Diurnal Cycle and Photoinhibition of Photosynthesis in Palm

Trachycarpus fortunei H. Wendl. under Winter and Summer Conditions

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A study was conducted to analyze the contribution of high irradiance and resulting photoinhibition to the decline in net photosynthesis in the leaves of palm Trachycarpus fortunei during summer and winter as well as at normal growth and low temperatures in field and laboratory conditions, respectively. Fluorescence induction measurements indicated that there was a 10% decrease in the \(\text{F}_\text{v}/\text{F}_\text{m}\) ratio in field conditions at midday during both summer and winter, due to the relatively low intensity of incident light resulting from the partial leaf segment folding. Fluorescence parameters completely recovered by the evening hours. In summer the midday decay was due to the decrease of \(\text{F}_\text{m}\), which probably represents a rapidly reversible component of photoinhibition by the protective down-regulation of PSII mediated by the xanthophyll cycle. In winter, however, the initial \(\text{F}_\text{v}/\text{F}_\text{m}\) ratio was 40% less than as measured in summer and its midday decline was associated with the decrease of \(\text{F}_\text{v}\), indicating the partial inactivation of PS II. The net \(\text{CO}_2\) assimilation rate followed the pattern of the \(\text{F}_\text{v}/\text{F}_\text{m}\) ratio but it could not recover due to the stomatal closure after midday. Comparing the fluorescence and gas exchange measurements we have concluded that the photoinhibition of \(T.\ fortunei\) represented by the \(\text{F}_\text{v}/\text{F}_\text{m}\) ratio changes is a regulatory adjustment of PS II efficiency to limiting carbon utilization and to limiting carbon availability imposed by stomatal closure. Leaves photoinhibited under laboratory conditions at growth temperature showed a substantial decrease of 50% in the \(\text{F}_\text{v}/\text{F}_\text{m}\) ratio due to the perpendicular exposure, but no apparent changes in \(\text{D}_{\text{P}}\), protein content could be detected. Phyotron grown plants exposed to cold stress (6°C) and low irradiance (250 \(\mu\text{mol m}^{-2} \text{s}^{-1}\)) under laboratory conditions showed a time related but much slower continuous decrease in \(\text{F}_\text{v}/\text{F}_\text{m}\) ratio. After high irradiance the recovery kinetics in the dark at normal growth temperature (28°C) strongly depended on the extent of the photoinhibition, while after low irradiance complete recovery occurred in 12 hours irrespective of the initial \(\text{F}_\text{v}/\text{F}_\text{m}\) value, independently from the time of cold treatment, indicating that at low light and cold treatments only reversible inactive PS II's were formed.

Introduction

Light is important to plants and to life on earth because it drives the process of photosynthesis. However, reduction in photosynthetic capacity called photoinhibition commonly occurs in nature when plants are exposed to solar radiation in excess of light energy necessary for photosynthetic or photorespiratory processes (Long \textit{et al.}, 1994; Krause \textit{et al.}, 1995). As the consequence of photoinhibition, midday decline of net \(\text{CO}_2\) assimilation (A), stomatal conductance (S) as well as the decrease of maximum fluorescence (\(\text{F}_\text{m}\)) and that of the \(\text{F}_\text{v}/\text{F}_\text{m}\) ratio have been reported during the growing season for dicotyledon trees (Joshi, 1995; Pathre \textit{et al.}, 1995; Krause \textit{et al.}, 1995, Faria \textit{et al.}, 1998), shrubs (Demmig-Adams \textit{et al.}, 1989) and vines (Chaves \textit{et al.}, 1987; Greer and Laing, 1992) of both tropical and temperate origins. Based on the recovery kinetics, it has been suggested that...
the midday decrease in PSII efficiency involves at least two processes, one related to the xanthophyll cycle (Demmig-Adams and Adams, 1996), the other to the D1 protein inactivation (Anderson et al., 1997).

The inhibitory effect of cold stress together with high irradiance has also been reported on photosynthesis and related physiological processes for both cold tolerant and chilling sensitive plants (Huner et al., 1993). For example, during winter and early spring evergreens such as conifers are subject to increased photoinhibition termed ‘photochilling’ in temperate climatic regions of the world (Bolhár-Nordenkampf and Lechner, 1988). Apparently the same is true in/from late autumn and/to early spring for a range of plants of subtropical origin like Eucalyptus (Ball et al., 1991), sugar cane (Bolhár-Nordenkampf et al., 1994) kiwifruit (Greer and Laing, 1992) grown under Mediterranean-type climates. Although, the key processes involved in the diurnal down regulation of photosynthesis have intensively been studied during the past decade, very little information is available on the high light induced alterations of physiological parameters in monocotyledon trees like the economically important palms, Arecaceae (Jayasekara and Jayasekara, 1995; Krause and Winter, 1996).

The Chinese windmill palm (Trachycarpus fortunei) used in our experiments is a characteristic landscape forming tree in South Europe, planted mainly in coastal towns, thus its significance in the tourism industry cannot be ruled out. It is one of the most frost tolerant species (Larcher et al., 1991) of an otherwise tropical family (Arecaceae) of great economic importance. In some of its native and introduced habitats the species is subjected to low temperatures and is able to withstand temperatures down to ~12 °C without any subsequent freeze damage (Larcher et al., 1991).

In the present study therefore, we have attempted to make a comparative analysis on the contribution of high irradiance and cold stress to the operation of diurnal cycle of photosynthesis in palm Trachycarpus fortunei during summer and winter conditions, respectively. For a better understanding of the underlying mechanism we also carried out both in vivo and in vitro photoinhibition experiments under laboratory conditions.

**Materials and Methods**

**Plant material**

For the field experiments, measurements were made on the 3 youngest fully expanded leaves of five 10 year old, trunkless Chinese windmill palms, by other name Chusan palm (Trachycarpus fortunei Wendl) growing in the Arboretum of the University of Horticulture and Food Industry, Budapest. Measurements were made on three consecutive days in February and July 1997 and 1998 during longer periods of anticyclonal weather conditions. For the in vivo and in vitro measurements under laboratory conditions, greenhouse grown 6 month old seedlings were used. Five days prior to the exposure to in vivo photoinhibitory conditions the seedlings were transferred to growth cabinets (Weiss Technik Bioclim 1600SP), where the illumination followed 16 hrs light/ 8 hrs dark cycles, the photon flux density (PFD) was 250 μmol m⁻² s⁻¹ and the day/night temperature was constantly 28 °C.

**Chloroplast isolation**

Intact chloroplasts were isolated using the protocol of Horváth et al. (1983).

**Photoinhibition and recovery under laboratory conditions**

Leaves were placed horizontally on wet filter paper. In vivo photoinhibition was induced by two 500-W metal-halide lamps with a photon flux density of 1.6–1.7 mmol m⁻² s⁻¹ upon exposure on the adaxial side of the leaves. Air temperature was kept at 6 °C in a cold room or 28 °C by placing a cold water bath between the light source and the leaves. For the recovery from photoinhibition, the plants were placed in complete darkness at a temperature of 25 °C. For in vitro photoinhibition, 1 ml of chloroplast suspension isolated from greenhouse grown leaves were placed in glass cuvettes with 1 mm thickness under the above conditions and illuminated for 5, 10, 20, 30 and 60 min.

**Chlorophyll fluorescence**

Chlorophyll fluorescence was recorded in both field and laboratory conditions with a portable fluorometer (Plant Efficiency Analyser, Hansatech Ltd., King’s Lynn, U. K.). After 15 minutes of dark
adaptation samples were excited with the actinic PFD of 1800 μmol m⁻² s⁻¹ at 650 nm. Fluorescence was detected for 120 sec and the usually used parameters: \( F_0, F_w, F_m \) and the \( F_v/F_m \) ratio were calculated (Bolhár-Nordenkampf and Öquist, 1993).

**Photosynthetic gas exchange**

The net photosynthesis of field grown palm leaves were measured in every second hour of sunlight days by LI-COR (LI-6200, USA) portable gas exchange system according to Winner et al. (1989). Total sunlight leaf parts were closed into a chamber of 250 cm³ with an area of 20–25 cm². The net photosynthesis values were calculated by the system and the average of six independent measurements were used.

**Stomatal conductivity**

Stomatal conductance was measured with a portable mass flow porometer (AP4 Delta-T Devices, Cambridge, U. K.) according to Tuba et al. (1998).

**Measurement of thermoluminescence**

The TL of leaf discs with a 10 mm diameter or 0.5 ml chloroplast suspension containing 125 μg chl were measured using an apparatus similar to that described earlier (Demeter et al., 1979). The light source was a 650 W tungsten lamp provided 10 W m⁻² irradiance on the surface of the sample. Samples were illuminated at the specified temperature and rapidly cooled down to −80 °C. TL was measured during heating the sample in darkness with a constant heating rate of 20 °C min⁻¹.

**Immunological detection of the \( D_1 \) protein**

The analysis was made on both \textit{in vivo} and \textit{in vitro} photoinhibited samples based on the work of Blake et al. (1984) and modified by Georgakopoulos and Argyroudi-Akoyunoglou (1997).

**Results and Discussion**

**Photoinhibition under field conditions**

Plants subject to high irradiance at normal growth and low temperatures exhibit different photoinhibitory responses which are resulted by various concomitant and subsequent molecular changes.
events (for reviews see Long et al. 1994; Huner et al. 1993; Krause 1994 and references therein). In order to obtain information about the photoinhibitory response of palm, \textit{T. fortunei}, the diurnal fluctuations of the fluorescence induction characteristics were compared in summer and in winter, respectively. As shown in Fig. 1 (top panels), in both cases the maximal light intensities were similar (1.7 and 1.5 mmol m\(^{-2}\) s\(^{-1}\)). The daily temperature regime in summer (25–32 °C) corresponded to the normal growth temperature, while in winter measurements plants were subjected to chilling or freezing temperatures (−3.2 to 5 °C). In both summer and winter, fluorescence measurements made under field conditions showed similar, only approximately a 10\% decrease in the PSII activity as indicated by the change of the F\(_{v}/F_m\) ratio (Fig. 1 middle panels). The inhibition was completely reversible and was fully recovered by the evening hours. In summer, the decrease in the F\(_{v}/F_m\) ratio was due to the decrease of F\(_m\) since F\(_0\) was practically unaltered during the day (Fig. 1 lower panels) which might be the result of the increase in xanthophyll cycle dependent energy dissipation in the antennae (Demmig-Adams and Adams, 1996; Jahn and Miehe, 1996). In winter, however, the F\(_{v}/F_m\) ratio was markedly lower than in summertime (Fig. 1 middle panels), which fitted well to earlier results obtained by the seasonal variation of the F\(_{v}/F_m\) (Bolhár-Nordenkampf and Lechner, 1988; Ot-tander et al., 1995) and explained by low temperature induced partial inactivation of PS II activity (Huner et al., 1993). The diurnal alteration of the F\(_{v}/F_m\) ratio was due to the changes in F\(_{v}\) (significant increase of F\(_0\) at midday with an unchanged F\(_m\)) which indicated that parallel with the energy dissipation mechanism, PS II inactivation also occurred (Krause, 1994; Schettger et al., 1994; Krause, 1988).

Since, according to the definition of Long et al. (1994) “light impairment of PS II is only photoinhibition if a decrease in the overall photosynthetic rate results”, we measured the diurnal fluctuation of the net CO\(_2\) assimilation in the field at normal growth conditions. As shown in Fig. 2A the net CO\(_2\) assimilation after a slight increase had the minimum value at similar midday hour as the F\(_{v}/F_m\) ratio but it could not return to the original value, just exhibited a second but much lower peak during the afternoon hours. Similar double peak diurnal curves were reported by Pathre et al. (1995) for different tree species.

Chaves et al. (1987) demonstrated a strong correlation between net photosynthesis and stomatal conductance in grapevine cultivars. In our case, however, the stomatal closure was not correlated with the net CO\(_2\) assimilation, because it declined only at midday when the CO\(_2\) gas exchange reached its minimum value and remained low during the rest of the day (Fig. 2B). The high stomatal conductance in the morning hours indicates that the decrease in the net CO\(_2\) fixation is the result of the intrinsic changes in the CO\(_2\) fixation rather than the CO\(_2\) limitation modulated by stomatal closure. The low net CO\(_2\) gas exchange in the afternoon hours is well explained by the stomatal closure (Chaves et al., 1987). Comparing the data of fluorescence induction measurements and the CO\(_2\) gas exchange, we have concluded that the photoinhibition of \textit{T. fortunei} under field conditions measured by the F\(_{v}/F_m\) ratio is a regulatory adjustment of PS II efficiency to limiting carbon utilization and to limiting carbon availability imposed by stomatal closure (Demmig-Adams and Adams, 1996; Faria et al., 1998).
**Photoinhibition under laboratory conditions**

After 120 min photoinhibition of 6 month old intact palm leaves induced at 28 °C under laboratory conditions, a 50% decrease was observed in the Fv/Fm ratio and more than 40% rise in the F0 value (Fig. 3). At low temperature (6 °C) similar changes were observed with a maximum deviation of 10% (data not shown). The decrease in the Fv/Fm ratio could not be the result of the limited carbon availability modulated by stomatal closure because stomatal conductance was increased due to the high water pressure during the experiment. The dramatic decrease in the TL intensity during photoinhibition indicated a substantial damage in PS II function which was not associated with any D1 degradation (data not shown). This is in agreement with the earlier observation that in vivo photoinhibition caused loss of photochemical activity without the degradation of D1 polypeptide (Cleland et al., 1990). Isolated T. fortunei chloroplasts under similar photoinhibitory conditions, however, completely lost the PS II activity with a concomitant D1 protein degradation (Fig. 4). The fact that PS II inactivation partially precedes the loss of D1 protein is in accordance with the general view that the impairment of PS II electron transport triggers the D1 degradation (for review see Andersson, 1992; Anderson et al., 1997; Long et al., 1994 and references therein). The difference between the in vivo and in vitro photoinhibition can be explained in the following way: In vivo mainly reversibly inactivated PS II are formed (Long et al., 1994) while in vitro the high O2 concentration of the suspension buffer enhances the formulation of the highly toxic oxygen radical (¹O2) resulting in a complete irreversible inhibition and/or destruction of PS II (Vass and Styring, 1993; Hideg et al., 1994).

This assumption seems to be verified by the recovery experiment (Fig. 5). The decreased Fv/Fm ratio of photoinhibited leaves could be restored in the dark but the rate of recovery was strongly dependent on the extent of photoinhibition. 20% decrease of the Fv/Fm ratio was practically recovered after 24 hours, while at 60% photoinhibition the Fv/Fm ratio was restored only in 70% even after 96 hours of dark recovery. In vitro no recovery could be found at any degree of photoinhibition (data not shown). Leaves subjected to low light (250 μmol m⁻² s⁻¹) at low (6 °C) temperature also showed a stepwise decrease in the Fv/Fm ratio but the rate of decrease was smaller by order of magnitude (Fig. 5. C). The Fv/Fm ratio was recovered in the dark within 12 hours independently from the extent of the Fv/Fm reduction similarly to the results of Janda et al., (1994). Our result is consistent with the earlier finding that at low light and low temperature conditions only reversibly inactivated PS II were formed (Briantis et al., 1992) and this form could not be converted to non-reversible inactive PS II centers (Long et al., 1994).

Comparing the different data obtained in field and laboratory conditions the difference between the results can be explained on the following way:
younger leaves are more sensitive to photoinhibition than mature leaves (Krause et al., 1995). Under field conditions mature leaves have a venation, a V fold of the leaf segments, thus irradiance reaches the leaves at different angles resulting in a significant decrease of the intensity of incident light. Whereas under laboratory conditions juvenile leaves of the seedlings lack the typical V-fold and high irradiance reached the leaves perpendicularly. Leaves in the field showed a further slight folding of the segments during the noon hours perhaps as a means of high irradiance avoidance which was also observed in other plants (Ludlow and Björkman, 1984).

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