



## Article

# Study on Heat Resistance of Peony Using Photosynthetic Indexes and Rapid Fluorescence Kinetics

Zhijun Li <sup>1,†</sup>, Wen Ji <sup>1,†</sup>, Erman Hong <sup>1</sup>, Zhen Fan <sup>2</sup>, Bangyu Lin <sup>1</sup>, Xuanze Xia <sup>1</sup>, Xia Chen <sup>1,\*</sup> and Xiangtao Zhu <sup>1,\*</sup><sup>1</sup> College of Jiyang, Zhejiang A&F University, Zhuji 311800, China<sup>2</sup> Garden Plant Protection Department, Linyi City Garden Environmental Protection Service Center, Linyi 276000, China

\* Correspondence: cx2912@zafu.edu.cn (X.C.); zxt8202@163.com (X.Z.)

† These authors contributed equally to this work.

**Abstract:** To investigate the effects of high-temperature stress on the chlorophyll fluorescence induction kinetics of peony and to determine indicators for the rapid screening of varieties responding to high temperatures, three four-year-old peony variety, ‘Fengdanbai’, ‘Huhong’, and ‘Yinhongqiaodui’, were selected as materials. The photosynthetic curves (Pn-PAR) and fast chlorophyll fluorescence curves (OJIP curves) of peony leaves were measured at different times under high-temperature stress conditions (40 °C), the changes in the photosynthetic characteristics of different peony varieties under high-temperature stress were analyzed, and the heat tolerance of peony was evaluated. The results showed that ‘Huhong’ grew well within 16 days, while all of the other varieties showed obvious wilting at 6–9 days. High temperatures damaged the structure and function of the photosystem of peony leaves, indicating that the maximum net photosynthetic rate (P<sub>nmax</sub>), apparent quantum efficiency (AQE), maximum photochemical efficiency (F<sub>v</sub>/F<sub>m</sub>), and photosynthetic performance index (PI<sub>ABS</sub>) all tended to decrease under high-temperature stress, while the rate of closing the PS II reaction center (Mo) and the absorption per reaction center (ABS/RC), the capture (TRo/RC), and the dissipation (Dio/RC) of light energy per reaction center showed an overall increasing trend. The ability to cope with high-temperature stress differed among varieties, and the heat tolerance was determined to be in the descending order of ‘Fengdanbai’ < ‘Yinhongqiaodui’ < ‘Huhong’. The correlation analysis among the parameters and the analysis of the morphological change patterns in peony leaves concluded that PI<sub>ABS</sub>, Dio/RC, and Mo could be used as indicators of peony tolerance to high-temperature stress. The results of the study can provide a basis for the screening of heat-tolerant peony species and peony heat defense in the Jiangnan area.

**Keywords:** peony; high-temperature stress; photosynthetic characteristic parameters; chlorophyll fluorescence kinetics



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## 1. Introduction

Peony (*Paeonia suffruticosa* Andr.) is a deciduous flowering shrub of the genus *Paeonia* in the family *Paeoniaceae*. The cultivation of peony originates from China. Peony is not only abundant in China but is also widely distributed. From the three provinces in northeast China to the border of southern Xinjiang and from the coast of the East China Sea to the Qinghai–Tibet Plateau, there are wild peony resources as well as areas of artificial cultivation. Due to the influence of climate, history, cultural customs, and many other factors, the distribution of the cultivated peony population in China has resulted in the Yellow River Basin being the main cultivation area. Peony can be roughly divided into three populations: the cold-tolerant peony represented by the purple-spot peony of Gansu province and the heat-resistant peony represented by the Tongling peony of Anhui province; peony plants from Luoyang, Henan Province; and peony plants from Caozhou, Shandong province. Peony has large and beautiful flowers as well as abundant flower colors and a pleasant fragrance, resulting in peony being known as the “king of flowers”. They

have a good condition of industrialization: the traditional culture of peonies with unique characteristics has created a good social atmosphere for the industrialization of peonies; the natural beauty of peonies and the rich variety peony resources lay the foundation for the industrialization of peonies, and the increasingly mature cultivation methods as well as the application technologies for peony cultivation provide the technical premises and guarantee peony industrialization. The broad domestic and international market prospects and huge developmental potential also represent positives for peony industrialization. Peony has ornamental, economic, and medicinal values. Due to different processing conditions, the peony alias Danpi can be divided into guudan or even Dan. It is commonly used as a material in Chinese medicine. It uses root bark for medicinal purposes. It has the functions of reducing fever and cooling blood, dispersing stasis, and passing through the meridian. It has effects on symptoms such as fever, hematemeses, amenorrhea, abdominal pain, stasis, hypertension, stroke, acute and chronic appendicitis, rhinitis, etc. Pharmacology shows that paeonol can lower blood pressure, in addition to analgesic, sedative, antipyretic, anti-inflammatory, and bactericidal activity. Currently, four large tree peony groups have been described, with nearly 1000 cultivars existing in China. Peony is widely used as a potted or cut flower, and the essential oil made from its petal components has medicinal properties, including anti-cancer, anti-inflammatory, and anti-oxidation activities. More recently, the seeds from some peony cultivars have been identified as excellent sources of  $\alpha$ -linolenic acid, so peony seeds can be used to produce plant oil. Peony plants prefer warm, cool, and dry environments, and the high temperatures in southern summers seriously affect the growth and development of peony, with some cultivars being on the verge of extinction [1–4]. Photosynthesis is the most basic and complex process of all green plants. High-temperature stress significantly reduces the photosynthetic capacity of plants and further restricts the growth and development of plants. Chloroplasts are the main organelle involved in photosynthesis, and the photosynthetic pigments, two photosystems (PS II and PS I), electron transport system, and CO<sub>2</sub> reduction pathway are important components of photosynthesis. The disruption of any part of the photosynthesis system is sufficient to inhibit the overall photosynthetic mechanism of plants [5,6], reducing stomatal conductance, the photochemical efficiency of reaction centers, the CO<sub>2</sub> assimilation capacity, and water-use efficiency [7]. Studies have shown that high-temperature stress can lead to PSII failure, reduce electron transport efficiency, and increase the production of reactive oxygen species [8]. For example, high temperatures reduce the photosynthetic capacity of cotton leaves and affect the growth and development of cotton [9]. The maximum photosynthetic rate ( $Pn_{max}$ ) and apparent quantum efficiency (AQE) of grape leaves were significantly decreased after high-temperature stress [10], and irreversible damage to peony chloroplasts and their photosynthetic machinery was found after high-temperature stress treatment, resulting in the relative content of chlorophyll (SPAD) and the PS II parameters of  $F_o$ ,  $F_m$ , and  $F_v/F_m$  as well as other chlorophyll fluorescence parameters being significantly reduced, resulting in the yellowing of plant leaves and a significant decrease in photosynthetic capacity [11]. Obviously, studying the structure and function of PSII can reflect the responses of plants to high-temperature stress, and the rapid chlorophyll fluorescence induction curve and fluorescence parameters can be used to analyze a lot of information about the primary photochemistry of PSII reaction centers [12]. In recent years, numerous studies have demonstrated that chlorophyll fluorescence analysis techniques can reflect the damage induced by high temperatures on plants and are an important technical tool for screening heat-tolerant plant species that are widely used in research on plant heat tolerance [13–16]. For example, by analyzing the relevant parameters of fast chlorophyll fluorescence-induced kinetic curves, it was found that the ability of PS II light energy capture and transfer to the downstream area of  $Q_A$  decreased more significantly in high-temperature-sensitive wheat varieties compared to high-temperature-insensitive wheat varieties and led to a rapid decrease in the comprehensive performance of photosynthesis, while the efficiency of electron transfer from the  $Q_A$  (the maximum rate of primary quinone receptor) receptor side to the end of PS I and the activity of the oxygen release complex did

not decrease significantly [17]. In conclusion, the rapid chlorophyll fluorescence induction kinetic analysis (JIP test) of PSII is an important tool to study thermal stability, which is of great significance when exploring the effects of heat stress and the damage mechanism and to rapidly identify the heat tolerance of different varieties of peony.

At present, studies on high-temperature stress in peony mainly focus on the effects of high-temperature stress on physiological characteristics [18,19] and the photosynthetic mechanism [20–23] of peony, with examples of findings being how high temperatures lead to a decrease in  $P_n$ , a decrease in the soluble protein content and SOD activity, and an increase in the proline content and MDA content in peony leaves; an increase in the electrical conductivity of peony leaves; the inhibition of chlorophyll synthesis or chlorophyll degradation; and a decreased photosynthetic rate decreased and increased lipid membrane permeability. However, there are no reports on the correlation between the photosynthetic characteristic curve, the kinetic curve of rapid chlorophyll fluorescence induction, and the heat tolerance of different peony varieties under high-temperature stress. In addition, there are few reports on the selection of photosynthetic fluorescence indexes in response to the heat resistance of peony, and the determination of these indexes is of great significance for the selection of heat-resistant varieties of peony, which could provide an effective reference for the introduction of peony varieties or for the evaluation of adaptability to high temperatures. Therefore, in this paper, three four-year-old peony varieties, 'Fengdanbai', 'Huhong', and 'Yinhongqiaodui', were used as the materials, and a high-temperature stress treatment was carried out via an artificial control test. Based on the previous analysis of the changes in the photosynthetic parameters and chlorophyll fluorescence kinetic indexes of 'Fengdanbai' under high-temperature stress [24], the response differences in the photosystems of different peony varieties under high-temperature stress was systematically studied by photosynthetic characteristic parameters and fluorescence parameters. Through a horizontal comparison of different peony varieties, the key photosynthetic characteristic parameters and fluorescence parameters of peony varieties responding to high temperatures were screened, providing a theoretical basis and important reference for the rapid screening of peony varieties with high-temperature tolerance.

## 2. Materials and Methods

### 2.1. Plant Materials and Experimental Design

In this experiment, four-year-old plants of peony varieties 'Fengdanbai', 'Huhong', and 'Yinhongqiaodui' from Heze, Shandong Province were selected to be placed in an artificial incubator from April to June 2021. They were planted in plastic basin from September to October in 2020 with a mixture of peat:sand:perlite (mass ratio: 5:3:2). In mid-May 2021, plants with basically the same growth and size were selected, with 6 plants per variety, 3 for control treatment and 3 for high temperature treatment. Fluorescence parameters were measured after 2 weeks of pretreatment as the index on day 0. Each variety was divided into two groups and moved into the artificial incubator at the same time: the experimental group (EG) at 40 °C/35 °C (day/night) and the control group (CK) at 25 °C/20 °C (day/night).

### 2.2. Electron Microscope Observation

The materials were dried using a freeze dryer (Yamato DC810, Tokyo, Japan), and gold was sprayed using an ion sputtering instrument (E-1045, Tokyo, Japan). The treated samples were observed using a COXEM EM-30 (Daejeon, Republic of Korea) scanning electron microscope. Leaf thickness was measured at 5 random fields under an electron microscope with 500 times magnification. All data were the average of 20 data points.

### 2.3. Determination of Photosynthetic Parameters

A Li-6400 portable photosynthesis measurement system (Li-COR Biosciences Inc., Lincoln, NE, USA) was used to measure the parameters of the light–response curves of the peony leaves from 8:00 to 12:00 every day. Three plants were selected from each variety,

and the third to fifth healthiest mature leaves without disease or insect pests were selected as test leaves and taken from the top to the bottom of each plant. Photosynthetically active radiation (PAR) was set at 11 horizontal gradients: 2000, 1500, 1200, 1000, 750, 500, 250, 150, 100, 50, and 0  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and preheating was carried out for 30 min before each measurement. Meanwhile, light adaptation of the measured blade was carried out for 30 min. The temperature of the Li-6400 leaf chamber was set at 25 °C; the CO<sub>2</sub> concentration was 400  $\mu\text{mol}\cdot\text{s}^{-1}$ ; the corresponding net photosynthetic rate (Pn) was determined; and the light–response curve was plotted.

The light saturation point (LSP), light compensation point (LCP), apparent quantum effect (AQE) and maximum net photosynthetic rate (Pn<sub>max</sub>) of different varieties of peony were obtained by fitting the simplified model of plant photosynthesis to light response mechanism [25]. Three non-adjacent leaves of each plant were selected for determination, and three replicates were completed. After measurement at 9:00–11:00 a.m., on day 0, the third fully expanded leaf below the top of different plants was selected and marked for repositioning, and six replicates of each treatment were completed.

#### 2.4. Determination of Chlorophyll Fluorescence Kinetic Parameters

The Handy PEA Plant efficiency analyzer (Hansatech, Norfolk, UK) was used to take measurements from 9:00 a.m. to 11:00 a.m. every day. At the same time, after the measurements taken on day 0, the third fully expanded leaf below the tops of the different plants was selected and marked for repositioning, and six replicates of each treatment were completed. The OJIP curve that was obtained was analyzed and was the fast chlorophyll fluorescence induction kinetics curve (OJIP curve) measured at 0–25 d (or plant death day) under high-temperature stress.

#### 2.5. Statistical Analyses

The data were subjected to one-way ANOVA and a test of variance using SPSS 20.0 software, and Microsoft Office Excel 2020 and Origin9 software were used for data processing and graphing.

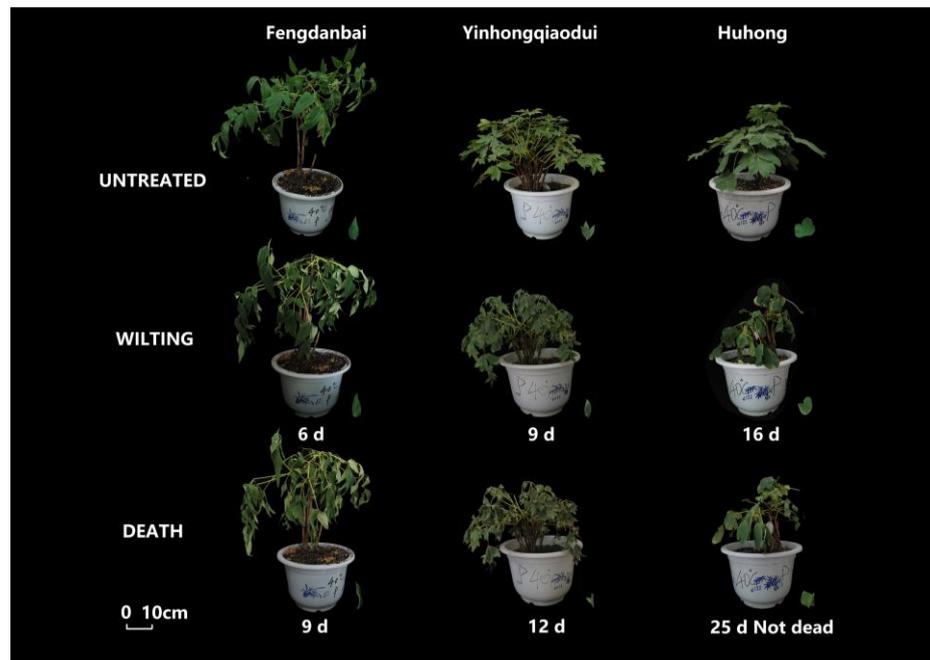
### 3. Results

#### 3.1. Changes in Leaf Morphology of Different Species of Peony under High-Temperature Stress

From Figure 1 and Table 1, it is clear that ‘Fengdanbai’ was the most sensitive to high-temperature stress, with leaf wilt appearing after 6 d of high-temperature stress treatment, and the shortest time to death (all leaves wilted and stems died) was 9 d; ‘Yinhongqiaodui’ had the second highest sensitivity to high-temperature stress; and ‘Huhong’ was the most tolerant to high temperatures, with leaf wilt appearing initially at 16 d under stress treatment, and death was not observed after 25 d under high-temperature conditions. Combined with the fact that summer temperatures in Zhejiang can reach above 35 °C (temperatures above 40 °C are rare) and last for approximately 30 d and the fact that the conditions of this experiment were the ultimate high temperature of 40 °C, we can confirm that ‘Huhong’ is the most heat-tolerant peony variety of the ones tested and that it can better adapt to the hot summer weather in Zhejiang.

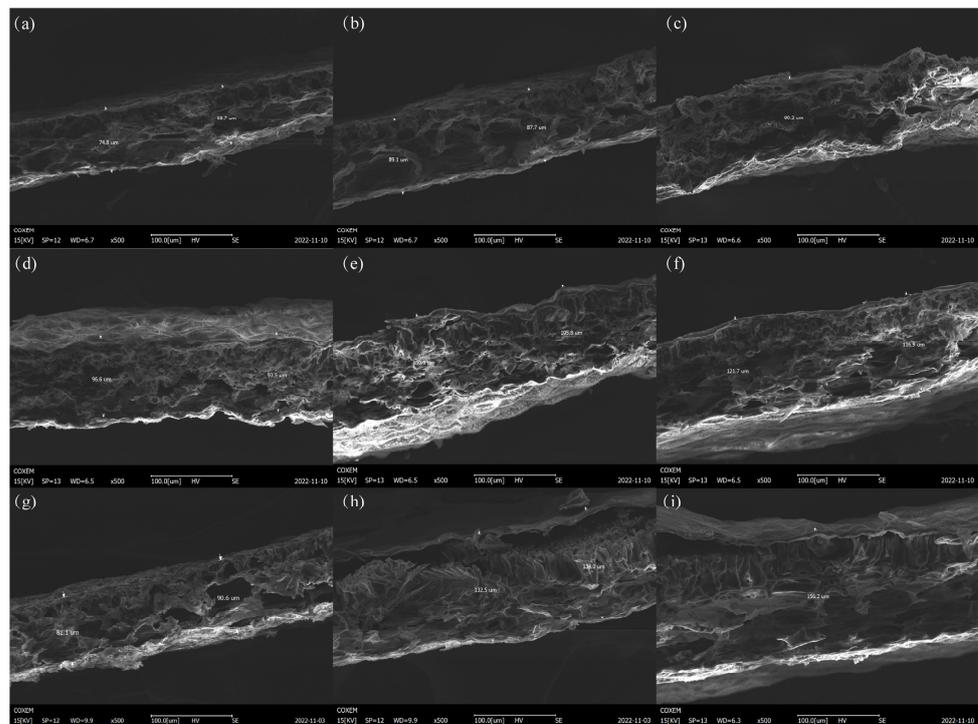
**Table 1.** Time to onset of leaf wilting and plant death in ‘Fengdanbai’ ‘Yinhongqiaodui’ and ‘Huhong’ cultivars of peony under high-temperature stress.

Cultivar	Origin	Time to Leaf Wilting (d)	Time of Plant Death (d)
Fengdanbai	Shandong	6	9
Huhong	Shandong	16	/
Yinhongqiaodui	Shandong	9	12



**Figure 1.** Morphological changes in ‘Fengdanbai’ ‘Yinhongqiaodui’ and ‘Huhong’ cultivars of peony under high-temperature stress. The experimental group was treated with a high temperature of 40 °C and the rest remained the same as the control group.

As shown in Table 2 and Figure 2, the leaf thicknesses of different peony varieties are different, and the changes in the leaf thickness under high-temperature stress are also different. With the extension of the high-temperature stress time, the thicknesses of leaves of all varieties increased, and the thickness of ‘Huhong’ demonstrating the largest increase.



**Figure 2.** Electron microscope observations of leaf thickness in ‘Fengdanbai’ ‘Yinhongqiaodui’ and ‘Huhong’ cultivars of peony. (a–c): leaf thickness of ‘Fengdanbai’ (untreated, wilting, death); (d–f): leaf thickness of ‘Yinhongqiaodui’; (g–i): leaf thickness of ‘Huhong’.

**Table 2.** The changes in the leaf thickness of Fengdanbai ‘Yinhongqiaodui’ and ‘Huhong peony cultivars under high-temperature stress.

	Fengdanbai ( $\mu\text{m}$ )	Huhong ( $\mu\text{m}$ )	Yinhongqiaodui ( $\mu\text{m}$ )
Untreated	71.47 $\pm$ 3.09 c	86.43 $\pm$ 4.86 c	95.30 $\pm$ 1.61 c
Wilting	88.07 $\pm$ 0.91 b	132.10 $\pm$ 2.13 b	107.47 $\pm$ 2.15 b
death	91.43 $\pm$ 1.23 a	150.53 $\pm$ 1.96 a	119.47 $\pm$ 2.42 a

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

### 3.2. Effects of High-Temperature Stress on Photosynthetic Characteristic Curve Parameters of Different Varieties of Peony Leaves

The photosynthetic parameters of the three peony varieties were obtained by fitting a simplified model of the peony light–response mechanism model [25], and as shown in Table 3, high-temperature stress had different effects on the characteristic parameters of the light–response curves of the different varieties of peony leaves. The light saturation point (LSP) index and maximum net photosynthetic rate ( $P_{n_{\max}}$ ) showed a decreasing trend as stress progressed. The apparent quantum efficiency (AQE) of Hu Hong and Yinhongqiao showed a downward trend, while that of Feng Dan decreased first and then increased. On the contrary, the light compensation point (LCP) showed an overall increasing trend under high-temperature stress. During the initial stage of stress (0–2 days), ‘Fengdanbai’ changed the most dramatically, with an increase of 218.30%. There was no similar pattern in the changes in the dark respiration rate ( $R_d$ ) in the leaves of different peony species. As shown in Table 4, the maximum net photosynthetic rate of different peony leaves under high-temperature stress was significantly and positively correlated with the change in the apparent quantum efficiency.

**Table 3.** Effect of high-temperature stress on the characteristic parameters of light–response curves of Fengdanbai ‘Yinhongqiaodui’ and ‘Huhong cultivars of peony leaves.

Cultivar	Stress Days	LSP ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	LCP ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	$R_d$ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	$P_{n_{\max}}$ ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	AQE ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
Fengdanbai	0	526 $\pm$ 22.79 b	0.3383 $\pm$ 0.10 d	0.0286 $\pm$ 0.11 d	5.9489 $\pm$ 0.14 a	0.0175 $\pm$ 0.0001 a
	2	591 $\pm$ 12.01 a	1.0768 $\pm$ 0.39 bc	0.0325 $\pm$ 0.01 c	1.3040 $\pm$ 0.05 d	0.0025 $\pm$ 0.0003 d
	5	567 $\pm$ 44.83 ab	1.3513 $\pm$ 0.20 a	0.0391 $\pm$ 0.02 b	1.6740 $\pm$ 0.47 c	0.0031 $\pm$ 0.0008 c
	6 *	502 $\pm$ 46.54 bc	1.1913 $\pm$ 0.02 b	0.0622 $\pm$ 0.01 a	2.0135 $\pm$ 0.05 b	0.0036 $\pm$ 0.0002 b
Huhong	0	501 $\pm$ 39.20 a	6.3132 $\pm$ 1.50 c	0.7930 $\pm$ 0.15 b	3.8829 $\pm$ 0.79 b	0.0100 $\pm$ 0.0022 ab
	2	476 $\pm$ 14.53 b	7.1531 $\pm$ 0.28 b	0.6958 $\pm$ 0.01 d	4.3902 $\pm$ 0.06 a	0.0114 $\pm$ 0.0001 a
	5	486 $\pm$ 51.65 ab	7.1175 $\pm$ 1.45 b	0.7792 $\pm$ 0.17 bc	3.5432 $\pm$ 0.14 c	0.0106 $\pm$ 0.0031 a
	16 *	484 $\pm$ 49.89 ab	7.9326 $\pm$ 1.64 a	0.8982 $\pm$ 0.44 a	3.7228 $\pm$ 0.10 b	0.0062 $\pm$ 0.0053 b
Yinhongqiaodui	0	426 $\pm$ 21.21 c	8.0760 $\pm$ 0.90 d	0.8302 $\pm$ 0.09 a	5.7344 $\pm$ 0.79 a	0.0300 $\pm$ 0.0137 a
	2	543 $\pm$ 87.88 b	14.7729 $\pm$ 0.89 c	0.6056 $\pm$ 0.19 b	3.9681 $\pm$ 0.81 b	0.0203 $\pm$ 0.0032 b
	5	612 $\pm$ 83.29 a	38.7643 $\pm$ 2.70 a	0.5933 $\pm$ 0.01 b	0.2534 $\pm$ 0.02 d	0.0048 $\pm$ 0.0006 c
	9 *	349 $\pm$ 21.24 d	30.5128 $\pm$ 4.79 b	0.6117 $\pm$ 0.16 b	0.2834 $\pm$ 0.05 c	0.0015 $\pm$ 0.0012 d

Note: Light saturation point index (LSP); light compensation point (LCP); dark respiration rate ( $R_d$ ); maximum net photosynthetic rate ( $P_{n_{\max}}$ ); apparent quantum efficiency (AQE). Different lowercase letters indicate significant differences at the  $p < 0.05$  level for different stress times for the same species. \* Indicates the day as the time of wilting of the plant.

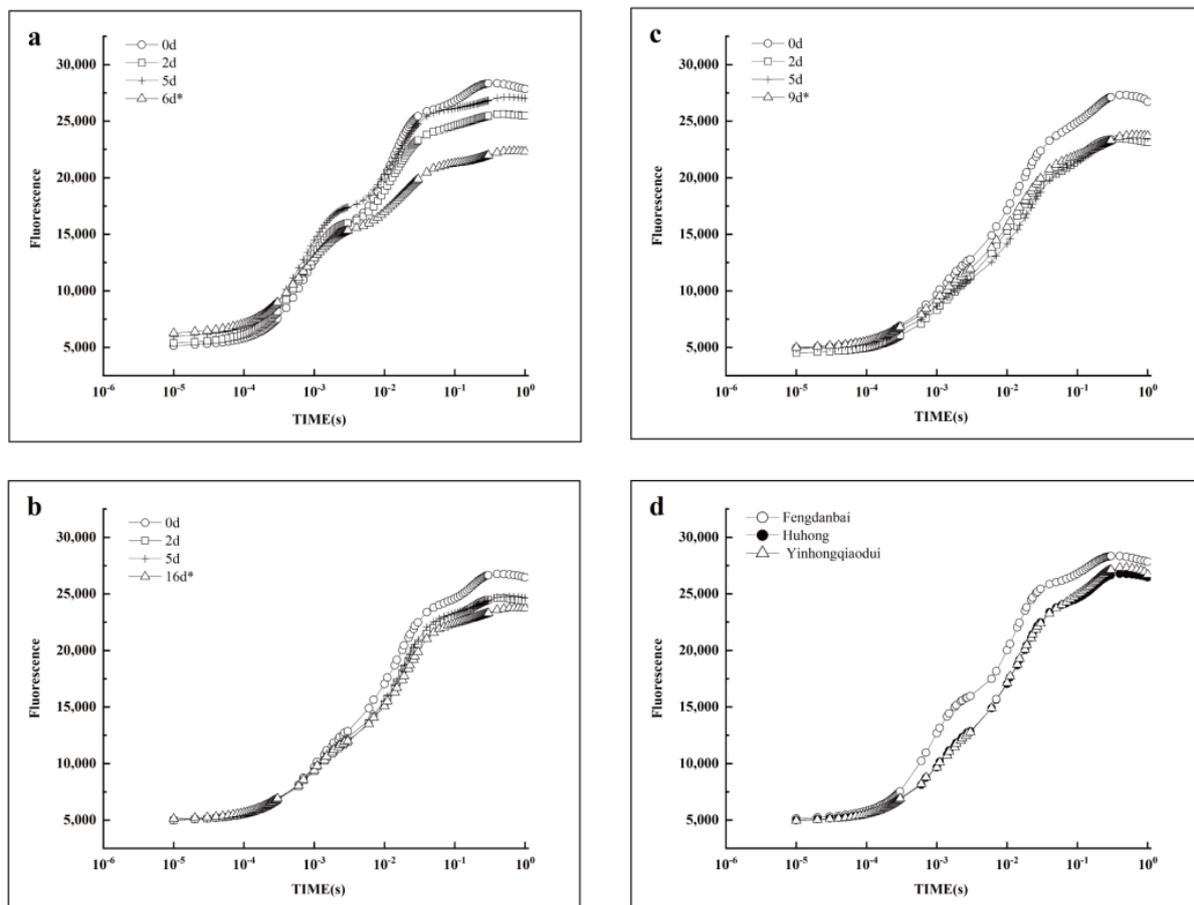
**Table 4.** Correlation analysis among characteristic parameters of light–response curves of Fengdanbai ‘Yinhongqiaodui’ and ‘Huhong cultivars of peony under high-temperature stress.

Index	LSP	Pn <sub>max</sub>	AQE	LCP	R <sub>d</sub>
LSP	1				
Pn <sub>max</sub>	−0.184	1			
AQE	−0.193	0.821 **	1		
LCP	−0.112	−0.537	−0.173	1	
R <sub>d</sub>	−0.461	0.250	0.321	0.387	1

Note: Light saturation point index (LSP); light compensation point (LCP); dark respiration rate (R<sub>d</sub>); maximum net photosynthetic rate (Pn<sub>max</sub>); apparent quantum efficiency (AQE). \*\* means extremely significant difference at 0.01 level.

**3.3. Effects of High-Temperature Stress on the Rapid Chlorophyll Fluorescence Induction Kinetic Curves of Different Species of Peony**

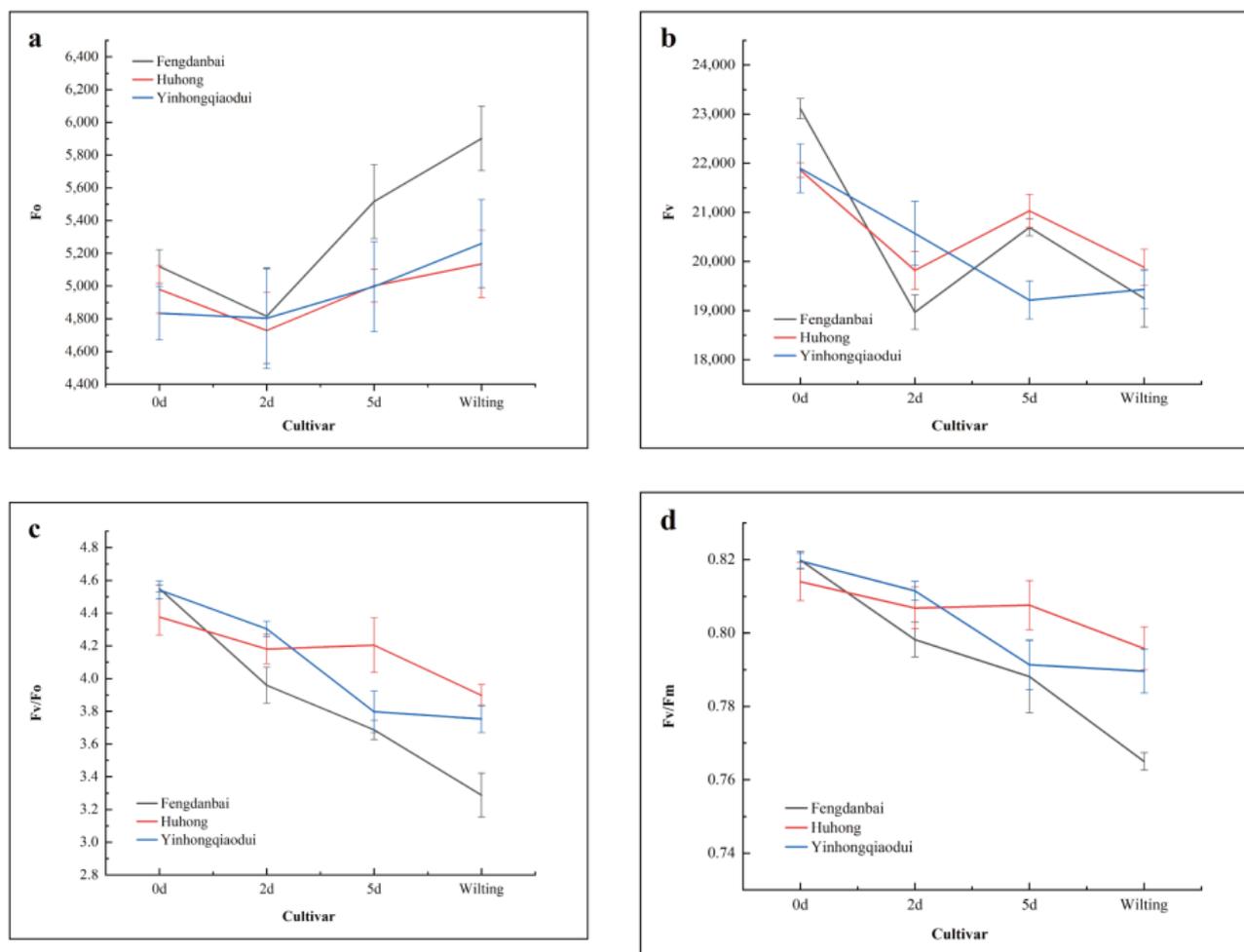
As shown in Figure 3, the OJIP curves of peony leaves changed significantly after high-temperature stress. The values of the J-I phase and the maximum fluorescence intensity (*p*) values in the OJIP curves of all peony species were lower than those at 0 h of treatment. Among them, the values of P for ‘Fengdanbai’, ‘Huhong’, and ‘Yinhongqiaodui’ decreased by 19.8%, 10.2%, and 11.2%, respectively, compared to those at 0 h of treatment. The J-I phase inflection point of ‘Huhong’ tended to be flat, while the J-I phase of ‘Fengdanbai’ had a significant inflection point.



**Figure 3.** Effects of high-temperature stress on the rapid chlorophyll fluorescence induction curves of ‘Fengdanbai’ ‘Yinhongqiaodui’ and ‘Huhong’ cultivars of peony (a–c): show the rapid chlorophyll fluorescence induction curves of ‘Fengdanbai’, ‘Huhong’, and ‘Yinhongqiaodui’, respectively; (d): shows the OJIP curves of the three peony varieties without stress. \* Indicates the day as the time of wilting of the plant.

### 3.4. Effects of High-Temperature Stress on the Basic Parameters of Chlorophyll Fluorescence of Different Varieties of Peony Leaves

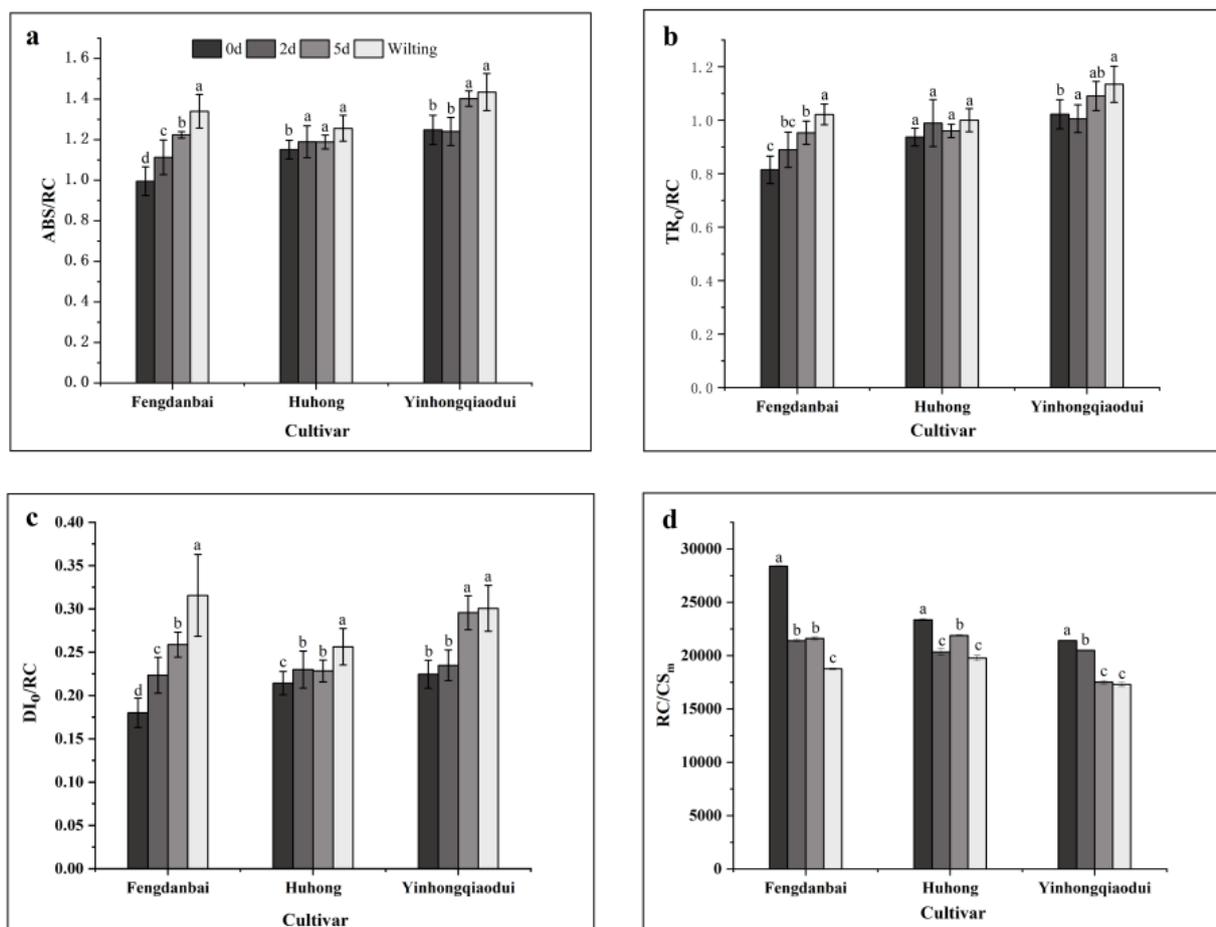
As shown in Figure 4, the  $F_o$  (minimal fluorescence) of all three peony varieties showed an increasing trend as the number of stress days increased, while  $F_v/F_o$  (the ratio of the photochemical reaction and non-photochemical quenching rates in the excitation energy utilization of reaction centers),  $F_v/F_m$  (maximum photochemical efficiency), and  $F_v$  (variable fluorescence) showed a decreasing trend. The trend of 'Fengdanbai' was more obvious than that in the other two varieties in these four indicators.



**Figure 4.** Effect of high-temperature stress on the basic parameters of chlorophyll fluorescence of 'Fengdanbai' 'Yinhongqiaodui' and 'Huhong' cultivars of peony leaves. Minimal fluorescence (all PS II RCs are assumed to be open) ( $F_o$ ; (a)); maximal variable fluorescence ( $F_v$ ; (b)); (c): the potential activity of PS II ( $F_v/F_o$ ; (c)); PS II primary light energy conversion efficiency ( $F_v/F_m$ ; (d)).

### 3.5. Effects of High-Temperature Stress on the Activity of PSII Reaction Centers of Peony Leaves of Different Species

As shown in Figure 5, as the high-temperature stress treatment time increased, the light energy absorbed (ABS/RC), captured (TRo/RC), and dissipated (Dio/RC) per unit reaction center of the leaves of the three peony species showed an increasing trend compared with that of CK, while the number of active reaction centers (RC/CS<sub>m</sub>) per unit leaf area decreased significantly, with the trend of 'Fengdanbai' being more significant.



**Figure 5.** Effects of high-temperature stress on specific activity parameters of ‘Fengdanbai’ ‘Yinhongqiaodui’ and ‘Huhong’ cultivars of peony leaves. Absorption flux (of antenna Chls) per RC (also a measure of PSII apparent antenna size) (ABS/RC; (a)); trapped energy flux (leading to  $Q_A$  reduction) per RC (TR<sub>o</sub>/RC; (b)); dissipated energy flux per RC (DI<sub>o</sub>/RC; (c)); the number of reaction centers per unit area at maximum fluorescence (RC/CS<sub>m</sub>; (d)). Different lowercase letters indicate significant differences at the  $p < 0.05$  level for different stress times for the same species.

### 3.6. Effects of High-Temperature Stress on the Sides of PSII Receptors in Leaves of Different Peony Species

As can be seen from Table 5, all of the indexes of the different peony varieties were significantly different when the wilting condition occurred compared to at 0 h of treatment. The  $\Psi_o$  (2 ms illumination has the openness of the active anyway center) and  $\phi_{Eo}$  (quantum yield for electron transfer (at  $t = 0$ )) of the leaves from all of the cultivars decreased significantly compared to at 0 h of treatment. On the contrary, Mo (the maximum rate at which the primary quinone receptor ( $Q_A$ ) was reduced) and  $V_j$  (the amount of reactive  $Q_A$ -accumulation) both show an upward trend.

### 3.7. Effects of High-Temperature Stress on the Photochemical Performance Index of Different Varieties of Peony Leaves

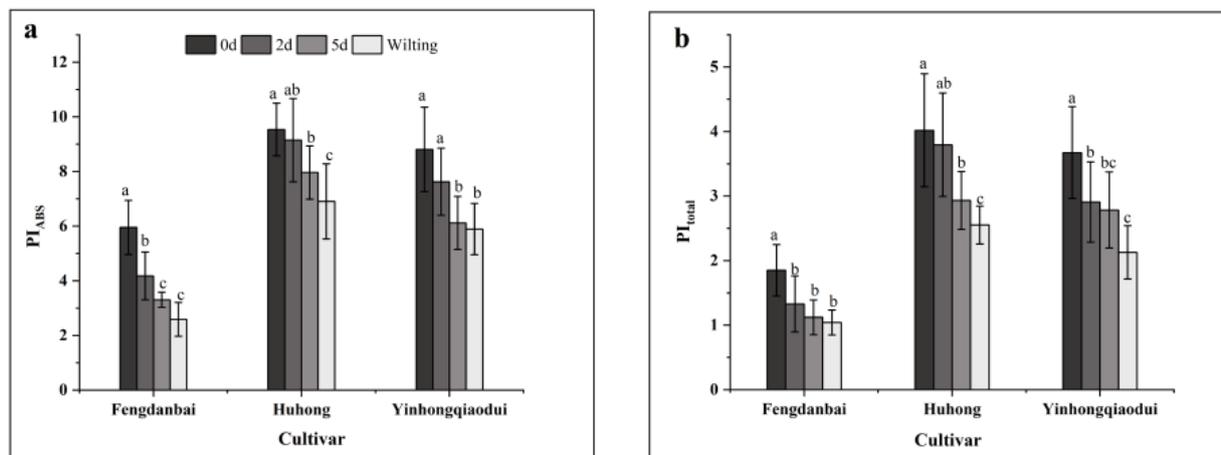
PI<sub>ABS</sub>, a PS II performance index based on light energy absorption, was at its maximum at 0 h of treatment (Figure 6) and showed a decreasing trend with the extension of the stress time. After 2 d of stress treatment, the PI<sub>ABS</sub> levels of ‘Fengdanbai’, ‘Huhong’, and ‘Yinhongqiaodui’ decreased by 29.86%, 4.11%, and 13.43%, respectively, indicating that ‘Fengdanbai’ caused strong inhibition of its photosynthetic mechanism during the early stages of high-temperature stress. PI<sub>total</sub> is the overall functional activity index, indicating PS II, PS I, and the inter-systemic electron transport chain. The PI<sub>total</sub> values of all of

the varieties showed a decreasing trend compared to treatment at 0 h, with ‘Fengdanbai’ showing the largest percentage decrease of 28.16% compared to CK at 2 d of stress treatment.

**Table 5.** Effects of high-temperature stress on PS II receptor-side parameters on the OJIP curves of Fengdanbai ‘Yinhongqiaodui’ and ‘Huhong cultivars of peony plants.

Cultivar	Stress Days	$M_o$	$V_j$	$\Psi_o$	$\Phi_{Eo}$
Fengdanbai	0	$0.359 \pm 0.041$ c	$0.438 \pm 0.021$ c	$0.562 \pm 0.021$ a	$0.452 \pm 0.030$ a
	2	$0.427 \pm 0.058$ b	$0.467 \pm 0.028$ b	$0.533 \pm 0.028$ b	$0.422 \pm 0.024$ ab
	5	$0.453 \pm 0.033$ b	$0.479 \pm 0.017$ b	$0.527 \pm 0.026$ bc	$0.410 \pm 0.012$ b
	6 *	$0.505 \pm 0.020$ a	$0.492 \pm 0.019$ a	$0.508 \pm 0.019$ c	$0.389 \pm 0.021$ c
Huhong	0	$0.276 \pm 0.010$ ab	$0.293 \pm 0.007$ b	$0.707 \pm 0.007$ b	$0.577 \pm 0.008$ b
	2	$0.297 \pm 0.060$ ab	$0.282 \pm 0.015$ b	$0.718 \pm 0.015$ a	$0.580 \pm 0.009$ a
	5	$0.297 \pm 0.014$ a	$0.307 \pm 0.010$ a	$0.693 \pm 0.010$ c	$0.560 \pm 0.012$ c
	16 *	$0.297 \pm 0.017$ a	$0.310 \pm 0.009$ a	$0.690 \pm 0.009$ c	$0.503 \pm 0.019$ d
Yinhongqiaodui	0	$0.304 \pm 0.034$ c	$0.296 \pm 0.023$ bc	$0.704 \pm 0.023$ a	$0.573 \pm 0.030$ a
	2	$0.318 \pm 0.033$ bc	$0.315 \pm 0.022$ ab	$0.685 \pm 0.022$ b	$0.556 \pm 0.016$ b
	5	$0.331 \pm 0.024$ b	$0.310 \pm 0.018$ b	$0.690 \pm 0.018$ ab	$0.512 \pm 0.124$ c
	9 *	$0.375 \pm 0.030$ a	$0.323 \pm 0.020$ a	$0.677 \pm 0.020$ bc	$0.544 \pm 0.030$ b

Note: Approximated initial (in  $ms^{-1}$ ) ( $M_o$ ); Standardized variable fluorescence at J point (2 ms) ( $V_j$ ); probability that a trapped exciton moves an electron into the electron transport chain beyond  $Q_A^-$  (at  $t = 0$ ) ( $\Psi_o$ ); quantum yield for electron transport (ET) (at  $t = 0$ ) ( $\Phi_{Eo}$ ). Different lowercase letters indicate significant differences at the  $p < 0.05$  level for different stress times for the same species. \* Indicates the day as the time of wilting of the plant.



**Figure 6.** Effects of high-temperature stress on PS II performance index for  $PI_{ABS}$  and  $PI_{total}$  values of ‘Fengdanbai’ ‘Yinhongqiaodui’ and ‘Huhong’ cultivars of peony leaves. Performance index showing absorption basis ( $PI_{ABS}$ ; (a)) and performance index (potential) for energy conservation from exciton to the reduction of PS I end acceptors ( $PI_{total}$ ; (b)). Different lowercase letters indicate significant differences at the  $p < 0.05$  level for different stress times for the same species.

### 3.8. Correlation of Chlorophyll Fluorescence Parameters of Peony Leaves under High-Temperature Stress

Correlation analysis was conducted on 14 kinetic parameters of chlorophyll fluorescence for peony leaves under high-temperature stress, and the results are shown in Table 6. Among the above indicators,  $F_v/F_m$  was significantly positively correlated with  $F_v$ ,  $F_v/F_o$ , and  $RC/CS_m$ ; significantly positively correlated with  $PI_{ABS}$ ; and significantly negatively correlated with  $ABS/RC$ ,  $TR_o/RC$ , and  $DI_o/RC$ .  $PI_{ABS}$ , a comprehensive index of the changes in the reaction optical system, is positively correlated with  $F_v/F_m$ ,  $F_v/F_o$ ,  $\Psi_o$ ,  $\Phi_{Eo}$ , and  $PI_{total}$  and negatively correlated with  $F_o$ ,  $M_o$ , and  $V_j$ . The cross correlation between the other indicators is obvious.

**Table 6.** Correlation of chlorophyll fluorescence parameters under high-temperature stress.

	Fo	Fv	Fv/Fm	Fv/Fo	ABS/RC	TRo/RC	DIo/RC	RC/CS <sub>m</sub>	Ψ <sub>o</sub>	φE <sub>o</sub>	Mo	V <sub>j</sub>	PI <sub>ABS</sub>	PI <sub>total</sub>
Fo	1													
Fv	0.371	1												
Fv/Fm	−0.321	0.730 **	1											
Fv/Fo	−0.330	0.727 **	0.999 **	1										
ABS/RC	−0.266	−0.850 **	−0.653 **	−0.640 *	1									
TRo/RC	−0.059	−0.660 *	−0.502 **	−0.496	0.842 **	1								
DIo/RC	0.174	−0.719 **	−0.784 **	−0.778 **	0.838 **	0.915 **	1							
RC/CS <sub>m</sub>	0.196	0.860 **	0.628 **	0.627 *	−0.907 **	−0.940 **	−0.899 **	1						
Ψ <sub>o</sub>	−0.636 *	−0.517	0.039	0.043	0.548	0.655 *	0.403	−0.669 *	1					
φE <sub>o</sub>	−0.661 *	−0.370	0.220	0.222	0.433	0.572	0.259	−0.545	0.961 **	1				
Mo	0.613 *	0.204	−0.320	−0.322	−0.216	−0.380	0.116	0.354	−0.915 **	−0.894 **	1			
V <sub>j</sub>	0.646 *	0.519	−0.041	−0.044	−0.551	−0.649 *	−0.397	0.665 *	−1.000 **	−0.961 **	0.915 **	1		
PI <sub>ABS</sub>	−0.824 **	−0.034	0.593 *	0.598 *	−0.023	−0.012	−0.322	−0.015	0.716 **	0.791 **	−0.817 **	−0.721 **	1	
PI <sub>total</sub>	−0.776 **	−0.187	0.437	0.439	0.182	0.251	−0.063	−0.268	0.848 **	0.882 **	−0.871 **	−0.850 **	0.937 **	1

Note: \* means significant difference at 0.05 level. \*\* means extremely significant difference at 0.01 level.

Significant relationships existed between Fo and Ψ<sub>o</sub>, φE<sub>o</sub>, Mo, and V<sub>j</sub>. Fundamental characteristics of chlorophyll fluorescence, such as Fv, Fv/Fm, and Fv/Fo, were strongly connected with the parameters of chlorophyll fluorescence's particular activity (ABS/RC, DIo/RC, and RC/CS<sub>m</sub>), but not with the parameters of Ψ<sub>o</sub>, φE<sub>o</sub>, Mo, or V<sub>j</sub>. Significant correlations between TRo/RC and RC/CS<sub>m</sub> and Ψ<sub>o</sub> and V<sub>j</sub> were found.

#### 4. Discussion

Abiotic stress factors such as extreme temperatures, droughts and floods, and salinity are influential factors that govern plant growth and development [26]. Studies have shown that the responses of plants to high-temperature stress is a complex and coordinated biological regulation process that results in changes in plant morphology [27], while high-temperature stress affects the physiological responses of plants and can severely damage their own adversity-control ability and produce heat damage [28]. Peony, which “likes mildness and avoids heat”, has a high light compensation point and a low light saturation point, and its effective use of light energy is narrow, so its photosynthetic physiology experiences stress under the high light and high temperature conditions during southern summers [23,29,30]. To enrich the number of peony varieties in Jiangnan, high temperature is one of the most important factors among all constraints, and therefore, it is imperative to select and breed excellent heat-resistant peony varieties. Based on the analysis of the photosynthetic parameters and the chlorophyll fluorescence kinetic indexes of ‘Fengdanbai’ under high-temperature stress [24], this study aimed to clarify the mechanism of peony's response and adaptation to high-temperature stress, determine the indexes that can quickly screen the varieties responding to high temperatures, and lay the foundation for screening peony varieties with high-temperature tolerance by comparing and analyzing the differences in the photosynthetic curve parameters and the chlorophyll fluorescence kinetic curves of different peony varieties under high-temperature stress.

The leaf thicknesses of the different peony varieties are different and can be used as one of the indexes to measure the heat resistance of plants. Generally, thicker leaves have slower water transpiration and experience less water loss. In hot environments, the rate of water loss can also be reduced to alleviate the damage incurred by high temperature to leaves [31]. In this study, the leaf thicknesses of the three peony cultivars increased as the high-temperature stress time was extended, with ‘Huhong’ increasing the most, which may have been due to the thickening of the leaves to plant reduce caused by the high temperatures.

Photosynthesis is sensitive to changes in temperature and plays an important role in plant growth and development, and studies have shown that high-temperature stress leads to photoinhibition in plants, damaging their photosystems and inactivating the Rubisco-activating enzyme for photosynthesis, resulting in a decrease in their ability to assimilate CO<sub>2</sub> and decreasing the photosynthetic rate [32–34]. In the present study, the characteristic parameters of the photosynthetic curves of peony leaves: Pn<sub>max</sub> and AQE,

showed an overall decreasing trend with high-temperature stress, and this result was consistent with the results of Yuanda Zhang et al. [35] and Chao Xu et al. [36], indicating that high-temperature stress has a significant inhibitory effect on photosynthesis in peony leaves. For  $Pn_{max}$ , a parameter that reflects the potential photosynthetic capacity of the plant, the higher this value is, the lower the probability of the photoinhibition response occurring in plants under strong light conditions [37], and usually, AQE is positively correlated with light energy utilization, and the higher the AQE value, the higher light energy utilization is [38]. In this study, high-temperature stress significantly limited the  $Pn_{max}$  of peony, and the AQE of most peony species decreased significantly at the beginning of the stress period. This suggests that its leaves were severely damaged by chloroplasts at 40°C and that fewer pigment protein complexes could absorb and convert the light energy in plants [11,24], while the short-term heat pressure did not significantly affect 'Huhong's light energy utilization capacity. This indicates that 'Huhong' is a more heat-tolerant species that can respond to high temperatures quickly in a relatively short period of time, and its ability to resist high-temperature stress during the early stages is stronger, while the 'Fengdanbai' variety needs cooling protection measures to prevent over-summering in the Jiangsu and Zhejiang regions.

LSP and LCP are two parameter indicators that can be used to gauge how well plants tolerate shade, which, in turn, can gauge how well plants react to high temperatures [39]. At the early stage of high-temperature stress, the LSP of 'Huhong' decreased significantly, and LCP increased significantly, indicating that both the low and high light adaptations of this variety were weakened and that the light adaptation range was reduced to different degrees to alleviate the damage caused by high-temperature stress, which is consistent with the findings of Heli Zhao et al. [40]. The reason for the decrease in  $Pn_{max}$  may be that high temperatures significantly reduce the accumulation of carbon in plants. All species undergo photosynthetic and respiratory adjustments to heat stress [41], which result in LCP being different from LSP.

PS II, located on the membranes of plant cysts, is the most heat-sensitive part of the electron transport chain, and its electron transfer process is evident under chlorophyll fluorescence detection [42,43]. The rise and fall of J-point fluorescence in the OJIP curve can reflect the electron transfer activity from  $Q_A$  to  $Q_B$  on the  $P_{680}$  receptor side [44]; the J-I point reflects the complete reduction of the reduced plastoquinone (PQ) pool during the electron transfer process; the P point is when  $Q_A$  is completely in the reduced state and the PS II reaction center is closed: when the fluorescence yield is maximum. Studies by Murata et al. [45] have shown that high temperatures can inhibit the synthesis of the  $D_1$  protein by accelerating the production of  $H_2O_2$ , resulting in a significant reduction in electron transport between  $Q_A$  and  $Q_B$ , resulting in light inhibition. The OJIP curve of the J-I phase in the peony leaves was significantly reduced, indicating that the total reduced plastoquinone (PQ) library of peony leaves decreased after high-temperature stress, the photosynthetic properties were impaired, and the electron transport process of PS II was indeed inhibited, which is similar to the conclusions of previous studies [40].

Donor-side and receptor-side activities can reflect the PS II activity in plants. High temperatures hinder PS II-related central vesicle energy transfer and can cause the inhibition of the electron transfer efficiency between the PS II donor side of cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* Miller) and the acceptor side of winter wheat (*Triticum aestivum* L.) and peony [36,40,46–48]. The increase in  $Mo$  and  $V_j$ , is a specific sign that reflects the blocked electron transfer from the PS II receptor side ( $Q_A$ ) to the secondary quinone receptor ( $Q_B$ ), indicating an increased rate of  $Q_A$  reduction [44,49]. In this experiment, the receptor sides of three different peony varieties were inhibited, with 'Fengdanbai' showing more drastic changes before the onset of wilting, and this expression was the most significant among the  $Mo$  indicators. This indicates that the PS II receptor side of 'Fengdanbai' was more severely damaged and that electron transfer was hindered at the early stages of high-temperature stress compared with the other varieties, while both 'Huhong' and 'Yinhongqiaodui' showed different degrees of response to high-temperature

stress, with 'Huhong' responding to high temperatures the most rapidly. High-temperature stress also led to a decrease in  $F_v/F_m$ ,  $\phi_{E_0}$ , and  $\Psi_0$  all of which indicated a decrease in the ability of the PS II receptor side  $Q_A$  to transfer electrons, suggesting a decrease in the opening of the active reaction centers, which is consistent with the findings of Jiale Yuan et al. [50].

While light energy is captured by the photosynthetic machinery for electron transfer, a portion of the energy is dissipated in the form of heat and fluorescence. These three products are in competition with each other, and changes in any one of them will lead to changes in the other two [42,51]. Under high-temperature stress, many higher plants turn off the PS II reaction center and deactivate it transiently. It reduces the conversion efficiency of the absorbed light energy by increasing heat dissipation and the dissipation of the fluorescent form of chlorophyll, alleviating the damage caused by excessive light energy transfer to PS II [46]. Nevertheless, partial inactivation of the reaction center increases the burden on the remaining active reaction centers [44]. In this experiment, the parameters  $ABS/RC$ ,  $TR_0/RC$ , and  $Dio/RC$  increased and  $RC/CS_m$  decreased, while the total light energy absorption per unit leaf area of the three peony species still showed an overall decreasing trend, indicating that the overall activity of the reaction centers on the leaves of these peony species decreased after the high-temperature stress and the overall light energy absorption decreased, consistent with the results of Yi Sun et al. [52] on alum root (*Heuchera micrantha* Douglas ex Lindl.). Due to the overall damage to the reaction centers on peony leaves under high-temperature stress, the number of active unit reaction centers decreased, resulting in a rise in the amount of light energy required to be absorbed and captured by the remaining unit reaction centers, which is consistent with the findings of Ji Wen et al. [24]. The increase in  $ABS/RC$  may be due to a decreased proportion of active RCs and an increased proportion of absorbed light energy, which may be related to the inactivation of RCs or to an increased antenna size. Previous studies [53] showed that the RC activity in plants is related to light inhibition, that is, reducing it more than the amount of light absorbed by the plant can result in the plant using its own photosystem.  $TR_0/RC$  represent the maximum speed at which the RC captures the excitons [54].  $TR_0/RC$  and  $DIO/RC$  were significantly higher, indicating that all of the  $Q_A$ -values were reduced and could not be reoxidized due to increased temperatures and excessive light exposure. When the reoxidation of  $Q_A$ -is suppressed,  $Q_A$ -cannot efficiently transfer electrons to  $Q_B$  because the inactive center cannot capture a large number of photons, and thus, this excess of photons is considered for energy dissipation [55]. Therefore, the inhibition of the overall activity of the reaction center also leads to an obstruction of the subsequent electron transfer process, and too much light energy is pooled and stored in the reaction center, resulting in the partial shutdown of the unit's reaction center and increased light energy dissipation in the antenna complex. This provides slight protection to the subsequent PS I and the whole electron transport chain, but the photosynthetic apparatus was damaged, and the photosynthetic capacity still showed a downward trend. Therefore, the damage degree to the reaction center of PS II unit could be inferred by the increased ratios of  $ABS/RC$ ,  $TR_0/RC$ , and  $Dio/RC$  indexes.

The number of active PS II reaction centers in 'Huhong' showed a significant increase after 5 d of treatment compared after 2 days of treatment, indicating that the inactivation of its PS II reaction centers was self-regulated in response to high-temperature stress, while most of the unit reaction centers of 'Fengdanbai' were closed by irreversible damage, probably due to the poor regulation ability of its photosynthetic mechanism under high-temperature stress, and the PS II reaction centers failed to respond to high-temperature stress and to regulate themselves in time after the onset of stress.

$PI_{ABS}$  includes three mutually independent parameters:  $RC/ABS$ ,  $\phi_{P_0}$ , and  $\Psi_0$ , which reflect the three components of PS II's active reaction center concentration, the primary photochemical reaction, and electron transfer as well as the photosystem activity in an integrated manner, thus making it more sensitive than  $F_v/F_m$  [24,44]. Previous studies have shown that  $PI_{ABS}$  and  $PI_{total}$  can accurately respond to the electron transfer between PS I and PS II and to the state of their photosynthetic systems [56]. Therefore,  $PI_{ABS}$  can be

used as a valid fluorescence parameter indicator for different species of peony in response to high-temperature stress [57]. Zhang et al. [58] showed that high-temperature stress decreased the  $PI_{ABS}$  of cyanobacteria, mainly due to damage on the donor side of PS II, over-reduction on the acceptor side, and lower energy transfer from the unit's PS II center. In the present study, the  $PI_{ABS}$  values showed a significant decreasing trend, and high-temperature stress led to a decrease in PS II activity in peony leaves with different degrees of reduction related to its degree of heat tolerance, similar to the results of previous studies [59].

## 5. Conclusions

The morphological structure, photosynthetic characteristic parameters, OJIP curves, PS II receptor side, photosynthetic performance indexes, and specific activity parameters of different heat-tolerant peony varieties were affected by high-temperature stress, and the varieties with stronger heat tolerance were less affected than those with weak heat tolerance. Under high-temperature stress, the photosystem of peony leaves was disrupted; this was further corroborated by the fast chlorophyll fluorescence results and by the fact that some of the PS II reaction centers were inactivated and that the amount of light energy required to be absorbed and captured by the remaining unit reaction centers increased. However, the overall activity of the reaction centers still decreased, and the photochemical efficiency subsequently decreased. The receptor-side PS II was inhibited, and its electron transfer capacity was decreased, so the photosynthetic mechanism was affected.

The ability of PS II's light energy capture and transfer to downstream of  $Q_A$  was more significantly reduced in the less heat-tolerant varieties than in the more heat-tolerant ones and further led to a rapid decrease in the comprehensive photosynthetic performance. Among all of the tested parameters, the  $PI_{ABS}$  index was more sensitive to the changes in the photosynthetic machinery in the peony varieties, while the specific activity parameter  $DIo/RC$  and the receptor-side parameter  $Mo$  showed significant correlations with the sensitivity to high-temperature stress. Therefore, the changes in the  $PI_{ABS}$ ,  $DIo/RC$ , and  $Mo$  parameters can reflect the physiological conditions of peony over time, and the heat tolerance and growth status of peony can be identified using these indexes. Therefore, in this study, the key photosynthetic characteristics and fluorescence parameters of peony in response to high temperatures were selected, which provided a theoretical basis and an important reference for the rapid screening of high-temperature-tolerant varieties.

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