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Heat Stress Recovery of Chlorophyll Fluorescence in Tomato (*Lycopersicon esculentum* Mill.) Leaves through Nitrogen Levels

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Abstract: To study the impact of nitrogen application on the photosynthetic structure and photosystem activity of tomato (*Lycopersicon esculentum* Mill.) leaves during the recovery stage after heat stress, the OJIP curve and JIP parameters were determined through a control experiment in an artificial climate chamber. The tomato variety was "Jinfen No. 1". Four day/night temperature levels (25 °C/15 °C as control CKT; 30 °C/20 °C, lightly high-temperature LHT; 35 °C/25 °C, moderate high-temperature MHT; 40 °C/30 °C, severe high-temperature SHT) were set for a duration of 7 days. Five nitrogen supply levels (N1–N5: 0, 1.3, 1.95, 2.6 and 3.75 g/plant, respectively; 2.6 g/plant is the recommended nitrogen application rate, CKTN4) were applied. The results showed that the O, K, J, I and P phases on the chlorophyll a fluorescence curve were significantly affected by different nitrogen treatments in heat stress recovery. Compared with CKT, with the increase in nitrogen supply, the fluorescence intensity of SHTN2-SHTN5 treatment increased significantly at P, I and J phases, while that of MHTN1-MHTN4 treatment decreased. The fluorescence intensity of SHTN5 and SHTN3 increased by 13.27% and 10.10% in the P phase, 13.52% and 12.1% in the I phase and 20.16% and 26.18% in the J phase, respectively. There were highly significant differences ($p < 0.01$) in the impact of high temperatures and nitrogen levels on the fluorescence parameters. On the 1st day after short-term heat stress, N had no significant effect on F_v/F_M, F_v, F_o and F_M; however, their interaction was significant ($p < 0.05$). On the 8th day, there were no significant interaction effects between HT and N for F_v/F_o, ABS/RC and DI_o/RC. F_v/F_o proved to be sensitive to the application of both high temperatures and nitrogen. Under all five nitrogen applications, temperature played a significant role in increasing DI_o/RC, especially for N2 and N3. The results indicated that decreasing the nitrogen application under SHT resulted in a higher number of active RCs and an increased value of specific energy flux (ABS/RC, TR_o/RC and DI_o/RC), indicating the enhanced ability of RC to reduce plastoquinone. The study provides a reference for the diagnosis of nitrogen nutrition under high-temperature stress using chlorophyll fluorescence methods.

Keywords: heat stress; nitrogen application; fluorescence characteristics; OJIP curves; tomato

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1. Introduction

Spring and summer are the main growing periods for greenhouse tomatoes. Enclosed cultivation spaces tend to create a high-temperature environment. Tomato is a predominant facility crop in China, accounting for 1/3 of all facility crops. Temperature plays a pivotal role in the cultivation of crops, including greenhouse tomatoes [1]. Tomato is a heat-loving vegetable that is especially sensitive to environmental temperature, with an optimal daytime temperature ranging from 24 to 26 °C and nighttime temperature from 15

to 17 °C [2]. Growth tends to deteriorate when the temperature exceeds 35 °C, and temperatures above 45 °C can lead to burning due to physiological drought, ultimately resulting in plant death [3]. In the context of global warming, the frequency of high-temperature heat damage to facility tomato has notably increased [4–6]. The occurrence of high temperatures inevitably affects the photosynthetic structure and photosystem function of facility crops, subsequently impacting their growth and development process [7]; this, in turn, affects the yield formation and quality of greenhouse crops [8].

The photosynthetic capacity of crop leaves is highly sensitive to heat stress, and it is partially or almost completely depressed by heat stress before other signs and symptoms appear. Photosystem II (PSII) reaction centers, CO_2 assimilation, and ATP complexes are the main sites susceptible to heat stress [9–14]. Among these, PSII is the most sensitive and vulnerable component when higher plants are exposed to heat stress [15]. Heat stress can induce various structural and functional changes in PSII, and in severe cases, lead to its damage [16]. High temperatures can alter the fluidity of the chloroplast-like cyst membrane, causing a decrease in PSII complex stability and facilitating its decomposition [17]. The fast chlorophyll fluorescence induction kinetic curve (OJIP curve) is a method used to quickly gather various information, such as the PSII photochemical activity, electron transport, and photosynthetic organ structure and status [18]. The JIP test is a quantitative method for analyzing changes in the OJIP curve, allowing reflection on light energy absorption, conversion, the activity of the acceptor and donor sides of the PSII reaction center, and dynamic alterations in the redox state of the electron transfer entities. A data analysis and processing method was established for creating fast chlorophyll fluorescence induction curves based on biofilm energy flow, and measuring the internal changes in the sample under a given physical state by calculating the energy flow and energy ratio [19]. Simplified energy flow model diagrams are widely used to distinguish the effects of low-temperature, drought, salt, waterlogging, and heat stress in particular on plants [20–22].

The application of nitrogen fertilizer is crucial for enhancing crop stress resistance [23]. Optimizing plant fertilizer management can alleviate the damage caused by heat stress to crop growth [24]. At present, little research has been conducted on the effect of nitrogen on the mitigation of heat stress in facility crops, and the related studies have mainly focused on field crops, such as wheat, rice and maize. Under high-temperature stress, nitrogen nutrition plays a pivotal role in ameliorating senescence in wheat [25,26], and influences the extent of the effect of heat stress on wheat grain weight [27,28]. Under appropriate nitrogen application, the rate of chlorophyll synthesis in plant leaves is remarkably accelerated, and the photosynthetic rate, actual photochemical efficiency and maximum photochemical efficiency of photosystem II (PSII) [29] are largely improved. Different nitrogen fertilization treatments have different impacts on the yield reduction rate of wheat grains under heat stress [30,31]. Under heat stress, nitrogen application can reduce the ear temperature by increasing the net photosynthetic rate and stomatal conductance of rice flag leaves, with higher nitrogen levels being more effective than medium nitrogen levels [32–34]. The JIP parameters and energy pipeline model showed that heat stress affects the photosystem II electron transfer chain more than chilling stress, with more pronounced changes observed in the fruit than in the leaves [35].

It is important to study the characteristics of the rapidly induced chlorophyll fluorescence curves of tomato leaves to further understand the effects of nitrogen application on the photosynthetic structure and photosystem of tomato leaves under different levels of heat stress. Chlorophyll fluorescence can reflect the absorption and utilization of light energy by tomato plants during light reactions and the hydrolysis with both the photosystem I (PSI) and photosystem II (PSII) complexes to release oxygen. The electron transfer downstream of the electron chain is related to the process of electron transfer from the PSII complex to the PSI complex. However, to the best of our knowledge, the mechanism by which heat stress affects photosynthetic electron carriers and the photosynthetic structure in tomato leaves have not been elucidated.

The aim of this study was to determine the chlorophyll fluorescence kinetic parameters of tomato leaves and to elucidate the effects different levels of nitrogen supply on photosystem activity under heat stress using OJIP curves. Meanwhile, based on various transient parameters of the OJIP, this study attempted to explain the site and mechanism of action of nitrogen implicated in photosystem activity and the photosynthetic capacity of tomato leaves under heat stress. Therefore, this study can provide data support for nitrogen application under a high-temperature environment in tomato production.

2. Materials and Methods

2.1. Plant Materials and Experimental Conditions

The experiment was carried out in a Venlo-type glass greenhouse in the Jiangsu Key Laboratory of Agricultural Meteorology at Nanjing University of Information Science and Technology (Nanjing, China). The greenhouse boasts a 5.0 m roof height, 4.5 m shoulder height, 9.6 m width and 30.0 m length. It is situated in a north–south orientation with an automatic skylight and side vents. The experimental soil was moderate loam with even fertility, pH 7.4, an organic matter content of 18.32 g/kg, a total nitrogen content of 0.86 g/kg, a total phosphate content of 0.75 g/kg, and a volumetric soil moisture content of 32.45% [36].

The tomato cultivar selected for this experiment was 'Jinfen No. 1'. Plants, measuring approximately 15 cm in height, were transplanted into flowerpots of 28 cm (height) × 34 cm (upper diameter) × 18 cm (bottom diameter) on 10 September 2021. After the successful growth of the tomato plants, they were treated with different levels of fertilizer on 16 September. Following the absorption of fertilizer by the tomato plants, the temperature treatment experiment was conducted on 20 September. The tomato plants in the artificial climate chamber are shown in Figure 1.

Figure 1. Artificial climate chamber and tomato plants in them.

Experiments were designed with two factors: temperature and nitrogen. The temperature was set at 4 levels, with day/night temperatures of 25 °C/15 °C (CKT), 30 °C/20 °C (LHT), 35 °C/25 °C (MHT) and 40 °C/30 °C (SHT). The daily minimum temperature of the artificial climate chamber was set at 5:00 am, and the daily maximum temperature was set at 14:00. The hourly change curve was based on the daily change in the greenhouse temperature in Nanjing, Jiangsu Province [34,37], as shown in Figure 2. Five levels of nitrogen fertilizer were applied to the soil. The purpose of using different nitrogen levels was to control the nitrogen content in the plant according to the amount of fertilizer applied, and to create the necessary gradient of nitrogen content. The nitrogen fertilizer application treatments were as follows: without nitrogen fertilizer (N1, 0 g/plant) treatment; 0.5 times the recommended nitrogen fertilizer (N2, 1.3 g/plant); 0.75 times the recommended nitrogen fertilizer (N3, 1.95 g/plant); the recommended nitrogen fertilizer (CKN4, 2.6 g/plant as control); and 1.25 times the recommended nitrogen fertilizer (N5, 3.25 g/plant). A sum of 20 treatments were designed for temperature and nitrogen (Table 1), and each treatment was replicated three times.

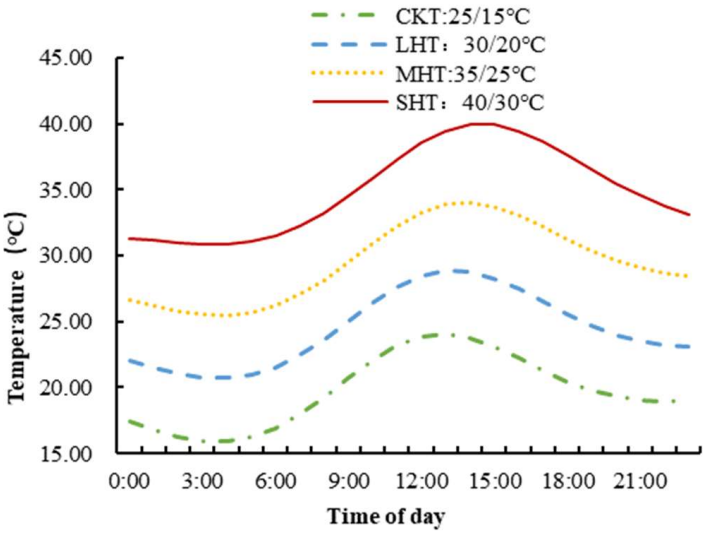

Figure 2. Air temperature dynamics of artificial climate chamber (24 h).

Table 1. Combination of nitrogen level and temperature level treatments for potted tomatoes.

Nitrogen Treatment (g·Plant^{-1})	**High Temperature Treatment (Day/Night)**			
	CKT (25 °C/15 °C)	**LHT (30 °C/20 °C)**	**MHT (35 °C/25 °C)**	**SHT (40 °C/30 °C)**
N1: 0N (0 g·plant^{-1})	CKTN1	LHTN1	MHTN1	SHTN1
N2: 0.5N (1.3 g·plant^{-1})	CKTN2	LHTN2	MHTN2	SHTN2
N3: 0.75N (1.95 g·plant^{-1})	CKTN3	LHTN3	MHTN3	SHTN3
N4: 1N (2.6 g·plant^{-1},CKN4)	CKTN4	LHTN4	MHTN4	SHTN4
N5: 1.25N (3.25 g·plant^{-1})	CKTN5	LHTN5	MHTN5	SHTN5

The environmental parameters of the artificial climate chamber were set as shown in Table 2. During the experiment, the soil moisture content of the potted tomato seedlings was maintained at 80% of the field water capacity. The potted tomatoes were placed in the artificial climate chamber (BDW 40, Conviron 6050, Canada) for 7 days, and then the potted plants were placed in a glass greenhouse. After that, samples were taken every 7 days to study the effect of a short-term high temperature on the fluorescence characteristics of tomato leaves during the flowering and fruiting period.

Table 2. Environmental parameters of artificial climate chamber.

Time of day	0:00	1:00	2:00	3:00	4:00	5:00	6:00	7:00	8:00	9:00	10:00	11:00
PAR (μmol m^{-2} s^{-1})	0	0	0	0	0	0	600	700	800	800	800	1000
Relative humidity (%)	67	66	68	69	71	73	73	74	70	65	65	65
Time of day	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00	21:00	22:00	23:00
PAR (μmol m^{-2} s^{-1})	1000	1000	1000	800	800	700	600	0	0	0	0	0
Relative humidity (%)	65	65	65	65	66	67	71	72	72	72	73	74

2.2. Evaluation of Chlorophll Fluorescence

After the high-temperature and nitrogen treatments, 3rd functional leaves from healthy plants top were selected and the chlorophyll fluorescence parameters were measured with a portable photosynthetic efficiency analyzer (Ltd Pocket PEA, Hansatech Inc., Pentney, UK) from 9:00 a.m. to 11:00 a.m. on the sampling day. After fixing special labels on the leaves to be measured and allowing them to adapt to the dark time for 30 min, the sensor was installed on the leaves to determine the chlorophyll fluorescence parameters. The chlorophyll a fluorescence transient was induced by a saturated luminous flux density

of 3500 µmol (photon) $m^{-2}s^{-1}$ emitted by three light-emitting domes (650 nm peak), producing fluorescence profiles ranging from F_o to F_m for all treatments [38].

2.3. Specific Energy Fluxes (per RC: Q_A − reducing PSII Reaction Center)

The OJIP curve can be used to analyze the activity of PSII reaction centers (each Q_A − reducing PSII reaction center: RC), which is represented by four activity parameters: the absorption flux per reaction center (ABS/RC), the trapped energy flux per reaction center (TR_o/RC), the electron transport flux per reaction center (ET_o/RC), and the dissipated energy flux per reaction center (DI_o/RC). The specific energy fluxes were calculated using the following equations.

$$ABS/RC = M_o \times (1 - V_J) \times (1/\varphi P_o) \quad (1)$$

$$TR_o/RC = M_o \times (1/V_J) \quad (2)$$

$$ET_o/RC = M_o \times (1/V_J) \times (1 - V_J) \quad (3)$$

$$DI_o/RC = ABS/RC - TR_o/RC \quad (4)$$

2.4. Heatmap

To further investigate the correlations between the parameters, a heatmap was plotted using Graphpad Prism version 9.5.0 for Windows (Graphpad Software, San Diego, CA, USA). The heatmap indicated the correlation matrix of the parameters, visualizing the strength and direction of the relationships between the parameters. The heatmap colors ranged from −1 (strong negative correlation) to 1 (strong positive correlation), with 0 denoting no correlation. Compared to regression plots, heatmaps provide a more comprehensive view of the data. In summary, the thermogram visualized the relationship between the chlorophyll fluorescence parameters and the heat stress of tomato leaves under different nitrogen application conditions. The results demonstrated the relationship between the parameters and deepened our understanding of the effects of heat stress under different nitrogen application conditions [39]. All abbreviations in the paper are in the Supplementary Table.

2.5. Statistical Analysis

Data were processed and plotted using Microsoft Excel for Windows. Data differences were analyzed using SPSS Statistics 26 (SPSS, Chicago, IL, USA), and Duncan's test was carried out for multiple comparisons (α = 0.05).

3. Results

3.1. The Chlorophyll Fluorescence (ChlF) Curve

3.1.1. ChlF Rise

The characteristics of the chlorophyll a fluorescence (ChlF) rise reflect the electron transport in the PSII complex reaction center, which further provides information about the photosynthetic efficiency and potential of tomato leaves. Chlorophyll fluorescence technology is the most reliable technique for evaluating PSII function and its overall photosynthesis performance in plants through chlorophyll fluorescence [40]. It is also the most dependable technique for understanding the physiological mechanism of plants under stressed environments [41]. The ChlF rise curve for all tomato leaves under the whole recovery period followed a typical OJIP curve when plotted on a logarithmic time scale (Figures 3–5), indicating that all treatments were photosynthetically active. However, it can also be found that different high-temperature/nitrogen combination treatments led to obvious differences in the O, K, J, I, and P phase.

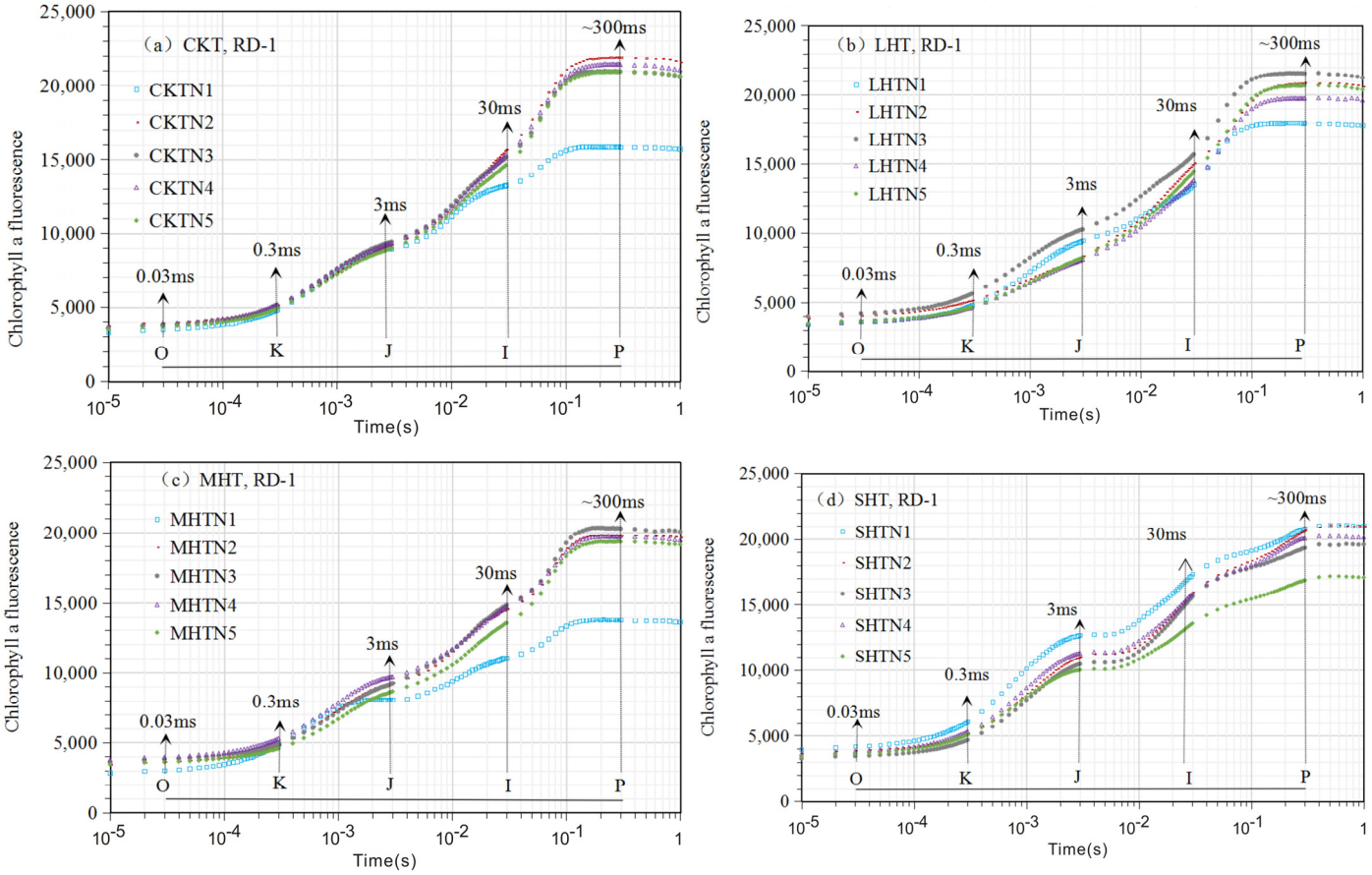

Figure 3. Comparison of OJIP steps of the fluorescence transient between the temperature-stress-treated and control tomato plants at different evaluation periods on recovery day 1 (RD-1). OJIP fluorescence curve under different combinations of temperature regimes [(**a**) control temperature (CKT: 25 °C/15 °C day/night); (**b**) lightly high temperature (LHT: 30 °C/20 °C day/night); (**c**) moderate high temperature (MHT: 35 °C/25 °C day/night); (**d**) severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKN4); N5: 1.25N (3.25 g·plant^{-1})].

On the first day of the recovery period, the OJIP curve under CKT, LHT, MHT and all nitrogen treatments had similar changes (Figure 3a–d), and the fluorescence intensity of the N1 treatment was significantly lower in the I and P phase than in the others. However, under SHT, the opposite situation occurred, and the fluorescence value of the SHTN1 treatment was higher than that of the other nitrogen treatments. Compared with control SHTN4, the fluorescence value of high-nitrogen treatment SHTN5 after J phase was lower than that of other treatments. The OJIP curves of SHTN2 and SHTN3 were similar to those of the control treatment. At the P phase, compared with CKTN4, the fluorescence value of SHTN1-SHTN5 was reduced, and the fluorescence value of the high-nitrogen treatment was decreased by 21.26%. At the I phase, compared with CKTN4, only SHTN5 decreased the fluorescence value by 11.40%, and SHTN1-SHTN4 increased the fluorescence value, among which SHTN1 increased the most, by 13.21%.

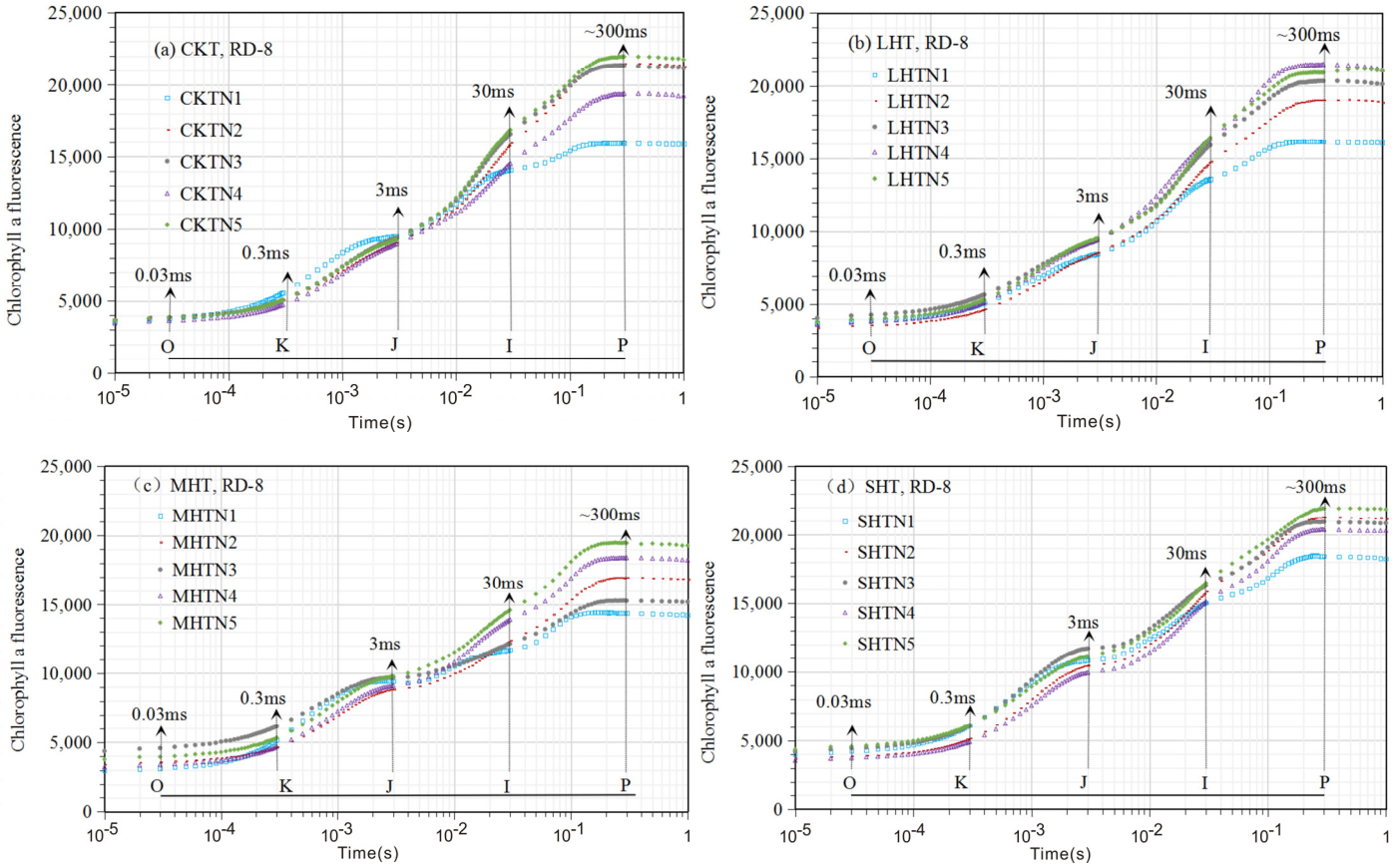

Figure 4. Comparison of OJIP steps of the fluorescence transient between the temperature-stress-treated and control tomato plants at different evaluation periods on recovery day 8 (RD-8). OJIP fluorescence curve under different combinations of temperature regimes [(**a**) control temperature (CKT: 25 °C/15 °C day/night); (**b**) lightly high temperature (LHT: 30 °C/20 °C day/night); (**c**) moderate high temperature (MHT: 35 °C/25 °C day/night); (**d**) severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKN4); N5: 1.25N (3.25 g·plant^{-1})].

On the 8th day of the recovery period, the chlorophyll fluorescence intensity of different nitrogen treatments was significantly different, with the minimum value obtained in the N1 treatment and the maximum value obtained in the N5 treatment (Figure 4a–d). Compared with CKTN4, nitrogen application increased the fluorescence intensity of SHTN2-SHTN5 in the P, I and J phases, and decreased that of MHTN1-MHTN4. At point P, SHTN5 increased by 13.27% and SHTN3 by 10.10%. At the I phase, SHTN5 increased by 13.52% and SHTN3 by 12.21%. At the J phase, SHTN5 increased by 20.16% and SHTN3 by 26.18%.

On the 15th day of the recovery period, the difference in the chlorophyll fluorescence intensity of the four temperature treatments gradually increased (Figure 5a–d). Under CKT, the fluorescence values were in the order of CKTN4 > CKTN5 > CKTN1 > CKTN3 > CKTN2. Compared with the 8th day, the fluorescence intensity of the N4 treatment was increased, while that of the N5 treatment was decreased. Under LHT, the LHTN3 and LHTN5 treatments showed higher fluorescence values than the other three nitrogen treatments. Under MHT treatment, the fluorescence intensity of the MHTN2 and MHTN3 treatments was lower than that of the MHTN4 treatment. Under the SHT treatment, the fluorescence intensity was as follows: SHTN5 > SHTN4 > SHTN2 > SHTN3 > SHTN1.

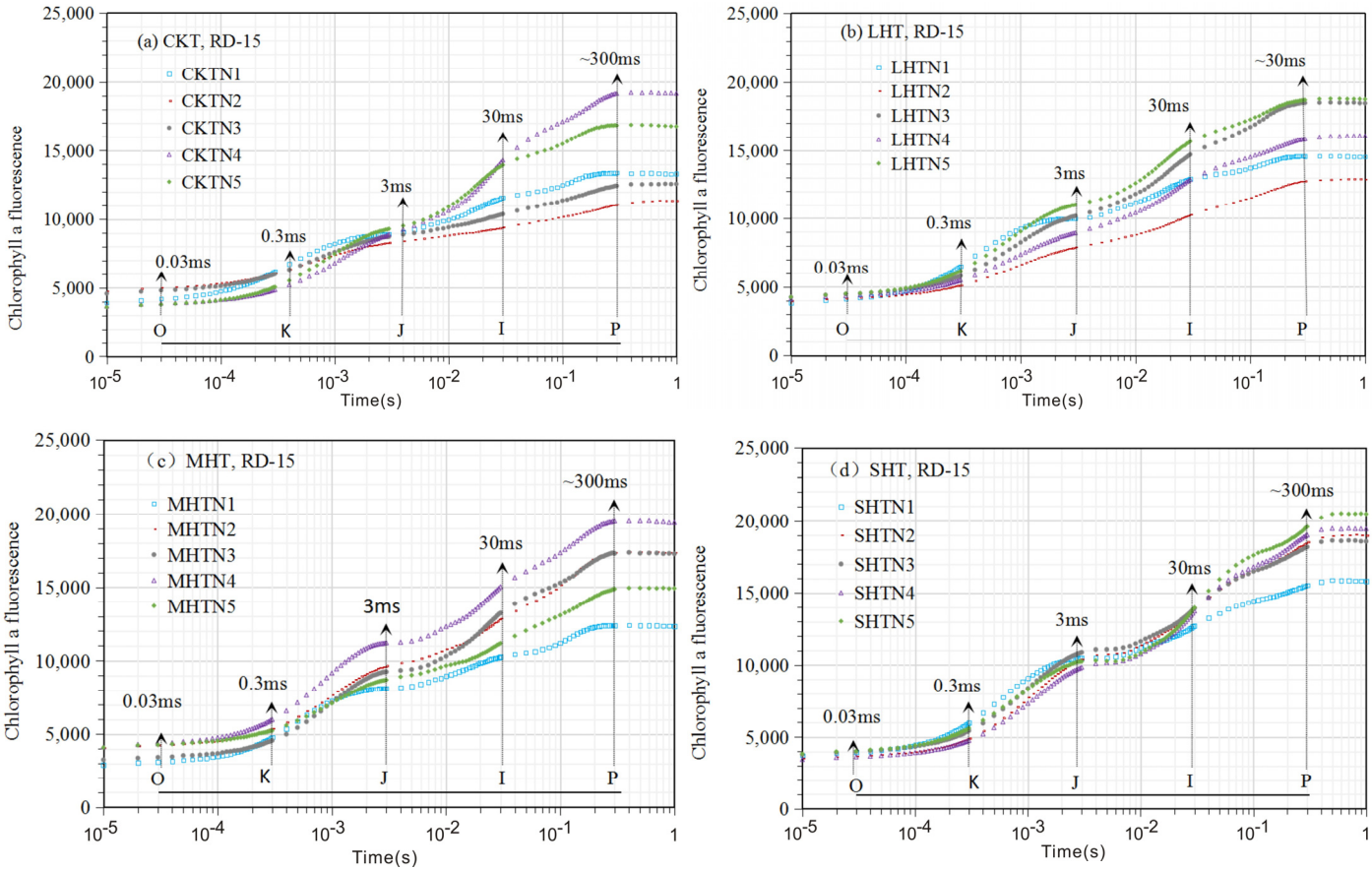

Figure 5. Comparison of OJIP steps of the fluorescence transient between the temperature-stress-treated and control tomato plants at different evaluation periods on recovery day 15 (RD-15). OJIP fluorescence curve under different combinations of temperature regimes [(**a**) control temperature (CKT: 25 °C/15 °C day/night); (**b**) lightly high temperature (LHT: 30 °C/20 °C day/night); (**c**) moderate high temperature (MHT: 35 °C/25 °C day/night); (**d**) severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKN4); N5: 1.25N (3.25 g·plant^{-1})].

3.1.2. The Relative Variable Fluorescence ΔV_t Changes in Tomato Leaves

Normalizing the chlorophyll fluorescence kinetic curve to the relative variable fluorescence $V_t = (F_t - F_o)(F_m - F_o)$ enables the quantitative analysis of differences. This normalization process fixes the maximum amplitude of the rise at one, which is convenient for comparing the different reduction rates of the terminal electron acceptor under different high temperatures and nitrogen application rates. The relative variable fluorescence difference $\Delta V_t = V_t - V_{t(control)}$ can be used to analyze the changes in the tomato leaf oxygen-evolving complex (OEC) in PSII. CKTN4 was used as a control in different recovery stages. Under different temperature treatments, the ΔV_t values under the N1 treatment were all greater than zero (Figures 6–8).

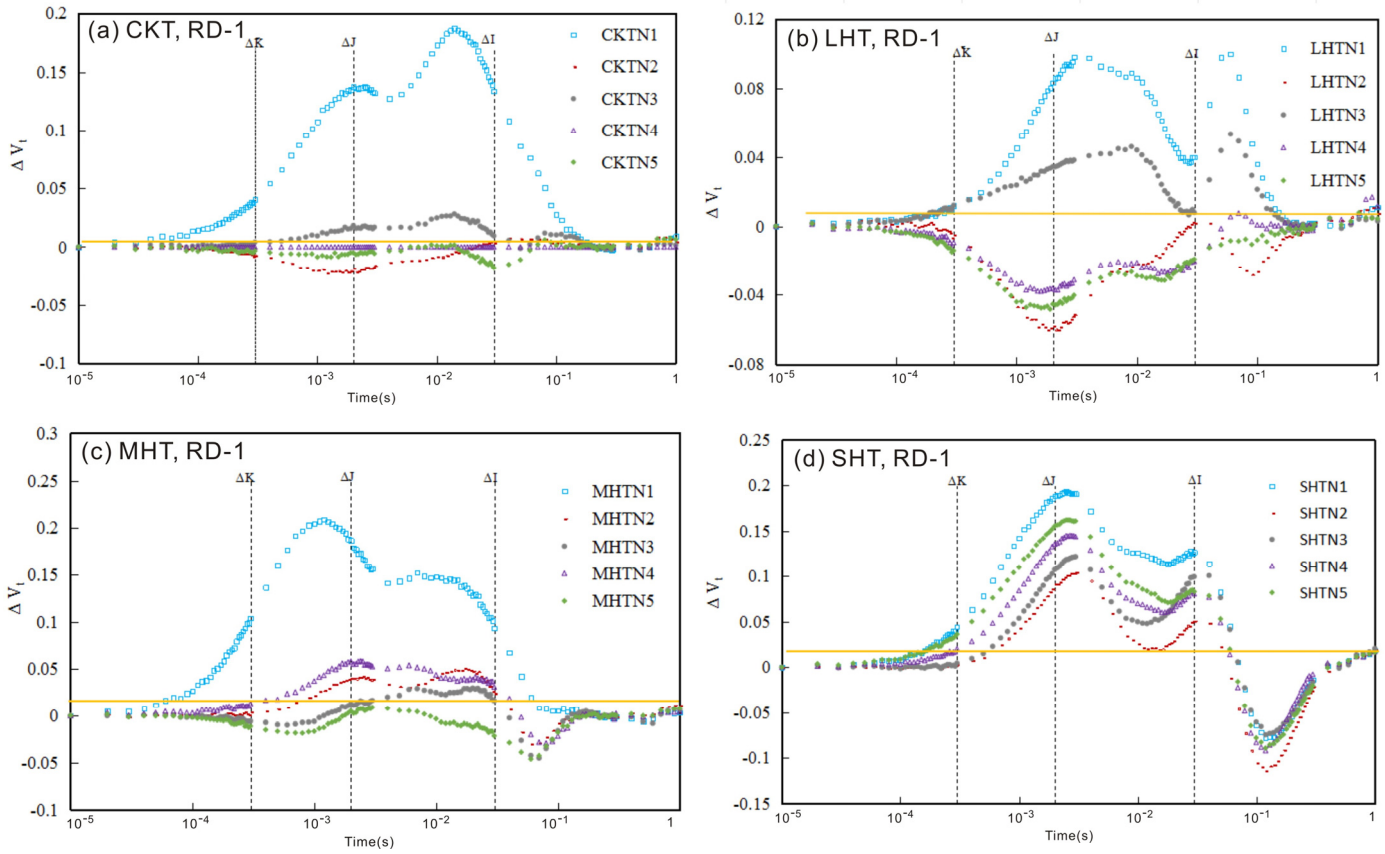

Figure 6. ΔV_t of tomato leaves on the 1st day of the recovery period. (**a**) ΔV_t of tomato leaves under CKT; (**b**) ΔV_t of tomato leaves under LHT; (**c**) ΔV_t of tomato leaves under MHT; (**d**) ΔV_t of tomato leaves under SHT. Note: $V_t = (F_t - F_o)(F_m - F_o)$, $\Delta V_t = V_t - V_{t(control)}$.

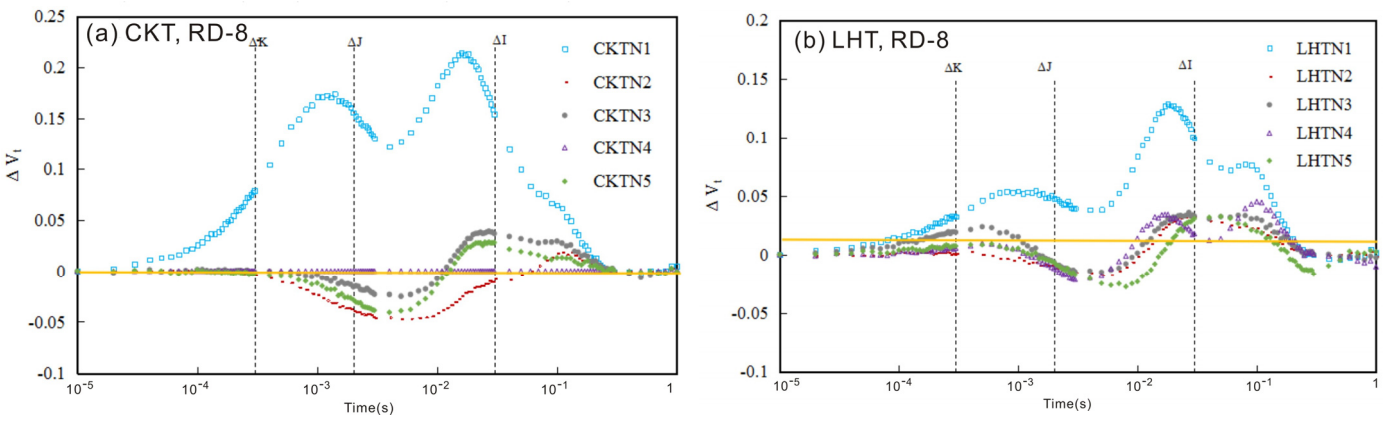

Figure 7. *Cont*.

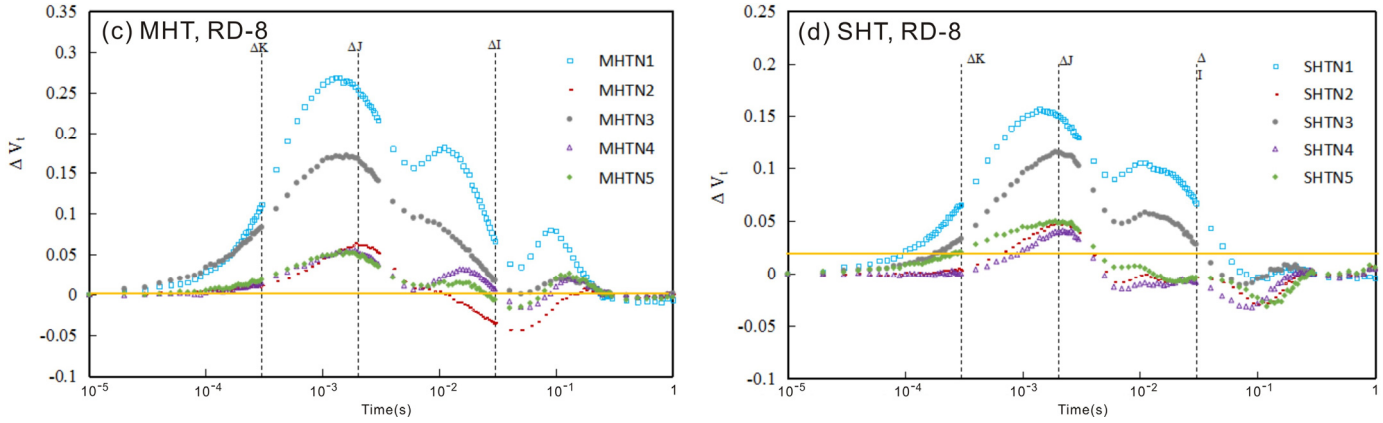

Figure 7. ΔV_t of tomato leaves on the 8th day of recovery. (**a**) ΔV_t of tomato leaves under CKT; (**b**) ΔV_t of tomato leaves under LHT; (**c**) ΔV_t of tomato leaves under MHT; (**d**) ΔV_t of tomato leaves under SHT. Note: $V_t = (F_t - F_o)(F_m - F_o)$, $\Delta V_t = V_t - V_{t(control)}$.

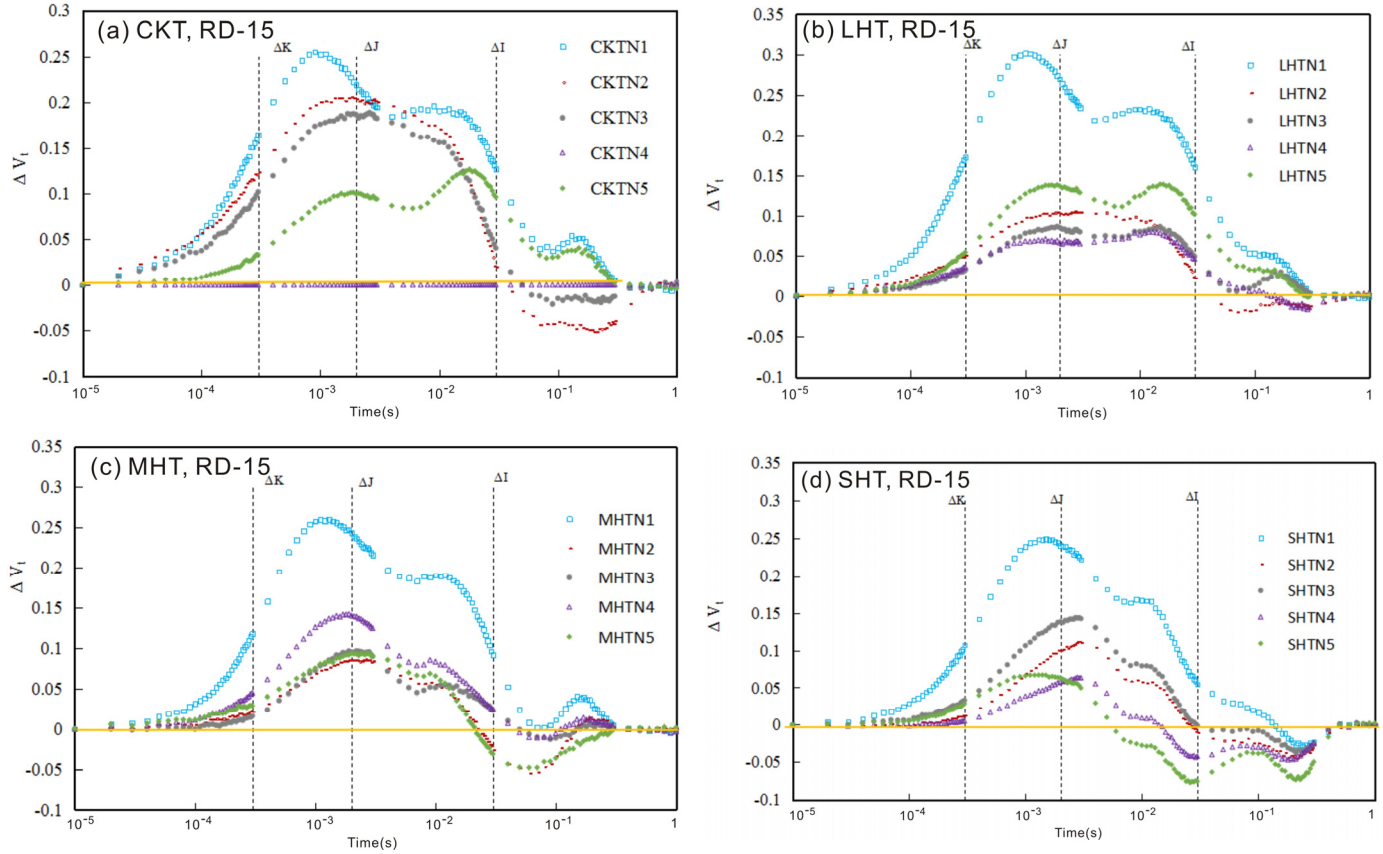

Figure 8. ΔV_t of tomato leaves on the 15th day in the recovery period. (**a**) ΔV_t of tomato leaves under CKT; (**b**) ΔV_t of tomato leaves under LHT; (**c**) ΔV_t of tomato leaves under MHT; (**d**) ΔV_t of tomato leaves under SHT. Note: $V_t = (F_t - F_o)(F_m - F_o)$, $\Delta V_t = V_t - V_{t(control)}$.

On the first day of the recovery period, $\Delta V_{t-CKTN3} > 0$ and $\Delta V_{t-CKTN2} > 0$, while $\Delta V_{t-CKTN5} < 0$ (Figure 6a–d). Compared with CKT, the change trend under the LHT treatment was more obvious, $\Delta V_{t-CKTN1} > 0$ and $\Delta V_{t-CKTN3} > 0$, and the peak value moved from ΔI to ΔJ. LHTN1 decreased by 47.31% compared with CKTN1, while LHTN3 increased by 70.4% compared with CKTN3. Under the LHT treatment, the other three groups showed a negative multi-peak fluctuation pattern, the maximum peak value was obvious, the waveforms of LHTN4 and LHTN5 were consistent, and LHTN2 approached zero at ΔI. Under

the MHT treatment, at ΔK, ΔJ and ΔI, $\Delta V_{t-MHTN1} > \Delta V_{t-MHTN4} > \Delta V_{t-MHTN2} > 0$. Between ΔK and ΔJ, $\Delta V_{t-MHTN5} < \Delta V_{t-MHTN3} < 0$. After ΔI, $\Delta V_{t-MHTN5} < \Delta V_{t-MHTN3} < \Delta V_{t-MHTN2} < \Delta V_{t-MHTN4} < 0$.

On day 8 of recovery, $\Delta V_{t-CKTN1} > 0$, $\Delta V_{t-CKTN1} > \Delta V_{t-CKTN3} > \Delta V_{t-CKTN5} > 0$ (Figure 7a–d). $\Delta V_{t-CKTN1}$ was bimodal, and the peak value was near ΔJ and ΔI. The application of nitrogen made the negative peak appear in the initial stage, while the positive peak decreased and shifted backward. Under LHT, the peak value of $\Delta V_{t-LHTN1}$ decreased positively and moved backward to the vicinity of ΔI. Under LHT, the peak value of $\Delta V_{t-CKTN1}$ decreased forward and shifted back to near ΔI. Under both MHT and SHT, the negative peak shifted back to ΔI due to nitrogen application. Under MHT, the area of the negative region was $\Delta V_{t-MHTN2} > \Delta V_{t-MHTN4} > \Delta V_{t-MHTN5} > 0$; under SHT, the area of the negative region was $\Delta V_{t-MHTN4} > \Delta V_{t-MHTN5} > \Delta V_{t-MHTN3} > 0$.

On day 15 of recovery, the ΔV_t values of each nitrogen application treatment were significantly different from the previous two recovery periods using the four temperature treatments, and the PSII status of the tomato leaves was restricted by the nitrogen application level at this time (Figure 8a–d). Under CKT and LHT, it was positive before ΔI, $\Delta V_{t-CKTN1} > \Delta V_{t-CKTN2} > \Delta V_{t-CKTN3} > \Delta V_{t-CKTN5} > \Delta V_{t-CKTN4}$, $\Delta V_{t-LHTN1} > \Delta V_{t-LHTN5} > \Delta V_{t-LHTN2} > \Delta V_{t-LHTN3} > \Delta V_{t-LHTN4}$. Both high nitrogen levels and low nitrogen levels can form stress and interact with temperature stress. Under heat stress, the addition of nitrogen decreases the ΔV_t value as a whole, and the positive value transitions to the negative value. Under SHT, the addition of nitrogen under heat stress can improve the energy connectivity of PSII.

3.2. Physical Biological Parameters from JIP-Test Equations

3.2.1. Basic Parameters

The fluorescence intensity recorded at 50 us is expressed as F_o, when all primary quinone accepters (Q_As) are in the open (oxidized) state. F_v is the maximal variable fluorescence, which can reflect the maximum electron transport potential of PSII. On the 1st day of the recovery period, the minimal fluorescence intensity (F_o) and the maximum fluorescence intensity (F_M) both increased as the nitrogen application increased. With the aggravation of heat stress, the F_v value of the low-nitrogen treatment augmented, and with the increase in the recovery time, the F_v value of the high-nitrogen treatment gradually increased (Table 3).

Table 3. Basic parameter changes in tomato plants under different combinations of high temperature and nitrogen (g·plant^{-1}).

Fluorescence Parameters	Sample Period	Treatments				
		CKTN1	CKTN2	CKTN3	CKTN4	CKTN5
F_v	RD-1	13,007 ± 375 g	17,267 ± 498 a	17,273 ± 499 a	16,578 ± 478 ab	17,005 ± 490 ab
	RD-8	12,235 ± 353 ef	16,143 ± 466 bcd	16,039 ± 463 bcd	15,638 ± 451 bcd	16,999 ± 490 ab
	RD-15	10,107 ± 291 gh	6473 ± 186 j	9768 ± 281 h	11,003 ± 317 efg	11,733 ± 338 ef
F_v/F_m	RD-1	0.79 ± 0.023 ab	0.82 ± 0.024 ab	0.82 ± 0.024 ab	0.82 ± 0.024 ab	0.83 ± 0.024 a
	RD-8	0.77 ± 0.022 a	0.80 ± 0.023 a	0.81 ± 0.024 a	0.80 ± 0.023 a	0.81 ± 0.024 a
	RD-15	0.73 ± 0.021 bcd	0.59 ± 0.017 e	0.67 ± 0.019 d	0.70 ± 0.020 cd	0.74 ± 0.021 abc
F_v/F_o	1st day	3.73 ± 0.108 hi	4.60 ± 0.133 abc	4.67 ± 0.135 ab	4.56 ± 0.132 abc	4.77 ± 138 a
	RD-8	3.38 ± 0.097 g	4.10 ± 0.118 cde	4.42 ± 0.127 abc	4.16 ± 0.120 cde	4.4 ± 0.127 abc
	RD-15	2.69 ± 0.078 ef	1.46 ± 0.042 h	2.06 ± 0.059 g	2.77 ± 0.079 def	2.98 ± 0.086 d

Table 3. *Cont.*

Fluorescence Parameters	Sample Period	Treatments				
		LHTN1	**LHTN2**	**LHTN3**	**LHTN4**	**LHTN5**
F_v	RD-1	14,012 ± 404 efg	16,385 ± 472 bcd	16,918 ± 488 ab	16,487 ± 475 abc	16,336 ± 471 bcd
	RD-8	11,471 ± 331 f	15,032 ± 433 cd	15,585 ± 449 bcd	17,805 ± 513 a	16,445 ± 474 bc
	RD-15	10,210 ± 294 gh	8285 ± 239 i	13,903 ± 401 bc	11,729 ± 338 ef	13,842 ± 399 bc
F_v/F_m	RD-1	0.79 ± 0.023 ab	0.82 ± 0.024 ab	0.82 ± 0.024 ab	0.83 ± 0.024 a	0.82 ± 0.024 ab
	RD-8	0.77 ± 0.022 a	0.80 ± 0.023 a	0.80 ± 0.023 a	0.82 ± 0.024 a	0.81 ± 0.024 a
	RD-15	0.74 ± 0.021 bcd	0.67 ± 0.020 d	0.77 ± 0.022 ab	0.73 ± 0.021 bcd	0.77 ± 0.022 ab
F_v/F_o	RD-1	3.90 ± 0.113 ghi	4.57 ± 0.132 abc	4.56 ± 0.132 abc	4.74 ± 0.137 a	4.64 ± 0.134 ab
	RD-8	3.34 ± 0.167 g	4.08 ± 0.096 cde	3.98 ± 0.117 def	4.56 ± 0.131 ab	4.39 ± 0.126 bc
	RD-15	2.78 ± 0.080 def	2.02 ± 0.058 g	3.40 ± 0.098 abc	2.76 ± 0.079 def	3.35 ± 0.096 bc
Fluorescence Parameters	**Sample Period**	**Treatments**				
		MHTN1	**MHTN2**	**MHTN3**	**MHTN4**	**MHTN5**
F_v	RD-1	10,905 ± 314 h	15,067 ± 434 cde	15,959 ± 460 bcd	16,020 ± 462 bcd	13,635 ± 393 fg
	RD-8	11,479 ± 331 f	13,506 ± 389 e	12,303 ± 355 ef	14,851 ± 428 d	15,934 ±459 bcd
	RD-15	10,614 ± 306 gh	12,895 ± 372 cd	12,056 ± 348 de	10,697 ± 308 fgh	10,080 ± 290 gh
F_v/F_m	RD-1	0.78 ± 0.023 ab	0.80 ± 0.023 ab	0.82 ± 0.024 ab	0.82 ± 0.024 ab	0.74 ± 0.021 b
	RD-8	0.80 ± 0.022 a	0.79 ± 0.022 a	0.77 ± 0.023 a	0.79 ± 0.023 a	0.81 ± 0.023 a
	RD-15	0.78 ± 0.022 ab	0.75 ± 0.022 abc	0.78 ± 0.022 ab	0.69 ± 0.020 cd	0.72 ± 0.021 bcd
F_v/F_o	RD-1	3.63 ± 0.105 i	4.08 ± 0.112 fgh	4.45 ± 0.129 bcd	4.52 ± 0.131 abc	3.27 ± 0.094 j
	RD-8	3.91 ± 0.113 ef	3.86 ± 0.112 ef	3.69 ± 0.106 fg	3.86 ± 0.111 ef	4.30 ± 0.124 bcd
	RD-15	3.45 ± 0.099 abc	2.94 ± 0.084 de	3.57 ± 0.103 bc	2.57 ± 0.074 f	2.53 ± 0.073 f
Fluorescence Parameters	**Sample Period**	**Treatments**				
		SHTN1	**SHTN2**	**SHTN3**	**SHTN4**	**SHTN5**
F_v	RD-1	15,761 ± 454 bcd	16,604 ± 479 ab	14,961 ± 431 def	15,602 ± 450 cd	13,852 ± 399 efg
	RD-8	13,220 ± 381 e	16,260 ± 469 bcd	15,840 ± 457 bcd	16,586 ± 478 ab	16,206 ± 467 bcd
	RD-15	11,826 ± 341 e	13,133 ± 379 c	13,880 ± 400 bc	14,284 ± 412 b	16,098 ± 292 a
F_v/F_m	RD-1	0.81 ± 0.023 ab	0.82 ± 0.024 ab	0.80 ± 0.023 ab	0.81 ± 0.023 ab	0.79 ± 0.023 ab
	RD-8	0.77 ± 0.022 a	0.83 ± 0.024 a	0.79 ± 0.024 a	0.80 ± 0.023 a	0.80 ± 0.023 a
	RD-15	0.77 ± 0.022 ab	0.75 ± 0.022 abc	0.79 ± 0.022 ab	0.77 ± 0.022 ab	0.80 ± 0.023 a
F_v/F_o	RD-1	4.20 ± 0.121 efg	4.59 ± 0.133 abc	4.08 ± 0.118 fgh	4.29 ± 0.124 cde	3.83 ± 0.111 ghi
	RD-8	3.40 ± 0.098 g	4.77 ± 0.137 a	3.87 ± 0.112 ef	4.06 ± 0.117 def	3.92 ± 0.113 ef
	RD-15	3.34 ± 0.096 bc	3.27 ± 0.094 c	3.67 ± 0.105 b	3.6 ± 0.103 bc	4.11 ± 0.118 a

Note: control temperature (CKT: 25 °C/15 °C day/night); lightly high temperature (LHT: 30 °C/20 °C day/night); moderate high temperature (MHT: 35 °C/25 °C day/night); severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKTN4); N5: 1.25N (3.25 g·plant^{-1})]. Different lowercase letters indicate significant differences among treatment at the $p < 0.05$ level by Ducan' test. Values are mean ± SD (n = 3)

The maximum primary yield of the photochemistry of PSII (F_v/F_o) is linked to the photosynthetic efficiency of the plant, and an increased value of F_v/F_o indicates normal PSII functioning. On the first day of the recovery period, the F_v/F_o ratio (ratio between the rate constants of the photochemical and nonphotochemical deactivation of exited chlorophyll molecules) increased with the decrease in the nitrogen application level, and the maximum values were CKTN5, LHTN4, MHTN4, SHTN2, respectively.

Variance analysis showed that different high-temperature and nitrogen treatments and their interactions significantly affected the chlorophyll fluorescence parameters of the tomato leaves (Table 3). There were significant differences ($p < 0.01$) in the responses of high temperatures to the nitrogen supply levels (Table 4). On the 1st day of the recovery period, nitrogen had no significant effect on F_v/F_M, F_v, F_o and F_M; however, their interaction was significant ($p < 0.05$). HT and N for F_o, F_M, F_v, F_o/F_M, F_v/F_M, F_v/F_o, ABS/RC and DI_o/RC had no significant interaction effects on day 8 of the recovery period.

Table 4. ANOVA results of different high-temperature/nitrogen combinations on PSII reaction center activity parameters for tomato leaves during the recovery period.

Sample Period	Source	df	F_o	F_M	F_V	F_O/F_M	F_V/F_M	F_V/F_O	ABS/RC	DI_O/RC	TR_O/RC	ET_O/RC
RD-1	High Temperature (HT)	4	**	**	**	**	**	**	**	**	**	**
	Nitrogen (N)	5	*	NS	NS	NS	NS	**	NS	NS	*	**
	HT × N	20	**	**	**	*	*	**	*	*	**	**
RD-8	High Temperature (HT)	4	NS	*	**	*	*	*	*	*	**	**
	Nitrogen (N)	5	NS	**	**	*	*	**	*	*	*	NS
	HT × N	20	NS	NS	NS	NS	NS	NS	NS	NS	*	**
RD-15	High Temperature (HT)	4	*	**	**	**	**	**	**	**	**	**
	Nitrogen (N)	5	*	**	**	**	**	**	**	**	**	NS
	HT × N	20	*	*	**	**	**	**	**	**	**	**

Note: ** and * indicate the significance level at $p < 0.01$ and $p < 0.05$, respectively; NS denotes non-significance. The abbreviations are in the Supplementary Documents. RD, recovery periods.

The quantum yield of primary photochemistry F_v/F_M (ϕP_o), which reflects the overall photosynthetic potential of active PSII reaction centers, was not significantly affected by nitrogen application. On the first day of the recovery period, nitrogen had no significant effect on F_v/F_M, F_v, F_o and F_M (Table 4).

According to the ANOVA results (Table 4), the effects of temperature changed the JIP test parameters significantly for the tomato leaves sampling on the 1st day of the recovery period. Heat stress induced an increase in F_o and decreased F_v/F_M. Under different temperature treatments, the N5 treatment caused a significant rise in F_v/F_o. Temperature, nitrogen application and their interaction effects significantly reduced ET_o/RC and increased TR_o/RC, especially for N3 and N4. Under all five nitrogen applications, temperature played a significant role in the increase in DI_o/RC, especially for N2 and N3.

However, on the 8th day of the recovery period, neither temperature nor nitrogen had a significant effect on F_o. Although temperature and nitrogen had certain significant effects on the fluorescence parameters ($p < 0.05$ or $p < 0.01$), their interaction did not have an effect on the fluorescence parameters, except TR_o/RC and ET_o/RC. Interestingly, temperature, nitrogen, and their interaction effects changed the JIP test parameters significantly for the tomato leaves sampling on the 15th day of the recovery period, especially for F_v, F_o/F_M, F_v/F_M, F_v/F_o, ABS/RC, DI_o/RC, TR_o/RC, and ET_o/RC.

3.2.2. Specific Energy Fluxes

The specific energy fluxes were analyzed to determine the photosynthetic performance of the active PSII reaction centers of the tomato leaves subjected to various nitrogen applications under different high temperatures during the recovery period (Figures 9–11).

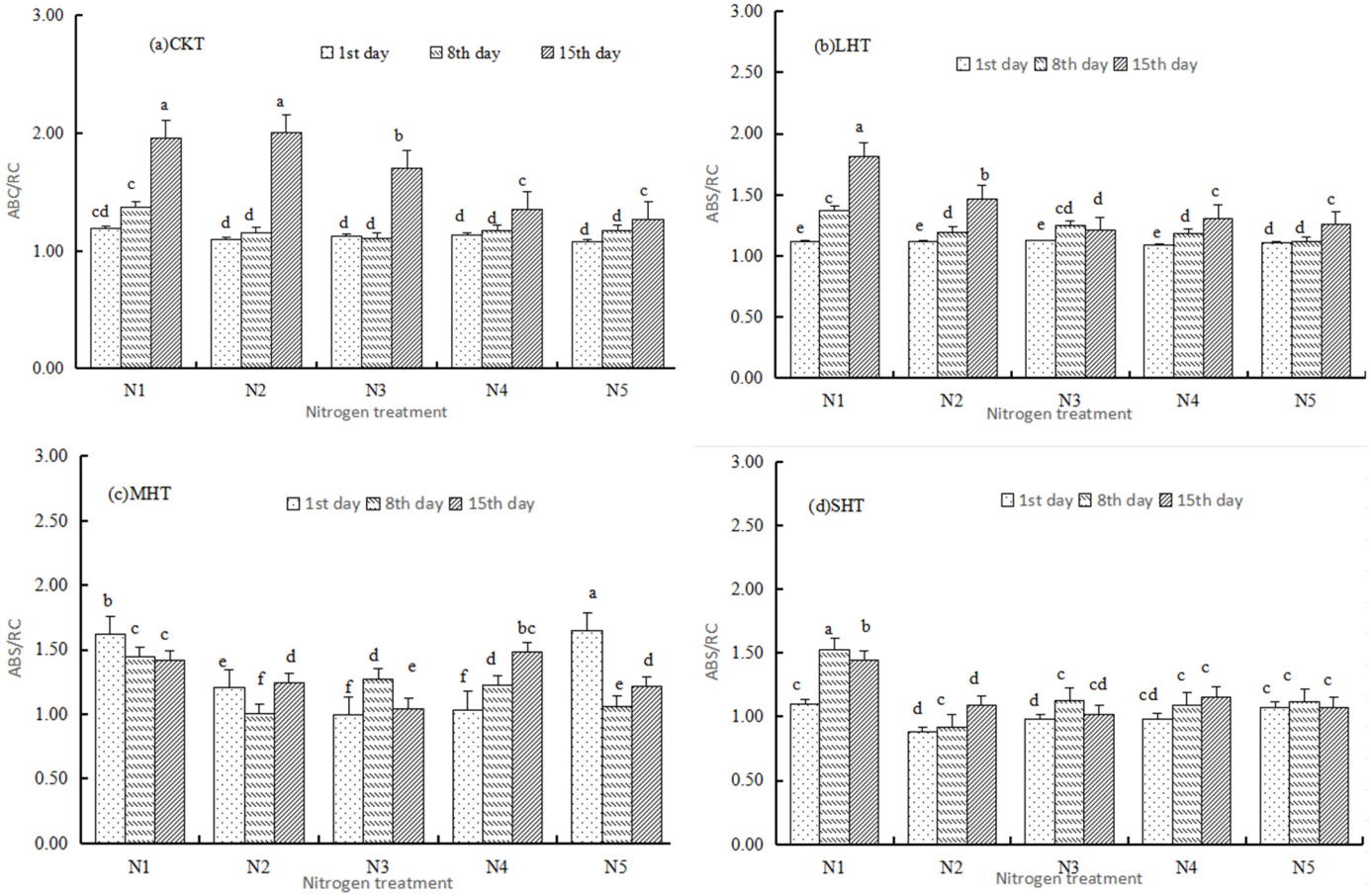

Figure 9. The PSII reaction center activity parameters (ABS/RC) of tomato leaves in the recovery period. (**a**) ABS/RC of tomato leaves under CKT; (**b**) ABS/RC of tomato leaves under LHT; (**c**) ABS/RC of tomato leaves under MHT; (**d**) ABS/RC of tomato leaves under SHT. Control temperature (CKT: 25 °C/15 °C day/night); lightly high temperature (LHT: 30 °C/20 °C day/night); moderate high temperature (MHT: 35 °C/25 °C day/night); severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKTN4); N5: 1.25N (3.25 g·plant^{-1})]. The abbreviations are in the Supplementary Documents. RD, recovery periods. Different lowercase letters indicate significant differences among treatment at the $p < 0.05$ level by Ducan' test.

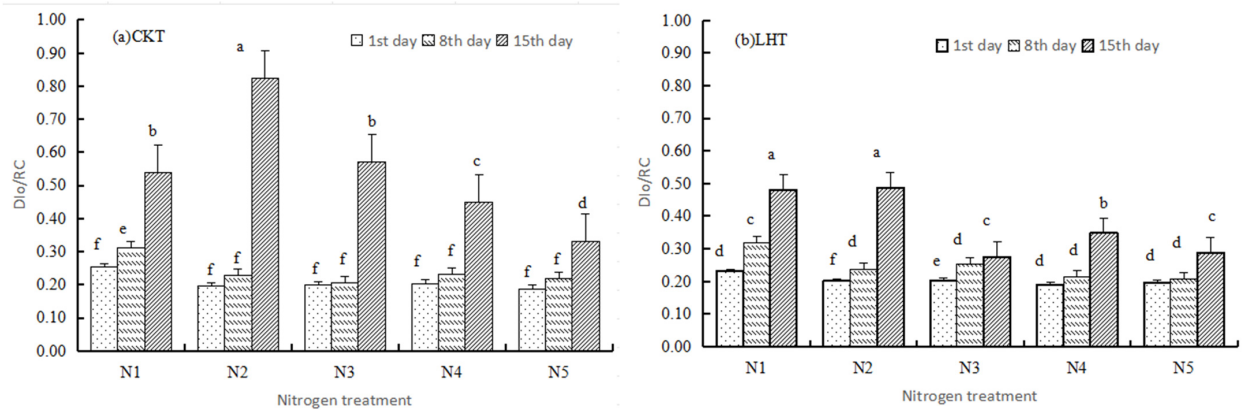

Figure 10. *Cont.*

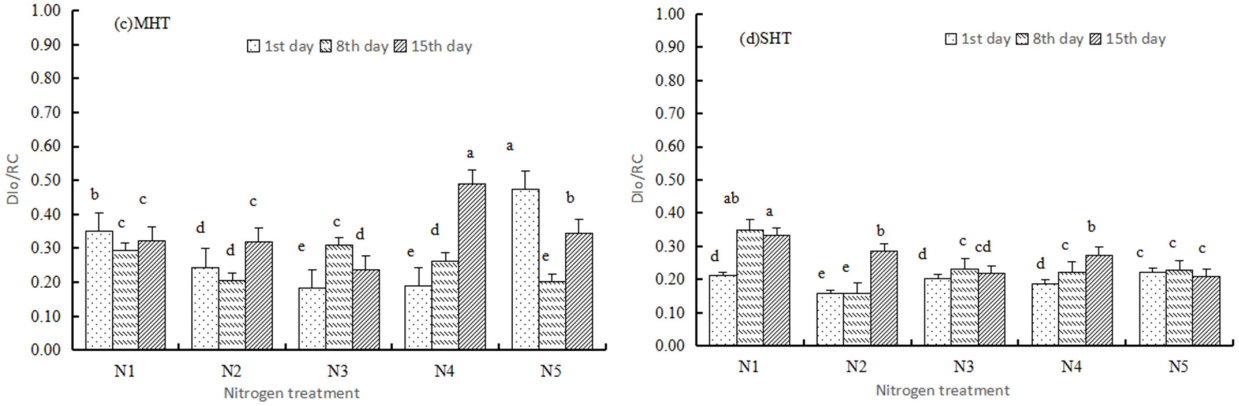

Figure 10. The PSII reaction center activity parameters (DI_o/RC) of tomato leaves in the recovery period. (**a**) DI_o/RC of tomato leaves under CKT; (**b**) DI_o/RC of tomato leaves under LHT; (**c**) DI_o/RC of tomato leaves under MHT; (**d**) DI_o/RC of tomato leaves under SHT. Control temperature (CKT: 25 °C/15 °C day/night); lightly high temperature (LHT: 30 °C/20 °C day/night); moderate high temperature (MHT: 35 °C/25 °C day/night); severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKTN4); N5: 1.25N (3.25 g·plant^{-1})]. The abbreviations are in the supplementary Documents. RD, recovery periods. Different lowercase letters indicate significant differences among treatment at the $p < 0.05$ level by Ducan' test.

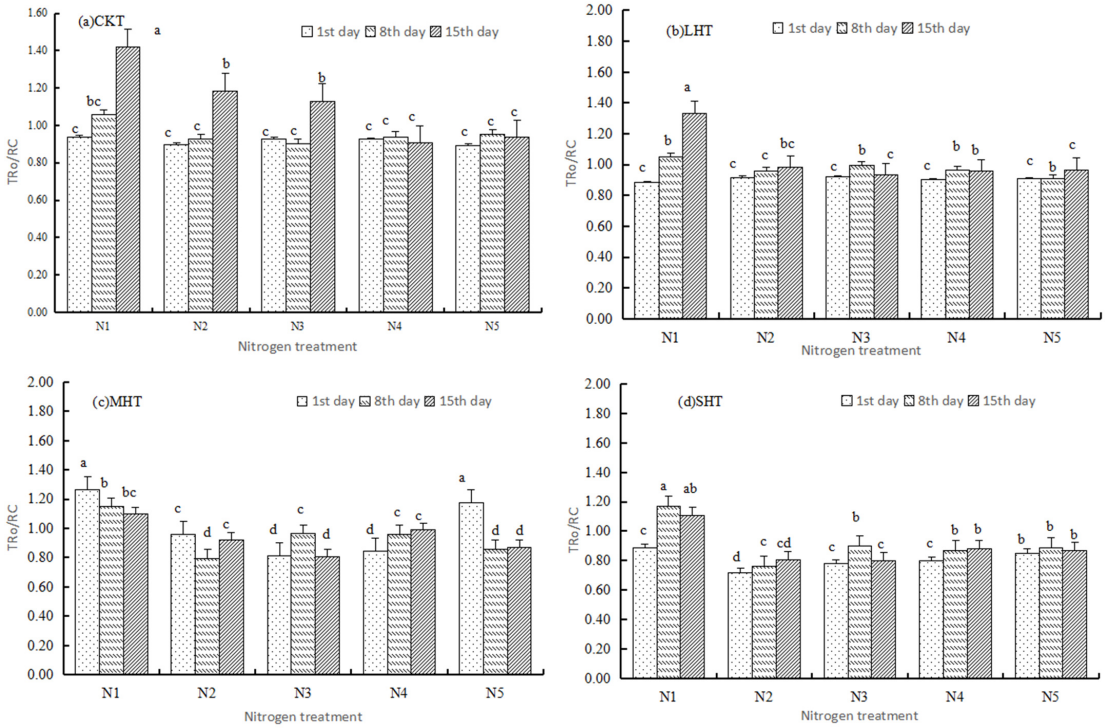

Figure 11. The PSII reaction center activity parameters (TR_o/RC) of tomato leaves in the recovery period. (**a**) TR_o/RC of tomato leaves under CKT; (**b**) TR_o/RC of tomato leaves under LHT; (**c**) TR_o/RC of tomato leaves under MHT; (**d**) TR_o/RC of tomato leaves under SHT. Control temperature (CKT: 25 °C/15 °C day/night); lightly high temperature (LHT: 30 °C/20 °C day/night); moderate high temperature (MHT: 35 °C/25 °C day/night); severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKTN4); N5: 1.25N (3.25 g·plant^{-1})]. The abbreviations are in the Supplementary Documents. RD, recovery periods. Different lowercase letters indicate significant differences among treatment at the $p < 0.05$ level by Ducan' test.

With the increase in heat stress, the ABS/RC value of the light energy absorbed by the reaction center gradually decreased. The ABS/RC values under the CKT and LHT treatments increased in the recovery period (Figure 9a–d). The absorption flux per center (ABS/RC) did not change significantly under the N1-N5 treatments on days 1 and 8 of the recovery period. Under MHT, the N1, N2 and N5 treatments decreased with the increase in the recovery period. Under SHT, the ABS/RC values of all treatments were low, and the differences were not remarkable.

The dissipated energy flux per reaction center (DI_o/RC) showed a trend of firstly increasing and later declining; and the DI_o/RC value was generally maximum under MHT, while under SHT, DI_o/RC followed the same trend as ABS/RC (Figure 10a–d).

A similar trend in TR_o/RC was shown, thereafter increasing as the nitrogen application increased (Figure 11a–d).

The changes in the electron transport flux per reaction center (ET_o/RC) under different nitrogen application rates after heat stress were different from those of ABS/RC, TR_o/RC and DI_o/RC (Figure 12a–d). Under CKT, with the extension of the recovery period, the ET_o/RC ratio of CKTN1 increased, but the ratio of CKTN2-CKTN5 decreased. On day 1 and day 8 of the recovery period, the ET_o/RC ratio increased with increasing nitrogen application, but on day 15 of the recovery period, the ET_o/RC ratio decreased (17.93%, compared with CKTN1). Under LHT, the ET_o/RC ratio of all treatments decreased gradually with the extension of the recovery period. The maximum ET_o/RC ratio of CKTN2 was 0.71 on day 1 of the recovery period, that of CKTN3 was 0.72 on day 8 of the recovery period (an increase of 4.41% compared with CKTN4 as the control), and that of CKTN4 was 0.62 on day 15 of the recovery period (a decrease of 8.82% compared with the control). Under MHT, with the extension of the recovery period, the ET_o/RC ratio of the CKTN1-CKTN2 treatment showed a trend of first decreasing and then increasing, while that of the CKTN3-CKTN5 treatment tended to increase and then decrease. The ratio of ET_o/RC was reduced by nitrogen application. Under SHT, on day 1 of the recovery period, the SHTN3 reduction effect of the low-nitrogen treatment was 7.66% higher than that of the high-nitrogen treatment.

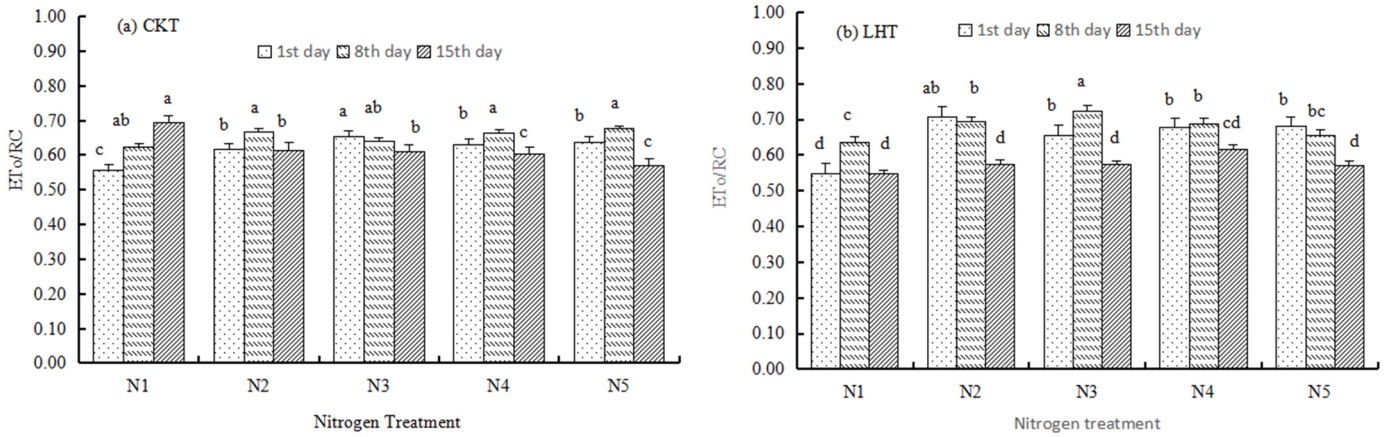

Figure 12. *Cont.*

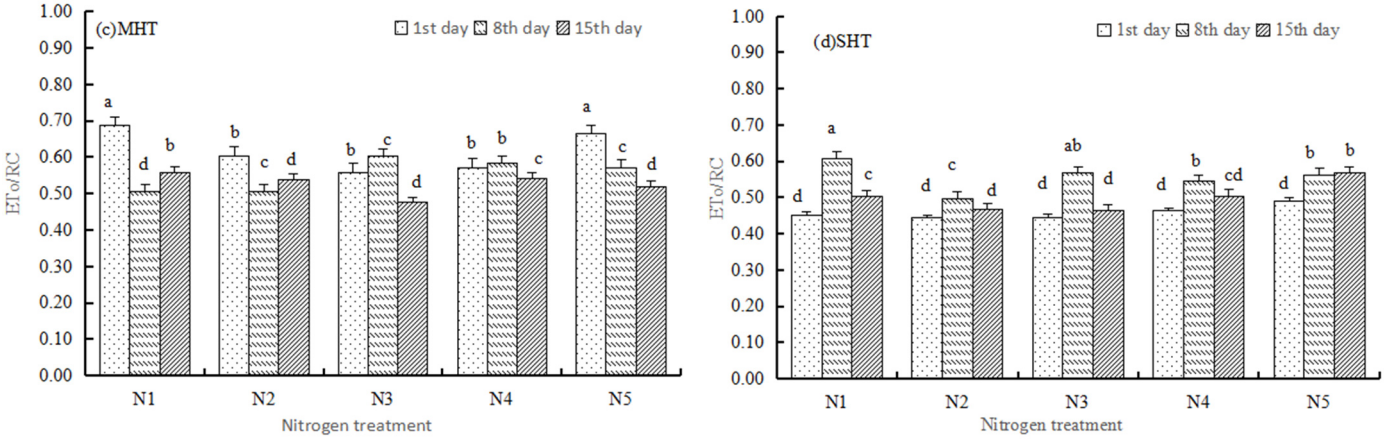

Figure 12. The PSII reaction center activity parameters (ET_o/RC) of tomato leaves in the recovery period. (**a**) ET_o/RC of tomato leaves under CKT; (**b**) ET_o/RC of tomato leaves under LHT; (**c**) ET_o/RC of tomato leaves under MHT; (**d**) ET_o/RC of tomato leaves under SHT. Control temperature (CKT: 25 °C/15 °C day/night); lightly high temperature (LHT: 30 °C/20 °C day/night); moderate high temperature (MHT: 35 °C/25 °C day/night); severe high temperature (SHT: 40 °C/30 °C day/night)] and nitrogen levels [N1: 0N (0 g·plant^{-1}); N2: 0.5N (1.3 g·plant^{-1}); N3: 0.75N (1.95 g·plant^{-1}); N4: 1N (2.6 g·plant^{-1}, CKTN4); N5: 1.25N (3.25 g·plant^{-1})]. The abbreviations are in the Supplementary Documents. RD, recovery periods. Different lowercase letters indicate significant differences among treatment at the $p < 0.05$ level by Ducan' test.

3.3. Performance Indexes

The findings revealed that nitrogen had a marked effect on all photosynthetic parameters, especially for performance indexes. PI_{abs} was significantly affected by the nitrogen application under different heat exposures, with values reaching zero in the fronds of tomato leaves at severe heat stress levels of 40 °C/30 °C. The lowest value of PI_{abs} was observed in tomato plants subjected to MHTN2. The general effects of different heat stress levels on photosynthetic parameters are shown in the form of a radar plot (Figure 13a–d). The values of PI and RC/CS tended to increase and then decrease as the recovery time increased, with the maximum occurring on day 15 for CKTN5, with a value of 1.35502, and the minimum occurring on day 1 for SHTN5, with a value of 0.37765.

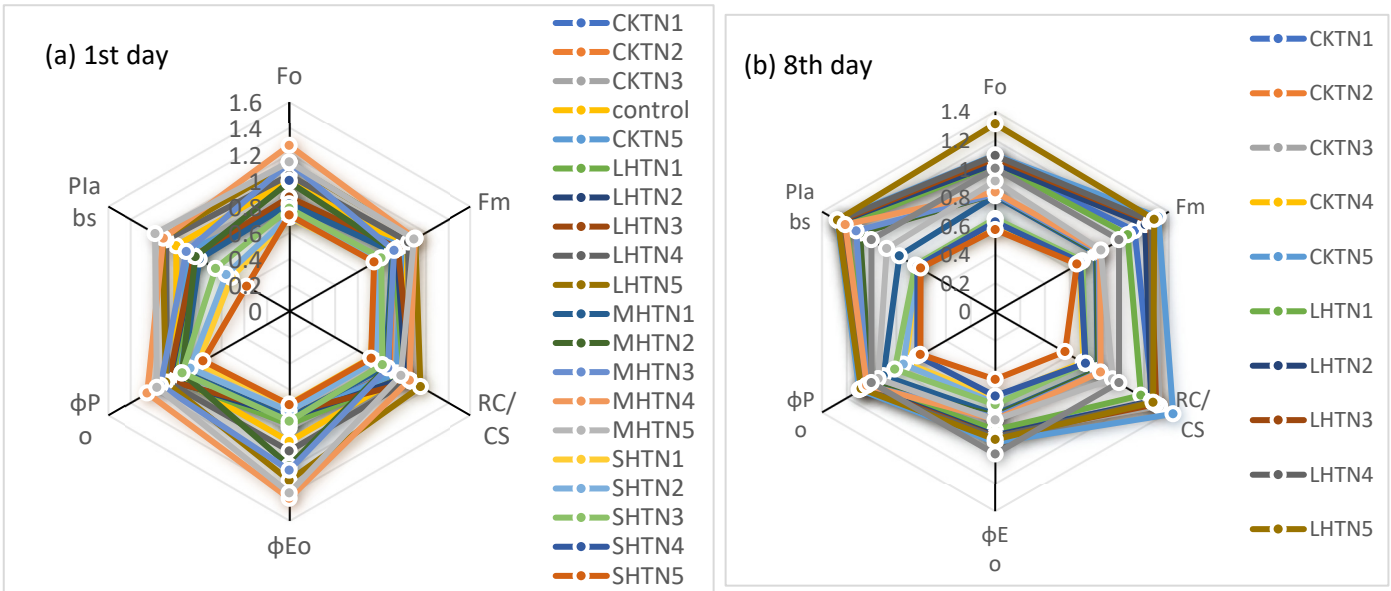

Figure 13. *Cont.*

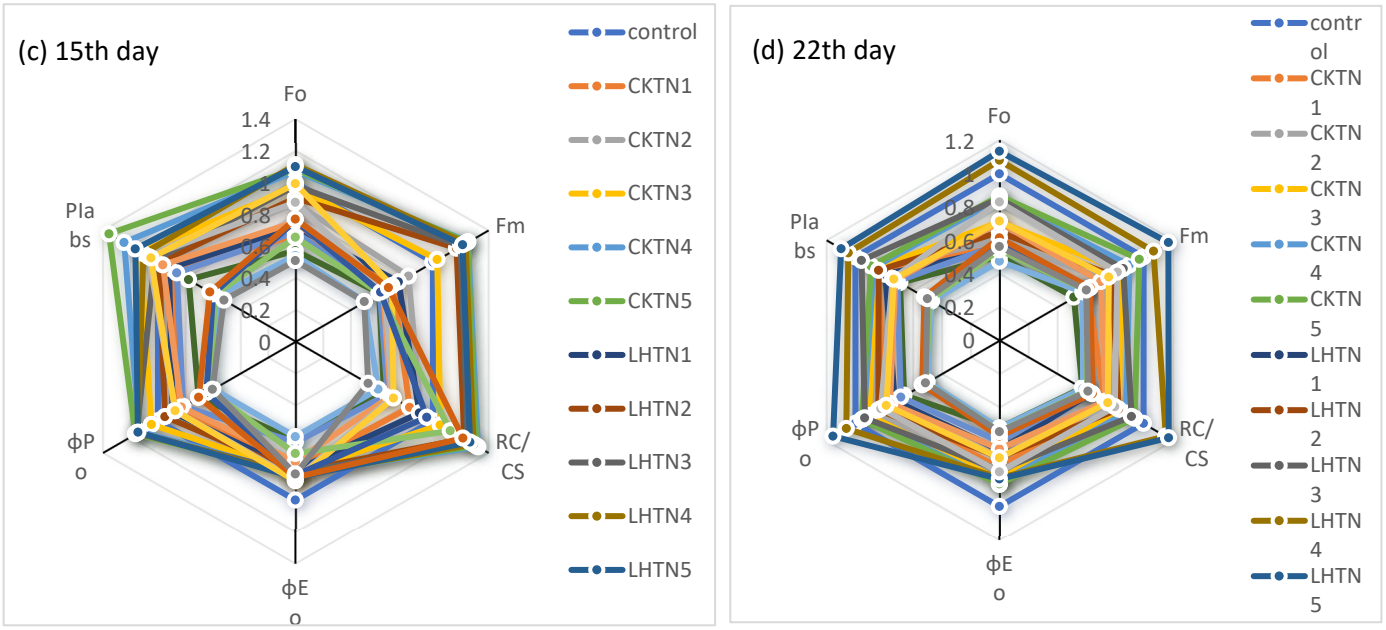

Figure 13. Radar plot of chosen JIP parameters (PI_{ABS}, RC/CS, φE$_o$, φP_o) of tomato leaves in recovery period. (**a**) Selected JIP parameters of tomato leaves on day 1 of recovery period; (**b**) selected JIP parameters of tomato leaves on day 8 of recovery period; (**c**) selected JIP parameters of tomato leaves on day 15 of recovery period; (**d**) selected JIP parameters of tomato leaves on day 22 of recovery period. The abbreviations are in the Supplementary Documents.

4. Discussion

The OJIP curves either decreased or increased due to nitrogen application under different high temperatures (Figures 3–5). There were significant differences ($p < 0.01$) among the responses of high temperatures to nitrogen supply levels for F_v/F_M; this is except for N on the 1st day, as well as for HT and N on the 8th day of the recovery period (Table 3). Therefore, F_v/F_M is not a sufficiently sensitive parameter for the assessment of nitrogen application under different heat stress levels. From 25 to 35 °C in tomato leaves and from 25 to 42 °C in the peels of apple fruit, the F_v/F_M ratio held constant while the temperature increased [42,43]. However, the use of OJIP curves is a more accurate and reliable approach than using the F_v/F_M ratio to measure the physiological damage caused by heat stress to the photosynthetic apparatus.

PSII is the primary site at which photoinhibition occurs and is located on the inner side of the cystoid membrane. High-temperature stress reduces the ability of tomato PSII reaction centers to capture and use light energy. The number of active centers per unit area and the proportion of absorbed light energy used for electron transfer decreased to various degrees [44]. At this time, the absorption of light energy by PSII reaction centers is mostly dissipated in the form of heat energy [45]. Under heat stress, nitrogen can enhance the adaptability of plants to heat stress by maintaining the optimal light energy conversion efficiency of PSII through heat dissipation [46]. Studies have shown that an appropriate amount of nitrogen fertilizer can improve the photosynthetic rate and the actual photochemical efficiency of PSII, but that excessive nitrogen fertilizer application has a negative effect [47]. The results of this study show that heat stress can significantly reduce F_o, F_m, F_v, F_v/F_o and F_v/F_m, indicating that high temperatures inhibit the light energy conversion efficiency of PSII in plants and weaken the potential activity of PSII on the 15th day of the tomato recovery period (Table 4, Figure 6).

Moderate nitrogen can improve the primary light energy conversion efficiency of tomato and enhance the potential activity of PSII to a certain extent after heat stress. Within a certain range, different nitrogen fertilization treatments, compared with the control, improved the F_o, F_m, F_v, F_v/F_o and F_v/F_m of leaves to different degrees as a whole, and

the nitrogen application rate of 1.95–2.6 g/plant performed better. Studies have shown that an appropriate increase in the nitrogen application rate in the later stage of wheat can increase its photosynthetic rate, photochemical efficiency and PSII activity, and at the same time reduce the heat dissipation of non-radiative energy, thereby improving the quantum efficiency of PSII [48]. The elevated F_o observed in spinach and rice is attributed to the irreversible dissociation of LHC II from the PSII complex, and the partial reversible inactivation of PSII. The decrease in F_m may be related to chlorophyll protein denaturation. In our study, with the delay of the tomato growth period after heat stress, the F_o/F_m of different nitrogen fertilization treatments showed an upward trend, and the appropriate nitrogen fertilization rate (1.95–2.6 g/plant) could relatively reduce the increase in the ratio, indicating that the appropriate application of nitrogen fertilizer can reduce the heat dissipation of light energy absorbed by PSII antenna pigments.

The heatmap graphically showed the interrelationships between different fluorescence parameters (Figure 14). Furthermore, it was able to determine the density of the active and inactive PSII reaction centers (RC/CS) and other indicators. Compared to the control, the addition of nitrogen increased the values of PI_{abs} and RC/CS under LHT, while the addition of nitrogen did not result in higher values of PI_{abs} and RC/CS than the control under SHT. However, low levels of nitrogen addition resulted in higher values of PI_{abs} and RC/CS than high levels of nitrogen addition. Heat stress also affected the shape of the OJIP curve, resulting in a decrease in F_m and an increase in F_o. The increase in F_o may have been caused by the dissociation of the light-trapping chromophore complex LHC II from the PSII complex, the deactivation of the PSII photochemical reaction, or the suppression of electron flux transfer from the reduced electron acceptor Q_A to Q_B [49]. F_v is a variable fluorescence that reflects PSII's maximum potential for electron transfer. Under severe heat stress, the tomato with a low nitrogen application rate had the highest F_v value in our study. The damage to the plant was minimal, but in the subsequent recovery process, sufficient nitrogen supply increased the electron transfer potential of tomato leaves, thereby improving the light energy absorption and utilization efficiency of tomato leaves in light reactions.

F_v/F_m is the efficiency with which the PSII reaction center captures the excitation energy [50], that is, the maximum photochemical efficiency. The photoelectron transport capacity and potential photochemical efficiency of plants are inhibited after heat stress [49]. Previously, it was believed that heat stress inhibited the PSII potential photochemical efficiency (F_v/F_m) and photochemical quenching (qP) [51–53]. In our study, under the four temperature treatments, the F_v/F_m values of the low-nitrogen treatment were the smallest, indicating that the maximum photochemical efficiency of the tomato leaves without nitrogen fertilizer was lower. F_v/F_o represents the potential photochemical activity of PSII and is proportional to the number of active reaction centers. Our study found that the potential photochemical activity of tomato leaves increased as the degree of heat stress increased, and that the nitrogen application level decreased at the early stage of the assay. On the 15th day of measurement, the maximum value of F_v/F_o under each temperature treatment was N5, which indicated that sufficient nitrogen could increase the number of active reaction centers in PSII of tomato leaves.

A large amount of primary photochemical information about the PSII reaction center can be analyzed using OJIP curves and fluorescence parameters [54]. The OJIP curve has four phases, namely O, J, I, and P, in the fluorescence rising stage. At the O point, the electron acceptor of PSII completely loses electrons and is oxidized. At this time, the acceptor side of PSII has the strongest ability to accept electrons. The fluorescence intensity increases at the J point, and Q_A accumulates on the acceptor side of the PSII reaction center; J~I represents the complete reduction of the reduced plastoquinone (PQ) pool during the electron transfer; at point P, Q_A fully enters in the reduced state, and the PSII reaction center is closed when the fluorescence yield is maximum [55,56]. Therefore, appropriate nitrogen application can improve PSII energy connectivity and strong stress resistance; under a moderate high-temperature treatment, with the increase in the nitrogen amount,

ΔV_t changes from a negative value to a positive value, indicating that an appropriate amount of nitrogen application can maintain the photosystem of tomato leaves in a good state. After nitrogen application exceeds a certain amount, the photosystem of tomato leaves will be damaged; under a severe high-temperature treatment, the ΔV_t values of all nitrogen application treatments in ΔK, ΔJ, and ΔI are greater than 0, which indicates that severe heat stress damages the PSII of tomato leaves, resulting in poor stability.

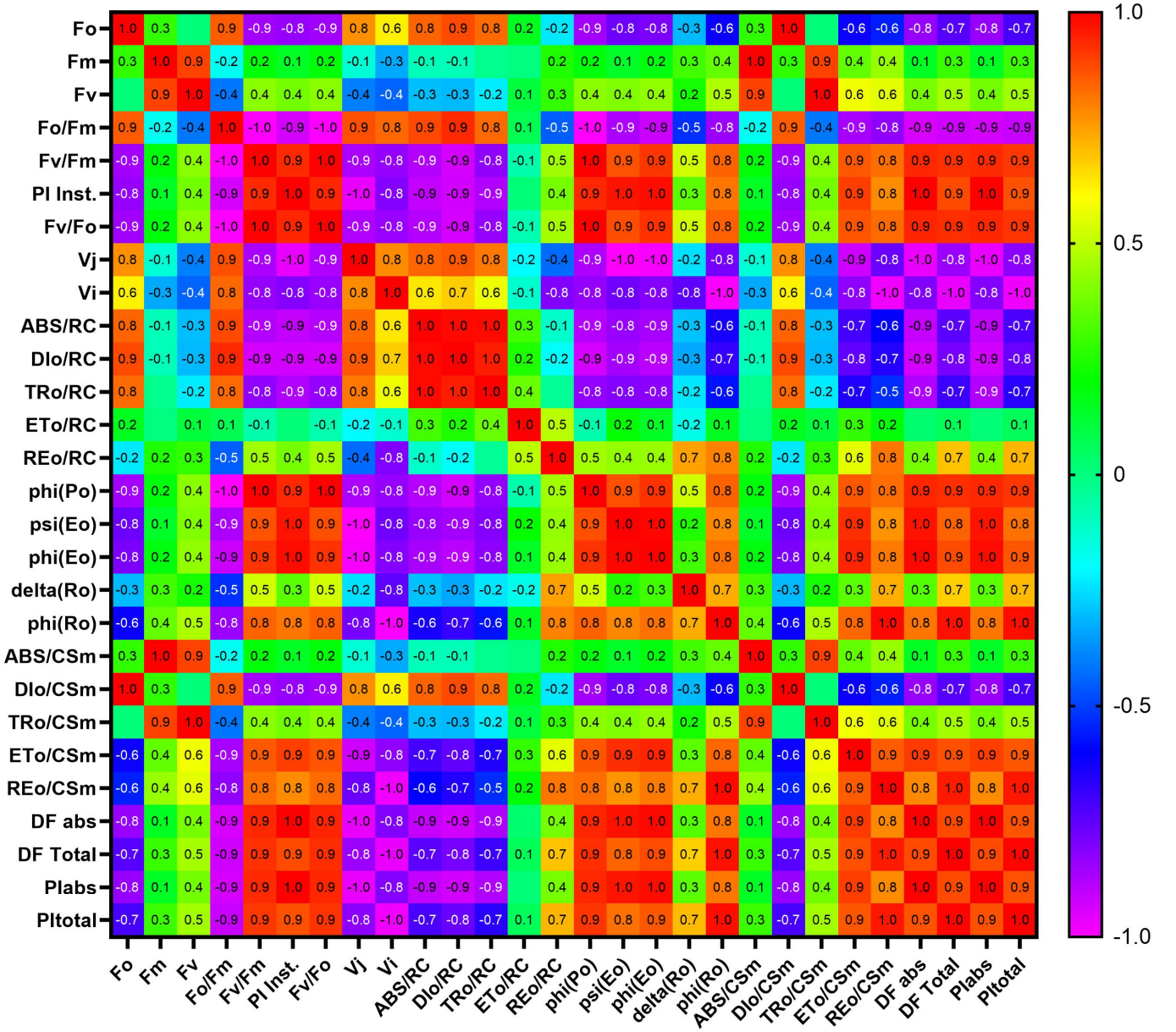

Figure 14. Heatmap representing the correlation matrix of several fluorescence parameters, obtained after using the JIP test for tomato leaves on the 1st day of the recovery period. The abbreviations are in the Supplementary Documents.

The K peak (300 μs) is an excellent indicator of heat stress, and can be used to indicate the dissociation of the oxygen-evolving complex OEC and the electron transfer between Pheo and the primary electron acceptor Q_A [43]. In wheat, 35 °C treatment had no effect on the net photosynthetic rate, while 45 °C treatment resulted in irreversible damage to the OEC [51]. The direct reason for the appearance of the K peak is that the outflow of electrons from P680 to the PSII electron acceptor far exceeds the inflow of electrons from the PSII donor side to P680. At the same time, the K peak is also affected by the change

in the energy relationship between photosystem II. Contrary to some of the above results, during the recovery period after heat stress, our OJIP curve showed obvious O, J, I, P site characteristics, but no obvious K peak. But there are obvious DI_o/RC dissipated energy changes. There is a threshold (intensity) for the energy dissipated by DI_o/RC, and there is an energy transfer phenomenon (waveform and frequency). If the threshold of energy dissipation is increased, the stress resistance of plants to heat stress can be improved.

High-temperature stress is most sensitive to the phase of electron transfer QA to PQH2, but evidence for this is lacking in research on tomatoes. We found that, based on RC, under severe heat stress, the values of ABS/RC, TR_o/RC and DI_o/RC increased significantly, but the values of ET_o/RC decreased significantly; this indicates that the blade reduces the energy share for electron transfer and increases the heat dissipation energy share to reduce high-temperature-induced damage. With the increase in heat stress, the greater the energy absorbed by the PSII reaction center, the greater the dissipated energy. Although the TR_o/RC value of the energy captured by the SHT treatment for reducing Q_A was significantly lower, the overall TR_o/RC value of the other three temperature treatments was not significantly different. Under CKT, sufficient nitrogen supply can enhance the ability of the PSII reaction center to capture electron transfer energy. Under SHT, appropriately reducing the nitrogen application rate can enhance the stress resistance of tomato plants, and in the subsequent recovery period, appropriately increasing the nitrogen application rate is helpful for the recovery of tomato plants.

5. Conclusions

We analyzed the effect of nitrogen application on OJIP curves under different heat stress conditions during the recovery period. With the deepening of heat stress, reducing the amount of nitrogen application was found to enhance the resistance of tomato plants. For CKT-SHT, all N1 treatments had ΔV_t values greater than zero at ΔK and ΔJ. In the recovery stage, the higher nitrogen level was beneficial to the recovery of tomato plants. F_v/F_o was found to be sensitive to the application of high temperatures and nitrogen. As the degree of heat stress increased, the nitrogen application level decreased, and the potential photochemical activity of tomato leaves increased. During the recovery period, sufficient nitrogen could increase the number of active reaction centers of PSII in tomato leaves, and enhance the ability of the PSII reaction center to capture energy for electron transfer, thus improving the activity of the PSII reaction center. Furthermore, further research needs to be conducted to clarify the mechanism. This is especially in combination with molecular biology methods, such as the use of transmission electron microscopy (TEM), in order to observe the cystoid structure of chloroplasts (<1 nm), determine the expression of heat stress genes, and analyze the co-localization of nitrogen and electron transport-related elements.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13122858/s1.

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