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Photoprotective functions of the rhodoxanthin accumulated in sun-exposed needles of *Cryptomeria japonica* in the winter

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Introduction

The needles in some conifers, especially species belonging to Taxodiaceae, change their color from green to reddish-brown during winter. The change is induced by the conversion from chloroplast to chromoplast, which contains reddish particles of rhodoxanthin (Ida and Hida 1981). Since the level of rhodoxanthin was enhanced by the combination of high light intensity and low temperature (Ida *et al.* 1991), it was supposed that the accumulation of rhodoxanthin involved in photoinhibition at low temperature (Weger *et al.* 1993, Han and Mukai 1999). However, the photoprotective role of rhodoxanthin and the physiological processes leading to rhodoxanthin accumulation have not yet, been characterized. In this study, we used the *Cryptomeria japonica* mutant that lacks the ability to accumulate rhodoxanthin (Oda *et al.* 1986). We compared the differences in pigment composition and chlorophyll fluorescence between the wild type and the mutant, and among the saplings of wild type grown under four the different light conditions.

Materials and Methods

The wild type (WT) and the mutant (MT) of *Cryptomeria japonica* had been planted in the experimental garden in Shizuoka University (34° 59'N 138°27'E). The two-year-old WT saplings were also used. The saplings were grown under four different light conditions. Different light conditions were produced by covering different numbers of aluminum-meshed shade sheet on the cubic frames (2×1.2×1.2m). Solar radiation in each light conditions was measured by PFD sensors (ISK-27 Koito, Tokyo, Japan) and recorded in a data logger (CR10x, Campbell Inc., U. K.). The relative light intensities in each attenuated light condition were 64% (single layered), 39% (double layered), and 13% (triple layered) of control (no sheet).

Photosynthetic rates were measured *in situ* at noon under natural environmental conditions, using a Compact CO₂/H₂O porometer (Walz, Effeltrich, Germany). Chlorophyll fluorescence was measured with a pulse-amplitude-modulation-type fluorometer (PAM 2000, Waltz, Effeltrich, Germany) using the south-facing needles uncovered by adjacent foliage. The measurement was performed both on the midday of the clear days (maximum PFD>1,000 mmol/m²/s) and on the following night. The fluorescence yield was calculated as $F_v/F_m = (F_m - F_o)/F_m$

After measurement of chlorophyll fluorescence and/or photosynthetic rate, the needles were immediately submerged in liquid nitrogen and stored at -80°C prior to use. Total pigments were extracted from the needles as described previously (Han and Mukai 1999). The amounts

of Chlorophyll (Chl) were analyzed by the method described by Arnon (1949). Carotenoid analysis was performed as described previously (Han and Mukai 1999) according to Gilmore and Yamamoto (1991) with slight modifications. In order to separate rhodoxanthin from the other major pigments, an endcapped ODS column (Wakosil 5C18, 4.6mm×250mm, Wako Pure Chem., Osaka, Japan) was used instead of non-endcapped one. This modification enabled to separate all of the major pigments extracted from reddish-brown needles. The authentic rhodoxanthin was extracted from alils of *Taxus cuspidata* and purified by thin layer chromatography according to Davis (1976). The total amount of xanthophyll cycle pigments (VAZ) was calculated as $VAZ = \text{violaxanthin (V)} + \text{Antheraxanthin (A)} + \text{zeaxanthin (Z)}$. The de-epoxidation status of the xanthophyll cycle pigments (DEPS, Schindler *et al.* 1994) was calculated as $DEPS = (Z + 0.5 \times A) / (V + A + Z)$.

Results and Discussion

The Fv/Fm in both WT and MT were decreased from August to February (Fig. 1). The decrease in Fv/Fm in MT was greater than that in WT. VAZ in both WT and MT were similar in August. In February, WT accumulated rhodoxanthin and increased VAZ slightly. By contrast, MT, which could not accumulate rhodoxanthin, increased VAZ significantly in February (Fig.1). The

photosynthetic rate and Chl content in WT were at higher levels than those in MT (data not shown). Therefore, WT had the ability of rhodoxanthin accumulation and was able to maintain photosynthetic rate, PSII efficiency and Chl content at the higher levels. In contrast, MT could protect the photosynthetic apparatus by the combination of decrease in Chl content and increase in energy dissipation via xanthophyll cycle. Thus, rhodoxanthin plays an important role in photoprotection.

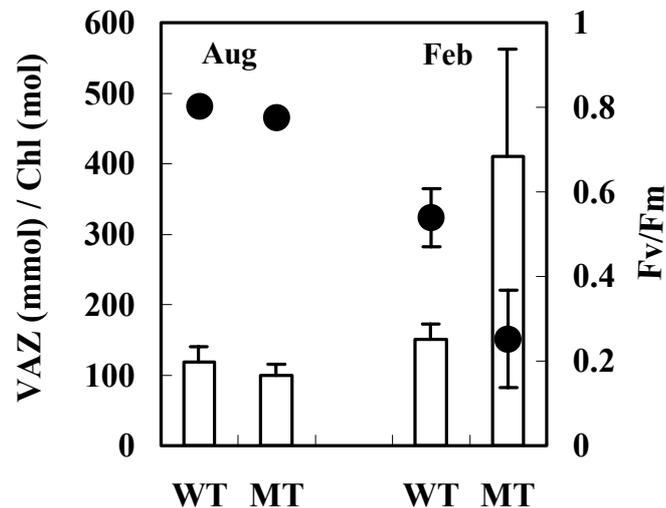


Fig. 1. Differences in PSII efficiency (Fv/Fm) and total amount of xanthophyll cycle pigments (VAZ) between WT and MT in August and February. The solid circles and the bars represent Fv/Fm and VAZ, respectively. Values are mean \pm SD for n=3 to 5.

The Fv/Fm value and pigment composition of WT were compared at the four different light conditions (Fig.2). The Fv/Fm values were at the highest levels in August. However, the Fv/Fm decreased at the higher light conditions (relative PFD 100% and 64%) in December, and thus differences in Fv/Fm values among the light conditions became much significant. The saplings under the higher light intensities accumulated large amount of rhodoxanthin, and the levels of rhodoxanthin were correlated with light intensities (Fig. 2). This result was consistent with the previous report (Ida *et al.* 1991). In addition, we observed that the decrease in Fv/Fm was closely related to the amount of rhodoxanthin.

The differences in the levels of VAZ were observed under the four light conditions in August (Fig.3). However, the levels of VAZ at two higher light intensities decreased to those at two lower light conditions in December (Fig.3). This result was different from those observed in other evergreen conifers (Adams and Dimmig-Adams 1994, Adams *et al.* 1995), in which the levels of VAZ increased in winter. Although the DEPS values at four light conditions were at the lowest levels in August, the DEPS at three higher light conditions increased prominently in December (Fig.3), indicating the presence of the nocturnal

retention of de-epoxide(s) (Z and A) in winter. This result was consistent with the previous results of other evergreen conifers (Adams and Dimmig-Adams 1994). In such conditions, the performance of xanthophyll cycle might be greatly suppressed.

The relationships among the decrease in Fv/Fm, the increase in rhodoxanthin and the increase in DEPS were observed in *C. japonica* during cold acclimation (Fig. 2. and 3). In

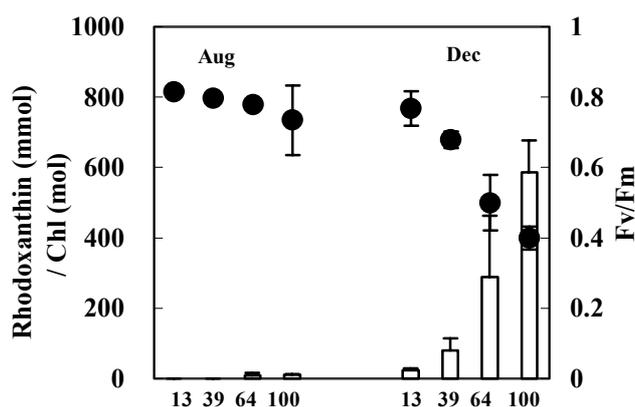


Fig. 2. Differences in PSII efficiency (Fv/Fm) and amount of rhodoxanthin among four light regimes in August and December. The solid circles and the bars represent Fv/Fm and amounts of rhodoxanthin, respectively. Values are mean \pm SD for n=5 to 6. The numbers in the bottom of the figure represent the relative PFD.

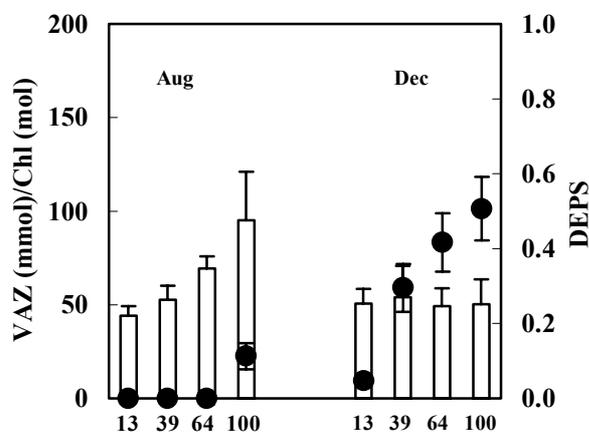


Fig. 3. Differences in total amount of xanthophyll cycle pigments (VAZ) and de-epoxidation status (DEPS) among four light regimes in August and December. The solid circles and the bars represent DEPS and VAZ, respectively. Values are mean \pm SD for n=5 to 6. The numbers in the bottom of the figure represent the relative PFD.

many evergreen plants, the relationship between nocturnal retention of de-epoxide(s) in cold days and depressed PSII efficiency has been demonstrated (Adams and Dimmig-Adams 1994, Adams *et al.* 1995). Czczuga (1987) proposed the converting pathway from zeaxanthin to rhodoxanthin via antheraxanthin. The combination of high light intensity and low temperature causes the increase in excess excitation energy, which facilitates to accumulate the large proportion of de-epoxide(s). It was supposed that nocturnal retention of de-epoxide(s), caused by decreased reverting efficiency at low temperature, led to increase in the precursor(s) for rhodoxanthin. Thus, the accumulation of rhodoxanthin was caused by photoinhibition. In addition, the degree of nocturnal retention of de-epoxide(s) was correlated with the level of rhodoxanthin. When large amount of rhodoxanthin was accumulated, VAZ at two higher light conditions decreased to the level of the weakest light intensity (Fig 2, 3). Weger *et al.* (1993) proposed that rhodoxanthin provides photodynamic screen, which attenuates the incident light intensity. The accumulation of rhodoxanthin within the cross section of single needle was highest in the sun-exposed side and it decreased toward the shady side (Ida *et al.* 1991). Together with the observation of Ida *et al.* (1991), our results indicated that the level of rhodoxanthin might be balanced with the capacity of energy dissipation via VAZ.

Acknowledgements

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