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Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings

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Abstract

Influence of manganese (Mn) toxicity on photosynthesis in ricebean (*Vigna umbellata*) was studied by the measurement of gas exchange characteristics and chlorophyll fluorescence parameters. The net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were reduced with increasing Mn concentration in nutrient solution. The reduction in g_s and E was more pronounced at 6 d of Mn treatment. However, P_N declined at 2 d of Mn treatment implying that the reduction in photosynthesis was not due to the direct effect of Mn on stomatal regulation. Mn did not affect the maximum efficiency of photosystem 2 (PS2) photochemistry (F_v/F_m). A reduction in photochemical quenching (q_P) and excitation capture efficiency of open PS2 (F_v'/F_m') with a concomitant increase in q_N was observed. This implies that reduced demand for ATP and NADPH due to the reduction in photosynthesis causes a down-regulation of PS2 photochemistry and thus a high pH gradient (increase in q_N) and limited electron transport (decreased q_P).

Additional key words: chlorophyll fluorescence induction; net photosynthetic rate; photosystem 2; photochemical and non-photochemical quenching; stomatal conductance; transpiration rate.

Introduction

Aluminum and manganese are two of the most abundant metals in the earth's crust and their solubility and specification is strongly affected by soil pH (Foy *et al.* 1984, Gilkes and McKenzie 1988). As toxic levels of Mn generally occur in soils of higher pH in the process of soil acidification, the toxic effects of Mn are expected to precede those of aluminium (Sumner *et al.* 1991, Kitao *et al.* 1997b). Mn toxicity leads to the reduction of growth in plants with or without visible foliar symptoms such as necrosis of chlorosis (Burke *et al.* 1990; Foy *et al.* 1978, Korcak 1988). Increase in polyphenol oxidase and reduction in photosynthesis are the most sensitive indicators of Mn toxicity (Nable *et al.* 1988).

The reduction in photosynthesis by excess leaf Mn is attributed to the peroxidative impairment of thylakoid membrane function (Panda *et al.* 1987), to reduction in photosynthetic enzyme activities (Loomis 1974, Vaughn and Duke 1984), or to modification of ribulose-1,5-bisphosphate carboxylase/oxygenase kinetics (Houtz *et al.* 1988, McDaniel and Toman 1994, Kitao *et al.* 1997b). Kitao *et al.* (1997a) found that the acumulation of Mn in leaves of *Betula ermanii*, *Alnus hirsuta*, *Ulmus davidiana*, and *Acer mono* resulted in decline of photosynthesis and carboxylation efficiency. Mn toxicity reduced the chlorophyll (Chl) content with a consequent reduction of leaf P_N in *Phaseolus vulgaris* plants (González and Lynch 1997). Macfie and Taylor (1992) found that CO_2 assimilation per unit of Chl declined

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Abbreviations: Chl = chlorophyll; E = transpiration rate; F_m, F_m' = maximum fluorescence of dark- and light-adapted leaves, respectively; F_v, F_v' = minimal fluorescence of dark- and light-adapted state, respectively; g_s = stomatal conductance; P_N = net photosynthetic rate; PS2 = photosystem 2; q_E = energy dependent quenching; q_I = quenching due to photoinhibition; q_P, q_N = coefficients of photochemical and non-photochemical quenching of variable fluorescence; Φ_e = efficiency of excitation energy capture by PS2 reaction centre.

more in the sensitive to Mn wheat cultivar than in the tolerant one. Excess Mn also affected physiological functions in leaves. Stomata were closed and *E* decreased (Suresh *et al.* 1987). Genotype variability to Mn toxicity was identified within and between *species* (Foy *et al.* 1978). We tried to assess the influence of Mn toxicity on *in vivo* photosynthetic response of ricebean (*Vigna umbellata*) seedlings using gas exchange and Chl fluorescence measurements.

Materials and methods

Seeds of ricebean (*Vigna umbellata* L.) were surface sterilised with 0.5 % $HgCl_2$ for 5 min and were germinated in autoclaved vermiculite at 28 °C. Ten days after germination, the plants were transferred to a growth chamber at 28 °C (photon flux density inside the chamber was 320 μmol m^{-2} s^{-1}). After further 8 d the plants were transferred to nutrient solution (Hewitt 1966) containing either normal (0.3 μM) or toxic (0.2, 0.4, and 0.6 mM) concentrations of manganese provided as $MnCl_2$.

P_N, *E*, and g_s were measured on first trifoliate leaves using *CIRS-1* (*PP Systems,* Hitchin, U.K.) portable photosynthesis measuring system fitted with broad leaf cuvette (exposed area 250 mm^2) at 0, 2, and 6 d after manganese treatment. Gas exchange measurements were made on four individual plants per treatment. The experiment was repeated twice. The values were analysed in Completely Randomized Design (CRD) with two factors and four replications. Chl *a* fluorescence was measured at room temperature on intact whole leaves at 0, 2, 6, and 10 d after Mn treatment using a dual-channel modulated fluorescence measuring system (*Hansatech,* King's Lynn, U.K.). The frequency of modulation was approximately 4.8 kHz and its intensity was about 1-2 μmol m^{-2} s^{-1}, insufficient to produce variable fluorescence. Both the saturating irradiance (8 000 μmol m^{-2} s^{-1}) and actinic irradiance (430 μmol m^{-2} s^{-1}) were provided by *Hansatech LS2* and *PLS 1* light sources, respectively. The minimal fluorescence yield of the light-adapted leaf (F_0') was determined by irradiating the leaves with far-red radiation (Genty *et al.* 1989). The efficiency of excitation capture by open PS2 reaction centres ($\Phi_e = F_v'/F_m$, where $F_v' = (F_m' - F_0')$, the coefficient of photochemical quenching [$q_P = (F_m' - F_s)/(F_m' - F_0')$], and the coefficient of non-photochemical quenching [$q_N = (F_m - F_m')/(F_m - F_0)$] were computed according to Schreiber *et al.* (1986) where F_m and F_m' are the maximal fluorescence in the dark and light adapted states, respectively, F_0 is the minimal fluorescence in the dark-adapted state, and F_s is the steady-state fluorescence in the light-adapted state.

Results and discussion

Mn toxicity symptoms are discernible in the first trifoliate leaf of ricebean seedlings after 4 d of Mn treatment. The leaves of excess Mn treated plants show clear signs of chlorosis and appearance of irregular brown spots on leaf lamina was also noticed. Reduction in shoot growth and appearance of chlorosis, crinkled leaves, and brown speckles were observed in different plant species as a result of Mn toxicity (Marschner 1995, González and Lynch 1997). Intervenal chlorosis, appearance of irregular brown spots, and puckering of the leaf surfaces was recorded in young expanding leaves of soybean (Andrew and Pieters 1976). Leaf curling and chlorosis was recorded in snap bean, soybean, cotton, and grasses (Foy 1984). In tobacco seedlings foliar chlorosis and necrotic spotting were observed (Petolino and Collins 1985). In *Vigna unguiculata* small dark brown spots spread all over the leaf lamina (Horst 1982).

The gas exchange indicated a significant reduction in P_N at high Mn concentration (Table 1). The reduction was more pronounced with increasing Mn concentration in the nutrient solution. Mn toxicity also reduced *E* and g_s (Table 1). The reduction was pronounced only at 6 d of Mn treatment.

P_N in young burley tobacco leaves declined with high Mn treatment (Nable *et al.* 1988). The reduction in P_N by high Mn concentration was due to reduced ribulose-1,5-bisphosphate carboxylase activity (Jordan and Ogren 1981, Houtz *et al.* 1988, McDaniel and Toman 1994, Kitao *et al.* 1997a,b). However, González and Lynch (1997) suggest that Mn toxicity reduced P_N in immature leaves of bean through reduction of Chl concentration. According to Okhi (1985), the decline in P_N by excess Mn was followed by reduction in *E* and Chl concentration. Suresh *et al.* (1987) reported a reduction in g_s and *E* with increasing Mn concentration and concluded that Mn interfered with stomatal regulation. However, González and Lynch (1997) observed that Mn toxicity reduced *E* and g_s only in young leaves with advanced leaf damage. No such reduction in g_s was observed in mature bean leaves that accumulated high

Mn amounts but were without any sign of chlorosis; they concluded that the effect of Mn on g_s might be a direct result of leaf damage.

Table 1. Effect of manganese toxicity on net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], transpiration rate, E [mmol m^{-2} s^{-1}], and stomatal conductance, g_s [mmol m^{-2} s^{-1}] of ricebean.

Time [d]	Mn^{2+} [mM]	P_N	E	g_s
0	0.0	12.5	5.02	328
	0.2	12.2	5.16	326
	0.4	12.9	5.12	320
	0.6	12.0	5.40	300
2	0.0	11.8	5.12	320
	0.2	09.0	5.00	324
	0.4	07.1	4.61	310
	0.6	06.3	4.94	306
6	0.0	11.6	5.31	322
	0.2	08.6	4.21	291
	0.4	06.0	4.11	289
	0.6	05.8	3.88	256
Days (d)	CD ($p = 0.05$)	0.91	0.60	15.66
	SEM ±	0.32	0.21	5.46
Mn^{2+} conc. (c)	CD ($p = 0.05$)	1.04	0.69	18.08
	SEM ±	0.37	0.24	6.31
d×c	CD (p = 0.05)	1.82	1.20	31.32
	SEM ±	0.63	0.90	10.91
Cv [%]		13.13	16.41	7.09

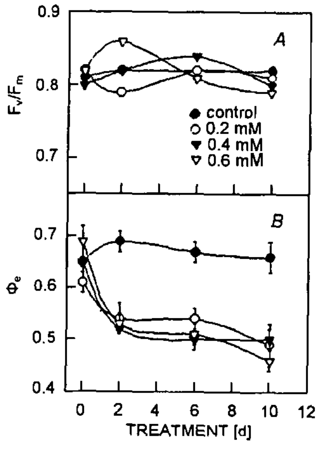

Fig. 1. Effects of manganese toxicity on (*A*) maximum efficiency of PS2 photochemistry (F_v/F_m), and (*B*) efficiency of excitation capture ($\Phi_e = F_v'/F_m'$). Means of 4 replications ± SE.

A 46.6 % reduction in P_N was observed in plants treated with 0.6 mM Mn for 2 d whereas the reductions in g_s and E were only 4.4 and 3.5 %, respectively (Table 1). This indicates that the reduction in P_N was not due to the direct effect of Mn on stomatal regulation. Nevertheless, significant reduction in E and g_s was observed at 6 d of Mn treatment.

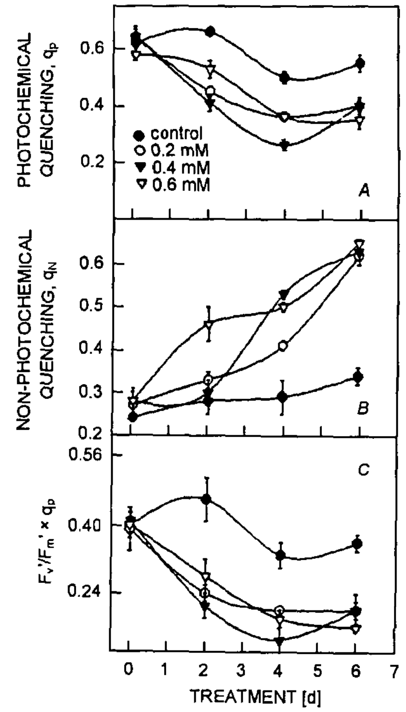

Fig. 2. Effects of manganese toxicity on (*A*) coefficient of photochemical quenching (q_P), (*B*) coefficient of non-photochemical quenching (q_N), and (*C*) *in vivo* PS2 quantum efficiency ($\Delta F/F_m' = F_v'/F_m' \times q_P$). Means of 4 replications ± SE.

As shown in Fig. 1*A*, Mn did not substantially affect the F_v/F_m ratio of dark-adapted leaves. However, a marked depression in F_v'/F_m' (Φ_e), which is a measure of PS2 photochemical efficiency, was observed (Fig. 1*B*). This suggests that light-induced non-photochemical quenching may be established in the leaves as a result of Mn treatment. Since the potential maximum efficiency of PS2 photochemistry, as represented by the F_v/F_m ratio (Butler 1978), was not affected by Mn toxicity, the quenching mechanism might not be due to inhibited photochemistry. Similar results were reported by Kitao *et al.* (1997b) in *Betula platyphylla* leaves. The photochemical and non-photochemical processes that bring about the relaxation of the excited Chl molecules to ground state were measured as coefficients of photochemical (q_P) and non-photochemical (q_N) quenching of variable fluorescence, respectively (Fig. 2*A*,*B*). A significant reduction in q_P with a concomitant increase in q_N was observed in plants at high Mn treatment. The reduction in q_P suggests that the energy consumption is inhibited. Excess Mn treatment increased the q_N in ricebean leaves (Fig. 2*B*). The non-photochemical quenching of fluorescence related to photo-inhibition (q_I) may be excluded since the ricebean plants were grown at moderate irradiance. The increase in q_N indicates an increased trans-thylakoid pH gradient under steady-state (Krause and Weis 1984, Rees and Horton 1990, Jacob 1995). Thus q_N should be referred to as q_E, non-photochemical quenching due to high-energy state (Genty *et al.* 1990, Krause and Weis 1991). This may cause down-regulation of PS2 to avoid over-reduction of Q_A (Genty *et al.* 1990). Carbon dioxide reduction in photosynthesis consumes the products (ATP and NADPH) of photosynthetic electron transport. The oxidised NADPH acts as the terminal electron acceptor and permits the photochemical de-excitation of reaction centres. Any factor, such as excess Mn, which interfered with the photosynthetic CO_2 reduction, will diminish the utilisation of photosynthetic energy and affect high-energy state. Non-photochemical quenching will modify the electron transport through PS2.

The reduction in photosynthesis could influence the photochemical efficiency during high exposure *via* an imbalance between radiant energy absorbed and the energy utilised by carbon metabolism (Gray *et al.* 1996). The *in vivo* PS2 quantum efficiency, $\Delta F/F_m'$ was reduced as a result of Mn treatment (Fig. 2*C*) mainly as a result of reduction in both F_v'/F_m' and q_P (Figs. 1*B* and 2*A*). The F_v'/F_m' (Φ_e) is a measure of efficiency of excitation capture by open PS2 (Schreiber *et al.* 1994). Reduction in this parameter is interpreted as the increase in thermal energy dissipation (Schindler and Lichtenthaler 1996, Demmig-Adams *et al.* 1995). Kitao *et al.* (1997a,b) concluded that the reduction in PS2 quantum efficiency by Mn toxicity might be due to the increase in thermal energy dissipation and decrease in faction of open PS2.

In conclusion, our present observations of gas exchange and Chl fluorescence suggest that Mn toxicity affects the activity of PCR cycle, and the primary photochemistry of the PS2 is not directly affected by Mn toxicity. The reduced demand for ATP and NADPH is probably due to the inhibition of CO_2 reduction, induces a down-regulation of PS2 photochemistry causing a high pH gradient (increase in q_N) and limited electron transport (decreased q_P).

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