Scientific Research Publishing

American Journal of Plant Sciences, 2017, 8, 328-339
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

# Photoinhibition of Leaves with Different Photosynthetic Carbon Assimilation Characteristics in Maize (*Zea mays*)

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**How to cite this paper:** Ruan, Y.Y., Li, X.Y., Wang, Y.P., Jiang, S.Q., Song, B., Guo, Z.Y., Zhang, A., Qi, Q., Zhang, L.J., Fan, J.J., Guan, Y.X., Cui, Z.H. and Zhu, Y.S. (2017) Photoinhibition of Leaves with Different Photosynthetic Carbon Assimilation Characteristics in Maize (*Zea mays*). *American Journal of Plant Sciences*, **8**, 328-339.
https://doi.org/10.4236/ajps.2017.83023

**Received:** December 3, 2016
**Accepted:** February 1, 2017
**Published:** February 4, 2017



## Abstract

Strong light decreases the rate of photosynthesis and assimilates production of crop plants. Plants with different carbon reduction cycles respond differently to strong light stress. However, variation in photoinhibition in leaves with different photosynthetic characteristics in maize is not clear. In this experiment, we used the first leaves (with an incomplete $C_4$ cycle) and fifth leaves (with a complete $C_4$ cycle) of maize plants as well as the fifth leaves ($C_3$ cycle) of tobacco plants as a reference to measure the photosynthetic rate ($P_N$) and chlorophyll a parameters under strong light stress. During treatment, $P_N$, the maximal fluorescence ($F_m$), the maximal quantum yield of PSII photochemistry ($F_v/F_m$), and the number of active photosystem II (PSII) reaction centers per excited cross-section ($RC/CS_m$) declined dramatically in all three types of leaves but to different degrees. $P_N$, $F_m$, $F_v/F_m$, and $RC/CS_m$ were less inhibited by strong light in $C_4$ leaves. The results showed that maize $C_4$ leaves with higher rates of photosynthesis are more tolerant to strong light stress than incomplete $C_4$ leaves, and the carbon reduction cycle is more important to photoprotection in $C_4$ leaves, while state transition is critical in incomplete $C_4$ leaves.

## Keywords

Fluorescence Transient, Photosystem II (PSII), Photoprotection, Light Stress, $C_4$ Photosynthesis

## 1. Introduction

Strong light is an important factor that reduces photosynthetic activity and lim-

its the production of assimilates in crop plants via a process called photoinhibition [1]. The longer the exposure to excess excitation energy, the more damage to the photosynthetic apparatus. To avoid this damage, plants have evolved a series of protective mechanisms [2] [3] [4] [5], including photochemical quenching, fluorescence quenching, and thermal dissipation of excess excitation energy. Photochemical quenching is related to the activity of photosystem II (PSII) reaction centers (RC), the efficiency of the electron transfer chain, and the capacity of the photosynthetic carbon cycle. As the terminal destination of excitation energy, the photosynthetic cycle affects the amount of surplus excitation energy absorbed by leaves.

Based on the pathway of photosynthetic carbon fixation, higher plants are classified into three types: $C_3$, $C_4$, and CAM. In $C_3$ plants, photosynthesis operates in mesophyll cells (MC) via PSII and ribulose bisphosphate carboxylase/oxygenase (Rubisco). $C_4$ plants evolved from $C_3$ plants [6] and have a higher carbon reduction efficiency. In typical $C_4$ plants, MC and vascular bundle sheath cells (BSC) in the leaves are arranged in specialized Kranz anatomy around vascular tissues. MC chloroplasts have higher PSII activity and lower Rubisco activity. In contrast, BSC chloroplasts have lower PSII activity and higher Rubisco activity [7] [8]. Additionally, $C_4$ photosynthetic enzymes are distributed in MC and BSC, which cooperate during $C_4$ photosynthesis.

The responses of plants with different photosynthetic pathways to strong light are different [9] [10] [11]. $C_4$ plants are less susceptible to strong light stress than $C_3$ plants [10]. The maximal photochemical efficiency of PSII ($F_v/F_m$) declined more slowly in $C_4$ maize than that in $C_3$ plants under strong light [12], while the efficiency of the $C_4$ photosynthetic cycle varies in maize leaves at different positions. The first to third leaves of maize have not completed the differentiation of MC and BSC and thus have a less efficient $C_4$ cycle, with lower activity of $C_4$ photosynthetic enzymes in MC and higher activity of PSII in BSC [13] [14]. However, how these maize leaves differ in photoinhibition is not clear. Knowing this difference and its cause would help to understand the mechanisms of strong light defense in plants. In this paper, we investigated the differences in photoinhibition among the first (incomplete $C_4$ cycle) and fifth (complete $C_4$ cycle) leaves of maize and the fifth leaves ($C_3$ cycle) of the $C_3$ plant tobacco as a reference and analyzed the basis of the differences.

## 2. Materials and Methods

### 2.1. Experimental Materials

Maize hybrid Zhengdan958 (a widely used Chinese hybrid) was crossed by Zheng58 and Chang7-2 inbred at Experimental Station of Shenyang Agricultrual University in the summer of 2012. Tobacco K326 were from plant immunity institute of Shenyang Agricultrual University. Both maize and tobacco were grown in pots in a growth chamber. The photon flux density (PFD) on the plant canopy was 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from metal halogen lamps with a 14 h/10h light/dark cycle at 24˚C/22˚C (day/night). The first (M1) and fifth (M5) fully expanded

leaves on maize plants and the fifth (T5) fully expanded leaves on tobacco were used for measurements.

### 2.2. Treatments

Plants were illuminated for 3 h at 28˚C and a PFD of 2000 μmol·m$^{-2}$·s$^{-1}$ as a strong light treatment. A distance of 0.5m above the top of plant were measured. The white light source was 400 W SON-T AGRO lamps (*Royal Dutch Philips Electronics Ltd.*, Amsterdam, Netherlands). Each treatment was repeated with six plants.

### 2.3. Photosynthetic Rate

Photosynthetic rate ($P_N$) was measured each hour during the light treatment using a potable photosynthesis system (*CIRAS*-1, *PP-system*, Hitchin, UK) in normal air from 8:00 am to 11:00 am.

### 2.4. Photorespiration Rate and Gross Photosynthetic Rate

The $P_n$ was measured at the end of the 3 h light treatment using the CIRAS-1 PP-system in normal air (21% $O_2$ + 75% $N_2$ + 380 μmol·mol $CO_2^{-1}$) and low-oxygen air (2% $O_2$ + 95% $N_2$ + 380 μmol·mol $CO_2^{-1}$). The photorespiration rate ($P_r$) was calculated as the difference between $P_N$ in low-oxygen and normal air, using the equation ($Pn2\%O_2$-$Pn21\%O_2$)/$Pn2\%O_2$ [15]. The $P_N$ in low-oxygen air was designated the gross photosynthetic rate ($GP_N$).

### 2.5. Chlorophyll *a* Fluorescence Parameters

We measured chlorophyll *a* fluorescence each hour during the light treatment with a Hand-PEA (Hansatech Instruments Limited, UK). After 20 min of dark adaptation, all sample leaves were immediately exposed to a saturating light pulse (3000 μmol·m$^{-2}$·s$^{-1}$) for 2 s. The fluorescence transients in each dark-adapted leaf were analyzed according to the JIP-test using the following parameters: 1) the initial fluorescence ($F_0$); 2) the maximal fluorescence ($F_m$); 3) the difference between $F_m$ and $F_0$ ($F_v$); 4) the maximal quantum yield of PSII photochemistry ($F_v/F_m$); 5) the quantum yield of fluorescence dissipation ($\Phi D_0$); and 6) the number of active PSII RC per excited cross-section ($CS_m$).

### 2.6. Statistical Analysis

Statistical analyses were performed using SPSS 11.5 (*IBM*, Chicago, IL, USA). Treatment means were subjected to two-way analysis of variance (ANOVA), and these values and their significant differences (measured by Duncan's significance test) are presented in the figures and table. Design of the experiments was completely randomized with six replications.

## 3. Results

### 3.1. Photosynthesis

The three types of leaves had different $P_N$ values under control light conditions

and varied in their responses to the strong light treatment (Figure 1). Under control light, M5 showed the highest $P_N$ (22 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), followed by M1 (18 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) and T5 (14 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). Under strong light, all three types of leaves showed a decrease in $P_N$, suggesting the occurrence of photoinhibition in all experimental materials. During the treatment period, $P_N$ of M5 declined slowly, by 6.8% in the first hour; M1 decreased more rapidly in the first hour (by 44.4%) and then more slowly. A similar pattern was observed in T5, but $P_N$ decreased more sharply (by 60.7%) in the first hour. During treatment, M5 maintained a consistently higher $P_N$ than did M1 and T5. These results suggested that $C_4$ leaves (M5) were more tolerant to strong light stress than leaves with an incomplete $C_4$ (M1) and $C_3$ leaves (T5).

### 3.2. Photorespiration and Gross Photosynthesis

The three types of leaves had different $P_r$ values at the end of the 3-h strong light treatment (Table 1). T5 showed the highest $P_r$ (2.87 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) and $P_r/GP_N$ ratio (43.50%), followed by M1 (2.60 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, 17.8%) and M5 (0.47 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, 2.24%). $GP_N$, the sum of $P_N$ and $P_r$, indicates the amount

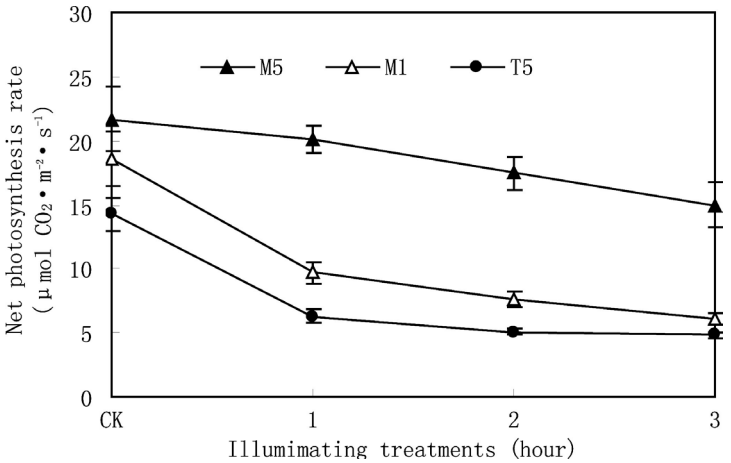


Figure 1. Changes in net photosynthesis rate ($P_n$) in leaves with different photosynthetic characteristics during strong light treatments. The sample leaves were subjected to strong light (2000 μmol·$m^{-2} \cdot s^{-1}$) for 3 h. ▲, maize fifth leaves (complete $C_4$ cycle, M5); △, maize first leaves (incomplete $C_4$ cycle, M1); ●, tobacco fifth leaves ($C_3$ cycle, T5). Mean ± SD of six replicates. Bars not seen are smaller than the size of the symbols.

Table 1. Photorespiration rates of leaves with different types of photosynthesis under strong light treatment.

| Materials | Gross photosynthetic rate ($GP_n$) in 2% $O_2$ (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | Net photosynthetic rate ($P_n$) in 21% $O_2$ (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | Photorespiration rate ($P_r$) (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | $P_r$ in 21% $O_2/GP_n$ in 2% $O_2$ (%) |
|---|---|---|---|---|
| Maize fifth leaves (M5) | 20.73 ± 0.15 a | 20.27 ± 0.32 a | 0.47 ± 0.06 b | 2.24 c |
| Maize first leaves (M1) | 14.57 ± 0.32 b | 11.97 ± 0.21 b | 2.60 ± 0.22 a | 17.80 b |
| Tobacco leaves (T5) | 6.57 ± 0.20 c | 3.70 ± 0.23 c | 2.87 ± 0.33 a | 43.50 a |

Note: Sample leaves were subjected to strong light (2000 μmol·$m^{-2} \cdot s^{-1}$) for 3 h and measured at the end of the light treatment. Each value in the table represents mean ± SD of six leaves. Maize fifth leaves have a complete $C_4$ cycle (M5), maize first leaves have an incomplete $C_4$ cycle (M1), and tobacco fifth leaves have a $C_3$ cycle (T5). Different letters above each column indicate significant differences at $P < 0.01$ (measured by Duncan's significance test). Values are means ± S.D. (n = 6).

of energy consumed via carbon reduction and the oxidation cycle in plants. Similar to the pattern seen with $P_N$, at the end of the treatment, M5 had the highest $GP_N$ (20.73 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), followed by M1 (14.57 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) and T5 (6.57 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). Despite the higher $P_r$ and $P_r/GP_N$ under strong light stress, $GP_N$ in the $C_3$ leaves (T5) and incomplete $C_4$ leaves (M1) was still lower than that in the $C_4$ leaves (M5).

### 3.3. $F_0$, $F_m$, and $F_v$

$F_0$ is measured when the PSII RC are completely open and represents the intrinsic loss of energy transfer from chlorophyll a to the RC in PSII. As shown in Figure 2(a), under control light, M1 showed the highest $F_0$ (251.33), followed by T5 (228.00) and M5 (181.67); all types of leaves experienced a slow decrease in $F_0$ under strong light. This experiment showed that $F_0$ was not very susceptible to strong light stress.

$F_m$ is measured when the RC of PSII are totally closed and represents the maximal amount of energy absorbed by chlorophyll a in PSII. As shown in Figure 2(b), under control light, T5 showed the highest $F_m$ (1355.00), followed by M1 (1116.00) and M5 (795.67). Under strong light, $F_m$ in all types of leaves decreased sharply in the first hour, by 49.77% (to 399.67) in M5, by 63.26% (to 410.00) in M1, and by 55.11% (to 608.25) in T5. The decline then slowed in M5 and M1 but continued rapidly in T5. The data demonstrated that $F_m$ in all three types of leaves was susceptible to strong light stress, but $C_4$ leaves (M5) were less vulnerable than incomplete $C_4$ leaves (M1) and $C_3$ leaves (T5).

$F_v$ is the difference between $F_m$ and $F_0$ and indicates the maximal amount of energy used by PSII photochemical reactions. Generally, the $C_4$ cycle has the highest capacity of excitation energy use among the three types of photosynthetic carbon reduction pathways. In this experiment (Figure 2(c)), under control light, T5 showed the highest $F_v$ (1127.00), followed by M1 (864.67) and M5 (614.00). The pattern was similar to that of $F_m$ under strong light. $F_v$ in all types of leaves decreased sharply in the first hour, to 250.50 (by 59.20%) in M5, to 172.5 (by 80.05%) in M1, and to 186.33 (by 83.47%) in T5, and then more slowly, suggesting that $F_v$ in $C_4$ leaves (M5) was less vulnerable to strong light stress than in incomplete $C_4$ leaves (M1) and $C_3$ leaves (T5). The decline in $F_v$ was mainly caused by changes in $F_m$.

### 3.4. $F_v/F_m$ and $\Phi D_0$

$F_v/F_m$ describes the efficiency of the PSII photochemical reaction. As shown in Figure 2(d), under control light, the value of $F_v/F_m$ was 0.771 in M5, 0.775 in M1 and 0.832 in T5. Under strong light, $F_v/F_m$ of all sample leaves declined sharply, but less so in M5, which reached its lowest value (0.531) in the second hour, than in M1 and T5, which reached their lowest values (0.221 and 0.173, respectively) in the third hour. Thus, in $F_v/F_m$, M5 was more tolerant to light stress than M1 and T5. The decline of $F_v/F_m$ in all types of leaves was attributed to the decrease in $F_m$.

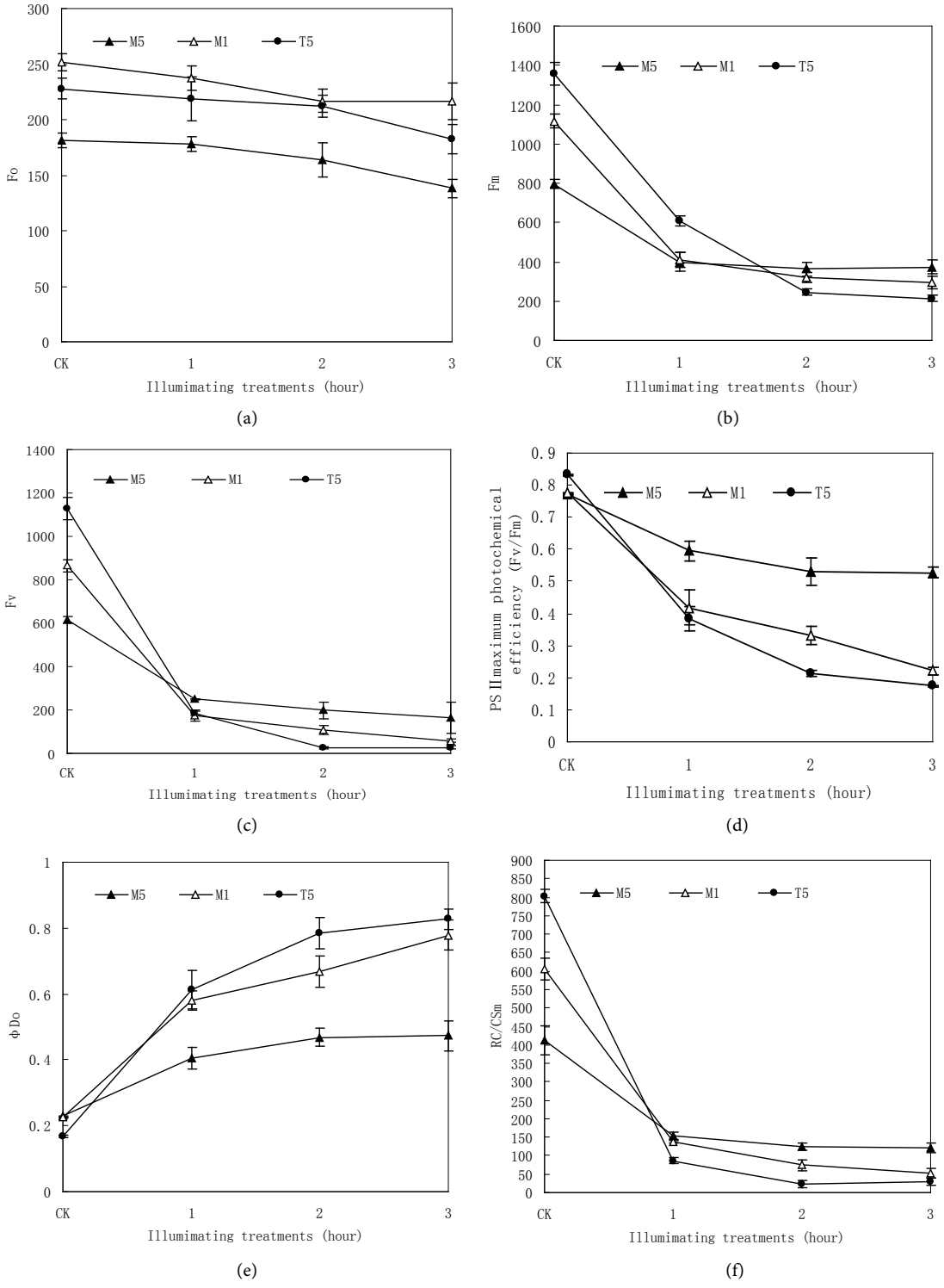


**Figure 2.** Changes in basic fluorescence indices in leaves with different photosynthetic characteristics during strong light and dark recovery treatments. (a) Initial fluorescence yield ($F_0$); (b) maximum chlorophyll fluorescence ($F_m$); (c) difference between $F_m$ and $F_0$ ($F_v$); (d) maximum photochemical efficiency of photosystem II ($F_v/F_m$); (e) fluorescence dissipation efficiency of light energy absorbed by photosystem II ($\Phi D_0 = F_0/F_m$); (f) number of active photosystem II reaction centers per excited cross-section ($RC/CS_m$). Sample leaves were subjected to strong light (2000 μmol·m$^{-2}$·s$^{-1}$) for 3 h and subsequent dark recovery for 3 h. ▲, maize fifth leaves (complete $C_4$ cycle, M5); △, maize first leaves (incomplete $C_4$ cycle, M1); ●, tobacco fifth leaves ($C_3$ cycle, T5). Mean ± SD of six replicates. Bars not seen are smaller than the size of the symbols.

Fluorescence dissipation ($\Phi D_0$) is $F_0/F_m$, representing the quantum yield of fluorescence dissipation of absorbed energy by harvesting pigments [16] [17]. An increase in $\Phi D_0$ can protect PSII against photodamage. As **Figure 2(e)** shows, before light treatment, $\Phi D_0$ was 0.228 in M5, 0.225 in M1, and 0.168 in T5. Under strong light stress, $\Phi D_0$ increased at different scales in the three types of leaves. The $\Phi D_0$ of M5, M1, and T5 increased by 107.15%, 245.71% and 391.29%, respectively. The increase under strong light was less sharp in $C_4$ leaves (M5) than in incomplete $C_4$ leaves (M1) and $C_3$ leaves (T5). However, the increases in $\Phi D_0$ were caused by a reduction in $F_m$, not by an increase in $F_0$, because $F_0$ declined under strong light. This result suggested that $\Phi D_0$ did not play a role in avoiding excess excitation energy accumulation in PSII under strong light in this experiment.

### 3.5. $RC/CS_m$

$RC/CS_m$ is the number of active PSII RC per excited cross-section, reflecting the inactivation state of PSII RC. The three types of leaves showed different levels of $RC/CS_m$ under control light, and all values declined dramatically, but at different scales, under strong light (**Figure 2(f)**). Under strong light, $RC/CS_m$ in M5 decreased from 411.25 to 122.28 (by 62.76%), in M1 from 605.31 to 52.50 (by 77.38%), and in T5 from 803.37 to 29.41 (by 89.34%). The decline of $RC/CS_m$ indicated that a number of RC were inactivated by excess excitation energy. In comparison, $C_4$ leaves (M5) had less active RC under control light but maintained more active RC under strong light than the incomplete $C_4$ leaves (M1) and $C_3$ leaves (T5).

## 4. Discussions

The light energy absorbed by leaves is mainly used to drive the photosynthetic carbon reduction cycle. Therefore, surplus energy is generated if carbon reduction is impeded or if light energy absorbed by leaves exceeds that consumed by carbon reduction. The resulting excess energy will lead to photoinhibition, that is, it impairs the photosynthetic apparatus and reduces the photosynthesis rate [1]. The amount of excess energy is related to photosynthetic efficiency. Under the same light intensity, leaves of $C_4$ plants photosynthesize more efficiently than leaves of $C_3$ plants, which means that more absorbed light energy flows into the carbon cycle and less excess energy is produced [10]. As a result, $C_4$ leaves will be less inhibited by strong light than $C_3$ leaves. In this study, under control light intensity, the $C_4$ leaves (M5) had the highest rate of photosynthesis, followed by leaves with an incomplete $C_4$ cycle (M1) and $C_3$ leaves (T5). Although photoinhibition occurred in all types of leaves under strong light, M5 leaves were more tolerant than M1 and T5 leaves. This result showed that the photosynthetic rate underlies photoinhibition defense in plants.

Photorespiration is a carbon oxidation cycle that consumes light energy like carbon reduction pathways [18]. Increased photorespiration rates have been observed under drought [19], high temperature [20], and strong light stress [9] and

are regarded as an important mechanism to prevent photoinhibition. In the present study, a decline in photosynthesis occurred in all types of leaves at the end of the light treatment, but the levels of decline in M1 and T5 were greater than in M5, and their photorespiration rates and the ratio of photorespiration to gross photosynthesis were much higher than those in M5. These results suggested that photorespiration played a larger role in photoinhibition defense in M1 and T5 leaves. Although the photorespiration rates increased in M1 and T5 leaves, the total energy consumption via carbon reduction and oxidation did not increase during photoinhibition. The gross photosynthetic rates at the end of light treatment were significantly lower than at the beginning of treatment. This means that the rise in energy consumption owing to photorespiration only partially compensates for the decline caused by photosynthesis. For $C_4$ leaves, although the photorespiration rate is very low, the $C_4$ cycle consumes more energy than the $C_3$ cycle and reduces the energy surplus.

$F_v/F_m$ is the photochemical reaction efficiency of PSII and can be used to describe the state of the PSII RC photodamage [17]. In this experiment, a decline in $F_v/F_m$ occurred in all types of leaves under strong light treatment, but $F_m$ decreased dramatically and $F_0$ reduced slowly. Because $F_v$ is the difference between $F_m$ and $F_0$, the decline in $F_v/F_m$ was caused by the decrease in $F_m$. $F_v/F_m$ declined less in M5 than in M1 and T5. This means that M5 maintained higher energy flow into the PSII RC under strong light. Given the higher rate of photosynthesis in M5 under light treatment, the energy entering PSII RC would be used to drive carbon reduction or other biochemical reactions. Hence, the dark reaction in M5 photosynthesis made a much larger contribution to avoiding energy surplus than in M1 and T5. Thus, the carbon reduction cycle played a more pivotal role in strong-light tolerance in $C_4$ leaves than in incomplete $C_4$ leaves.

$F_0/F_m$ ($\Phi D_0$) indicates the ratio of fluorescence dissipation via light-harvesting pigments [16] [17]. However, the rise in $F_0/F_m$ is not simply regarded as an increase in energy dissipation and exerting a role in photoprotection, because the ratio will rise when $F_m$ decreases, even if $F_0$ decreases during strong light treatment and thus will not contribute to reducing excess energy. In the present study, both $F_m$ and $F_0$ declined in all three leaf types, and $F_m$ decreased more than $F_0$ under strong light. Consequently, $F_0/F_m$ is not suitable to represent energy dissipation via fluorescence release under strong light.

$F_0$ is generated during the process of transferring light energy from the light-harvesting complex IIs to the PSII RC. The variation in $F_0$ under strong light in this experiment was inconsistent with changes under other stress conditions, such as high temperature and salt stress [21] [22], when $F_0$ usually rises. The decline in $F_0$ under light treatment may be owing to the dramatic decline in $F_m$, which decreased the energy flow from the light-harvesting complex IIs to PSII RC. The rise in $F_0$ under high temperature and salinity may have resulted from conformational changes in PSII supercomplexes.

The $F_m$ decline under strong light is mainly caused by state transition. In this process, light-harvesting complex IIs dissociate from PSII RC so as to reduce the

energy supply to the latter. Therefore, state transition is considered a pivotal mechanism to protect PSII under light stress [23] [24]. Here, we used the decline rate in $F_m$ to estimate the variation in state transition. Among the three types of leaves, T5 showed the highest rate of decline (53.66%) in $F_m$, followed by M1 (45.97%) and then M5 (22.96%). Hence, we deduced that state transition was more crucial to preventing photodamage to PSII RC in $C_3$ leaves (T5) and incomplete $C_4$ leaves (M1) than in $C_4$ leaves (M5).

In PSII RC, D1 proteins are extremely vulnerable to photooxidative damage [25]. Therefore, the activity of RC is very susceptible to strong light stress. In this experiment, all three leaf types showed a sharp decline in $RC/CS_m$ after strong light treatment. The $RC/CS_m$ of M5 decreased the least (62.76%), followed by M1 (77.38%) and then T5 (89.34%). We used $F_v/RC$ to analyze the variation in energy flow passing through PSII centers and found that it decreased after treatment by strong light. In control light conditions, $F_v/RC$ in M5, M1, and T5 were 1.493, 1.428, and 1.403, respectively. At the end of light treatment, M5 had the highest $F_v/RC$ (1.330), followed by M1 (1.116) and T5 (0.930). These results showed that RC in incomplete $C_4$ leaves in maize was susceptible to strong light, similar to $C_3$ leaves.

## 5. Conclusion

In conclusion, $C_4$ maize leaves, with a higher rate of photosynthesis, are more tolerant to strong light stress than incomplete $C_4$ leaves, and their PSII RC are less susceptible to intense radiation. In photoprotection, the carbon reduction cycle has an important role in $C_4$ leaves, while state transition is pivotal in incomplete $C_4$ leaves. Further investigation will be required to explain the underlying mechanisms of PSII reaction center susceptibility to strong light in maize incomplete $C_4$ leaves. Interestingly, at present some genus contains both C3, C4 and C3-C4 intermediate species [26] [27] [28] [29], and some genus changes from C3 to C4 in different environments [30] [31]. The studies of these materials under strong light will provide more direct adaptability differences between C3 and C4 pathway.

## Acknowledgements

This work was supported by the Technology Pillar Program of Liaoning Province, China (2015103001), the Natural Science Foundation of China (31000673), the Science and Technology Development of Liaoning Province, China (2014208001), the PhD research startup foundation of Liaoning Province (201501063), the Youth Foundation of Bioscience and Biotechnology College of Shenyang Agricultural University (2015).

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