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# Effects of β-carotene feeding on chlorophyll fluorescence, zeaxanthin content, and D1 protein turnover in rice (*Oryza sativa* L.) leaves exposed to high irradiance

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**Abstract.** To examine the mechanism of photoprotective effect of exogenous β-carotene against photoinhibition under strong light conditions, leaves of rice (*Oryza sativa* L.) were fed with 30 mmol/L β-carotene through the transpiration stream at a PPFD of 20 μmol $m^{-2}$ $s^{-1}$ for 3 h. The leaves were then exposed to strong light at a PPFD of 2000 μmol $m^{-2}$ $s^{-1}$ for another 3 h. The photo-induced decrease in activity of PS2 in β-carotene fed leaves, as estimated in terms of the changes of Fv/Fm, photochemical quenching (qP), and PSII electron transport rate ($J_F$), was smaller than that in control leaves during exposure to strong illumination. However, the presence of chloramphenicol produced no significant difference. Furthermore, feeding of β-carotene increased endogenous β-carotene content. In addition, the content of zeaxanthin and the xanthophyll cycle pigments pool size (V+A+Z) were increased in comparison with control leaves when the leaves were exposed to high light for 3 h. However, no significant difference was observed in the presence of chloramphenicol. These results indicate that the photoprotective effect of β-carotene feeding can be partially explained by the conversion of β-carotene to zeaxanthin, a process involved in the rapid D1 protein turnover during the reassembly of PS2 in high irradiance.

**Keywords:** β-Carotene; Chlorophyll fluorescence; D1 protein turnover; High irradiance; Xanthophyll cycle; Zeaxanthin.

**Abbreviations: A,** antheraxanthin; **β-Car,** β-carotene; **CAP**, chloramphenicol; **DTT**, dithiothreitol; **Fv/Fm**, maximum photochemical efficiency of PS2; **PPFD**, photosynthetic photon flux density; **PS2**, photosystem 2; **qP**, Photochemical quenching; **qN**, non-photochemical quenching; $\mathbf{J_F}$, PSII electron transport rate; **V**, violaxanthin; **Z**, zeaxanthin.

## Introduction

The inhibition of photosynthetic activity by high irradiance is a long-known phenomenon (Kandler and Sironval, 1959). Plants have developed a wide range of mechanisms that ameliorate photoinactivation by converting excitation energy harmlessly into heat, thereby preventing the formation of reactive oxygen species and protecting PS2 against photoinactivation during high light stress (for an overview see Niyogi, 1999).

As a result of adaptation to environmental conditions, higher plants possess several enzymatic and non-enzymatic scavenging systems to minimize the deleterious effects of reactive oxygen species. β-Car is an important membrane-bound antioxidant in plant tissue that can quench $^1O_2$ produced from interaction of $^3$P680 and $O_2$ in the PS2 reaction center (Telfer et al., 1994). There are two β-Car molecules in the reaction center of PS2 bound to the D1 and D2 protein (Nanba and Satoh, 1987), but they are not involved in the triplet quenching of P680 (Telfer et al., 1994), and they seem to be lost and hydroxylated to zeaxanthin during the degradation of damaged D1 protein under high irradiance (Trebst and Depka, 1997). The content of β-Car decreased, while the relative content of pigments of the xanthophyll cycle was almost stable and the extent of photoinhibition increased during the senescence of rice leaves (Yang et al., 2001). Deo and Biswall (2001) suggested that β-Car may contribute to the assembly and stability of the D1 protein during senescence and water stress in clusterbean cotyledons. As shown earlier by Markgraf and Oelmueller (1991), β-Car was obligatory in the assembly of PS2 in the greening of etiolated tissue. Recently, the role of the β-Car in the rapid turnover and assembly of the D1 protein into the PS2 center of a green alga has been studied under photoinhibitory conditions (Depka et al., 1998; Deo and Biswal, 2001).

That β-Car is destroyed under high light has been recognized for decades. The reduction of the β-Car pool (Demmig-Adams, 1990), in parallel to an increase of the xanthophyll cycle pool size was reported (Trebst and Depka, 1997). However, little information is known about the relationship among β-Car biosynthesis, zeaxanthin formation, and D1 protein turnover under high light. In the present paper, we report exogenous feeding of β-Car on the

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photoinhibition and pigment and the possible protective role of β-Car under high light stress.

## Materials and Methods

### *Plant Material and Growth Conditions*

Rice (*Oryza sativa* L.) cv. Shanyou 63 was grown under a 14 h photoperiod at 25°C. The PPFD during growth was about 120 μmol $m^{-2}$ $s^{-1}$. Fully expanded leaves of 4-week-old plants were used.

### *Photoinhibition Treatments*

Leaf segments (2-cm long) were floated on water at room temperature (25°C) in petri dishes with the adaxial side facing up. A 3 h exposure was performed by PPFD of 2000 μmol $m^{-2}$ $s^{-1}$ from a 1000-W halogen light source passing through a 10 cm deep water bath.

### *Chemical Treatments*

Leaves were excised at the base and allowed to take up a 50 mmol/L phosphate buffer (pH 7.0) containing 30 mmol/L β-Car (made as a stock solution in 100% ethanol) through the transpiration stream at a PPFD of about 20 μmol $m^{-2}$ $s^{-1}$ and 25°C for 3 h. Control leaves were treated similarly but without β-Car. To inhibit violaxanthin deepoxidase activity and chloroplast-encoded protein synthesis, leaves were vacuum infiltrated with 5 mmol/L DTT, an inhibitor of deepoxidase (Demmig-Adama et al., 1990), and 3 mmol/L CAP, chloroplast-encoded protein synthesis inhibitor (Okada et al., 1991). Immediately after β-Car treatment control leaves were infiltrated with water.

### *Pigment Analysis*

Leaf segments for pigment analysis were frozen in liquid nitrogen and ground to a powder for analysis. Extraction and HPLC analysis of carotenoid composition were carried out following the procedure of Gilmore and Yamamoto (1991). The pigment content was calculated using the conversion factors published (Gilmore and Yamamoto, 1991).

### *Chlorophyll a Fluorescence Measurements*

Chlorophyll *a* fluorescence was measured at room temperature with a pulse-modulated fluorometer (PAM 101/102/103, Walz, Effeltrich, Germany). The minimal (dark) (Fo) and maximal (Fm) fluorescence yield was measured under weak modulated light (0.04 μmol $m^{-2}$ $s^{-1}$), which followed a 1-s pulse of saturating light (5000 μmol $m^{-2}$ $s^{-1}$). The ratio Fv/Fm as a measure of the maximum photochemical efficiency of PSII was calculated. Photochemical quenching (qP) and non-photochemical quenching (qN) were calculated according to Schreiber et al. (1986) and Vankooten and Snel (1990). The relative PSII electron transport rate ($J_F$) was determined as (1-Fs/Fm')*0.5*PPFD*leaf absorptance according to Genty et al. (1990), where Fs is the steady state fluorescence yield, 0.5 is a factor assuming an equal distribution of absorbed photons between PSII and PSI, and leaf absorptance is taken as 0.84.

## Results

The effect of exogenous β-Car on the changes of PS2 photochemical activity, expressed as Fv/Fm, was observed (Figure 1) in rice leaves during strong light exposure. In comparison with control, β-Car fed rice leaves displayed a significant protection for PSII from photoinhibition as indicated by a smaller decrease of Fv/Fm. The extent of photoinhibition was considerably enhanced in the presence of DTT or CAP, but less photoinhibition was found in β-Car treated leaves than in control leaves in the presence of DTT (Figure 1). However, little difference in Fv/Fm between control and β-Car treated leaves was observed in the presence of CAP.

To determine whether the protection against photoinhibition induced by the feeding of β-Car was related to energy dissipation, we monitored the changes in both qP and qN during exposure of leaves to strong light with a PPFD of 2000 μmol $m^{-2}$ $s^{-1}$ (Table 1). Irrespective of the β-Car treatment, a decrease in qP and an increase in qN occurred under high light. qP and $J_F$ did not differ markedly between β-Car-treated and control rice leaves prior to the exposure to strong light, whereas the qP and $J_F$ showed a 32% and 39% difference, respectively, between β-Car-treated and control leaves during subsequent exposure to strong light for 3 h. In contrast, no significant differences in the values of qN in between control and β-Car-treated leaves were observed before or during exposure to strong light.

Depka et al. (1998) reported that the loss of β-Car correlated with zeaxanthin formation under high light

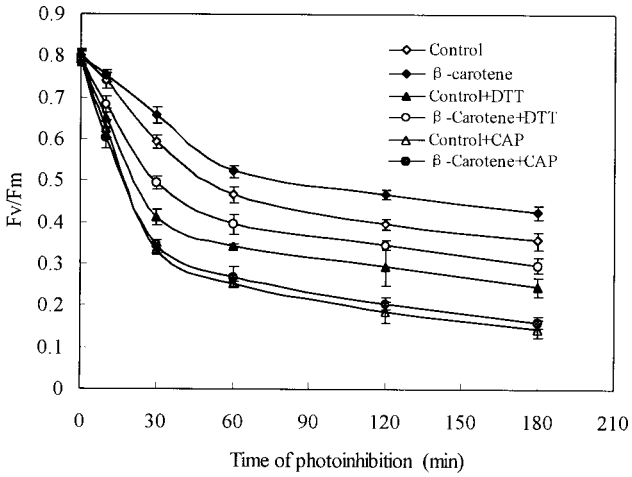


**Figure 1.** Changes in PSII photochemical efficiency (Fv/Fm) during high light treatment for up to 3 h. To inhibit violaxanthin deepoxidase and chloroplast-encoded protein synthesis, leaves were vacuum infiltrated with 5 mmol/L DTT and 3 mmol/L CAP immediately at the end of the β-Carotene treatment. Error bars represent standard errors (n=4-5).

**Table 1.** Effects of exogenous β-carotene on changes in the photochemical quenching (qP), PSII electron transport rate ($J_F$), and nonphotochemical quenching (qN) in rice leaves during high light treatment for up to 3 h. Values are the mean±SD obtained from four measurements.

| HL (min) | qP | | qN | | $J_F$ | |
|---|---|---|---|---|---|---|
| | Control | Treatment | Control | Treatment | Control | Treatment |
| 0 | 0.71±0.03 | 0.72±0.04 | 0.48±0.02 | 0.46±0.03 | 92.7±5.3 | 93.4±5.9 |
| 60 | 0.42±0.03 | 0.48±0.03 | 0.69±0.04 | 0.62±0.03 | 44.6±2.8 | 52.6±3.5 |
| 120 | 0.34±0.04 | 0.41±0.03 | 0.75±0.05 | 0.67±0.04 | 32.9±2.5 | 38.2±3.1 |
| 180 | 0.28±0.05 | 0.37±0.04 | 0.78±0.05 | 0.71±0.06 | 22.7±1.8 | 31.6±2.2 |

conditions. Thus, we compared the carotenoid composition in control with that in β-Car-treated leaves exposed to high light for 30 min and 180 min (Figure 2). The content of β-Car in rice leaves was increased by exogenous β-Car prior to exposure to high light, but there was little difference in the xanthophyll cycle pool between control and β-Car-treated leaves at exposure time between 0 and 30 min. Zeaxanthin content increased rapidly when exposed to high light for 30 min. As time of exposure was prolonged to 180 min, however, the xanthophyll pool size increased by 9 mmol/mol chl(a+b) and 16 mmol/mol chl (a+b) in control and β-Car-treated leaves, respectively, whereas the content of β-Car decreased by 12 mmol/ mol chl(a+b) and 19 mmol/mol chl(a+b), respectively. Thus our results showed that β-Car feeding increased the xanthophyll cycle pool size.

In order to ascertain whether exogenous β-Car feeding increased the rapid turnover of D1 protein, the effect of CAP on pigment composition was determined. The loss of β-Car and the increase in size of the xanthophyll cycle pool were less different between control and β-Car-fed leaves in the presence of CAP under high light stress (Figure 2).

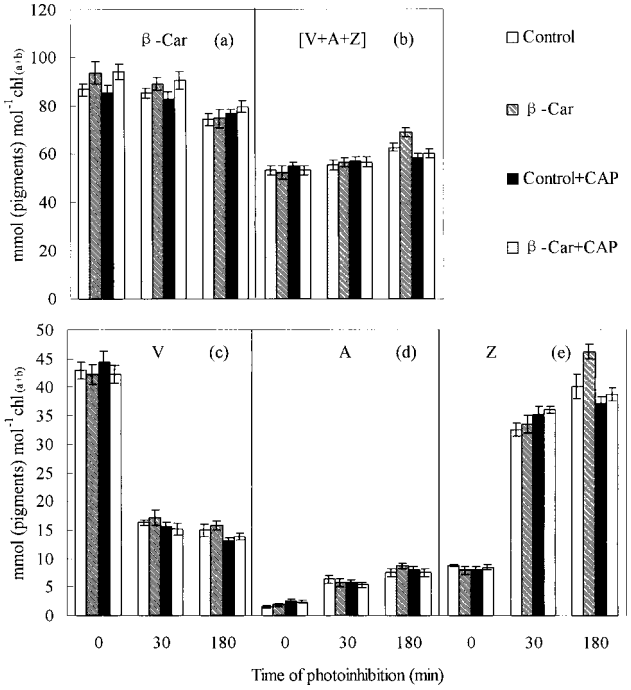


**Figure 2.** Efffects of exogenous β-carotene and chloramphenicol (CAP) on changes in the contents of β-Car (a), the sum of [V+A+Z] (b), violaxanthin (c), antheraxanthin (d) and zeaxanthin (e) in rice leaves during a 3 h high light treatment. Values represent the means±SD obtained from three replicates of one sample.

## Discussion

Our results clearly demonstrate the important role β-Car plays in the protection of PS2 against high light stress in rice leaves. We monitored changes in Fv/Fm (Figure 1) and $J_F$ (Table 1) during exposure to strong light. The decrease in both PS2 activity and $J_F$ in rice leaves treated with β-Car was slower than that in control leaves, suggesting that β-Car-fed leaves may be better able to repair photo-induced PSII inactivation than control leaves.

Mitigation of photoinhibition may be effected by two broad processes: (a) avoidance of over-excitation of the PS2 reaction center by increased thermal dissipation of excitation energy, particularly via qN in association with xanthophyll cycle activity (Long and Humphries, 1994); (b) a cycle of PS2 reaction center inactivation and repair. It is well known that the D1 protein is involved in the photo-induced inactivation and repair of the PS2 complex; the loss of D1 protein follows inactivation of electron transport (Zer and Ohad, 1995; Keren et al., 1997; Melis, 1999). While no marked difference was observed in the change of qN between β-Car-fed and control leaves (Table 1), $J_F$ and qP decreased less in β-Car-treated leaves than in control leaves (Table 1). Little difference in the extent of photoinhibition was obtained between control and β-Car-treated leaves exposed to strong light in the present of CAP, which inhibits protein synthesis in chloroplasts (Figure 1). Thus, the results indicate that protection of PS2 by exogenous β-Car is related to photochemical quenching and D1 protein turnover.

Carotenoids are essential constituents of chlorophyll (chl)-binding proteins in all-higher plants and perform several functions in photosynthetic membranes. The most important is preventing the formation of singlet oxygen and protecting Chls by quenching their triplet states via thermal dissipation of energy. Additionally, carotenoids play a central structural role for chl-binding proteins of both the

antenna system and the reaction center (Cogdell and Frank, 1987; Paulsen, 1997). Recent reports have shown that β-Car is essential for the assembly of D1 protein during its turnover in the formation of functional PS2 complexes in C*hlamydomonas reinhardtii* under high light conditions (Trebst and Depka, 1997; Depka et al., 1998). Our results show more zeaxanthin is formed than can be accounted for from the loss of violaxanthin both in control and β-Car treated leaves, a finding consistent with the results of Trebst and Depka (1997). Moreover, exogenous feeding of β-Car increased the endogenous β-Car content. Most importantly, after exposing leaves to high light for 180 min, the content of additional zeaxanthin and the size of the xanthophyll cycle pool of β-Car in treated leaves were much higher than those of control, and β-Car-fed leaves suffered a much greater loss of β-Car. However, no significant difference was observed in zeaxanthin content or the xanthophyll cycle pool size in the presence of CAP (Figure 2). Thus, the protective effect of exogenous β-Car feeding can be partially explained by an increase in endogenous β-Car, which was hydroxylated to give an increased zeaxanthin content, and this conversion is somehow involved in the rapid D1 protein turnover for the reassembly of PS2 under high light stress. Further investigation is necessary to define the details of the β-Car to zeaxanthin conversion, and the involvement of the process in the rapid D1 protein turnover for the reassembly of PS2.

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# 高光下水稻葉片餵飼β-胡蘿蔔素對葉綠素螢光、玉米黃質和D1 蛋白周轉的影響

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本文以水稻葉片爲材料，研究外源β胡蘿蔔素防禦強光光抑制的機制。在常溫弱光（20 μmol $m^{-2}s^{-1}$）下，通過蒸發作用餵飼 30 mmol/L β胡蘿蔔素 3 h 後，立即在強光（2000 μmol $m^{-2}s^{-1}$）下照射。在強光處理過程中，β胡蘿蔔素處理的葉片，其光系統 II 的活性（以 Fv/Fm，光化學淬滅和光系統 II 電子傳遞速率表示）下降的程度比對照低；在CAP存在下，二者無明顯差異。而且外源β胡蘿蔔素餵飼增加了內源β- 胡蘿蔔素含量，經 3 h 強光處理後比對照有更高的玉米黃質含量和葉黃素迴圈庫尺寸，而 CAP 存在下，二者也無明顯差異。這些結果暗示，外源β胡蘿蔔素光保護作用部分原因可能是β胡蘿蔔素羥化形成玉米黃質，此過程參與光系 II 重組裝過程中 D1 蛋白周轉。

**關鍵詞：**β胡蘿蔔素；葉綠素螢光；AD1 蛋白周轉；高光；葉黃素迴圈庫；玉米黃質。