



Photosynthetic responses of *Eulophia dentata*, *Bletilla formosana*, and *Saccharum spontaneum* under various photosynthetic photon flux density conditions

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Abstract

This study aimed to determine the photosynthetic performance and differences in chlorophyll fluorescence (ChlF) parameters between *Eulophia dentata* and its companion species *Bletilla formosana* and *Saccharum spontaneum* when subjected to different photosynthetic photon flux density (PPFDs). Leaf surfaces were then illuminated with 50, 100 (low PPFDs), 300, 500, 800 (moderate PPFDs); 1,000; 1,500; and 2,000 (high PPFDs) $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$, and the ChlF parameters were measured during the whole process. Increasing nonphotochemical quenching of ChlF and decreasing potential quantum efficiency of PSII, actual quantum efficiency of PSII, and quantum efficiency ratio of PSII in dark recovery from 0–60 min were observed in all leaves. A significant and negative relationship was detected between energy-dependent quenching (q_E) and photoinhibition percent in three species under specific PPFD conditions, whereas a significant and positive relationship was detected between photoinhibitory quenching (q_I) and photoinhibition percent. The q_E and q_I can be easily measured in the field and provide useful ecological indexes for *E. dentata* species restoration, habitat creation, and monitoring.

Keywords: *Bletilla*; chlorophyll fluorescence; *Eulophia*; light intensity; photoinhibition; *Saccharum*.

Introduction

Eulophia dentata Ames, a critically endangered plant in Taiwan, is endemic there. It grows on sandy banks along

the rivers and is distributed from 0–250 m in Miaoli and Yilan, Central Taiwan (24°17'34.6"N 120°50'02.1"E). This wild population is substantially threatened as a result of habitat losses from a forest fire, extreme weather, and

Highlights

- *E. dentata* and *S. spontaneum* adapt their photosynthesis to high and moderate PPFDs
- NPQ can be divided into photoprotection (q_E , q_Z , q_T) and photoinhibition (q_I)
- The photoprotection mode of *E. dentata* and *S. spontaneum* is dominated by q_E mediation

Received 1 May 2022

Accepted 22 November 2022

Published online 16 December 2022

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Abbreviations: ChlF – chlorophyll fluorescence; ETR – electron transport rate; F_0 – minimal ChlF; F_m – maximal ChlF; F_v/F_m – maximal quantum efficiency of PSII for dark-adapted states; NPQ – nonphotochemical quenching; q_E – energy-dependent quenching; q_I – photoinhibition quenching; q_T – state transition quenching; q_Z – zeaxanthin-dependent quenching; $\Delta F/F_m'$ – actual PSII efficiency; Φ_{PSII} – effective quantum yield of PSII for light-adapted states.

Acknowledgements: This work was partially supported by the Taiwan Government Department for Endemic Species Research Institute.

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Conflict of interest: The authors declare that they have no conflict of interest.

severe flooding in the Zhuolan Grand Canyon, Miaoli, causing a sharp decline in its population. Only two native habitat units were found 80 years ago, and the number of plants of this species is now approximately 250 (Editorial Committee of the Red List of Taiwan Plants 2017). Narrow distribution and vulnerability to disturbance make their native habitat conservation to be extremely urgent. Alternatively, *in vitro* techniques, including seed germination, micropropagation, meristem culture, and callus culture have been undertaken for *E. dentata* plant propagation, germplasm preservation, and restoration in Taiwan (Chang *et al.* 2014).

E. dentata plants commonly grow together with *Bletilla formosana* and *Saccharum spontaneum* in the current sampling area (Fig. 1S, *supplement*). We also observed that *B. formosana* grew along ecotones between forests and fields that do not have graminoid competition, while *E. dentata* grew in the habitat of *S. spontaneum* plants, leading to *E. dentata* plants receiving partial shade in the open field. We assume that *E. dentata* plants were approximately 50% shaded by tall *S. spontaneum* grass plants, therefore exhibiting shade tolerance. *Saccharum spontaneum* L., a perennial rhizomatous tall grass of the family Poaceae, is known to invade abandoned and pastoral lands in many tropical countries and is often planted in riverbeds in Taiwan to suppress dust in the area. In addition to grass vegetation studies, *S. spontaneum* attracts attention for its potential in the ecological restoration and stabilization of barren fly ash dumps (Pandey *et al.* 2015). Recently, the optimized pretreatment of *S. spontaneum* biomass with enzymatic saccharification has been developed to convert it into an ethanol biofuel through a consolidated bioprocess (Vaid *et al.* 2021). In addition, Li *et al.* (2021) reported the polyploid characteristics of dehydration-responsive element binding proteins (DREBs) and functions relative to the photosynthesis of *S. spontaneum* and plant development during drought stress. *Bletilla* is a common perennial herb that has been used in folk and traditional medicine for the treatment of bleeding, colds, esophagitis, erosive gastritis, and burns (Lin *et al.* 2016). *Bletilla formosana* (Hayata) Schltr. is the only member of the genus found in Taiwan on mountain slopes up to 2,200 m altitude, and has been utilized as Chinese medicinal and ornamental material (Wu *et al.* 2010). *B. formosana* is currently widely distributed throughout Taiwan and is important for the sustainable development of the orchid industry in conserving the biodiversity of precious orchid genetic resources.

Ecophysiological studies require knowledge of the photosynthetic rates of plants under different environmental conditions, particularly of a broad range of light intensity (photosynthetic photon flux density, PPFD). A photosynthetic light response can be used to assess the ability to capture light and understand the optimal ambient PPFD conditions of plants. Ecophysiological responses to excess sunlight vary among *E. dentata*, *B. formosana*, and *S. spontaneum*, and may significantly affect the survival rate and spatial distribution of *E. dentata* plants. In full sun-exposed habitats, leaves

often absorb considerably more photons than can be utilized, the excess absorbed energy often resulting in reducing the photochemical efficiency of PSII (Φ_{PSII}). Photoinhibition of photosynthesis occurs when PPFD exceeds the capacity/activity of the photosynthetic electron transport chain in the chloroplast, leading to the inactivation of and damage to PSII (Orekhova *et al.* 2021); in addition, at high temperatures, plants absorb excess light energy and inhibit photosynthesis (Colom *et al.* 2003, Kalapchieva *et al.* 2019). Furthermore, excessively high irradiance may result in photoinhibition, which is characterized by a loss of PSII activity and a light-dependent reduction, thus requiring the dissipation of excess excitation energy (Portela *et al.* 2019). Application of the ChlF fluorescence-based methods allows us to obtain information about the functions of the photosynthetic apparatus, and fluorescence is often used in physiological studies to investigate plant's response to various environmental stresses in controlled environments and the field (Kałużewicz *et al.* 2018). Unadapted temperature ranges have impacts on the physiological activities of plants, especially in high temperature/high light and low temperature/high light ranges, when the radiant energy captured by the photosynthetic pigment of the plant exceeds the carbon fixation rates; here, the rate of utilization of absorbed energy is low, and excess energy is generated. Dissipation in other ways may cause damage to photosynthetic organs, and this excess energy is often eliminated by loss as heat, through the well-known xanthophyll cycle (Tüffers *et al.* 1999, Adams *et al.* 2004). Thus, nonphotochemical quenching (NPQ) plays an important role in photoprotection because it quenches excess energy and dissipates it safely as heat (Murchie and Niyogi 2011). Moreover, plants exposed to high light also show decreases or adjustments in leaf photosynthetic pigment content, providing an important photoprotective mechanism (Souza *et al.* 2017).

Considering the limited number of *E. dentata* individuals at the sampling site, chlorophyll fluorescence (ChlF) was used to analyze the characteristics of photosynthesis in *E. dentata* plants grown under various light environments. ChlF measurements have been proposed to evaluate the conditions of plants in ecological systems and have been successfully applied in the physiological profiling of invasive plant species for ecological restoration (Bussotti *et al.* 2020, Pandey *et al.* 2020); further, many related parameters, such as energy-dependent quenching (q_E), photoinhibitory quenching (q_I), and zeaxanthin-dependent quenching (q_Z) have been used to detect the partitioning of light energy to alternative dissipative mechanisms (Guidi *et al.* 2019). The parameters derived from fluorescence kinetics, obtained using PAM-fluorescence methods, reflect the photosynthetic potential and potential for photochemical dissipation, and also demonstrate the percentage of PSII that is open and its effectiveness in capturing photon energy by light-harvesting complex (LHC) II and the subsequent transfer of quanta (Moya *et al.* 2019). However, no study has described its ecophysiological response under controlled irradiation conditions, and the function of the photosynthetic

apparatus has not yet been examined for the occurrence of ChlF indicators in *E. dentata* and its *B. formosana* and *S. spontaneum* companion species under field conditions to explain the development and distribution of *E. dentata*. Various PPFD levels may be applied for ChlF measurements to determine the actual state of the photosynthetic apparatus and the photoreceptor involved in *E. dentata* leaves. Efforts to gain an understanding of the photosynthetic characteristics of *E. dentata* leaves could benefit field cultivation management. Therefore, it is urgent to regulate, prioritize for management, and monitor this nationally endangered species for potentially threatened eradication. In the present study, we analyzed ChlF parameters in *E. dentata*, *B. formosana*, and *S. spontaneum*, growing in the field, to understand whether they can acclimate to intense light conditions and have higher physiological plasticity toward PPFD and shade tolerance. The mechanisms of capture, transfer, and dissipation of excitation energy were studied, through ChlF measurements in *E. dentata*, *B. formosana*, and *S. spontaneum* leaves in response to varying PPFD, and to check if *E. dentata* leaves exhibit strong PPFD adjustments in photosynthesis. Our study of *E. dentata* not only recognizes its ecological distinctness but highlights its critical conservation status. In addition, our research shows that the relationships of ChlF indices can be used for ecophysiological research in *E. dentata*, and that these parameters can be considered selection indices for examining the growth of *E. dentata* species grown under artificial light illumination.

Materials and methods

Plant materials and light treatments: We searched for and monitored *E. dentata* plants growing in Zhuolan Grand Canyon, Miaoli (24°17'34.6"N, 120°50'02.1"E), for the past three years, and discovered only 15 when we revisited the habitat site in April 2021. We selected five individuals of *E. dentata* plants, 20–30 cm tall, five plants of *B. formosana*, 30–50 cm tall, and five plants of *S. spontaneum*, 40–300 cm tall, within the riparian lands at Miaoli, Taiwan (Fig. 1S). These plants were studied in the field, and vegetation and habitat types were recorded on the sites from July to August 2021. The climate there is humid subtropical, with a mean annual rainfall of 1,000 mm, a mean annual air temperature of 26.5°C, and mean PPFDs of 1,000–1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, as recorded from January to December 2012–2022 (Fig. 2S, *supplement*). The upper leaves of the above-mentioned three species were selected for measuring ChlF parameters, and the light environment was 60–80% of the largest PPFD in the growing area.

Determination of ChlF parameters under fixed light intensity: Measurements were taken from September to October 2021. Five plants of each species per light treatment were used for ChlF measurements, and one upper fully open leaf per plant was used. Measurements were initially made on the dark-adapted leaf, after which the leaf surface was illuminated with 50, 100, 300,

500, 800; 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD using a portable fluorescence photosynthesis analyzer (*MINI-PAM-II*, Heinz Walz, Effeltrich, Germany). Overnight dark-adapted plants were exposed to light stepwise from low to high levels of PPFD, and ChlF parameters were measured during 60 min of irradiation and dark adaptation for 30 min. One data point was recorded at each 2-min interval over a 90-min period, followed by calculating the parameters listed below.

The potential and actual quantum efficiency of PSII and electron transport rate: The potential quantum efficiency of PSII (F_v/F_m) is calculated as $(F_m - F_0)/F_m$, and the actual PSII efficiency ($\Delta F/F_m'$) is the effective quantum yield of linear electron flux through PSII, which is used to express the ability of PSII to perform photochemistry (Demmig-Adams *et al.* 1996). Values of the minimal (F_0) and maximal ChlF (F_m) of dark-adapted samples were determined using modulated irradiation of a weak light-emitting diode beam (measuring light) and saturating pulse, respectively. F_m' is the maximal level of fluorescence during illumination as determined by applying a saturating flash. The photochemical Φ_{PSII} was calculated as $(F_m' - F_t)/F_m'$, where F_t is the steady-state level of fluorescence excited by actinic light of the applied PPFD levels (Maxwell and Johnson 2000). Furthermore, the degree of photoinhibition is calculated as the relative value of F_v/F_m after 30 min of dark adaptation, where the F_v/F_m value of the same leaves before illumination was taken as 100%. The apparent rate of the photosynthetic electron transport rate (ETR) of PSII was obtained as $\text{ETR} = \Delta F/F_m' \times \text{PPFD} \times 0.5 \times \alpha$, where factor 0.5 implies equal excitation of both PSII and PSI; α is leaf absorption, and we used the mean 'default' value of 0.84 for green leaves (Björkman and Demmig-Adams 1995). The following effective quantum yields were measured using the instant light-response curve program. From these data, several parameters were computed based on modulated fluorescence kinetics (see below).

Nonphotochemical quenching and its components: The NPQ coefficient and its components were calculated as $\text{NPQ} = (F_m - F_m')/F_m'$ (Weng *et al.* 2011). Energy-dependent quenching (q_E) as NPQ is largely the dominant high-energy form, which is calculated as $(F_{mD2} - F_{m60}')/F_{m60}'$ (Johnson and Ruban 2011). However, photoinhibitory quenching (q_I) is NPQ due to decreased CO_2 fixation, which is calculated as $(F_m - F_{mD30})/F_{m60}'$ (Müller *et al.* 2001). In addition, the part after the reaction of q_E is $(q_z + q_T)$, and is calculated as $(F_{mD30} - F_{mD2})/F_{m60}'$ (Nilkens *et al.* 2010). The F_{m60}' is the maximum fluorescence value of leaves at 60 min of light exposure. Both F_{mD2} and F_{mD30} are the F_m values measured at 2 and 30 min, respectively, after dark recovery (Wang *et al.* 2022). Measurements were recorded with *WinControl-3* software (Heinz Walz).

Statistical analysis: All PPFD treatments were arranged in a completely randomized design, and all ChlF parameters were subjected to a single-factor analysis of variance

(ANOVA) to determine whether a significant difference level of $p \leq 0.05$ using *PASW (Statistical Product and Service Solutions) Statistics 18* software (*PASW 18*, IBM, USA) existed between different treatments. Five leaves (one leaf per plant) were measured in each PPFD treatment (for a total of five replicates), and data from each leaf represented one replicate in the statistical analyses. Regression analyses were used to examine relationships among q_E , q_I , and photoinhibition (in %). Model datasets were based on at least five leaves at each PPFD level. Several models were tested, including the linear regression model, which was selected for the interpretation of the relationships between ChlF parameters and PPFD. All models were evaluated for the goodness of fit by the graphical analysis of residuals and by computing correlation coefficients at a significance level of $p \leq 0.05$ for ChlF parameters, and the linear regression model performance was considered to be the most suitable.

Results

Time-course changes in the light induction of ETR, NPQ, F_v/F_m , and Φ_{PSII} : The ETR values of *E. dentata* plants under 800; 1,000; and 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were significantly higher than the other PPFD treatments; ETR suddenly increased at the beginning of a short illumination time (2 min) and reached its peak at 60 min as time increased (Fig. 1A). Similar trends were observed in the ETR of *B. formosana* (Fig. 1B) and *S. spontaneum* (Fig. 1C) plants, but ETR values under 800; 1,000; and 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments at 60 min were remarkably lower [$< 70 \mu\text{mol(electron)} \text{ m}^{-2} \text{s}^{-1}$] compared to *E. dentata* under the same light conditions [$> 80 \mu\text{mol(electron)} \text{ m}^{-2} \text{s}^{-1}$]. This indicates that high PPFD limited *B. formosana* and *S. spontaneum* leaf growth and development, but that *E. dentata* plants could be grown under a specific and optimal light intensity and be adapted to less than

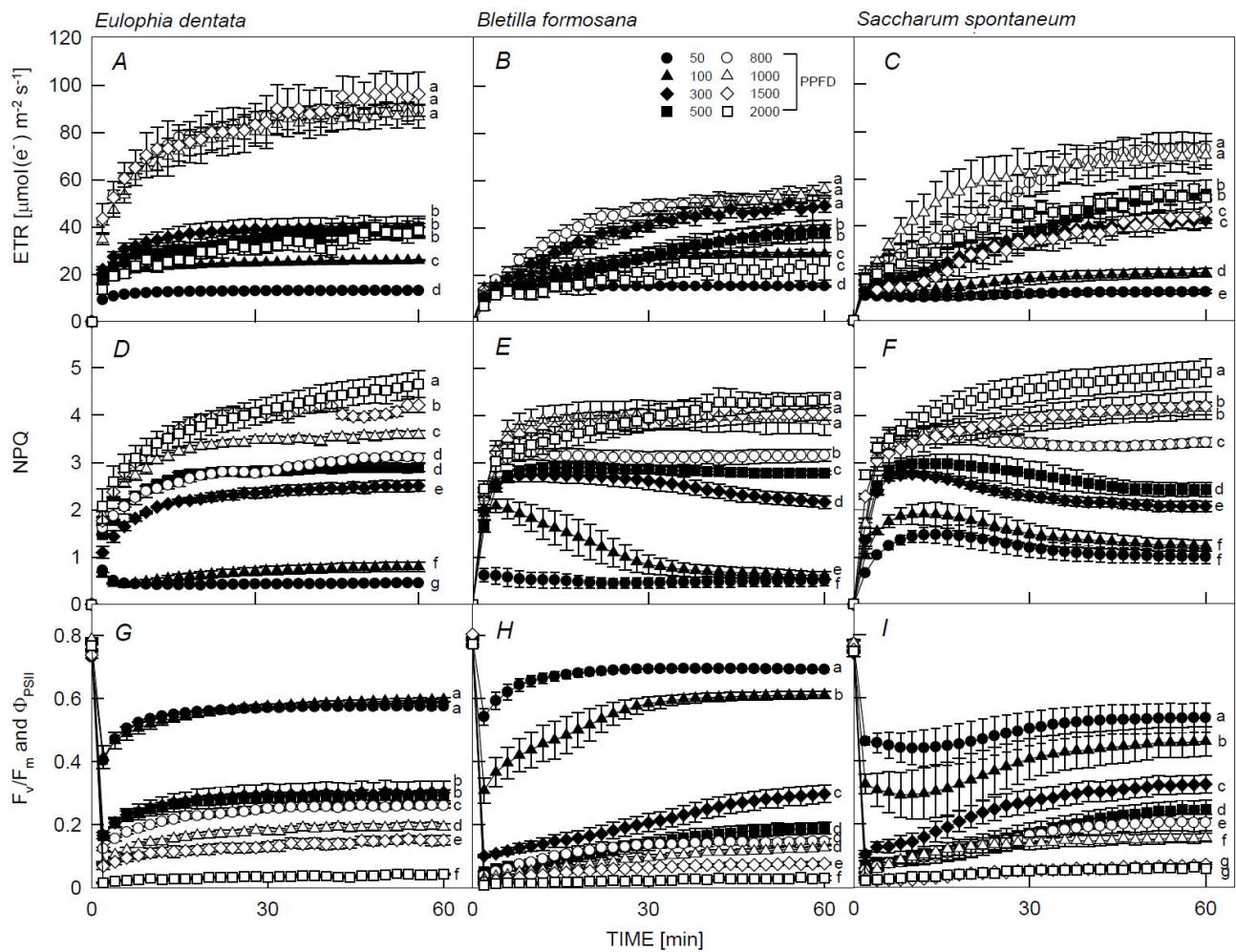


Fig. 1. Time-course variations in the electron transport rate (ETR), nonphotochemical quenching (NPQ), and F_v/F_m and PSII efficiency (Φ_{PSII}) of *Eulophia dentata* (A,D,G), *Bletilla formosana* (B,E,H), and *Saccharum spontaneum* (C,F,I), respectively. Measurements were made at 25°C under 50, 100, 300, 500, 800, 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD during the 60 min of light induction. Each point represents the mean of five leaves, and data are means \pm standard errors. Different letters indicate significant differences in Tukey's HSD analyses at eight illuminations ($P < 0.05$).

1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. However, all the tested plants were intolerant to 2,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. In addition, the ETR values of all the three species, used here, under the 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ remained low [$< 10 \mu\text{mol}(\text{electron}) \text{m}^{-2} \text{s}^{-1}$] as exposure time increased, and no reduction of electron transport was detectable at longer illumination times compared to the other PPFD treatments, which gradually increased thereafter. Moreover, Fig. 1D–F shows the NPQ values of the three species sharply increasing and continuing to linearly increase until 60 min under 1,000; 1,500; and 2,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. These values were significantly higher (> 3.5) than those under the other PPFD treatments (< 3), suggesting that these tested plants suffered high PPFD stress and that an optimal NPQ could be maintained in these three species exposed to less than 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Notably, NPQ values for *B. formosana* under 100 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and *S. spontaneum* leaves under PPFD of 50, 100, 300, and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ peaked (ranging 2–3) in the first 2–5 min, and then dropped remarkably thereafter during photoinhibition processes (Fig. 1E,F). Lastly, the slow phases of both F_v/F_m and Φ_{PSII} of the three species were found to increase slowly with prolonged illumination after the first 2 min under all the PPFD treatments (Fig. 1F–H). Almost no contribution of the slow phase was detectable, and the maximum amplitude was reached after 60 min of illumination. Significantly higher F_v/F_m and Φ_{PSII} levels were detected in all the tested plants under 50 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (ranged 0.42–0.65) compared to other PPFD treatments, in particular, the 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment, where F_v/F_m and Φ_{PSII} values were close to 0. This indicated that increasing the light led to a reduction in the F_v/F_m and Φ_{PSII} values in the three species, especially those subjected to high PPFD treatments [1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$], where the values dropped below 0.2.

Responses of $\Delta F/F_m'$ and F_v/F_m during 30 min of darkness after ceasing illumination: Both $\Delta F/F_m'$ and F_v/F_m [%] values of the three species in all PPFD treatments

rapidly increased right after stopping illumination during the dark period; this was followed by a gradual increase and then no change after 20 min of darkness. Both $\Delta F/F_m'$ and F_v/F_m [%] levels of *E. dentata* (Fig. 2A), *B. formosana* (Fig. 2B), and *S. spontaneum* (Fig. 2C) in the 50 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ recovered to 90–99% after 2 min of darkness (D2) and thereafter. This exhibited significantly higher photoprotection compared to samples under 800; 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments, with 60–80% recovery after 30 min of darkness (D30), indicating that these plants could be grown under low PPFD conditions. However, in 300, 500, 800; 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, both the $\Delta F/F_m'$ and F_v/F_m [%] of *B. formosana* plants (Fig. 2B) recovered to 28.8–66.6% at D2. This was significantly lower in comparison to *E. dentata* (Fig. 2A) and *S. spontaneum* (Fig. 2C), which were 45.6–81.5% at D2, suggesting that the photosynthetic system recovery abilities of *E. dentata* and *S. spontaneum* were higher than those of *B. formosana*.

The fractions of NPQ under PPFD treatments and after 30 min of darkness: All the three species, used in this study, in all PPFD treatments substantially increased their NPQ values, and their increases seemed unlikely to be related to photoprotection (q_E and $q_Z + q_T$), but rather linked to photoinhibition (q_I), and q_I values which increased as PPFD increased from 100 to 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3). In other words, the q_E and $q_Z + q_T$ values of three species did not contribute significantly to NPQ in all PPFD treatments. All leaves had significantly higher NPQ levels (ranging 3–5) in 800; 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ compared to those below 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (< 2.7).

Relations of photoinhibition to q_E and q_I values under PPFD treatments for 60 min: A significant and negative correlation with the r^2 value of 0.707 ($p < 0.05$) was detected in the degree of photoinhibition and q_E value of *E. dentata* plants under PPFDs above 100 μmol

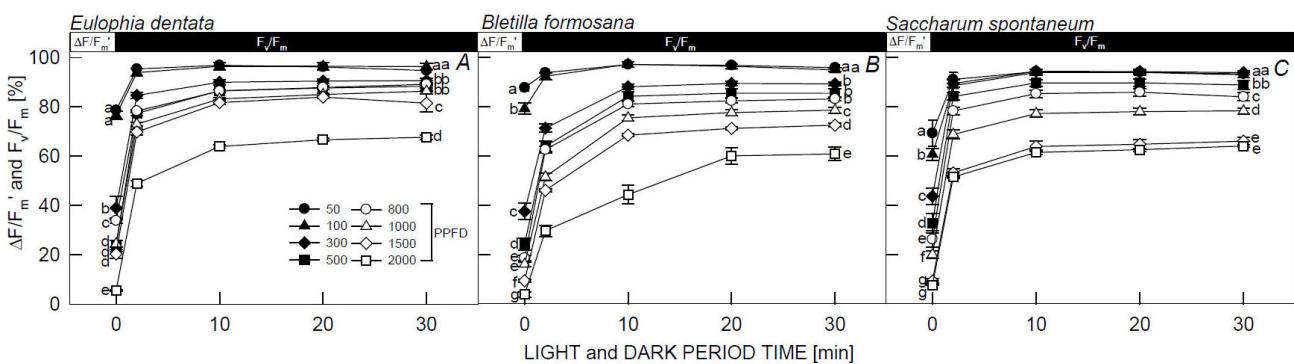


Fig. 2. Responses of the relative values of actual PSII efficiency ($\Delta F/F_m'$) and potential quantum efficiency of PSII (F_v/F_m value of the same leaves before illumination being 100%) in *Eulophia dentata* (A), *Bletilla formosana* (B), and *Saccharum spontaneum* (C). These ChlF parameters were obtained at artificial illumination of 60 min (L60) and a subsequent dark recovery for 2 min (D2), 10 min (D10), 20 min (D20), and 30 min (D30) periods under 50, 100, 300, 500, 800, 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD at 25°C. Each point represents the mean of five leaves, and data are means \pm standard errors. Different letters indicate significant differences in Tukey's HSD analyses at eight illuminations ($P < 0.05$).

$\text{m}^{-2} \text{ s}^{-1}$ (Fig. 4A), implying that *E. dentata* plants were more sensitive to PPFD conditions above 100 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, and there might not be a need to adjust the q_E value of *E. dentata* plants under 50 and 100 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Furthermore, significant and highly negative relationships were also detected between the degree of photoinhibition and q_E with an r^2 value of 0.993 ($p<0.01$) in *S. spontaneum* plants subjected to PPFDs higher than 500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig. 4E). In addition, the degree of photoinhibition in *E. dentata* plants was lower than that in *S. spontaneum* plants (Fig. 4A,E). Nevertheless, significant and highly positive correlations were observed between the degree of photoinhibition and q_I values of *E. dentata*, *B. formosana*, and *S. spontaneum* plants under all PPFD treatments, with r^2 values of 0.910 ($p<0.001$), 0.967 ($p<0.0001$), and 0.967 ($p<0.0001$), respectively (Fig. 4B,D,F). The increased % of photoinhibition led to increases in q_I values and decreases in q_E values due to photoinhibition and greater energy dissipation.

Discussion

Photosynthetic light responses were used to assess the ability of plants, selected for this study, to capture light and understand their optimal PPFD habitat conditions; in particular, we examined the interactive effect of PPFD on the growth and development of *E. dentata*. Measuring ChlF yields gives specific information about photochemical efficiency and heat dissipation (Papageorgiou and Govindjee 2014), and the exposure of plant leaves to illuminations that exceed photosynthetic capacity leads to photoinhibition of the electron transport system (see Demmig-Adams *et al.* 2006). Measurements were obtained initially after dark adaptation, after which overnight dark-adapted leaves were exposed to 50, 100 (low PPFDs), 300, 500, 800 (moderate PPFDs); 1,000; 1,500; and 2,000 (high PPFDs) $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for 0–60 min of light and 30 min of the dark. Generally, the ETR values

of the three species increased as PPFDs increased, except for leaves exposed to 2,000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. As PPFDs increased from 50 to 2,000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, increasing NPQ and decreasing F_v/F_m , Φ_{PSII} , $\Delta F/F_m'$, and the degree of photoinhibition from 0–60 min were observed in all leaves, indicating that thermal energy dissipation took place in antennae as PPFDs increased. All three species, used here, had low photosynthetic rates and needed to dissipate excess energy, in high light, to protect themselves; thus, all three species exhibited high downregulation of PSII efficiency (cf. Zulfugarov *et al.* 2007). A similar pattern on F_v/F_m and Φ_{PSII} of four invasive plant species has been found in north India (Pandey *et al.* 2020). Plants have developed numerous adaptive systems, including morphological and physiological modifications in defense against PPFD stress. When plants were exposed to high PPFD conditions, excess PSII energy increased and led to increases in NPQ values and decreases in F_v/F_m , Φ_{PSII} , $\Delta F/F_m'$, and the degree of photoinhibition due to greater energy dissipation. Notably, the NPQ of *E. dentata* and *S. spontaneum* leaves were relatively higher than that of *B. formosana* leaves under 1,500 and 2,000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ at 60 min, indicating that *E. dentata* and *S. spontaneum* had more nonphotochemical quenching with greater damage from photooxidation compared to that in *B. formosana*. Therefore, *E. dentata* and *S. spontaneum* plants appear to be adapted to less than 1,500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, whereas *B. formosana* plants are adapted to less than 800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Generally, the suitable temperature for photosynthesis of C₃-type plants (*E. dentata* and *B. formosana*) is ~25°C, while that of C₄-type (*S. spontaneum*) plants is ~35°C. When the plants stay in an appropriate temperature range, the efficiency of PSII rises as temperature increases (Pastenes and Horton 1996). In this study, carried out from September to October 2021, the average temperature was between 23.9–26.6°C and the high temperature was between 32.6–33.9°C, which was not high enough for the plants to reach photoinhibition. The reason for the higher

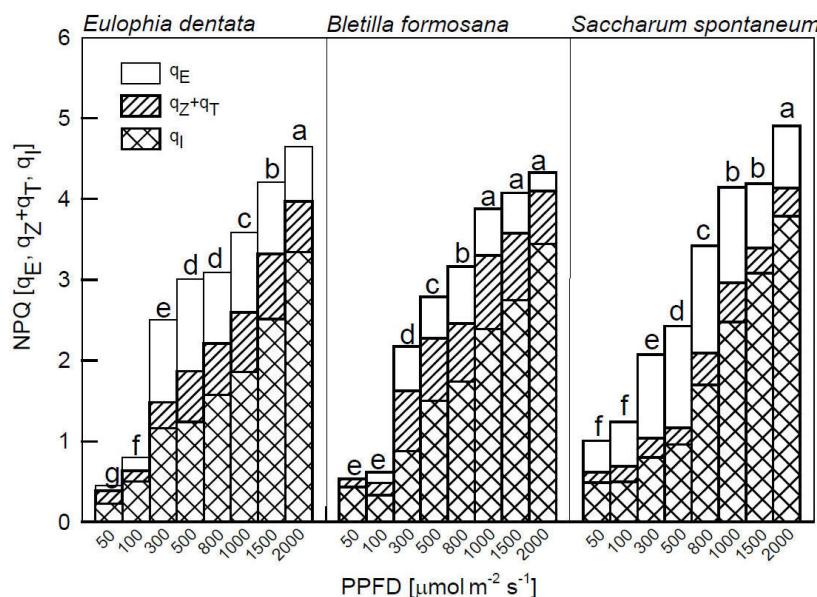


Fig. 3. Composition of nonphotochemical quenching in q_E , $q_z + q_T$, and q_I fractions of *Eulophia dentata*, *Bletilla formosana*, and *Saccharum spontaneum*. Measurements were made at 25°C under 50, 100, 300, 500, 800, 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD for 60 min and after a dark period for 30 min. Different letters indicate significant differences in Tukey's HSD analyses at eight illuminations ($P<0.05$). Each datum represents the mean of five leaves. q_E – energy-dependent quenching; q_I – photoinhibition quenching; q_z – zeaxanthin-dependent quenching.

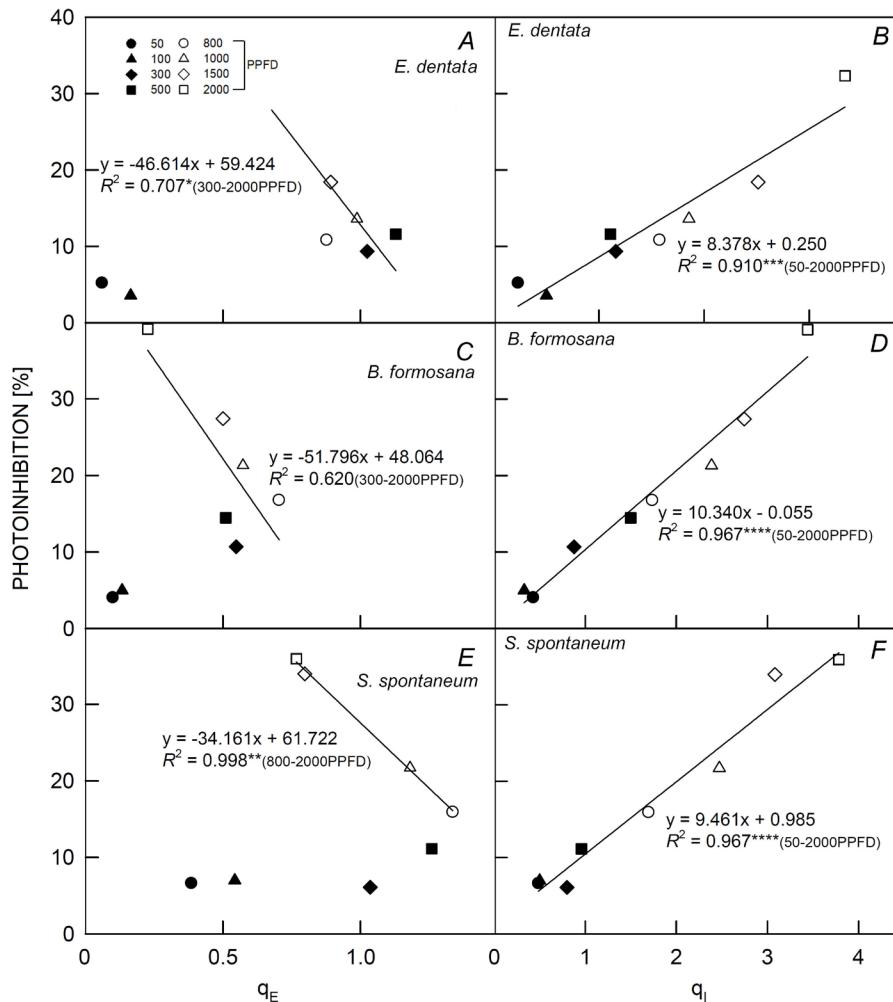


Fig. 4. Relationships between photoinhibition [%] and energy-dependent quenching (q_E) and photoinhibition quenching (q_I) of *Eulophia dentata* (A,B), *Bletilla formosana* (C,D), and *Saccharum spontaneum* (E,F). Measurements were made at 25°C under 50, 100, 300, 500, 800, 1,000; 1,500; and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD for 60 min. Each symbol represents the average of five leaves from one plant, and five plants were randomly selected for each light treatment. Each ChlF index was calculated using different leaf data ($n = 8$) from the model's validation datasets. The determination coefficient (r^2) and significance of the regression are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

PSII performance of *S. spontaneum* may lead to the higher adaptation temperature range of C₄-type plants.

Lower ETR and NPQ but higher F_v/F_m and Φ_{PSII} at low PPFDs were detected in all the three species, used in this study, compared to moderate and high PPFDs, suggesting that these three species adapt well favoring low PPFDs. The tested plants appeared to be sensitive to high PPFDs, which caused serious photoinhibition and photodamage. Furthermore, both the percent changes in $\Delta F/F_m$ and F_v/F_m recovered faster in *E. dentata* and *S. spontaneum* than that in *B. formosana* leaves at moderate and high PPFD conditions from D2 to D20 (Fig. 2). However, under treatment of darkness until reaching D30, all the three species, used here, seemed to adapt to low PPFDs with higher percent changes in $\Delta F/F_m$ and in F_v/F_m for photoprotection, indicating that protective mechanisms in all the three species with these higher values might prevent their leaves from suffering an excessive

reduction in PSII acceptors, avoid excessive energy absorption, and respond with higher PSII photochemical efficiencies. The effect of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *B. formosana* was overcome and plants underwent adaptive changes in physiology after 30 min of the dark period, quickly making up for the damage caused by the PPFD stress. ETR is a parameter to evaluate PSII efficiency, absorbing light, and the relative rate of electron transport through PSII. Thus, the elevated percent changes in ETR, $\Delta F/F_m$, and F_v/F_m of *E. dentata* and *S. spontaneum* leaves may help plants avoid high PPFD damage from excess energy. We suggest that the capacity and involvement of photo-protective mechanisms might vary seasonally, which may protect *E. dentata* and *S. spontaneum* from direct radiation.

During the 60-min light induction experiments, not only were all F_v/F_m and Φ_{PSII} values of the three species lesser than 0.8 (Fig. 1), but q_I values were higher than other parameters (Fig. 3), indicating that there was

photoinhibition and that high PPFD conditions were not suitable for the growth of the plants in this study. High PPFD decreases the ability of photosynthetic systems to utilize incident photons, thus leading to photoinhibition and reduced quantum yields in photochemistry and ChlF (Dewir *et al.* 2015). Low F_v/F_m and Φ_{PSII} can be interpreted as resulting from changes in reaction centers to quenching by excess light or depression after exposure to high PPFDs, which causes suppression of the electron transfer chain (Wong *et al.* 2012). These decreases in F_v/F_m and Φ_{PSII} reflect the increased thermal dissipation of excess excitation energy before it reaches the reaction centers. Plants adapt their photosynthesis in response to prevailing light irradiances, and the sensitivity of photosynthesis to PPFDs was found to vary among the tested plants. We found relatively higher q_l values in *S. spontaneum* plants than that in *E. dentata* and *B. formosana* plants under 1,500 and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatments (Fig. 3), indicating that *S. spontaneum* plants tend to drive photosynthetic ETR to quench energy, even when photoinhibition occurs there, when there is excess light. Although the photosynthetic system of the three species, examined in this paper, was dominated by q_l during photoinhibition, the photosynthetic system of *E. dentata* and *S. spontaneum* plants had higher q_E levels with more productive and greater photoprotective ability compared to that of *B. formosana* plants under moderate and high PPFD conditions. These results suggest that the photosynthetic system recovery of *E. dentata* and *S. spontaneum* is higher than that in *B. formosana* (Fig. 2). The observed light stress tolerance may be directly linked to the coordinated response of ETR, NPQ, q_E , q_l , F_v/F_m , and Φ_{PSII} , and could help in creating better future control methods to alleviate predicted adverse effects of global warming. This ability could prove to be important in PPFD stress tolerance because the net photodissipative capacity of these plant leaves could further increase under unfavorable conditions. These indicators respond to changes in PPFDs, and the combined analysis of these indicators provides accurate estimates of changes in the photosynthetic flux of the plant canopies and is much more significant for *E. dentata*, *B. formosana*, and *S. spontaneum* species diversity. An optimal strategy for regulation, by PPFD, is expected to help us in designing growth chambers and greenhouse light environments for growing *E. dentata* plants. The knowledge of these changes would also enable the development of models for planning optimal processing times for different growth and development stages to match the specific needs of PPFDs, and for selecting the most suitable plant species composition and structure of stands to minimize the impact of stressful environments and climate change on these species. However, these data would still reflect the physiological attributes that contribute to our perception of plant ecophysiology and their subsequent growth and development in open fields.

NPQ is associated with xanthophyll cycle-dependent energy quenching (see Demmig-Adams *et al.* 2006) and photoinhibition, leading to *S. spontaneum* plants showing more tolerance than *E. dentata* and *B. formosana*.

Further, *B. formosana* plants had a lower q_E and the xanthophyll cycle also maintained the same proportion; the q_E is associated with the violaxanthin to zeaxanthin transformation and thermal dissipation, and is a fast-activated and rapidly reversible component, while q_l and q_T are associated with conformational changes in LHC and PSII structure (see e.g., Giudici 2019). The increase in NPQ of the xanthophyll cycle is caused by the change in the structure of the PSII antenna system (*i.e.*, in the change in the rate of dissipation of heat from excess light energy). After the completion of the reaction in the subunit PsbS, zeaxanthin is combined with the PsbS protein to dissipate H^+ s, and q_T (phosphorylation shift-dependent quenching) shows the phosphorylation shift of LHCII between PSII and PSI (Malnoë 2018). Higher NPQ is a mechanism to protect plants from photoinhibition and photooxidation damages (Malnoë 2018). The higher the q_E value, the stronger the photoprotection mechanism. The fastest and most important component of NPQ is q_E , whereas the slowest reaction component of NPQ is q_l , which is related to photoinhibition or slow reversible recovery of the PSII reaction center. Stress decreases the ability of photosynthetic systems to utilize incident photons, thus leading to photoinhibition and reduced quantum yields in photochemistry and ChlF. Conversely, under low PPFDs that limit photosynthesis, zeaxanthin is converted to violaxanthin, and the reverse reaction occurs at the high PPFDs that exceed the level of light that can be consumed by photochemistry (Demmig-Adams *et al.* 2020). More work needs to be done to explore the photosynthetic mechanisms of NPQ, its exact location in the peripheral antenna of PSII, and its regulation and synergy with other quenching components (Malnoë 2018).

The susceptibility of photosynthesis to photoinhibition strongly depends on PPFDs. The larger the q_E is, the lower the % of photoinhibition is, but at a higher q_l (Fig. 4). Higher % of photoinhibition was detected under high PPFD conditions with higher q_E and q_l values compared to moderate and low PPFD conditions. The slope of q_E , related to % of photoinhibition, shows the photoprotection effect of q_E in all the tested plants. Significant and negative relationships were detected between q_E and % of photoinhibition in *E. dentata* and *S. spontaneum* plants under specific PPFD conditions, but significant and positive relationships were detected between q_l and % of photoinhibition in all three species under all PPFD conditions, indicating the differences in adjusting the path of energy flow between q_E and q_l . The path of energy flow to q_l was used mainly for photoinhibition at this stage, but our data show that all our samples may remain photochemically active and able to maintain lower q_l under low illumination. Further, *E. dentata* and *S. spontaneum* plants may be adjusting the path of energy flow absorption using q_E due to a photoprotective mechanism. In this study, plants were grown in the field and had high photoprotection at high PPFD conditions, with 60–80% PPFD in the habitat area, and these plants may adjust their path of energy flow using nonphotochemical quenching. PSII was destabilized in the field-collected samples of all the plants used in

this study experiencing high PPFDs, which may have resulted in a physical separation between LHCII and PSII reaction centers leading to an increase in F_v/F_m , Φ_{PSII} , and NPQ at different rates (cf. Makarenko *et al.* 2016). The susceptibility of photosynthesis to photoinhibition strongly depends on the PPFDs to which the plant is exposed during growth (Rosa-Manzano *et al.* 2015). During photosynthesis, changes in photoinhibition were mainly affected by q_I and q_E , followed by photoprotection. As % of photoinhibition and q_I levels increased, plants must have increased their q_E to cope with the negative effects of high light. Simple evaluations of photosynthesis can be made and relationships between the quenching of Chl *a* excited state (by heat) and photosynthetic efficiency can be estimated with these photosynthesis parameters, as they are highly sensitive indicators and provide quick means for identifying the physiological condition of plants (cf. Wang *et al.* 2022). Therefore, q_E and q_I can be used as an indicator of photoprotection and photoinhibition, respectively.

Our results are useful in efforts to predict the photosynthetic responses to light in *E. dentata*, *B. formosana*, and *S. spontaneum*, and are expected to provide a theoretical basis for afforestation in *E. dentata* plantations using native species. The habitats of *E. dentata* in the subtropics undergo greater PPFD changes and plants are less likely to experience high PPFDs. In field cultivation, choosing a suitable region or using artificial shading must be considered to avoid photoinhibition resulting from exposure to high PPFD. All of the above contribute to the moderation of the distribution of absorbed excitation energy in PSII and the better maintenance of the normal operations of the photosystem. These ChlF parameters could be used for the rapid monitoring and early detection of high or low PPFDs suitable for future regeneration. For instance, the impacts of changing q_E or q_I in *E. dentata* over time are affected by PPFD applications, and the balance between high or low PPFDs in the tested plants is crucial for determining the steady-state level of q_E or q_I . This balance can ameliorate high or low PPFDs and can be used as a substitute technology for the regeneration of *E. dentata*. Since q_E or q_I can be easily measured in the field, these values provide a useful ecological index for *E. dentata* restoration, habitat creation, and construction monitoring.

Conclusions: PAM chlorophyll fluorometry was used to monitor PSII efficiency in plant leaves under various PPFD for the abundance of *E. dentata*, *B. formosana*, and *S. spontaneum* plants growing in a subtropical region. Plants at a given PPFD showed variable ChlF values after 0–60 min of light, followed by 30 min of darkness. These plants displayed different capacities for protective mechanisms for avoiding damage to their photosynthetic apparatus when acclimating to various PPFD conditions. ETR values in *E. dentata* plants were elevated under 800; 1,000; and 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Higher NPQ levels in *E. dentata* and *S. spontaneum* plants were detected under 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ compared to those in *B. formosana* plants that had higher F_v/F_m and Φ_{PSII} levels than the

E. dentata and *S. spontaneum* plants under 50 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. These results suggest that *E. dentata* and *S. spontaneum* plants adapt their photosynthesis to high and moderate PPFDs, whereas *B. formosana* species acclimates to moderate to low PPFDs. Moreover, q_I increased and q_E decreased as % of photoinhibition increased, and higher % of photoinhibition was observed in *B. formosana* and *S. spontaneum* plants compared to *E. dentata* plants under high PPFDs. Our results indicate that plants acclimate to dynamic changes in light conditions, and thus can be used to study photosynthetic productivity and provide for ecophysiological research in *E. dentata*. Both q_I and q_E are useful for predicting changes in the performance and distribution of *E. dentata* plants and reflect the physiological attributes that contribute to our perception of *E. dentata* growth in the field.

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