Study on the Relationship between Delayed Fluorescence and Photosynthetic Capability at Elevated Temperature in Higher Plants

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Abstract. With the continuous elevation of the global temperature, high-temperature stress has been a major environmental factor that affects plant growth and productivity. Effects of short-term heat temperature stress on light-induced delayed fluorescence (DF) decay kinetic curve, intensity and emission spectrum have been investigated in C3 soybean (Jing Huang No.3) and C4 maize (Yun Xi No.5081) species. The temperature responses of DF decay kinetic curve from two different species show that decay rate characteristics are affected by high temperature. The spectroscopy measurements indicate that heat stress influence the shape of DF emission spectra of two species, especially the peak intensities at 685nm and 730nm. Moreover, our results clearly demonstrate that DF intensity of each plant positively correlated with F730/F685 of DF emission spectra at elevated temperatures. In addition, the net photosynthetic rate ($P_n$) of samples has the same temperature response with DF intensity and F730/F685. Based on these results, we can conclude that there is an excellent correlation between F730/F685 of DF emission spectra, DF intensity and $P_n$ in both C3 and C4 plants. Therefore, we proposed that the F730/F685 of DF emission spectrum can be used to measure the photosynthetic capability of higher plants to heat stress.

1. Introduction
There are many factors to restrict the growth and production of crops and forest plants, such as salinity, UVB radiation, drought, low or high temperature, etc. With the predicted greenhouse-induced rise in global surface temperature possibly already under way, the extremely temperature has been a normal weather in many cities. Accordingly, the effects of elevated temperatures on plant growth and ecosystem function have become a major area of concern. As we know, the high temperature affects the photosynthesis of plant, changes the structure and function of the photosynthetic apparatus, and causes various damages in thylakoid membranes [1]. There are some biochemical methods to examine the plant responses to increasing temperature. At present, the chlorophyll fluorescence techniques and photosynthetic capability detection are the main approaches for measuring plant stress responses. However, photosynthetic capability detection based on gas exchange technique is easy to interference.
from environmental factors [2]. Although chlorophyll fluorescence technique is a fast method of detection of plant stress response, there are some difficulties in practical application [3]. In recent years, some researchers proposed that delayed fluorescence (DF) can be a useful and effective method to detect the plant stress response, such as high temperature, salt and UVB stress, etc [4,5]. Accordingly, the analysis of DF behaviour can be useful in assessing the state of the photosynthetic processes.

In 1951, Strehler and Arnold firstly reported the DF, also referred as “delayed luminescence (DL)” and “delayed light emission (DLE)”, which is a long-live low level photon emission by plant after being illuminated with light and put into darkness [6]. After that, many scholars were eager to find out the characteristics of DF, the relationship between DF and environmental stress, and the biochemical representation of DF.

For years, researchers have been reported some DF characteristics such as origination of the peak at 730nm in DF emission spectrum [7], correlation between DF intensity and net photosynthetic rate (\(P_n\)) [4,5] and the environmental stress of plant based on DF [5,8], and so on. However, the responses of decay characteristics of DF signal and emission spectrum to stress has not been researched.

The objectives of this work were to investigate the variation of decay characteristics of DF decay signal and the DF emission spectrum at elevated temperatures. Moreover, the relationships between DF intensity, peak intensity of DF emission spectra and \(P_n\) of two different samples were researched. Experimental results indicated that the variation of decay characteristics of DF decay signals leads to the variation of DF intensity at elevated temperatures. The high temperature varied the peak intensities of DF emission spectrum. Additionally, results demonstrated that peak ratio (F730/F685) of DF emission spectrum, DF intensity and \(P_n\) response to temperature in a same way in both C3 and C4 samples.

2. Materials and Methods

2.1. Plant material and elevated temperature
Seeds of soybean (Glycine max (L.) Jing Huang No.3) and maize (Zea May L. Yun Xi 5081) were grown in a plant growth chamber (Conviron, model E7/2, Winnipeg, Canada) under a photoperiod of 14 hours with a light intensity of 400µmol photons m\(^{-2}\)s\(^{-1}\). The growth relative humidity (RH) or temperature was controlled as 70/80% (day/night) or 26°C/22°C (day/night), respectively. After a week, the seedlings were thinned out in the uniform size. Two to four plants were maintained per pot. The elevated temperatures were carried by water bath. The aluminium cuvette containing the detached leaves of samples were dipped into a water bath, and after incubation for 30min, DF intensity, \(P_n\), and DF spectrum was measured at the temperatures indicated, respectively. The experimental temperatures were set as 25°C, 30°C, 35°C, 40°C, 45°C and 50°C. For all experiments, the fourth or fifth leaf was used.

2.2. Measurements of delayed fluorescence (DF) decay signal and intensity
DF decay signals and intensities were measured by home-made portable DF detection system in our laboratory [4]. The excitation light was a set of LED (\(\lambda=660\)nm), and the light intensity was controlled within the range between 0 and 3000µmol photons m\(^{-2}\)s\(^{-1}\). The DF decay signal was collected by an ultra-high-sensitive Channel Photomultiplier DC-Module (MP963, Perkin-Elmer, Wiesbaden, Germany). In this paper, the DF intensity was integrated within 1 seconds of DF decay signal and registered as count per second (cps). The technical details of the system are described in reference [4]. Each experiment was replicated at least five times.

2.3. Measurements of net photosynthesis rate (\(P_n\))
The plant net photosynthetic rate (\(P_n\)) according to traditional interpretation, is used to represent plant photosynthetic capability. In this study, \(P_n\) was measured by a portable photosynthesis system (Model: LI-COR 6400, Lincoln, Nebraska, USA). In the experiment, a fixed concentration of CO\(_2\)
(1250–1350ppm) was chosen, which is saturated for C3 and C4 plants [9]. The temperature or humidity of system’s chamber was controlled at 26±0.5°C or 75%, respectively. Each experiment was replicated at least five times.

2.4. Measurements of delayed fluorescence (DF) spectrum
The DF emission spectra of samples were measured by a fluorescence spectrometer LS55 (Perkin-Elmer, USA). The DF emission spectrum measurements were carried out under phosphorescence model. DF emission spectrum was excited at 660nm and recorded from 670 to 760nm. Each experiment was replicated at least five times.

3. Results and Discussion

3.1. DF decay signals response to elevated temperature
Figure 1 shows the responses of DF decay signals to elevated temperatures. As shown in figure 1, with increasing of temperature, the initial intensity and decay rate of DF decay signal decreased obviously, especially at higher temperature.

![Figure 1](image)

**Figure 1.** The responses of DF decay signals to elevated temperature in maize (Yun Xi No. 5081) (a) and soybean (Jing Huang No.3) (b) leaves

To better research the DF decay characteristics at elevated temperature, we used curve fitting to analyse the DF decay signals in figure 1. In our recent report, we proposed that DF decay signal can be simplified as a three components decay kinetic model was shown as following [10]:

\[ I_{DF}(t) = I_1 e^{-\frac{t}{\tau_1}} + I_2 e^{-\frac{t}{\tau_2}} + I_3 e^{-\frac{t}{\tau_3}} \]  

In equation (1), \( I_1 e^{-\frac{t}{\tau_1}} \), \( I_2 e^{-\frac{t}{\tau_2}} \), and \( I_3 e^{-\frac{t}{\tau_3}} \) are corresponding to the electron reverse of three components, \( Q_A^- \), \( Q_B^- \), and \( F^- \), respectively. Although \( I_3 e^{-\frac{t}{\tau_3}} \) comes from the cyclic electron flow around PSI, the research testified that the third component is almost a constant [10]. Accordingly, the equation (1) can be further simplified as a double exponential:

\[ I_{DF}(t) = I_1 e^{-\frac{t}{\tau_1}} + I_2 e^{-\frac{t}{\tau_2}} + C \]  

\( \tau_1 \), which is related to the electron recombination of \( P_{680}^+ \), and \( Q_A^- \), is called decay rate constant of the fast decay component. \( \tau_2 \), which is related to the electron recombination of \( Q_A^- \) and \( Q_B^- \), is called decay rate constant of slow decay component. \( I_1 \) and \( I_2 \) are portion to \( Q_A^- \) and \( Q_B^- \). And C
represents the total of the DF component which comes from the cyclic electron flow around PSI and not negligible noise. We fitted the DF decay signals which are shown in figure 1 using the equation (2), and the fitting results are listed in table 1.

| Sample          | Temperature (°C) | $R^2$  | $C$    | $I_1$  | $\tau_1$ | $I_2$  | $\tau_2$ |
|-----------------|-----------------|--------|--------|--------|----------|--------|----------|
| Maize (Yun Xi No. 5081) | 25               | 0.989  | 251.831 | 480.923 | -0.066   | 506.669 | -0.271   |
|                 | 30               | 0.994  | 245.733 | 590.932 | -0.064   | 524.979 | -0.267   |
|                 | 35               | 0.994  | 277.839 | 392.429 | -0.066   | 458.082 | -0.267   |
|                 | 40               | 0.994  | 243.855 | 325.485 | -0.060   | 366.380 | -0.262   |
|                 | 45               | 0.989  | 230.114 | 288.555 | -0.058   | 304.001 | -0.270   |
|                 | 50               | 0.993  | 236.696 | 166.315 | -0.060   | 254.496 | -0.263   |
| Soybean (Jing Huang No.3) | 25               | 0.994  | 151.664 | 519.067 | -0.058   | 602.125 | -0.263   |
|                 | 30               | 0.989  | 166.856 | 480.932 | -0.066   | 566.661 | -0.271   |
|                 | 35               | 0.988  | 136.670 | 415.831 | -0.069   | 500.958 | -0.288   |
|                 | 40               | 0.991  | 169.383 | 332.630 | -0.050   | 349.992 | -0.273   |
|                 | 45               | 0.987  | 136.989 | 290.833 | -0.071   | 217.259 | -0.291   |
|                 | 50               | 0.979  | 140.368 | 113.802 | -0.070   | 132.497 | -0.310   |

It is clearly that two components fit for the DF decay signals at elevated temperatures of two samples were very excellent ($R^2 \equiv 0.979$). Moreover, the results listed in table 1 shown that values of $I_1$ and $I_2$ decreased when temperature is above 25°C for soybean sample. $\tau_1$ and $\tau_2$ are slightly different as the noise. The results indicated that under high temperature, the electron recombination of $P_{680}$ and $Q_A^-$, $Q_A^-$ and $Q_B^{2-}$ are greatly restrained. The inhibition leaded to the decrease of the $[Q_A^-]$ and $[Q_B^{2-}]$, accordingly deceased the values of $I_1$ and $I_2$. According to equation (2) and table (1), we concluded that the decreasing of values of $I_1$ and $I_2$ are the main factor which lead to the deceasing of $I_{DF}(t)$.

The similar results were observed in maize sample. As is known, C4 plant species have a higher temperature optimum for photosynthesis than C3 plants. Accordingly, maximum values of $I_1$ and $I_2$ on elevated temperature is observed at 30°C for maize sample and at 25°C for soybean sample, respectively.

3.2. DF spectra responses to elevated temperature

The DF spectra clusters of two samples are shown in figure 2(a) and (b). Data were normalized at the peak of 685nm.
Figure 2. The responses of DF emission spectra to elevated temperature in maize (Yun Xi No. 5081) (a) and soybean (Jing Huang No.3) (b) detached leaves

In room temperature, the DF emission spectrum, in turn, exhibits two maxima in the 685nm and 730nm region. Figure 2(a) shows the changes in DF emission spectrum of maize samples exposed to elevated temperatures. As shown in figure 2(a), the peak intensity at 730nm attained its maximum when the temperature was 30°C. Above 30°C, with increasing temperature, the peak intensity at 730nm continued to decrease. However, as shown in figure 2(b), the temperatures higher, the peak intensity at 730nm lower.

3.3. Correlation between DF intensity, $Pn$ and DF emission spectrum

From the results of 3.1 and 3.2, we observed a surprised phenomenon that the response of DF intensity and emission spectra to elevated temperature is similar. For the maize sample, when temperature was 30°C, the DF intensity and peak intensity at 730nm respectively attained the maximum. However, for the soybean sample, with increasing temperature, both them decreased. For better analyse the response of DF emission spectrum to elevated temperature, we researched the ratio of peak at 730nm to 685nm, which was marked as $F_{730/685}$. We hypothesized that DF intensity and $F_{730/685}$ of emission spectrum has some correlation. To verify the hypothesis, the correlations between DF intensity, $F_{730/685}$ and $Pn$ of two samples at elevated temperature were researched. As shown in figure 3(a) and 3(b), the DF intensity exhibited a good agreement with the $F_{730/685}$ for both maize and soybean samples in response to elevated temperatures. Moreover, the results showed that the $F_{730/685}$ and $Pn$ responded to elevated temperatures in a same way in both two samples (figure 3(a) and 3(b) insert maps). Similar experiments were performed for the leaves of two soybean species (Ke Feng No.1 and You Chun No.4) and three maize species (Jin Dan No.36, Ji No.853 and Yun Xi No. 422). The similar results have been found in the five different samples. All the results suggested that $F_{730/685}$ has the same response with DF intensity and $Pn$ to elevated temperature in both C3 and C4 plants.
Figure 3. Effect of elevated temperature on DF intensity and F730/F685 in maize (a) and soybean (b) leaves. Effect of elevated temperature on $P_n$ and F730/F685 in maize and soybean samples is respectively shown in the inset.

4. Conclusion

With the detached leaves of maize and soybean as testing samples, we have studied the variations of decay characteristics of DF decay signals and peak intensity of DF emission spectra under high temperature conditions. The results clearly show that, the high temperature inhibit the electron recombination of $P_{680}^+$ and $Q_A^-$, $Q_A^-$ and $Q_B^{2-}$, accordingly decrease the amplitudes of fast and slow decay components. Therefore, the DF intensity decreased with increasing temperature. The spectroscopic analysis results demonstrated that the high temperature affect the peak intensity of DF emission spectra. Moreover, the response of F730/F685 of DF emission spectra to elevated temperature is the same as that of DF intensity and $P_n$. Since the correlation between DF intensity and $P_n$ under stress has been reported before. Therefore, we proposed that the F730/F685 of DF emission spectrum can be used as an effective test for the state of photosynthetic apparatus and truly reflects the plant photosynthetic ability under high temperature stress.

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