PHOTOSYNTHETICA 45 (2): 208-213, 2007

Photosystem 2 activity of *Citrus volkameriana* (L.) leaves as affected by Mn nutrition and irradiance

I.E. PAPADAKIS[*,***], A. GIANNAKOULA[**], C.P. ANTONOPOULOU[*], M. MOUSTAKAS[**], E. AVRAMAKI[*], and I.N. THERIOS[*]

Department of Horticulture, School of Agriculture, Aristotle University, 541 24 Thessaloniki, Greece[*]
Department of Botany, School of Biology, Aristotle University, 541 24 Thessaloniki, Greece[**]

Abstract

Citrus volkameriana (L.) plants were grown for 43 d in nutrient solutions containing 0, 2, 14, 98, or 686 μM Mn (Mn_0, Mn_2, Mn_{14}, Mn_{98}, and Mn_{686}, respectively). To adequately investigate the combined effects of Mn nutrition and irradiance on photosystem 2 (PS2) activity, irradiance response curves for electron transport rate (ETR), non-photochemical quenching (q_N), photochemical quenching (q_P), and real photochemical efficiency of PS2 (Φ_{PS2}) were recorded under 10 different irradiances (66, 96, 136, 226, 336, 536, 811, 1 211, 1 911, and 3 111 μmol m^{-2} s^{-1}, I_{66} to I_{3111}, respectively) generated with the *PAM-2000* fluorometer. Leaf chlorophyll content was significantly lower under Mn excess (Mn_{686}) compared to Mn_0; its highest values were recorded in the treatments Mn_2–Mn_{98}. However, ETR and Φ_{PS2} values were significantly lower under Mn_0 compared to the other Mn treatments, when plants were exposed to irradiances ≥96 μmol m^{-2} s^{-1}. Furthermore, Mn_0 plants had significantly higher values of q_N and lower values of q_P at irradiances ≤226 and ≥336 μmol m^{-2} s^{-1}, respectively, than those grown under Mn_2–Mn_{686}. Irrespective of Mn treatment, the values of Φ_{PS2} and q_N decreased, while those of q_P increased progressively by increasing irradiance from I_{136} to I_{3111}. Finally, Mn_2–Mn_{98} plants were less sensitive to photoinhibition of photosynthesis (≥811 μmol m^{-2} s^{-1}) than the Mn_{686} (≥536 μmol m^{-2} s^{-1}) and Mn_0 (≥336 μmol m^{-2} s^{-1}) ones.

Additional key words: chlorophyll fluorescence; deficiency; photoinhibition; photosystem 2; toxicity.

Introduction

Mn deficiency and toxicity are two nutritional disorders affecting the growth of many plant species. Most studies concerning these two disorders have been limited to evaluations of growth, yield, visual symptoms, and photosynthetic rates. In general, photosynthetic rates, growth, and yield of plants suffer from Mn deficiency and those in plants grown in soils with excessive Mn availability are lower compared to plants grown under adequate Mn (Ohki 1981, 1985, Nable *et al.* 1988, Macfie and Taylor 1992, González and Lynch 1997, 1999, Subrahmanyam and Rathore 2000, Singh *et al.* 2001, Henriques 2003). However, no information exists concerning the effects of Mn toxicity on *in vivo* photosystem 2 (PS2) activity which can be measured easily using fluorometers, in spite of their increased physiological significance. Instead, little information exists about the characteristics of PS2 of some plant species suffering from Mn deficiency (Jiang *et al.* 2002, Henriques 2003). Furthermore, no study exists in which both the effects of Mn deficiency and toxicity on various PS2 activity parameters have been investigated together under the same experimental conditions. This is why we tried to find whether the Mn status of plants affects the irradiance in which the phenomenon of photoinhibition occurs.

Chlorophyll (Chl) fluorescence measurement is rapid, extremely sensitive, and non-destructive since it can be performed on intact, attached leaves. It is an important tool in the study of photosynthesis, in particular the functioning of PS2 (Schreiber *et al.* 1995). In general, fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson 2000). The fluorescence measurements also allow predictions of specific physical, energetic, and dynamic parameters of a sample under a given situation. The yield of Chl fluorescence emission from photosynthetic organisms is determined by two distinct processes, photochemical (q_P) and non-photochemical (q_N) quenching (Genty *et al.* 1989, Havaux *et al.* 1991). Efforts to quantify the relationships between these

Received 22 March 2006, *accepted* 31 August 2006.
[***]Corresponding author; fax: +30-2310-402-705, e-mail: jpapad2004@yahoo.gr
Acknowledgements: We thank the State Scholarships Foundation of Greece (I.K.Y.) for supporting this work.

fluorescence quenching processes and electron transport *in vivo* have led to the proposal that a component of non-photochemical quenching associated with activation of thylakoids is associated with a thermal deactivation of PS2 excitation, and consequently produces a decrease in the quantum efficiency of PS2 photochemistry (Genty *et al.* 1990). Overall, this technique improved understanding of photochemical and non-photochemical processes occurring in thylakoid membranes of chloroplasts (Roháček 2002). Finally, a relatively new fluorescence method assessing the state of the photosynthetic apparatus of plants is the photon response curves where many physiological parameters associated with the function of PS2 are recorded across different irradiances (White and Critchley 1999).

The objective of the present study was to investigate the effects of Mn nutrition on PS2 activity of *C. volkameriana* (L.) plants under different irradiances.

Materials and methods

Plants and Mn treatments: Seedlings of *Citrus volkameriana* (L.) with a pair of leaves were grown in white plastic pots containing 1 200 cm^3 of modified Hoagland No. 2 nutrient solution (Hoagland and Arnon 1950), prepared with double distilled water. All macronutrients and micronutrients, except Mn, were supplied at half strength of this nutrient solution. The Mn was supplied at six concentrations (treatments), *i.e.* 0, 2, 4, 98, and 686 μM (Mn_0, Mn_2, Mn_{14}, Mn_{98}, and Mn_{686}, respectively). For each treatment, 5 pots (replications) were used. In each pot, 5 seedlings were grown. The seedlings were grown for 43 d in a growth chamber under constant environmental conditions (temperature 25±1 ºC; irradiance 270–300 μmol m^{-2} s^{-1}; light : dark, 16 : 8 h), which were controlled electronically. The liquid solution (pH 5.8) of each pot was aerated on a 24-h basis with air pumps. The solution volume was maintained constant by daily addition of double-distilled water to cover the quantity of water lost by transpiration. Every fourth day, the solutions were renewed completely.

Chl fluorescence: Forty-three days after the beginning of Mn treatments, the youngest and fully expanded leaf blade of 5 plants from each treatment was used for determination of various parameters related to PS2 activity. *In vivo* PS2-Chl fluorescence was measured by a modulated (1.6 kHz) and low-intensity beam from light-emitting diodes (excitation wavelength 655 nm, detection above 700 nm) using a portable pulse-amplitude-modulated fluorometer (*PAM-2000*; *Walz*, Effeltrich, Germany), as described by Schreiber *et al.* (1986). The minimum fluorescence yield (F_0) of the plants adapted to darkness was determined under weak red modulated radiation. The mid-part of the front was held in the leaf clip of the fluorometer at a standard distance from the optic fibre probe and a weak 5-s far-red (735 nm) pulse was sent to fully oxidize the electron transport chain. The maximum fluorescence yield (F_m) of the dark-adapted plants was reached by exposing PS2 to a saturating pulse (0.8 s) of "white light". The difference between F_m and F_0 gave variable fluorescence (F_v). The maximum quantum yield of PS2 photochemistry was calculated as the ratio of variable fluorescence to maximal fluorescence (F_v/F_m) and represents the efficiency of open PS2 in the dark-adapted state. We also calculated the ratio between the parameters F_v and F_0 (F_v/F_0).

After these dark measurements, the plants were exposed to increasing actinic irradiances (66, 96, 136, 226, 336, 536, 811, 1 211, 1 911, and 3 111 μmol m^{-2} s^{-1} marked as I with the respective index). At the end of each irradiation period that lasted 60 s, the operating PS2 efficiency (Φ_{PS2}), the electron transport rate (ETR), q_P and q_N were determined. The value of Φ_{PS2}, which is the average photochemical efficiency of PS2 units in the light (including closed and open ones), was determined by the equation: $\Phi_{PS2} = \Delta F/F_m' = (F_m' - F_t)/F_m'$ (Genty *et al.* 1989), with measurements under actinic irradiation of the steady state fluorescence yield (F_t) and of the maximum fluorescence yield (F_m') obtained using a 0.8-s saturating pulse. Furthermore, ETR and thus the overall photosynthetic capacity *in vivo* (Genty *et al.* 1989) was calculated by the equation: $ETR = \Phi_{PS2} \times PFDa \times 0.5$, where PFDa was the absorbed photosynthetic flux density [μmol m^{-2} s^{-1}] measured using an integrating sphere, and 0.5 is a constant that accounts for partitioning of energy between PS2 and PS1 (Maxwell and Johnson 2000). Furthermore, q_N, which is related to energy dissipation by other means than photochemistry and fluorescence, was calculated according to the equation: $q_N = (F_m - F_m')/F_m'$, using the initial F_m measured after the long darkness period and using the F_m' measured after irradiation (Bilger and Björkman 1990). Another widely used fluorescence parameter measuring photochemistry is q_P which was calculated as: $q_P = (F_m' - F_t')/(F_m' - F_0')$ (Maxwell and Johnson 2000).

Chl content: Chl of the same leaves, which were used for the measurements of various fluorescence parameters, was extracted with ethanol (96 %) after incubation in a water bath (78 ºC). Chl amount was calculated according to Wintermans and Mots (1965) and expressed on a dry mass (DM) basis.

Statistical analysis: The data were subjected to analysis of variance (ANOVA) using the *SPSS 11.0.1 for Windows* statistical package (*SPSS*, Chicago, USA). For comparison of the means, the Duncan's multiple range test ($p \leq 0.05$) was employed.

Results

Mn deficiency and toxicity symptoms in the leaves: At the end of the experiment, Mn_0 and Mn_{686} plants presented visible symptoms of Mn deficiency (leaves with mild interveinal chlorosis) and toxicity (small, crinkled, and very chlorotic leaves), respectively.

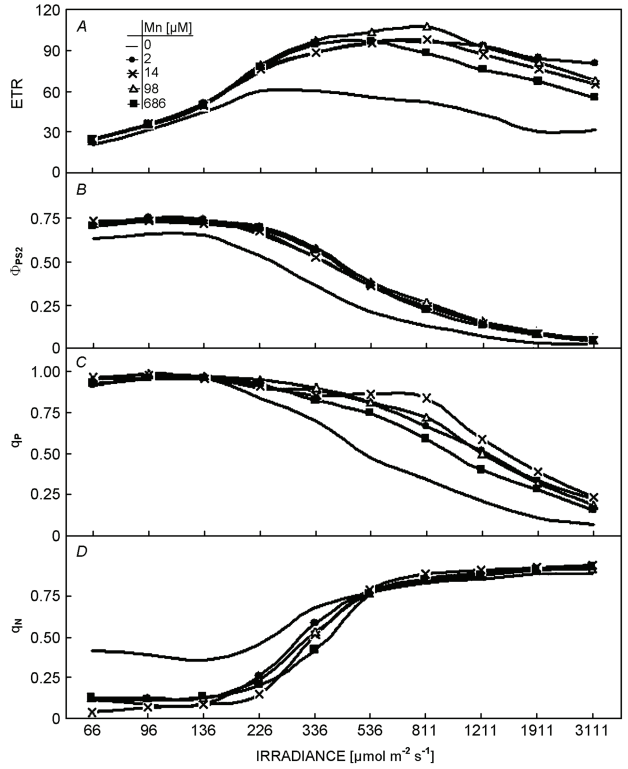

Fig. 1. Effects of Mn concentration in the nutrient solution [μM] and irradiance [μmol m^{-2} s^{-1}] on the fluctuation of ETR (*A*), Φ_{PS2} (*B*), q_P (*C*), and q_N (*D*) values.

Chl fluorescence parameters measured in the leaves of dark-adapted plants, *i.e.* F_v, F_m, F_0, F_v/F_m, and F_v/F_0, were not significantly affected by Mn concentration in the nutrient solution (data not shown). The fluctuation of the other fluorescence parameters (ETR, Φ_{PS2}, q_N, and q_P) in relation to 10 different irradiances and to Mn concentration in the nutrient solution are shown in Fig. 1. The analytical values of these parameters with the statistical analysis concerning all irradiances across the different Mn treatments are also presented in the Table 1.

For all Mn treatments, the increment of irradiance resulted initially in a progressive increase of ETR up to a maximum value, which was Mn-dependent; further increase of irradiance caused a progressive decrease of ETR (Fig. 1). The maximum ETR values were recorded at I_{336}, I_{536}, and I_{811} in Mn_0, Mn_{686}, and Mn_2–Mn_{98} plants, respectively. In general, ETR values were significantly lower in the treatment Mn_0 compared to the other Mn treatments for all tested irradiances except for I_{66}, where

Table 1. ETR, Φ_{PS2}, q_N, and q_P values as affected by Mn concentration [μM] in the nutrient solution and irradiance I [μmol m^{-2} s^{-1}] where the plants were exposed. For each irradiance, the values of each parameter marked with the same letter(s) do not differ from each other for $p \leq 0.05$ (Duncan's multiple range test); $n = 5$.

Irradiance	Mn	ETR	Φ_{PS2}	q_P	q_N
I_{66}	Mn_0	20.8a	0.631a	0.956a	0.418b
	Mn_2	23.4a	0.708a	0.916a	0.118a
	Mn_{14}	24.4a	0.738a	0.965a	0.040a
	Mn_{98}	23.9a	0.724a	0.942a	0.125a
	Mn_{686}	23.3a	0.706a	0.920a	0.124a
I_{96}	Mn_0	31.6a	0.659a	0.950a	0.395b
	Mn_2	36.1b	0.751b	0.964a	0.085a
	Mn_{14}	35.5b	0.739b	0.973a	0.070a
	Mn_{98}	36.3b	0.755b	0.982a	0.126a
	Mn_{686}	35.3b	0.736b	0.957a	0.116a
I_{136}	Mn_0	44.5a	0.655a	0.958a	0.355b
	Mn_2	50.7b	0.745b	0.957a	0.091a
	Mn_{14}	49.2b	0.723b	0.956a	0.084a
	Mn_{98}	50.6b	0.744b	0.969a	0.127a
	Mn_{686}	49.5b	0.728b	0.950a	0.133a
I_{226}	Mn_0	60.3a	0.533a	0.837a	0.457b
	Mn_2	75.0b	0.664b	0.901a	0.256a
	Mn_{14}	76.2b	0.675b	0.909a	0.152a
	Mn_{98}	79.5b	0.704b	0.947a	0.237a
	Mn_{686}	77.8b	0.689b	0.918a	0.204a
I_{336}	Mn_0	60.5a	0.360a	0.692a	0.679a
	Mn_2	88.5b	0.527b	0.879b	0.580a
	Mn_{14}	88.8b	0.529b	0.852b	0.513a
	Mn_{98}	96.9b	0.577b	0.900b	0.530a
	Mn_{686}	94.7b	0.564b	0.821b	0.419a
I_{536}	Mn_0	56.1a	0.209a	0.478a	0.774a
	Mn_2	96.0b	0.358b	0.805b	0.780a
	Mn_{14}	95.9b	0.358b	0.863b	0.791a
	Mn_{98}	103.6b	0.387b	0.816b	0.763a
	Mn_{686}	96.1b	0.359b	0.746b	0.754a
I_{811}	Mn_0	52.1a	0.129a	0.342a	0.825a
	Mn_2	96.7bc	0.239bc	0.661b	0.849a
	Mn_{14}	97.9bc	0.241bc	0.838c	0.880a
	Mn_{98}	107.3c	0.265c	0.723bc	0.847a
	Mn_{686}	87.9b	0.217b	0.587b	0.842a
I_{1211}	Mn_0	42.5a	0.070a	0.209a	0.852a
	Mn_2	93.2c	0.154c	0.516bc	0.888a
	Mn_{14}	86.7bc	0.144bc	0.589c	0.905a
	Mn_{98}	92.3c	0.153c	0.500bc	0.879a
	Mn_{686}	75.7b	0.125b	0.399b	0.876a
I_{1911}	Mn_0	30.3a	0.032a	0.109a	0.883a
	Mn_2	84.4c	0.089c	0.336b	0.904a
	Mn_{14}	76.8bc	0.081bc	0.391b	0.922a
	Mn_{98}	81.0c	0.085c	0.323b	0.903a
	Mn_{686}	67.4b	0.075b	0.285b	0.903a
I_{3111}	Mn_0	31.4a	0.021a	0.071a	0.884a
	Mn_2	80.5d	0.052d	0.230b	0.922a
	Mn_{14}	65.7c	0.042c	0.238b	0.936a
	Mn_{98}	67.6c	0.043c	0.187b	0.917a
	Mn_{686}	54.4b	0.035b	0.159b	0.921a

non-significant differences were observed between the Mn treatments (Table 1).

The increase of irradiance caused an analogous decrease of real photochemical efficiency of PS2 (Φ_{PS2}) and q_P, irrespective of Mn concentration in the nutrient solution. The opposite was observed concerning the values of q_N; they were increased with increase of irradiance (Fig. 1). For irradiances ranging from I_{96} to I_{3111}, Φ_{PS2} values were significantly lower in the plants grown in solutions containing 0 μM Mn compared to the other Mn treatments. Finally, Mn_0 plants had considerably higher values of q_N at irradiances ≤226 μmol m^{-2} s^{-1} and lower values of q_P at irradiances ≥336 μmol m^{-2} s^{-1}, compared to the treatments Mn_2–Mn_{686} (Table 1).

Leaf Chl content per dry mass was significantly lower in the Mn_{686} plants than in the Mn_0 ones. Contrary to these two Mn treatments, significantly higher Chl content was found in the Mn_2, Mn_{14}, and Mn_{98} leaves; the differences between these treatments were non-significant (Fig. 2).

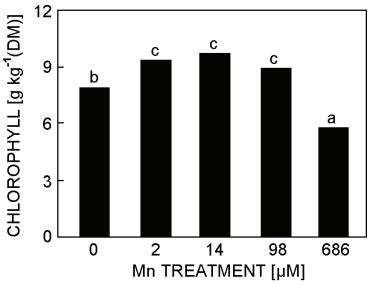

Fig. 2. Total chlorophyll contents in the Mn_0–Mn_{686} leaves, $n = 5$. The means marked with the same letter(s) do not differ significantly from each other for $p \leq 0.05$ (Duncan's multiple range test).

Discussion

The values of ETR and Φ_{PS2} were considerably lower in Mn_0 plants than in the Mn_2–Mn_{686} ones for I_{96}–I_{3111}. A large part of Mn entering into cytoplasm moves and binds in the outer side of thylakoid membranes of chloroplasts (González and Lynch 1999, Lidon and Teixeira 2000a,b), affecting their structure and function (Nable *et al.* 1988, Kitao *et al.* 1997). Indeed, the portion of chloroplast Mn binding strongly in thylakoids participates in the water splitting reaction; while the portion of chloroplast Mn binding very strongly (more strongly than the previous mentioned portion) in thylakoid membranes affects the function of the thylakoid bound electron transport chain (Kabata-Pendias and Pendias 2001). Therefore, the decreased ETR and Φ_{PS2} at Mn_0 was rather related to the negative effects of low Mn availability on the reaction of water photolysis (Clairmont *et al.* 1986, Marschner 1995, Fageria *et al.* 1997, González *et al.* 1998) and on the functionality of the thylakoid-bound electron transport chain from PS2 to PS1. The analytical fluorescence data presented in Table 1 show that up to I_{226}, q_N was significantly higher under Mn_0 compared to the other Mn treatments. In other words, the decreased Φ_{PS2} of Mn deficient plants (Mn_0) under low irradiances ($\leq I_{226}$) was due to the fact that high percentage of excess photon energy of PS2 was dissipated as heat (q_N); under such irradiances there were no significant differences between the five Mn treatments concerning the values of q_P. The increased losses of excitation energy of PS2 in the form of heat are considered to be a photoprotection mechanism of plant cells. The quantity of Mn in leaf cells of the Mn_0 plants was not enough to cover the needs of photosynthetic apparatus and thus the plants were adapted to I_{270}–I_{300} existing in the growth chamber. This means that Mn-deficient plants developed a mechanism by which the excitation energy of PS2 was dissipated as heat, protecting thus their photosynthetic apparatus from photoinhibition under I lower than I_{270}–I_{300}. In general, the decrease of Φ_{PS2} and increase of q_N is associated with the xanthophyll pigment cycle that provides photoprotection of photosystem by the dissipation of excess absorbed photon energy (Demmig-Adams and Adams 1992). Furthermore, the fluorescence parameters (F_v/F_m, F_v/F_0) measured in dark-adapted *C. volkameriana* plants were not affected significantly by the change of Mn concentration in the nutrient solution. Yoo *et al.* (2003) also reported that in many studies a decrease in the Φ_{PS2} has been observed but with no changes in F_v/F_m. In these studies, the reduction of measured efficiency in the light but the maintenance of PS2 efficiency in dark-adapted leaves was also coupled with an increase in q_N. This suggests that accessory pigments, particularly carotenoids, may participate in photoprotection of PS2. Instead, at I≥336 μmol m^{-2} s^{-1}, the reduced values of Φ_{PS2} that were observed in *C. volkameriana* Mn_0 plants compared to the other Mn treatments, could be probably ascribed to the increase of the percentage of close-reduced PS2 reaction centres (decreased values of q_P) due to photoinhibition. The closed reaction centres can not utilize effectively any part of the photon energy.

The irradiance at which the highest ETR was recorded or, in other words, the point of photon energy saturation beyond which any further increase of electromagnetic irradiation results in reduced ETR, was significantly affected by Mn treatment. Indeed, the photoinhibition of photosynthesis was observed at lower irradiances (I_{336}) in the treatment Mn_0 than Mn_{686} (I_{536}). The corresponding irradiance for all the other Mn treatments (Mn_2–Mn_{98}) was I_{811}. The higher sensitivity of Mn_0 plants to photoinhibition could be mainly ascribed to the previously mentioned negative effects of low Mn on photosynthesis, and secondly to other causes such as the lower leaf Chl content. Since no differences were observed between Mn_2, Mn_{14}, Mn_{98}, and Mn_{686} concerning the values of q_P and q_N, the fact that the photoinhibition in the Mn_{686}

plants occurred under lower irradiance (I_{536}) than in the Mn_2–Mn_{98} plants (I_{811}) was mainly due to the leaf Chl content, that was too low under Mn_{686}. If some other limiting factors, except Chl content, existing under Mn_{686}, their negative effects on plant photosynthetic performance would be expected at lower irradiances too, as it happened in the treatment Mn_0. Furthermore, González and Lynch (1997, 1999) suggest that Mn toxicity diminishes photosynthetic capacity of common bean leaves through a reduction of total Chl content.

Mn is not a structural component of Chl molecule and according to Campbell and Nable (1988) no evidence exists for its direct involvement in the biochemical pathways of biosynthesis and/or destruction of Chl. However, leaves with low Mn content are chlorotic because of Chl losses (Lerer and Bar-Akiva 1979, Kriedemann *et al.* 1985, Ohki 1985, Mercer and Graham 1987, Singh *et al.* 2001, Henriques 2003). The improper function under Mn deficiency not only of the water splitting reaction but also of the photosynthetic electron transport chain increases the probability of oxidative stress for leaf chloroplasts. Under such stress, molecular O_2 operates as an alternative acceptor for non-utilized electrons and photon energy (Cakmak and Romheld 1997), resulting thus in the generation of reactive oxygen species (Cakmak 1994). The ability of reactive oxygen species to cause photooxidative damages to organic molecules could probably explain the reductions of leaf Chl content under Mn_0. On the other hand, Horst (1988) reported that under high Mn concentrations, Chl biosynthesis is depressed, since Fe availability in the leaf tissues decreases sharply.

In general, the values of ETR, Φ_{PS2}, q_N, and q_P did not differ significantly between the treatments Mn_2, Mn_{14}, Mn_{98}, and Mn_{686}, although leaf Chl contents were considerably lower under Mn_{686} than under Mn_2–Mn_{98}. In other words, despite the severe chlorosis observed in the Mn_{686} leaves, the remaining leaf Chl was sufficient for the effective absorption and utilization of irradiances up to I_{536}. However, at higher irradiances ($\geq I_{811}$), the values of Φ_{PS2} and ETR in the Mn_{686} plants were somewhat lower than those of Mn_2, Mn_{14}, and Mn_{98} plants; the difference became greater as irradiance increased. Furthermore, Chl contents and saturation irradiance in the treatments Mn_2, Mn_{14}, and Mn_{98} had considerably higher values than those of the Mn_{686} plants. Consequently, at $I \geq 811$ μmol m^{-2} s^{-1} Chl of the Mn_{686} leaves was not enough to absorb the photon energy at the same degree as in the Mn_2, Mn_{14}, and Mn_{98} plants. The low irradiances (I_{270}–I_{300}) in the growth chamber, where the plants were grown for 43 d, did not contribute to the increment of cell oxidative stress. Probably, this was the primary reason explaining why the functional integrity of photosynthetic apparatus was not affected negatively under Mn_{686}.

References

Bilger, W., Björkman, O.: Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. – Photosynth. Res. **25**: 173-185, 1990.

Cakmak, I.: Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves, but not in phosphorous-deficient leaves. – J. exp. Bot. **45**: 1259-1266, 1994.

Cakmak, I., Romheld, V.: Boron deficiency-induced impairments of cellular fractions in plants. – Plant Soil **193**: 71-83, 1997.

Campbell, L.C., Nable, R.O.: Physiological functions of manganese in plants. – In: Graham, R.D., Hannam, J., Uren, N.C. (ed.): Manganese in Soils and Plants. Pp. 139-154. Kluwer Academic Publishers, Dordrecht 1988.

Clairmont, K.B., Hagar, W.G., Davis, E.A.: Manganese toxicity to chlorophyll synthesis in tobacco callus. – Plant Physiol. **80**: 291-293, 1986.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Fageria, N.K., Baligar, V.C., Jones, C.A.: Growth and Mineral Nutrition of Field Crops. – Marcel Dekker, New York 1997.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Genty, B., Harbinson, J., Briantais, J.-M., Baker, N.R.: The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem 2 photochemistry in leaves. – Photosynth. Res. **25**: 249-257, 1990.

González, A., Lynch, J.P.: Effects of manganese toxicity on leaf assimilation of contrasting common bean genotypes. – Physiol. Plant. **101**: 872-880, 1997.

González, A., Lynch, J.P.: Subcellular and tissue compartmentation in bean leaves under Mn toxicity stress. – Aust. J. Plant Physiol. **26**: 811-822, 1999.

González, A., Steffen, K.L., Lynch, J.P.: Light and excess manganese – Implications for oxidative stress in common bean. – Plant Physiol. **118**: 493-504. 1998.

Havaux, M., Strasser, R.J., Greppin, H.: A theoretical and experimental analysis of the q_P and q_N coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. – Photosynth. Res. **27**: 41-55, 1991.

Henriques, F.S.: Gas exchange, chlorophyll *a* fluorescence kinetics and lipid peroxidation of pecan leaves with varying manganese concentrations. – Plant Sci. **165**: 239-244, 2003.

Hoagland, D.R., Arnon, D.I.: The water culture method for growing plants without soil. – California Agriculture Experimental Station Circular 374, 1950.

Horst, W.J.: The physiology of manganese toxicity. – In: Graham, R.D., Hannam, J., Uren, N.C. (ed.): Manganese in Soils and Plants. Pp. 175-188. Kluwer Academic Publishers, Dordrecht 1988.

Jiang, C.-D., Gao, H.-Y., Zou, Q.: Characteristics of photosynthetic apparatus in Mn-starved maize leaves. – Photosynthetica **40**: 209-213, 2002.

Kabata-Pendias, A., Pendias, H.: Trace Elements in Soils and Plants. – CRC Press, Florida 2001.

Kitao, M., Lei, T.T., Koike, T.: Effects of manganese toxicity on photosynthesis of white birch (*Betula platyphylla* var. Japonica) seedlings. – Physiol. Plant. **101**: 249-256, 1997.

Kriedemann, P.E., Graham, R.D., Wiskich, J.T.: Photosynthetic dysfunction and *in vivo* changes in chlorophyll *a* fluorescence from manganese-deficient wheat leaves. – Aust. J. agr. Res. **36**: 157-169, 1985.

Lerer, M., Bar-Akiva, A.: Effect of manganese deficiency on chloroplasts of lemon leaves. – Physiol. Plant. **47**: 163-166, 1979.

Lidon, F.C., Teixeira, M.G.: Oxy radicals production and control in the chloroplast of Mn-treated rice. – Plant Sci. **152**: 7-15. 2000a.

Lidon, F.C., Teixeira, M.G.: Rice tolerance to excess Mn: Implications in the chloroplast lamellae and synthesis of a novel Mn protein. – Plant Physiol. Biochem. **38**: 969-978, 2000b.

Macfie, S.M., Taylor, G.J.: The effects of excess manganese on photosynthetic rate and concentration of chlorophyll in *Triticum aestivum* grown in solution culture. – Physiol. Plant. **85**: 467-475, 1992.

Marschner. H.: Mineral Nutrition of Higher Plants. – Academic Press, London 1995.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. – J. exp. Bot. **51**: 659-668, 2000.

Mercer, N.E., Graham, R.D.: Genotypic variation for Mn efficiency in wheat. – J. Plant Nutr. **10**: 2049-2055, 1987.

Nable, R.O., Houtz, R.L., Cheniae, G.M.: Early inhibition of photosynthesis during development of Mn toxicity in tobacco. – Plant Physiol. **86**: 1136-1142, 1988.

Ohki, K.: Manganese critical levels for soybean growth and physiological processes. – J. Plant Nutr. **3**: 271-284, 1981.

Ohki, K.: Manganese deficiency and toxicity effects on photosynthesis, chlorophyll and transpiration in wheat. – Crop Sci. **25**: 187-191, 1985.

Roháček, K.: Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. – Photosynthetica **40**: 13-29, 2002.

Schreiber, U., Hormann, H., Neubauer, C., Klughammer, C.: Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. – Aust. J. Plant Physiol. **22**: 209-220, 1995.

Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. – Photosynth. Res. **10**: 51-62, 1986.

Singh, P., Misra, A., Srivastava, N.K.: Influence of Mn deficiency on growth, chlorophyll content, physiology, and essential monoterpene oil(s) in genotypes of spearmint (*Mentha spicata*). – Photosynthetica **39**: 473-746, 2001.

Subrahmanyam, D., Rathore, V.S.: Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. – Photosynthetica **38**: 449-453, 2000.

White, A.J., Critchley, C.: Rapid light curves: A new fluorescence method to assess the state of the photosynthetic apparatus. – Photosynth. Res. **59**: 63-72, 1999.

Wintermans, J.F.G.M., de Mots, A.: Spectrophotometric characteristics of chlorophylls *a* and *b* and their pheophytins in ethanol. – Biochim. biophys. Acta **109**: 448-453, 1965.

Yoo, S.D., Greer, D.H., Laing, W.A., McManus, M.T.: Changes in photosynthetic efficiency and carotenoid composition in leaves of white clover at different developmental stages. – Plant Physiol. Biochem. **41**: 887-893, 2003.