The responses of photosynthetic gas exchange and chlorophyll a fluorescence to changes of irradiance and temperature in two species of Miscanthus

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Abstract

Photosynthetic CO₂ uptake and chlorophyll (Chl) a fluorescence of C₄ perennial grasses, Miscanthus floridulus (Labill) Warb and M. transmorrisonensis Hayata, from altitudes in central Taiwan of 390 and 2700 m, respectively, were studied at 10 and 25 °C to find if the species differ in their photosynthetic responses to a low temperature, and whether their photosystems 2 become more susceptible to the photoinhibition at low temperatures. For both species, the maximum photosynthetic rate (P_max) was reduced when the leaves were exposed to 10 °C. At irradiances higher than 400 μmol m⁻² s⁻¹, the values of F_v/F_m were reduced in both species at 10 °C but not at 25 °C, which indicated the photoinhibition at 10 °C. Reductions in P_max and the values of F_v/F_m at 10 °C were lesser in M. transmorrisonensis than in M. floridulus.

Additional key words: fluorescence quenching; photosystem 2.

Introduction

Low temperature is an important environmental stress which makes photosynthesis more sensitive so that even a low photon flux density (PFD) may cause photoinhibi-

**Miscanthus** spp., the perennial C₄ grasses, are widely distributed in Taiwan from river banks to the high mountain area. In central Taiwan, *M. floridulus* is the dominant grass at elevations below 2000 m, whereas *M. transmorrisonensis* dominates habitats above 2000 m (Chou et al. 1991). Response of isozymes to the temperature was different in two populations of *Miscanthus* sampled from altitudes of 780 and 2700 m, and *Miscanthus* plants growing at 780 m could not survive when transplanted to a habitat of 2700 m (Chou and Chang 1988). Hence, *M. floridulus* might be more sensitive to low temperature than *M. transmorrisonensis*. However, the influence of low temperature on photosynthesis of these two species has not been studied.

The amount and kinetics of Chl fluorescence emitted from leaves upon an actinic irradiation are a probe of the primary photochemistry of photosynthesis (Krause and Weis 1991). In particular, the linear relationship between quantum yield and the ratio of variable fluorescence to maximum fluorescence ($F_v/F_m$) (Adams et al. 1990) suggest that $F_v/F_m$ can monitor the photosynthetic carbon assimilation (Björkman 1987). A reduction in $F_v/F_m$ of dark-adapted leaves indicates photoinhibition of PS2. In addition, an information on photosynthetic electron transport activity may be obtained with a pulse amplitude modulated fluorescence fluorometer. For example, the photochemical quenching coefficient, $q_p$, indicates the proportion of reduced to oxidized state of the primary electron acceptor $Q_A$ of PS2 (Schreiber et al. 1994), and the status of $Q_A$ is related to photoinhibitory damage (Havaux 1987).

The aims of present study were to understand the effect of chilling temperature on photosynthesis of *M. floridulus* and *M. transmorrisonensis*, and to compare the responses of photosynthetic gas exchange and Chl a fluorescence of this species pair to some short-term changes in temperature and irradiance.

**Materials and methods**

**Plants**: Field-grown *M. floridulus* and *M. transmorrisonensis* plants were collected, respectively, from sites located at the elevations of 390 m near Shui-Li and 2700 m in Yushan National Park (23°29′N, 120°48′E), Nantou county, in central Taiwan. They were transplanted into 4000 cm³ plastic pots filled with vermiculite:soil (1:1), and grown in a glasshouse under natural daylight, watered every day, and fertilized with inorganic fertilizer (N:P:K of 20:20:20) once every two weeks from April to July of 1996. During the experiment, the day/night temperature and photoperiod were about 30/25 °C and 12 h, respectively.

**Photosynthetic CO₂ uptake** was measured using a steady-state open gas exchange system (Pacaya 9900, DGO, La Jolla, CA, USA). The youngest fully expanded leaf from each plant was used. The response of photosynthetic CO₂ uptake rate ($P$)
to photosynthetic photon flux density (PPFD) was assayed by enclosing the leaf in a
cuvette under a fiber irradiator (FL-400, Walz, Germany). Steady-state rates of \( P \)
were recorded after equilibration at each successive irradiance. The cuvette
conditions were: air temperature of 25 or 10 °C, leaf to air water vapour concen-
tration difference of 1.0-1.2 kPa. The air temperature was controlled by Peltier
heat exchange units mounted on the underside of the cuvette.

Chl fluorescence quenching analysis: Leaves were enclosed in the same cuvette as
for the measurement of gas exchange. After a 10 min irradiation, quenching of
variable Chl a fluorescence was analyzed using a portable, pulse amplitude
modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany). The minimal
fluorescence yield of the light-adapted leaf (\( F_{m}^{'} \)) was determined by irradiating the
leaves with far-red radiation of about 150 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The values of \( F_{m}^{'} /F_{m}^{'} \) and
the coefficient of photochemical quenching, \( q_{p} = (F_{m}^{'} - F_{i}^{'})(F_{m}^{'} - F_{0}^{'}), \) were
computed (Schreiber et al. 1986), where \( F_{m}^{'} \) was the maximal fluorescence, \( F_{0}^{'} \)
was the minimal fluorescence, and \( F_{i}^{'} \) was the steady-state fluorescence in the light-
adapted state. The proportion of the oxidized to the reduced reaction centres (\( Q_{A} \))
was estimated as \( 1 - q_{p} \).

Induction of photoinhibition: The plant attached leaves were placed in the same
cuvette as mentioned above. Actinic radiation was provided by a fiber irradiator
(FL-400, Walz, Germany) located 2 cm above the top of the cuvette. Following 2 h
irradiation, the leaves were dark-adapted for 40 min at the exposure temperature
(10 or 25 °C) before \( F_{m}^{'} /F_{m}^{'} \) measurements were taken. The frequency of
modulation and the saturating PFD were 600 Hz and 5000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \),
respectively. To elucidate the time course of changes in \( F_{m}^{'} /F_{m}^{'} \), plants were
transferred to a growth chamber (Conviron GC 108, Canada) inside which the air
temperature was kept at 10 °C, the relative humidity was 90 %, and the PFD was
400 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) on the leaf level.

Results

Photosynthetic gas exchange: The rate of irradiance saturated CO\(_{2}\) uptake (\( P_{\text{max}} \))
was depressed by lowering the temperature from 25 to 10 °C (Fig. 1). No
significant difference was found in \( P_{\text{max}} \) of \( M. \) floridus and \( M. \) transmorrisonensis when \( P \) was measured at 25 °C. Compared to the values
measured at 25 °C, the reduction in \( P_{\text{max}} \) at 10 °C was greater in \( M. \) floridus than
in \( M. \) transmorrisonensis, 50 and 34 % reduction, respectively. As a result, at an
air temperature of 10 °C, \( P_{\text{max}} \) of \( M. \) transmorrisonensis was significantly higher
than that of \( M. \) floridus, \( 174 \pm 5 \) vs \( 128 \pm 17 \) \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (mean ± s.e., \( n \)
= 5, \( t \)-test, \( p < 0.05 \)).

Quenching analysis of Chl a fluorescence: The irradiance response of Chl
fluorescence showed that the quantum yield parameter (\( F_{m}^{'} /F_{m}^{'} \)) and photochemical
quenching component ($q_p$) were lower when the irradiance was higher, and the reduction was more sensitive to irradiance at 10 than at 25 °C (Fig. 2A,B). As a result, the proportion of reduced reaction centres ($Q_A - 1 - q_p$) was higher in leaves exposed to 10 than 25 °C (Fig. 2C). No significant difference was found in $q_p$ and $F_v'/F_m'$ between leaves of M. floridulus and M. transmorrisonensis exposed to 25 °C. At 10 °C, leaves of M. transmorrisonensis had higher $F_v'/F_m'$ and $q_p$ values, but lower proportion of reduced reaction centres $Q_A$ than those of M. floridulus throughout the range of PFD tested.

![Fig. 1. Response of photosynthetic gas exchange to irradiance, PFD in M. floridulus (■, ■) and M. transmorrisonensis (□, ●) at 10 (filled) and 25 (open) °C. Values are means from five different plants; standard deviations are indicated by bars, if larger than symbols. In both species, the rate of PFD-saturated CO₂ uptake was significantly depressed by lowering the temperature from 25 to 10 °C (t-test, p < 0.05).

Induction of photoinhibition: Over the range of PFD no significant difference was found in $F_v'/F_m'$ either between the species pair, or among PFD treatments when the leaves were exposed to 25 °C. A rise in PFD decreased the $F_v'/F_m'$ value of 10 °C exposed leaves indicating photoinhibition. At PFD values 0 and 225 μmol m⁻² s⁻¹, there was no significant difference in $F_v'/F_m'$ between the leaves exposed to 10 and 25 °C. At a PFD > 450 μmol m⁻² s⁻¹, M. floridulus leaves exposed to 10 °C had a significantly lower $F_v'/F_m'$ than those exposed to 25 °C, and the reduction in $F_v'/F_m'$ increased with increasing PFD. The response of $F_v'/F_m'$ in leaves of M. transmorrisonensis exposed to 10 °C showed a similar trend as that of M. floridulus. Even so, the reduction in $F_v'/F_m'$ was greater in M. floridulus than in M. transmorrisonensis. Consequently, at a PFD > 450 μmol m⁻² s⁻¹ and temperature of 10 °C, M. floridulus leaves always had a significantly lower $F_v'/F_m'$ value than M. transmorrisonensis compared at the same PFD. Measuring of the time course of changes in $F_v'/F_m'$ of M. floridulus and M. transmorrisonensis leaves exposed to 10 °C and 400 μmol m⁻² s⁻¹ of PFD revealed an initial rapid decrease in $F_v'/F_m'$ followed by an exponential decline to approach a steady-state in 10 h (Fig. 4). $F_v'/F_m'$ value of M. floridulus leaves declined more rapidly than that of M. transmorrisonensis leaves.
Fig. 2. Response of effective quantum yield of photosystem 2, Fv'/Fm', photochemical quenching component, qP, and the reduction state of primary electron acceptor, QA at 25 (open) and 10 (filled) °C in *M. floridulus* (■, ■) and *M. transmorrisonensis* (☐, ○) leaves. Each point is the average of 5 replicates. Bars represent s.d.

Fig. 3. Effects of irradiance (PFD) on the Fv/Fm of *M. floridulus* (first two columns) and *M. transmorrisonensis* (columns three and four) leaves exposed to 10 (second and fourth columns) and 25 °C (first and third columns) under each PFD for 2 h. Each point is the average of 5 replicates. Bars represent s.d.
Fig. 4. Time course of changes in $F_v/F_m$ of *M. floridus* (■) and *M. transmorisonensis* (●) leaves at 10 °C and under an irradiance of 400 μmol m$^{-2}$ s$^{-1}$. Values are means of five replicates (± s.d.).

**Discussion**

Measurement of light responses of the photosynthetic CO$_2$ uptake revealed that the photosynthetic capacity was reduced with the leaves exposed to an air temperature of 10 °C (Fig. 1). The light response of the fluorescence components, $F_v/F_m$, qP and $Q_A$, showed a similar pattern (Fig. 2). In a simultaneous measurement of the photosynthetic capacity and fluorescence components of barley, Ottander et al. (1993) found that the depression of photosynthesis at a lower temperature resulted in a significantly increased proportion of reduced $Q_A$. Thus, the increase in $Q_A$ of *Miscanthus* leaves at 10 °C may be due to the lower capacity of photosynthesis at 10 °C than at 25 °C.

Photoinhibition occurs when the leaves are exposed to irradiances in excess of what can be utilized in photosynthesis (Powles 1984, Barber and Andersson 1992). In addition, the susceptibility of photosynthesis to inhibition increases with an increasing proportion of reduced $Q_A$ (Ottander et al. 1993). Thus, the result that reduction in $F_v/F_m$ occurred in 10 °C exposed leaves but not in 25 °C treated ones within the PFD range could be due to the effects of low temperature on the photosynthetic capacity (Fig. 1). Consequently, a higher proportion of reduced reaction centres was accumulated in 10 °C exposed leaves (Fig. 2C). A higher proportion of reduced $Q_A$ increased the susceptibility of *Miscanthus* leaves to photoinhibition. Similar results were found in *Zea mays*, where low temperature caused a concomitant reduction in maximum photosynthetic capacity and in the ability of the plant to utilize high irradiances for photosynthesis (Nie et al. 1992).

Reduction of the Chl fluorescence under a low temperature stress has also been observed in chilling-sensitive plants such as bean (Hetherington et al. 1989), tomato (Brüggemann et al. 1989), maize (Hetherington et al. 1989, Janda et al. 1994, Haldimann et al. 1996), and also in a warm season Zoysia grass (Okawara and Kaneko 1995).
Comparing the results of the tested species pair, a higher reduction in $F_o/F_m$ was measured in *M. floridulus* than in *M. transmorrisonensis* when exposed to 10 °C (Figs. 3 and 4). Thus, at a chilling temperature *M. floridulus* was more sensitive to photoinhibition than *M. transmorrisonensis*. The results from Chl fluorescence quenching analysis and gas exchange measurements suggest a possible mechanism for this difference. Because the reduction of photosynthetic capacity at a chilling temperature was higher in *M. floridulus* than in *M. transmorrisonensis* (Fig. 1), a higher proportion of reduced reaction centres was accumulated in the former species than in the latter one (Fig. 2). A higher proportion of reduced reaction centres of *M. floridulus* that increased at the chilling temperature, increased the susceptibility of this species to photoinhibition in comparison with *M. transmorrisonensis*.

As a conclusion, our study shows that chilling temperature reduced the capacity of photosynthesis of *M. floridulus* and *M. transmorrisonensis* causing an increased excitation pressure of the reaction centres of PS2, as expressed by increased reduction state of $Q_A$, hence increased the susceptibility of PS2 to photoinhibition. The reduction of photosynthetic capacity was higher in *M. floridulus* than in *M. transmorrisonensis*, and as a result, the photosynthetic apparatus of the former species was more susceptible to photoinhibition than that of the latter species.

References


