学 位 論 文 内 容 の 要 旨/Dissertation Abstract

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学 位 論 文 題 名/Title of Dissertation

An integrated study on the molecular mechanisms for sustained photosynthetic thermal dissipation in overwintering yew leaves
（越冬中のイチイの葉における光合成の熱放散の分子メカニズムに関する統合的研究）

In boreal regions, overwintering evergreens face the challenges of maintaining photosynthetic function and reducing damage from excess light energy under low temperatures and high light. When excess sunlight is absorbed by chlorophylls, it can lead to the generation of reactive oxygen species (ROS), which can damage various cellular components. To avoid those damages, overwintering evergreens employ a mechanism known as sustained thermal dissipation. This mechanism allows them to dissipate the majority of absorbed light energy as heat, providing photoprotection in winter.

Several hypotheses have been proposed regarding sustained thermal dissipation in evergreens, including the accumulation of pigments with thermal dissipation capacity, the reorganization of photosystems (PSs), the degradation of PS subunits, and protein phosphorylation triggered by low-temperature stress. To verify these hypotheses and reveal molecular mechanism of sustained thermal dissipation, I analyzed the photosynthetic apparatus, pigment dynamics, and transcriptomic characteristics of its leaves throughout the year with the leaves of yew (*Taxus cuspidata*) from sun and shade sides and of different ages, and examined the relationship with these physiological and molecular traits.

To better determine the timing of sustained thermal dissipation induction, I measured Y(II) and Fv/Fm, which represents the actual photosynthetic efficiency under light conditions and the maximum photosynthetic efficiency, respectively, in the field. Y(II) and Fv/Fm parameters showed a gradual decline during autumn. By late November, they dropped significantly and stayed low until late March, indicating activation of a thermal dissipation mechanism. Since Y(II) and Fv/Fm showed similar values on the shaded side, Y(II) was mainly used as an indicator for the induction of sustained thermal dissipation in this study. In contrast to previous studies that measured Y(II) on a monthly or seasonal basis, the weekly measurements in this study provided unprecedented temporal resolution, capturing dynamic changes in Y(II) over time and revealing a clear linear relationship with temperature.

Since chlorophylls exist stoichiometrically within PSs, changes in chlorophyll composition reflect PS composition. While chlorophyll *a* and *b* remained stable year-round, carotenoids such as zeaxanthin and lutein accumulated significantly during winter, showing a strong linear correlation with the decline in Y(II). Although zeaxanthin accumulation in winter is well-documented, its binding site and functional mechanisms remain unclear. It is plausible that specific proteins provide binding sites for zeaxanthin, facilitating its role in thermal dissipation.

Notably, key PSII subunits, such as the D1 protein, and the light-harvesting complex of PSII (LHCII) remained relatively stable across all seasons in yew. Unlike previous studies reporting a significant reduction in PSII amounts during winter in other

evergreen species, no such decrease was observed in yew, making it an ideal model for studying sustained thermal dissipation under stable PSII protein conditions, without the confounding effects of PSII protein reduction. This stability, along with pigment data, suggests that typical photoinhibition, characterized by D1 degradation, did not occur in yew. Despite the decline in PSII efficiency, the constant PSII levels indicate that yew relies on alternative protective mechanisms, such as sustained thermal dissipation, rather than repair-driven recovery mechanisms commonly observed in other species.

The core findings of this study were derived from year-round transcriptomic analysis. First, despite significant differences in light intensity between sun and shade leaves, their transcriptomic expression patterns were remarkably similar. Second, early light-induced protein (ELIP) exhibited a strong winter-induced expression pattern, with ELIP transcripts comprising 20% of the total transcriptome during winter. ELIP protein levels were nearly equivalent to those of major LHCII. Unlike a previous study that relied on limited time points, this study revealed the seasonal dynamics of ELIP protein and mRNA expression and their correlation with sustained thermal dissipation. Structure prediction showed that ELIP is similar to LHC, with appropriate binding sites for chlorophyll and carotenoids. Sucrose density gradient fractionation and pigment analysis further revealed that carotenoids, particularly zeaxanthin and lutein, were predominantly localized in the LHC and ELIP fractions during winter.

Previous studies offer differing views on the location of thermal dissipation, with some suggesting it occurs within the PSII core and others proposing it takes place in a PSII–PSI supercomplex, where PSI acts as the quencher. Using a faster fluorescence measurement, this study revealed rapid energy dissipation in the PSII antenna during winter, indicated by a shortened chlorophyll fluorescence lifetime, suggesting that thermal dissipation primarily occurs in the PSII antenna rather than the PSII core or PSI.

Based on these findings, I propose that ELIP binds chlorophyll and zeaxanthin, and functions as a quencher or regulator within the PSII antenna, promoting energy dissipation during winter. Combined with the accumulation and redistribution of carotenoids, this mechanism highlights the adaptive strategies of boreal evergreens to extreme environments. By dissipating excess excitation energy as heat, evergreens reduce ROS production and maintain photosynthetic stability. This study advances the understanding of sustained thermal dissipation and underscores the roles of protein dynamics, pigment biochemistry, and transcriptomic reprogramming in photoprotection.