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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 CO_2 uptake and chlorophyll (Chl) *a* fluorescence of C_4 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 photoinhi-

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Abbreviations: Chl - chlorophyll; F_0' - minimal fluorescence in light-adapted state; F_m - maximum fluorescence in dark-adapted state; F_m' - maximum fluorescence in light state; F_v - variable fluorescence in dark-adapted state; F_v' - variable fluorescence in light state; F_t - steady-state fluorescence in the light-adapted state; P_{max} - photosaturated photosynthetic rate; PFD - photon flux density; PS - photosystem; Q_A - primary electron acceptor of photosystem 2; q_P - photochemical quenching.

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bition (Öquist *et al.* 1987, Öquist and Huner 1991). Plants are more susceptible to photoinhibition under a low temperature (Hetherington *et al.* 1989, Janda *et al.* 1994, Krause 1994).

Miscanthus spp., the perennial C_4 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 cm^3 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 CO_2 uptake was measured using a steady-state open gas exchange system (*Pacsys 9900, DDG*, La Jolla, CA, USA). The youngest fully expanded leaf from each plant was used. The response of photosynthetic CO_2 uptake rate (*P*) to photosynthetic photon flux density (PFD) 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 concentration 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_0') was determined by irradiating the leaves with far-red radiation of about 150 µmol m^{-2} s^{-1}. The values of F_v'/F_m' and the coefficient of photochemical quenching, $q_P = (F_m' - F_t)/(F_m' - F_0')$, were computed (Schreiber *et al*. 1986), where F_m' was the maximal fluorescence, F_0' was the minimal fluorescence, and F_t 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_v/F_m measurements were taken. The frequency of modulation and the saturating PFD were 600 Hz and 5000 µmol m^{-2} s^{-1}, respectively. To elucidate the time course of changes in F_v/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 µmol m^{-2} s^{-1} on the leaf level.

Results

Photosynthetic gas exchange: The rate of irradiance saturated CO_2 uptake (P_{max}) was depressed by lowering the temperature from 25 to 10 °C (Fig. 1). No significant difference was found in P_{max} of *M. floridulus* and *M. transmorrisonensis* when P was measured at 25 °C. Compared to the values measured at 25 °C, the reduction in P_{max} at 10 °C was greater in *M. floridulus* than in *M. transmorrisonensis*, 50 and 34 % reduction, respectively. As a result, at an air temperature of 10 °C, P_{max} of *M. transmorrisonensis* was significantly higher than that of *M. floridulus*, 17.4 ± 0.5 *vs*. 12.8 ± 1.7 µmol m^{-2} s^{-1} (mean ± s.d., $n = 5$, t-test, $p < 0.05$).

Quenching analysis of Chl *a* fluorescence: The irradiance response of Chl fluorescence showed that the quantum yield parameter (F_v'/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. 2*A,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. 2*C*). 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.

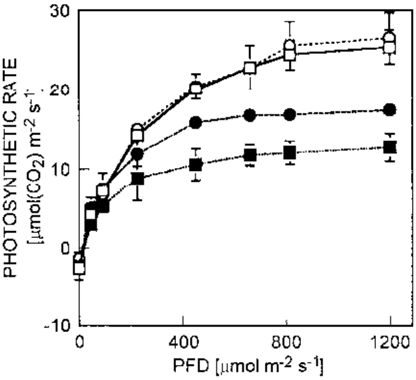

Fig. 1. Response of photosynthetic gas exchange to irradiance, PFD in *M. floridulus* (□, ■) and *M. transmorrisonensis* (○, ●) at 10 (*filled*) and 25 (*open*) °C. Values are means from five different plants; standard deviations are indicated by bars, if larger than symbols. In both species, the rate of PFD-saturated CO_2 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.

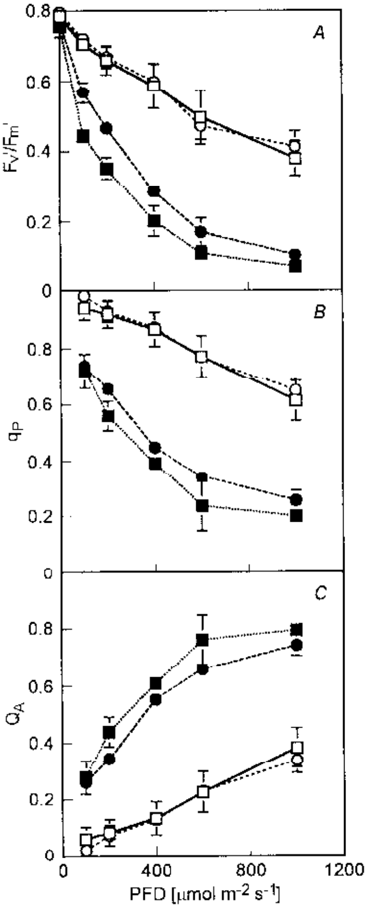

Fig. 2. Response of effective quantum yield of photosystem 2, F_v'/F_m', photochemical quenching component, q_P, and the reduction state of primary electron acceptor, Q_A at 25 (*open*) and 10 (*filled*) °C in *M. floridulus* (□, ■) and *M. transmorrisonensis* (○, ●) leaves. Each point is the average of 5 replicates. Bars represent s.d.

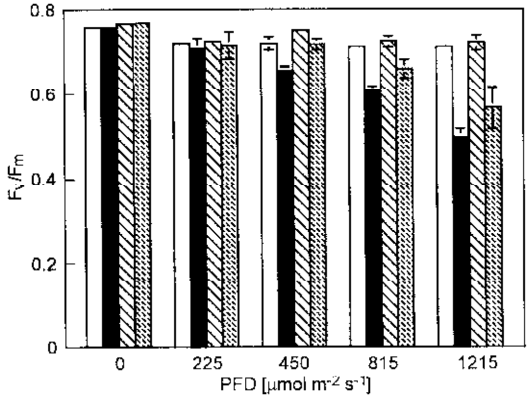

Fig. 3. Effects of irradiance (PFD) on the F_v/F_m of *M. floridulus* (*first two columns*) and *M. transmorrisonensis* (*columns three and four*) leaves exposed to 10 (*second and fourth columns*) and 25 °C (*first and third columns*) under each PFD for 2 h. Each point is the average of 5 replicates. Bars represent s.d.

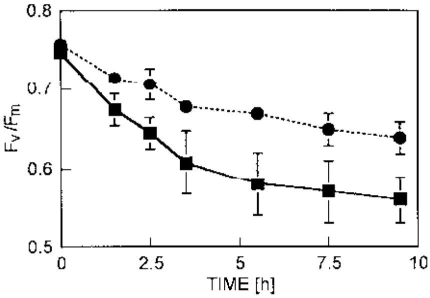

Fig. 4. Time course of changes in F_v/F_m of *M. floridulus* (■) and *M. transmorrisonensis* (●) leaves at 10 °C and under an irradiance of 400 μmol m^{-2} s^{-1}. Values are means of five replicates (± s.d.).

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. 2*C*). 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_V/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.

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