{50\mu s}) \equiv 4(F_{300\mu s} - F_{50\mu s})/(F_M - F_{50\mu s})$	Approximated initial slope (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence F_V
Specific energy fluxes [per QA-reducing PSII reaction center (RC)]	
$ABS/RC = M_0 (1/V_j)(1/\varphi_{P_0})$	Absorption flux (of antenna Chls) per RC
$TR_O/RC = M_0 (1/V_j)$	Trapped energy flux per RC (at $t = 0$)
$ET_O/RC = M_0 (1/V_j)\psi E_0$	Electron transport flux (further than Q_A^-) per RC (at $t = 0$)
$DI_O/RC = (ABS/RC) - (TR_O/RC)$	Dissipated energy flux per RC (at $t = 0$)
Yields or flux ratios	
$\varphi E_0 \equiv ET_O/ABS = [1 - (F_0/F_M)]\psi E_0$	Quantum yield for electron transport (ET)(at $t = 0$)
$\Psi_0 = ET_O/TR_O = (1 - V_j)$	Probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at $t = 0$)
$\varphi D_0 = 1 - \varphi P_0 = (F_0/F_M)$	Quantum ratio used for heat dissipation
Performance indexes (products of terms expressing partial potentials at steps of energy bifurcations)	
$PI_{ABS} \equiv (RC/ABS)[\varphi_{P_0}/(1 - \varphi_{P_0})][\Psi_0/(1 - \Psi_0)]$	Performance index on absorption basis
$PI_{total} \equiv PI_{ABS}[\delta_{R_0}/(1 - \delta_{R_0})]$	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors

2.2.3. Statistical Analysis

The SPSS 25 software (IBM, Armonk, New York, NY, USA) was used for one-way analysis of variance, and the Excel and Origin2021 software (OriginLab, Northampton, MA, USA) were used for data processing and graphing. Fisher's least significant difference was used for mean comparison ($p < 0.05$).

3. Results

3.1. Effects of High-Temperature Stress on Leaf Photosynthetic Characteristics of Peony

As shown in Table 2, with prolonged treatment time, the photosynthetic parameters of the peony leaves in both control and high-temperature groups changed. The net photosynthetic rate (Pn) and the stomatal conductance (Gs) of the peony leaves showed a decreasing trend, and the differences were all significant. When treated with high temperatures for three days, the Pn and the Gs of the peony leaves in the high-temperature group, as compared to day 0, decreased by 88.2% and 87.8%, respectively, and the control group decreased by 13.7% and 31.3%, respectively. When treated with high temperatures for five days, the Pn and the Gs of the peony leaves in the high-temperature group, as compared to day 0, decreased by 91.9% and 83.9%, respectively, and the control group decreased by 46.7% and 41.8%, respectively; with prolonged treatment time, the intercellular CO₂ concentration generally showed a trend of first decreasing and then increasing. When treated at 25 °C for 3 d, the Ci decreased by 15.8%, as compared to day 0, and decreased by 40.0% at high temperatures. When treated at 25 °C for 5 d, the Ci increased by 17.8% and increased by 11.7% at high temperatures. When treated with high temperatures for 5 days, the Tr of the peony leaves in the high-temperature group, as compared to day 0, decreased by 71.2%, and the control group decreased by 22.6%. When treated for 7 d, Tr decreased by 74.2% at high temperatures and decreased by 66.2% at 25 °C.

Table 2. Effects of high-temperature stress on leaf light and parameters of peony.

Time (d)	Temperature	Pn ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Gs ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Ci ($\mu\text{mol}/\text{mol}$)	Tr ($\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$)
0	25 °C	5.8203 ± 0.3775 a	0.0582 ± 0.0042 a	248.4497 ± 1.9420 b	0.5802 ± 0.0188 a
	40 °C	5.2338 ± 0.3429 a	0.0596 ± 0.0025 a	367.6925 ± 22.0826 ab	0.5540 ± 0.0380 a
1	25 °C	5.6017 ± 0.1824 a	0.0628 ± 0.0059 a	269.3729 ± 8.4733 ab	0.5490 ± 0.0536 a
	40 °C	3.5051 ± 0.0621 b	0.0369 ± 0.0013 c	333.0420 ± 46.4777 ab	0.3848 ± 0.0240 b
2	25 °C	2.5803 ± 0.2868 d	0.0205 ± 0.0016 c	209.1131 ± 36.8020 b	0.3635 ± 0.0168 d
	40 °C	1.2469 ± 0.3513 b	0.0197 ± 0.0007 d	325.2725 ± 23.8841 ab	0.1829 ± 0.0169 c
3	25 °C	5.0258 ± 0.1830 b	0.0400 ± 0.0028 b	223.9212 ± 21.8206 b	0.5054 ± 0.0197 b
	40 °C	0.6174 ± 0.0740 c	0.0073 ± 0.0010 de	220.6303 ± 30.8073 b	0.1056 ± 0.0205 d
4	25 °C	4.7060 ± 0.1808 b	0.0373 ± 0.0013 b	244.7754 ± 1.4064 b	0.3919 ± 0.0063 d
	40 °C	0.4703 ± 0.0967 c	0.0046 ± 0.0011 e	286.5971 ± 64.4177 b	0.0616 ± 0.0171 d
5	25 °C	3.1041 ± 0.3254 c	0.0339 ± 0.0032 b	292.7151 ± 16.2667 a	0.4493 ± 0.03893 c
	40 °C	0.4253 ± 0.0912 c	0.0096 ± 0.0034 d	410.0798 ± 123.7733 a	0.1596 ± 0.0594 cd
6	25 °C	1.4205 ± 0.1654 e	0.0156 ± 0.0008 cd	267.4682 ± 9.7356 ab	0.2411 ± 0.0118 e
	40 °C	0.3069 ± 0.0630 c	0.0227 ± 0.0037 b	266.7287 ± 25.6768 b	0.4007 ± 0.0793 b
7	25 °C	1.0134 ± 0.1410 e	0.0127 ± 0.0014 d	292.9464 ± 27.4262 a	0.1962 ± 0.0238 f
	40 °C	−0.1586 ± 0.0276 d	0.0086 ± 0.0011 d	302.4088 ± 120.2667 ab	0.1429 ± 0.0154 cd

Note: Different letters in the same column indicate significant differences at the 0.05 level.

3.2. Effects of High-Temperature Stress on the Rapid Chlorophyll Fluorescence Characteristics of the Peony Leaves

3.2.1. Changes in Chlorophyll Fluorescence Parameters

High-temperature stress had obvious effects on the fast chlorophyll fluorescence parameters of the peony plants (Figure 1). The maximum photochemical efficiency (F_V/F_M)

and leaf photochemical performance index (PI_{abs}) were both lower than those of the control and gradually decreased with prolonged high-temperature stress (Figure 1A,B). Under high-temperature stress, the light energy absorbed by the unit reaction center (ABS/RC), the energy captured by the unit reaction center for Q_A reduction (TR_O/RC), the energy captured by the unit reaction center for electron transport (ET_O/RC), and the energy dissipated by the unit reaction center (DI_O/RC) were higher than those of the control, and when the exposure to high temperatures was extended, there was an overall upward trend (Figure 1C–F).

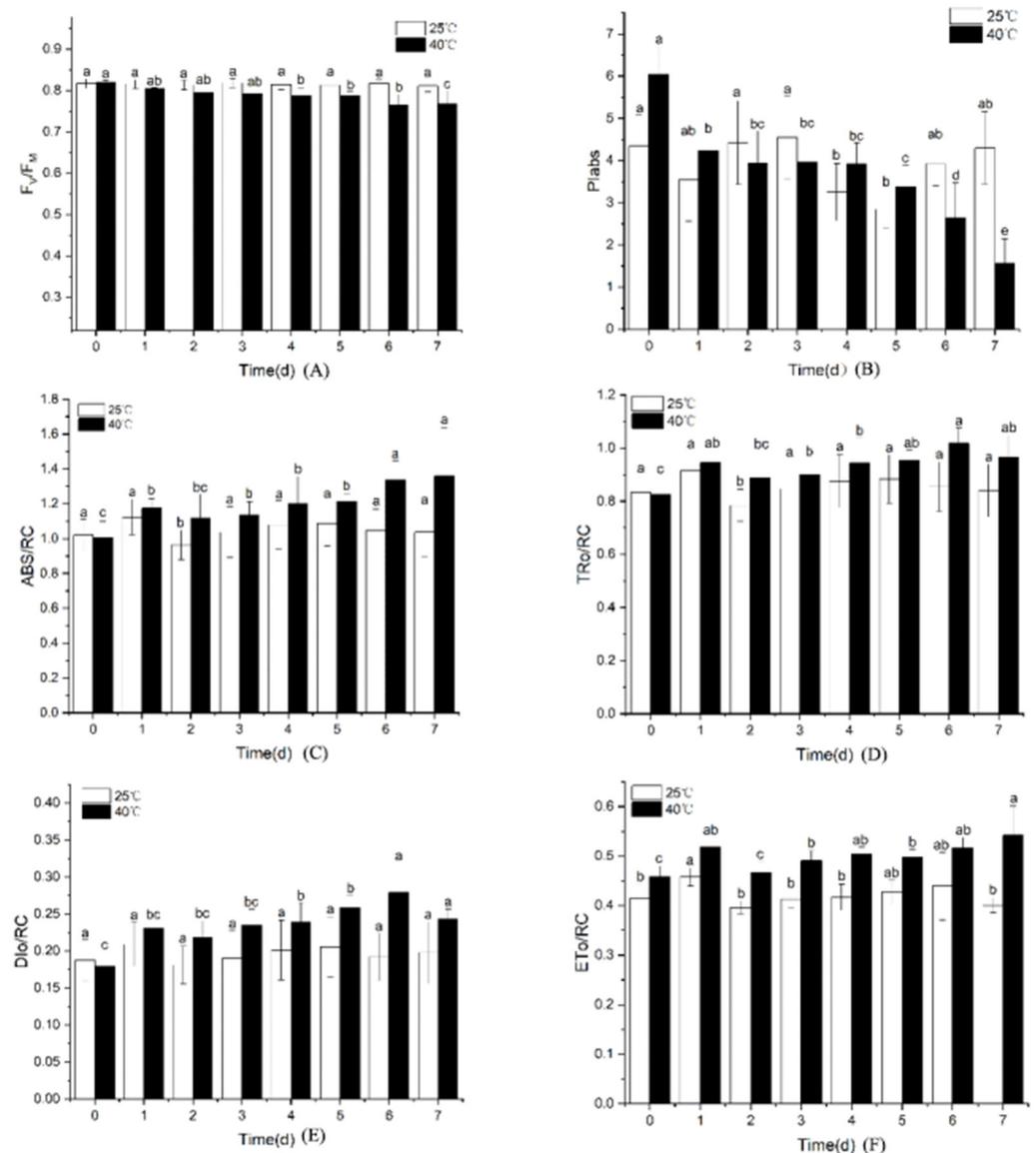


Figure 1. Effects of high-temperature stress on chlorophyll fluorescence characteristics of tree peony leaves. (A,B) Effects of high-temperature stress on photochemical performance index (PI_{abs}) and the maximal quantum efficiency of PSII (F_v/F_m) of peony leaves; (C–F) Effect of high-temperature stress on the activity of PSII reaction center in leaves of peony. The energy flux absorbed by a single active reaction center (ABS/RC), the excitation energy flux captured by each active reaction center at the beginning of illumination of the dark-adapted sample (i.e., $T = 0$ (TR_O/RC)), the total energy dissipated by a single active reaction center (DI_O/RC), and the flux of electrons from each active center at $T = 0$ (ET_O/RC). Different letters in the same column indicate significant differences at the 0.05 level.

3.2.2. Effects of High-Temperature Stress on V_J and Quantum Yield

With extended time under high-temperature stress, the relative variable fluorescence (V_J) of the J phase showed an overall increase but then decreased. The probability of captured excitons transferring electrons to other electron acceptors beyond Q_A in the electron transport chain (Ψ_o) showed an overall decrease and then increased. The quantum ratio (ϕDo) used for heat dissipation generally increased. The quantum yield (ϕEo) used for electron transport decreased with increased exposure to high temperatures (Table 3). Under extended periods of high-temperature stress, the overall difference between V_J and Ψ_o was not significant, while the difference between ϕDo and ϕEo was significant. The V_J and quantum yield of the control group did not change significantly with prolonged treatment time.

Table 3. Effects of high-temperature stress on leaf quantum yield of tree peony.

Time (d)	Temperature	V_J	Ψ_o	ϕEo	ϕDo
0	25 °C	0.4992 ± 0.0564 a	0.5008 ± 0.0564 a	0.4097 ± 0.0505 a	0.1831 ± 0.0110 a
	40 °C	0.4406 ± 0.0348 b	0.5594 ± 0.0348 a	0.4580 ± 0.0312 a	0.1815 ± 0.0056 e
1	25 °C	0.4959 ± 0.0521 a	0.4959 ± 0.0521 a	0.4113 ± 0.0478 a	0.1852 ± 0.0105 a
	40 °C	0.4519 ± 0.0136 b	0.5481 ± 0.0136 ab	0.4404 ± 0.0103 ab	0.1964 ± 0.0036 ce
2	25 °C	0.4908 ± 0.0483 a	0.5092 ± 0.0483 a	0.4145 ± 0.0446 a	0.1870 ± 0.0114 a
	40 °C	0.4716 ± 0.0483 ab	0.5284 ± 0.0483 ab	0.4203 ± 0.0438 bc	0.2055 ± 0.0132 ce
3	25 °C	0.5038 ± 0.0673 a	0.4962 ± 0.0673 a	0.4066 ± 0.0599 a	0.1820 ± 0.0109 a
	40 °C	0.4509 ± 0.0497 b	0.5491 ± 0.0497 ab	0.4354 ± 0.0400 ab	0.2071 ± 0.0096 c
4	25 °C	0.5159 ± 0.0575 a	0.4842 ± 0.0757 a	0.3953 ± 0.0671 a	0.1854 ± 0.0133 a
	40 °C	0.4636 ± 0.0373 ab	0.5364 ± 0.0373 ab	0.4230 ± 0.0376 b	0.2124 ± 0.0197 c
5	25 °C	0.5082 ± 0.0715 a	0.4918 ± 0.0715 a	0.4007 ± 0.0648 a	0.1873 ± 0.0153 a
	40 °C	0.4776 ± 0.0271 ab	0.5224 ± 0.0271 b	0.4111 ± 0.0207 bc	0.2130 ± 0.0097 bc
6	25 °C	0.4858 ± 0.0473 a	0.5142 ± 0.0473 a	0.4201 ± 0.0389 a	0.1830 ± 0.0092 a
	40 °C	0.4916 ± 0.0251 a	0.5084 ± 0.0251 b	0.3889 ± 0.0295 c	0.2358 ± 0.0265 b
7	25 °C	0.5154 ± 0.0665 a	0.4846 ± 0.0665 a	0.3939 ± 0.0597 a	0.1890 ± 0.0140 a
	40 °C	0.4586 ± 0.0296 ab	0.5414 ± 0.0296 ab	0.3924 ± 0.0439 bc	0.2737 ± 0.0875 a

Note: Different letters in the same column indicate significant differences at the 0.05 level. The relative variable fluorescence of J phase (V_J), the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at $t = 0$) (Ψ_o), the quantum yield for electron transport (ET) (at $t = 0$), and the quantum ratio of heat dissipation (ϕDo).

3.2.3. Kinetic Curve of Rapid Chlorophyll Fluorescence Induction

A plant's rapid chlorophyll fluorescence induction kinetics curve (OJIP) can reflect a large amount of photochemical information about the PSII reaction center and can reflect the changes in the electron redox of the donor side, the acceptor side, and the reaction center of PSII in the photoreaction [11]. As shown in Figure 2A, at 25 °C, an obvious K point (300 μ s) appeared, and the OJIP curve was deformed into an OKJIP curve (Figure 2A). However, no significant change in the fluorescence value of the peony leaves occurred, indicating that the peony leaves that were exposed to the oxygen-evolving complex (OEC) were damaged to a certain extent, but the photosynthetic apparatus of the leaves was not seriously damaged. As shown in Figure 2B, when the duration of the high-temperature stress increased to 6 d, the fluorescence value of the peony leaves decreased at varying degrees, and an obvious inflection point K appeared in the curve under high-temperature stress, indicating that the oxygen-evolving complex of the peony leaves was seriously damaged under high temperature; while the fluorescence value of the peony leaves changed into a straight line distribution at seven days, indicating that peony leaves were seriously damaged by high temperatures.

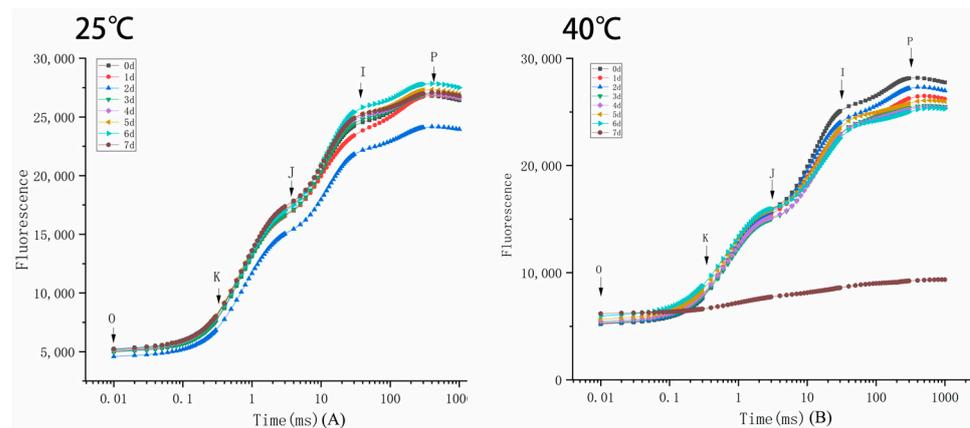


Figure 2. Effects of high-temperature stress on kinetic OJIP curve rapid chlorophyll fluorescence induction in peony. (A): OJIP curves under control treatment; (B): OJIP curves under high temperature treatment.

Figure 3B shows the ΔV_t curve that was based on the control fluorescence value. The results showed that from the beginning of the high temperature treatment to day 6, the chlorophyll fluorescence intensity of the peony leaves under high-temperature stress increased rapidly at approximately 300 μs after illumination, and there was an obvious characteristic site K point. The ΔV_K was greater than 0, and the ΔV_K value showed an upward trend with increased periods of high temperature treatments, indicating that the damage to the donor side of PSII in peony leaves was deeper, and the ability to provide electrons downstream was weaker. Figure 3A shows that K point also appeared in the control group, but its ΔV_K value increased significantly less than that in the high-temperature group, indicating that the damage suffered by the control group was significantly lower than that of the high-temperature group. The ΔV_J values of the peony leaves under high temperature treatment were all greater than 0. When $\Delta V_J > 0$ indicated Q_A -accumulation, it suggested that high-temperature stress inhibited the electron transfer from Q_A to Q_B in the peony leaves. After 7 days of high temperature treatment, the values of ΔV_K and ΔV_J increased sharply, indicating that the donor side of PSII in the peony leaves was severely damaged, the oxygen-evolving complex (OEC) was seriously damaged, and the electron transfer from Q_A to Q_B was also severely inhibited.

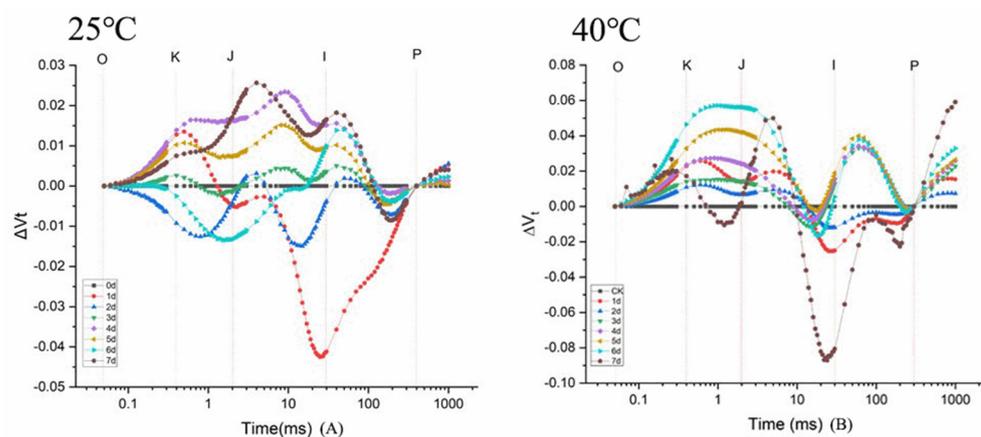


Figure 3. Effect of high-temperature stress on the relative variable fluorescence ($\Delta V_t = (F_t - F_0)/(F_m - F_0) - V_t$ (control)) of peony. (A): the relative variable fluorescence under control treatment; (B) the relative variable fluorescence under high temperature treatment.

Figure 4A,B show the kinetic curve ΔW_{OJ} of chlorophyll fluorescence data at 50 μs^{-2} ms. When the K-band was greater than 0, it indicated that the OEC, oxygen-evolving complex, had been inactivated. The inactivation of the oxygen-evolving complex began on the first

day of high-temperature stress. With the prolonged high-temperature treatment time, the degree of inactivation of OEC gradually increased. Under treatment of 25 °C, the ΔW_{OJ} value increased with time, but the increase was much smaller than that of the high temperature treatment. The magnitude was much smaller than that of the high temperature treatment. Figure 4C,D show the normalized W_{IP} between phase I (30 ms) and phase P (300 ms). The time point at $W_{IP} = 0.5$ (the half-life of the rising curve) reflected the reduction rate of the electron acceptor library at the end of PSI. The results showed that the half-life after high temperature treatment was shorter than that of the control, which reflected that high-temperature stress accelerated the reduction rate of the terminal electron library.

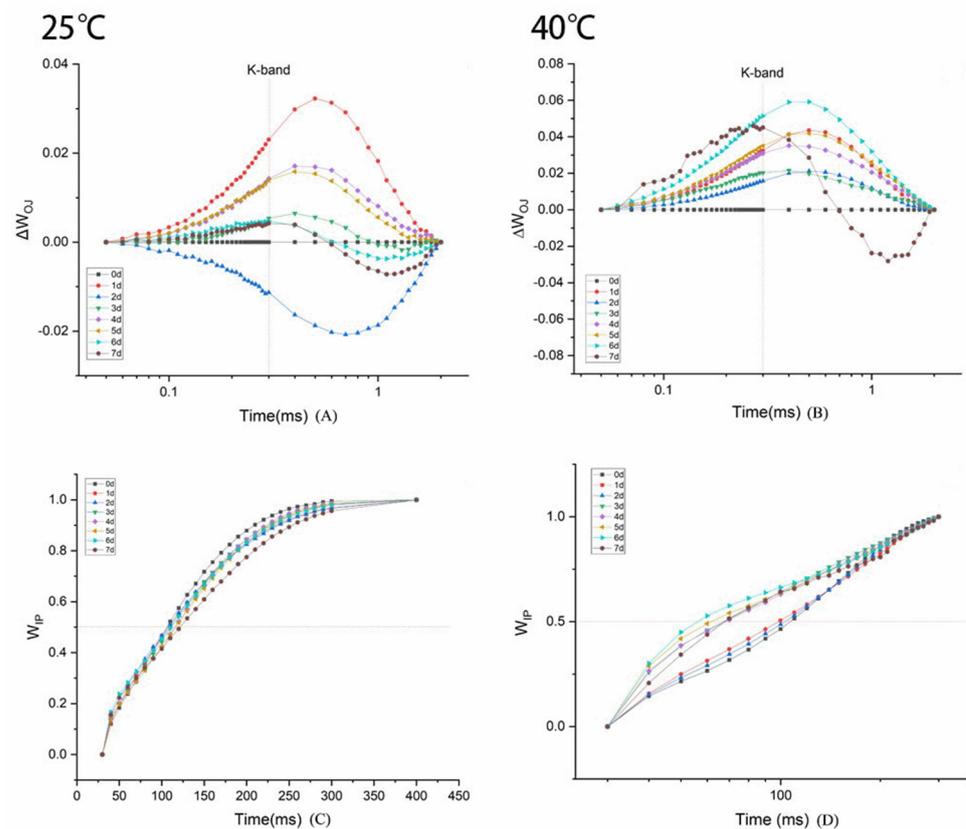


Figure 4. Effects of high-temperature stress on O–J phase kinetic curve and I–P phase kinetic curve of peony. (A,B) Effect of high-temperature stress on O–J phase dynamics curve of peony (double normalized by O–step (50 μ s) and J–step (2 ms) to show K–band); (C,D) Effect of high-temperature stress on the kinetic curve of I–P phase in peony (double normalized by I–step (30 ms) and P–step (300 ms) to show the reduction rate of the terminal electron receptor on the PSI receptor side).

4. Discussion

4.1. Effect of High Temperature on Photosynthetic Characteristics of the Peony Leaves

The high temperature in summer limits the normal growth of plants, causing a series of changes in plant morphology, anatomy, physiology, biochemistry, and phenology [24]. Plants are prone to photoinhibition under high temperatures [25]. In this experiment, the Pn and the Gs of the peony leaves showed decreasing trends with extended high temperature exposure. The mechanism of the net photosynthetic rate decrease may be different for different cultivars under various time periods of high temperature. Gao et al. [26] suggested that the limiting factors of photosynthesis were divided into stomatal restriction and non-stomatal restriction. In the process of gas exchange, when Gs and Ci decrease simultaneously, the decrease in Pn was affected by stomatal limitation; when the decrease in Pn was accompanied by an increase in Ci, it indicated that the decrease in Pn was influenced by the non-stomatal restriction of the decrease in the activity of the photosynthetic

mechanism. [27] In this study, when the high temperature treatment time was less than three days, the C_i of the peony leaves decreased with the decrease in G_s , indicating that the P_n of the leaves was restricted by the stomata. When the high temperature treatment time was more than three days, the C_i increased with the decrease in G_s , indicating that the P_n decrease was restricted by the non-stomata. By our calculations, when the plants were treated for three days at high temperatures, the 40 °C stomatal restriction value was 0.4484; when the high-temperature treatment lasted for five days, the 40 °C non-stomatal restriction value was 42,716.6458. With extended exposure to high temperatures, T_r first decreased and then increased. Ma et al. [28] found that with the increase in external temperatures, plants use stomatal closure through feedback regulation to reduce water loss caused by transpiration, and while under continuous high temperatures, plants accelerate transpiration by increasing stomata to mitigate the heat stress and avoid damage to their photosynthetic structure.

4.2. The Effect of High Temperature on the Rapid Chlorophyll Fluorescence Characteristics of Peony Leaves

High-temperature stress not only affected the photosynthetic electron transfer and the photosynthetic phosphorylation in leaves but also damaged the photosynthetic machinery [29]. F_v/F_m is an indicator of the PSII light energy conversion rate [30]. Plant leaf F_v/F_m referred to the maximum photochemical efficiency of PSII that was not subject to environmental stress and had undergone sufficient dark adaptation under normal circumstances, generally constant at 0.80–0.85 [31]. Fatemeh et al. [32] found that F_v/F_m decreased when plants were stressed, and F_v/F_m decline was an important indicator of light inhibition [33]. In this study, with increased high temperature treatment time, the F_v/F_m decreased and varied significantly, which was consistent with the above results and indicated that high-temperature stress caused light inhibition in peony leaves. PIabs is a performance index based on the absorption of light energy that can comprehensively reflect the efficiency of the PSII reaction center. In this experiment, the leaves of the peony plants showed a gradually decreasing trend with prolonged high temperature duration, which indicated that the light suppression of the peony under high-temperature stress decreased, and the light energy conversion rate and electron conversion capacity were reduced. Previous studies have shown that PIabs could accurately reflect the overall state of plant photosynthetic machinery and were more sensitive to certain stresses than F_v/F_m [34]. Liu et al. [35] found that the changes in PIabs in the F_v/F_m of pepper leaves were more sensitive to temperature stress, but the sensitivity of PIabs was higher, which was also confirmed in this study.

Chlorophyll fluorescence parameters can effectively reflect the absorption, transmission, distribution and other utilization of light energy by plants during photosynthesis [36], and contain a lot of information about the structure and function of the photosynthetic mechanism [37]. Ψ_o and ϕE_o reflected the degree of damage caused by high-temperature stress to the PSII receptor side; in the study, ϕE_o showed an overall downward trend with increased high temperature treatment time, and ϕD_o showed an upward trend, which indicated that the electron transport capacity of the PSII receptor side was decreasing at high temperature. This was similar to the results of Liu [38]. The rapid chlorophyll fluorescence induction kinetic curve accurately reflected the absorption, transformation, and dissipation of light energy by plant photosynthetic organs, as compared to the active parameters [39,40]. Due to the destruction of the PSII structure, the electron transmission and energy conversion were greatly affected [41], and previous studies have shown that thermal stress affects electron transport, and the Q_A-Q_B process of photosynthetic electron transport is inhibited, thereby disrupting the function of PSII [42,43]. ABS/RC , TR_O/RC , ET_O/RC , and DI_O/RC showed upward trends with the increase in high temperature exposure, which showed that after the high-temperature stress was triggered, the inactivation or cleavage of the reaction center per unit area of the leaf and the efficiency of the remaining active reaction centers were promoted to better dissipate the energy in the electron transport chain. When

the energy imbalance affected the electron transport, the V_J value reflected the number of PSII reaction centers closed at the J point, that is, the accumulation of Q_A^- [44]. The V_J increase indicated that the reaction center was closed to a high degree and suggested that the transmission of photosynthetic electrons from Q_A to Q_B had been inhibited. The large accumulation of Q_A^- indicated that high-temperature stress had inhibited the electron transport on the PSII receptor side. The subsequent V_J decline may have been caused by severe damage to the photosynthetic structure of the blade.

The rapid chlorophyll fluorescence induction kinetic curve contained information regarding the original photochemical reaction of the PSII reaction center. Through the analysis of chlorophyll fluorescence parameters, the state changes of plant photosynthetic mechanisms under environmental stress can be determined. Typical rapid chlorophyll fluorescence kinetic curves include O, J, I, and P phases in the fluorescence ascending stage [45]. However, when the donor site of the PSII of the plant has been damaged, the chlorophyll fluorescence intensity of leaves increased rapidly at approximately 300 μ s of illumination, and the special sites of the K point appeared [43]. By plotting the rapid chlorophyll fluorescence induction kinetic curve, we found that the K point was more significant with increased exposure to high temperatures, which indicated that high-temperature stress could lead to the appearance of the K point, and the donor site of the PSII in the peony leaves was damaged under high temperature treatment [46], indicating that the oxygen release complex was damaged [47]. High temperature can also lead to PSI photoinhibition. Under high-temperature stress, PSI photoinhibition has a more serious impact on plant growth than PSII photoinhibition, and PSI photoinhibition seriously affects CO_2 absorption, photoprotection and plant growth [48,49]. The maximum amplitude of fluorescence increase reflected the size of the PSI side terminal electron receptor pool, indicating that the high-temperature treatment in this study resulted in an increase in the PSI receptor side terminal electron receptor pool, possibly due to the accumulation of superoxide in the chloroplast that accelerated the photoinhibition of PSI [50]. W_{IP} reflected the reduction rate of the PSI receptor side end-electron acceptor; W_{IP} equal to 0.5 reflected the half-life of the rising curve [51]. In this test, the half-life was shorter as the duration of the high temperature was extended, which indicated that the high temperature had accelerated the reduction rate of the terminal electrons on the PSI receptor side [52].

5. Conclusions

In this study, the analytical technique of rapid chlorophyll fluorescence induction kinetic curve (JIP-test) was used to investigate the photosynthetic characteristics and chlorophyll fluorescence characteristics of peony leaves. The analysis of photosynthetic and chlorophyll fluorescence parameters showed that with the prolongation of the high temperature duration, the photosynthetic rate and photochemical properties of peony leaves decreased, the PSII reaction center was partially inactivated, the donor and acceptor sides of the PSII reaction center, and the aerobic complex was inactivated. High-temperature stress reduces the electron transport capacity of the PSII receptor side and increases the reduction rate of the PSI receptor side terminal electrons. The results provide a reference for understanding the photosynthetic characteristics and tolerance of peony plants under heat stress.

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