Stress physiology of palm trees II. The effect of heavy metals and high irradiance on the photosynthesis of palm Trachycarpus fortunei

INTERNATIONAL JOURNAL OF HORTICULTURAL SCIENCE
AGROINFORM Publishing House, Hungary

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Key words: chlorophyll fluorescence, photoinhibition, heavy metals, Arecaceae, Trachycarpus

Summary: A study was carried out to analyse the individual and combined effects of heavy metal toxicity and high irradiance on the photosynthetic characteristics of young, fully expanded leaves of palm seedling *Trachycarpus fortunei* under laboratory conditions. Heavy metals were found to inhibit both the light and dark reactions of photosynthesis and the inhibition was more affected in the light than in the dark. Single photoinhibitory conditions caused a 60 % decrease in the electron transport activity after 120 min of light exposure which was completely reversible in the dark. In contrast, the combined effect of high light and heavy metal treatment resulted in a 90 % decrease in the activity, but no reversible recovery in the dark could be detected. This indicated that the simultaneous effect of these two stress factors led to irreversible damages of the photosynthetic machinery and as a consequence caused the general destruction of the plant.
Abbreviations and symbols: F_o: initial chlorophyll fluorescence; F_m: maximum total fluorescence; F_v: variable fluorescence; ΔF_i: intermediate level of fluorescence induction; PSII: photosystem 2.

Introduction

Environmental pollutants like heavy metals, organic chemicals, poisoning gases as well as other natural biotic and abiotic stress factors (extreme low and high temperatures, high light and UV radiations, viral and bacterial infections etc.) affect the physiological processes of plants at various levels and as a consequence decrease their growth and productivity (*Tuba* and *Csintalan* 1998, *Grillo* and *Leone* 1995).

Heavy metals as one of the most dangerous environmental pollutants inhibit both light and dark reactions of photosynthesis by interacting with different sites of electron transport and/or affecting the function and structure of various enzymes of the Calvin cycle, (*Krupa* and *Baszynski* 1995). High light irradiance as natural stress factor also inhibits photosynthesis which is generally called photoinhibition. During photoinhibition both the donor and acceptor sides of photosystem 2 (PSII) are damaged, stomata movement is affected and as a consequence of these events CO_2 fixation is inhibited (*Anderson* 1992, *Long* et al. 1994, *Anderson* et al. 1997). Recent studies under Cu toxicity have indicated that heavy metals may induce enhancement of photoinhibition and/or photoinhibition may contribute significantly to heavy metal toxicity (*Patsikka* et al. 1998). Therefore, detailed analysis of physiological responses of economically important crops in such stress conditions is one of the crucial goals of research.

Palm trees populate all continents of our planet and are present at most different geographic zones from the Mediterranean to the tropics (*Uhl* et al. 1987). Their significance is unavoidable in various areas of economy. As food suppliers they are important in agronomy and food industry (*Ruddle* et al. 1978; *Tomlinson* 1990), or as raw material resources in oil (*Davies* et al. 1985; *Hartley* 1967), and textile industries (*Dransfield* 1979). Their importance can also be considered in the "tourism industry" since they are major landscape forming trees of many popular touristic places (*Meerow* 1992). The O_2 producing and CO_2 absorbing capacities of palm tree vegetation also cannot be left out of consideration (*Jayasekara* and *Jayasekara* 1995). Besides the works of *Chase* and *Broschat* (1991) and *Neményi* et. al (1997), no other study deals with the sensitivity or tolerance of palms to heavy metal pollution.

Although palms mostly populate areas of the planet where the high light irradiance is predominant, interestingly no studies concerning with the photoinhibitory responses of palms are available in the literature.

In the present study, therefore, we have attempted to analyse the effect of heavy metals and high irradiance as stress factors on the photosynthetic machinery of palm *Trachycarpus fortunei*. The inhibition of photosynthesis was characterised by the alteration of the fluorescence induction parameters and by the changes in the protein composition of thylakoids. The degree of the photoinhibitory damage was estimated by the dark recovery of the F_v/F_m ratio after photoinhibition.

Material and methods

Plant material: Greenhouse grown 6 month old seedlings of Chinese windmill palm, by other name Chusan palm (*Trachycarpus fortunei* Wendl) were used for measurements. Before the application of heavy metals and/or light exposure to photoinhibitory conditions, the seedlings were placed into a hydroponic half Hoagland's culture medium *(Droppa* et al. 1985) and acclimated for a week in a growth cabinet (Weiss Technik Bioclim 1600SP) where the illumination followed 16 h light/8 h dark cycles, the light intensity was 150 µmol m^{-2} s^{-1} and the day/night temperature was constantly 28 C.

Heavy metal treatment: After one week of acclimation the seedlings were transferred at the beginning of the dark period to a fresh half Hoagland`s medium (pH 5.5), supplemented with different heavy metals. The applied concentration ranges were as follows: 5-6.5 mM Cd^{2+}, 6.5-20 mM Cu^{2+}, 20-40 mM Ni^{2+}, 40-60 mM Zn^{2+} *(Neményi* et al. 1997).

Photoinhibition and recovery: Leaves of seedlings were placed horizontally on wet filter paper. Light source was supplied by two 500 W metal-halide lamps. Photon flux density was 1.6–1.7 mmol m^{-2} s^{-1} upon exposure on the adaxial side of leaves. Air temperature was held at 28 °C by placing a cold water bath between the light source and the leaves. The extent of photoinhibition was estimated after 30, 60, 90 and 120 min of illumination. For the recovery from photoinhibition, plants were placed in complete darkness at 25 °C.

Gel electrophoresis of thylakoid membrane proteins: Electrophoretic analysis of chloroplast proteins was carried out using the protocol of *Chua* (1980).

Chlorophyll fluorescence: Chlorophyll fluorescence was measured with a portable fluorometer (Plant Efficiency Analyser, Hansatech Ltd., King`s Lynn, U.K.). After 15 min of dark adaptation samples were excited with an actinic light intensity of 1.8 mmol m^{-2} s^{-1} at 650 nm. Fluorescence was detected for 120 sec and the generally used parameters: the F_v/F_m; F_d/F_s and $\Delta F_i/F_v$ ratios were calculated (Bolhár-Nordenkampf and Öquist, 1993).

Results and discussion

The efficiency of the photosynthetic machinery can be characterised by the various parameters of the fluorescence induction kinetics measured upon illumination of intact leaves *(Fig. 1)*. Amongst them the ratio of the maximal and variable fluorescence (F_v/F_m) has a typical range of 0.75–0.85 and it is proportional with the quantum yield of photochemistry, while the ratio of the fluorescence decrease to the steady state level (F_d/F_s) characterises the carboxylation capacity of the leaf (Bolhár-Nordenkampf et al. 1994). The intermediate fluorescence level (F_i) is related to the non-functional primary quinone acceptors (Q_A) of PSII, thus the $\Delta F_i/F_v$ ratio represents the degree of inhibition within PSII. The stress induced alteration in the photosynthetic activity is well reflected by the changes of these parameters.

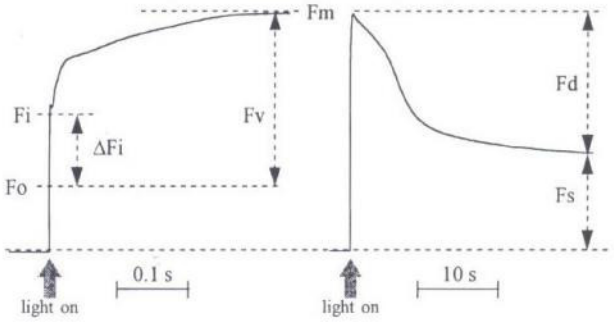

Figure 1 Typical traces of the fast and slow fluorescence induction kinetics of *T. fortunei* leaves. F_o : non-variable fluorescence; F_i : intermediate fluorescence level; F_m : total fluorescence yield; F_v : variable fluorescence yield; F_d : fluorescence decrease; F_s : steady state fluorescence level.

As shown in *Fig. 2*, all heavy metals applied decreased both the electron transport activity and the carboxylation capacity of *T. fortunei* leaves represented by the F_v/F_m and F_d/F_s ratios, respectively. All treatments can be defined with a single decreasing curve, except the higher (20mM) concentration of Cu, which shows a more defined decline. This is probably due to the fast and complete degradation of the photosynthetic electron transport chain due to the high Cu concentration build up within the plant cells *(Neményi* et al. 1997).

This seems to be verified by the proportional increase of the intermediate fluorescence level to the variable fluorescence ($\Delta F_i/F_v$). As shown in *Fig. 3* all heavy metal treatments exhibited a gradual increase of the $\Delta F_i/F_v$ ratio by the progress of the incubation time which indicated the enhanced inhibition of PSII *(Hideg* et al. 1986). In contrast, the high, 20 mM Cu treatment resulted in a much faster increase in the $\Delta F_i/F_v$ ratio and after 24 hours the PSII activity was completely inhibited, which was followed by a marked decline in the DF_i/F_v ratio. This decline can be explained by the general disorganisation of the chloroplasts due to the high Cu concentration induced oxidative damage of the thylakoid membrane *(Droppa* and *Horváth* 1990;

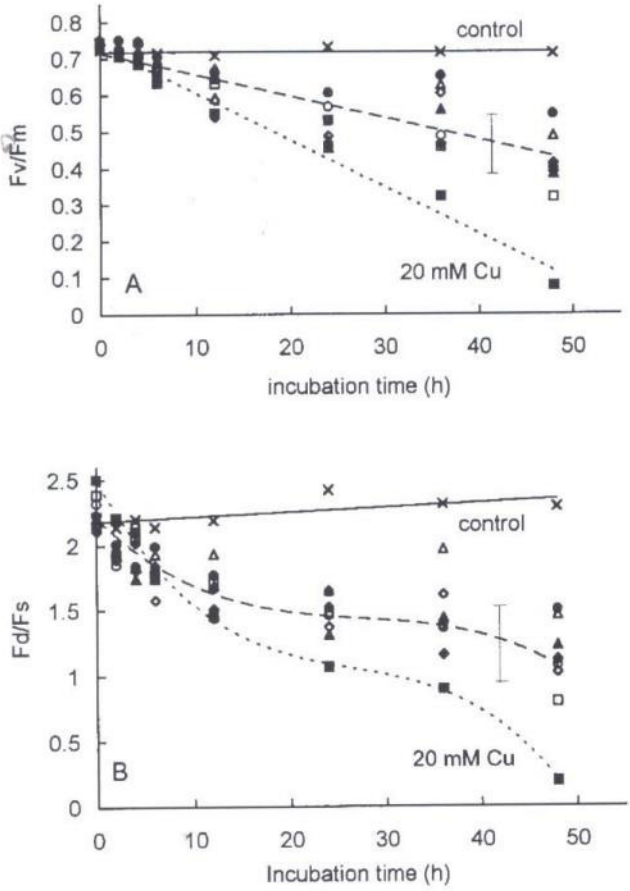

Figure 2 Changes of the photosynthetic activity of *T. fortunei* leaves treated with various heavy metals as characterized by the F_v/F_m and F_d/F_s ratios of fluorescence induction kinetics. Bar represents the average standard deviation of data points. Control (X); 5 mM Cd^{2+} (O); 6.5 mM Cd^{2+}(●); 6.5 mM Cu^{2+} ( ); 20 mM Cu^{2+}(■); 20 mM Ni^{2+} (Δ); 40 mM Ni^{2+} (▲); 40 mM Zn^{2+} (◊); 60 mM Zn^{2+} (♦)

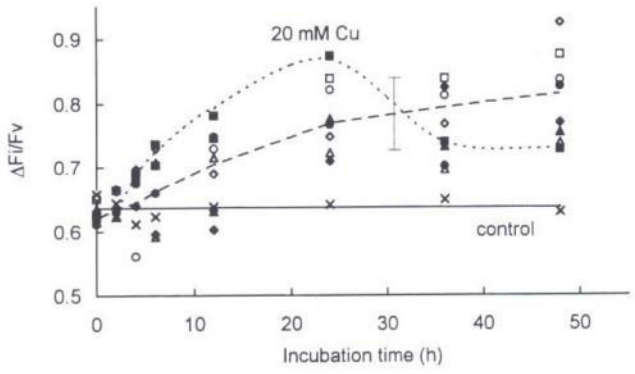

Figure 3 The extent of the inhibition within photosystem 2 of heavy metal treated *T. fortunei* leaves. Bar represents the average standard deviation of data points. Control (X); 5 mM Cd^{2+} (O); 6.5 mM Cd^{2+}(●); 6.5 mM Cu^{2+} ( ); 20 mM Cu^{2+}(■); 20 mM Ni^{2+} (Δ); 40 mM Ni^{2+} (▲); 40 mM Zn^{2+} (◊); 60 mM Zn^{2+} (♦)

Krupa and *Baszynski* 1995). In agreement with our conclusion, the polypeptide pattern of the heavy metal treated samples at 48 hours also showed that only the high concentration of Cu caused the general destruction of thylakoid proteins *(Fig. 4)*. Other heavy metals had only minor effects (data not shown) which can be accounted to partial inhibition of PSII. This result compares well to the earlier observation, that only 20 mM Cu resulted in a dramatic decrease in the chlorophyll content of *T. fortunei* leaves treated with various heavy metals *(Neményi* et al. 1997). The reason why the lower concentration (6.5 mM) of Cu did not decrease the chlorophyll content significantly is explained by the fact that the amount of accumulated Cu in these leaves after 48 hours still did not exceed the Cu concentrations of leaves exposed to high Cu concentration for 12 h *(Neményi* et al.).

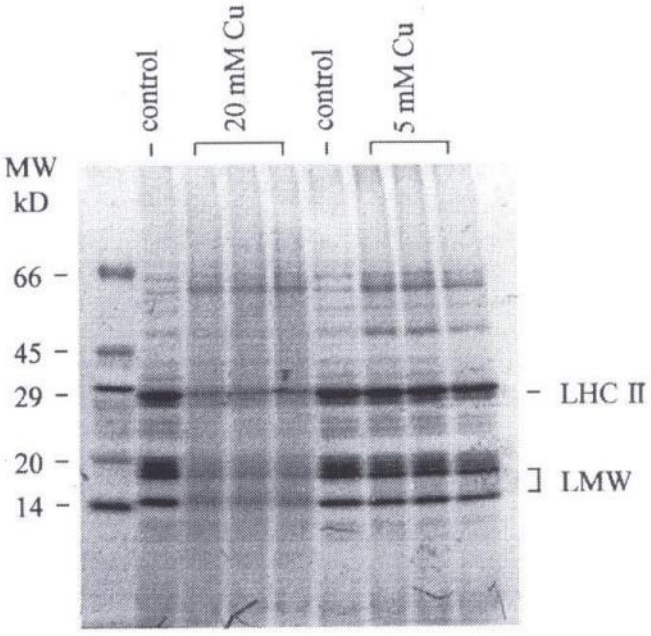

Figure 4 Gel electrophoretic patterns of Cu poisoned *T. fortunei* thylakoid membranes after 48 hours of heavy metal treatment. LHC: Light harvesting pigment-protein complex, LMW: low molecular weight polypeptides, MW: molecular weight.

From the data we have concluded that all heavy metal applied inhibit both the light and dark reactions of photosynthesis and they have a specific inhibitory site within PSII. The high, 20 mM Cu concentration caused a general destruction of the photosynthetic membrane system. From *Fig. 2* it is also evident that in the decreasing fluorescence curves there is relaxation between the 16th and 24th hour of treatment. this coincides with the dark period of the 16h light/ 8h dark photoperiod and indicates that the effect of the heavy metals is more pronounced under illumination than in the dark. This result fits to the general view that photodestruction caused by the light generated oxidative free radicals *(Droppa* and *Horváth* 1990; *Hideg* et al. 1994). Since the heavy metal induced inhibition of photosynthesis was more pronounced in the light than in the dark, the synergistic effect of photoinhibition and heavy metal treatment was analysed.

In control leaves high light (1.6–1.7 mmol m^{-2} s^{-1})

photoinhibitory conditions caused a substantial, 60% decrease in F_v/F_m values after 120 min of light exposure, whereas the combined effect of high light and heavy metal treatments after the same exposure time resulted in a 90% decrease of the fluorescence rise *(Fig. 5)*. The 10–15% decrease in the F_v/F_m ratio of the heavy metal treated leaves before high light exposure was due to the moderate heavy metal inhibition in the dark. The dark recovery which is a measure of the reversible inhibition occurred in 24 or 48 hours after 30 or 60 min of photoinhibitory treatment respectively, in either the absence or presence of heavy metals. While under heavy metal toxicity the photoinhibitory exposure of 120 min caused irreparable damage and no recovery occurred. This result fits well to earlier observations where photoinhibition was found to enhance the heavy metal toxicity *(Patsikka* et al. 1998). Our results indicate that the synergetic effect of these two stress factors results in the irreversible damage of the photosynthetic apparatus and as a consequence the destruction of the plant.

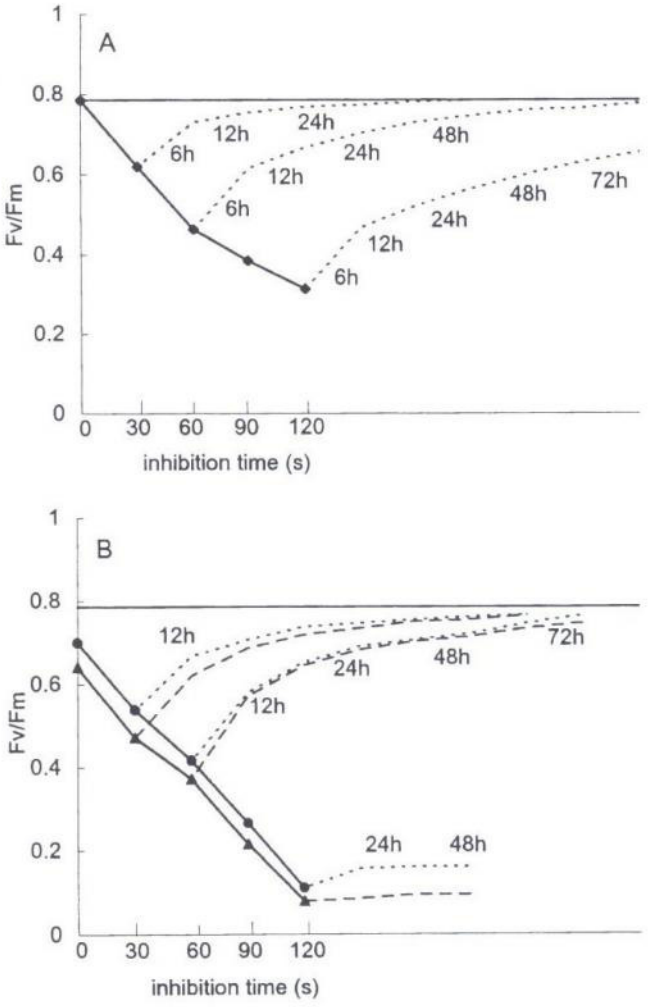

Figure 5 High light induced photoinhibition (solid line) and its dark recovery (dashed line) in control (A) and heavy metal treated (B) leaves of *T. fortunei*.

Acknowledgement: This work was partially supported by the Hungarian National Science Research Fund (T 026075, T026078), the Hungarian-Spanish Intergovernmental Exchange Program (E-19/97) and the Hungarian Academy of Sciences.

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