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Effect of manganese toxicity on growth and N_2 fixation in groundnut, *Arachis hypogaea*

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Summary

The development of manganese (Mn) toxicity symptoms and its effects on the growth, nodulation, and nitrogen fixation of groundnut genotypes were examined using a quartz-sand/solution culture system. The 11 genotypes tested all accumulated considerable concentrations of manganese (1.04 – 3.07 mg g^{-1} dry matter) when supplied with 15 μg Mn ml^{-1} of nutrient solution daily. Toxicity symptoms differed between genotypes: some showed no visual effects, some produced marginal leaf spots, and others developed marginal leaf spots coupled with an inward rolling of the margins of the younger leaves. The growth of one genotype (ICG 5394) grown with inorganic nitrogen as its source of N was more severely affected by Mn toxicity than when dependent on symbiotic fixation for its nitrogen.

Introduction

High concentrations of manganese in plant tissues are often associated with acid conditions in the soil (Munns, 1977). The responses of legumes to Mn-toxicity are different if they are grown wholly with inorganic nitrogen (N) or rely upon symbiotic fixation of gaseous N_2 (Andrew, 1978). Low pH reduces nodulation, N_2 fixation, and dry matter production in many legumes, but groundnut (*Arachis hypogaea*) is listed as an acid-tolerant species (Andrew, 1976, 1978). Mn-toxicity induced by high concentrations, causes chlorotic leaf margins in groundnut (Morris & Pierre, 1949). This short research note reports that different Mn-toxicity symptoms occur in different genotypes of groundnut, and effects of Mn on groundnut genotypes utilising mineral nitrogen or gaseous nitrogen (N_2) as the source of nitrogen.

Materials and Methods

The genotypes used were Robut 33-1, TMV 2, ICG 5394, JL 24, J 11, Argentine, Kadiri 71-1, NC Ac 2821, PI 259747, MH 2 and Gangapuri.

The experiments were conducted in a glasshouse in which day temperatures ranged from 23°C to 33°C and night temperatures from 10°C to 20°C. Plants were grown in a sterilised quartz-sand (Epex Enterprises, India). Before sowing, seeds were sterilised for 5 min with aqueous hydrogen peroxide (5%), thoroughly washed with sterile water, and treated with Thiram (3 g kg^{-1} seed). Seeds were inoculated with *Bradyrhizobium* (strain NC 43.3) at a rate

of 10^8 cells seed^{-1} (Nambiar, Ravishankar & Dart, 1983), and eight seeds sown in each pot (15 cm diam.). The resulting seedlings were thinned to three per pot 5-6 days after sowing. Pot surfaces were then covered with a 5 mm layer of sterilised gravel to reduce surface contaminants. A sterilised nutrient solutions containing 1 mM $CaCl_2$, 0.5 mM KH_2PO_4, 0.25 mM $MgSO_4.7H_2O$, 0.25 mM K_2SO_4, 10 μM $C_6H_5O_7$ $Fe.2H_2O$, 1 μM $MnSO_4.4H_2O$, 2 μM H_3BO_3, 0.5 μM $ZnSO_4.7H_2O$, 0.2 μM $CuSO_4.5H_2O$, 0.1 μM $CoSO_4.7H_2O$ and 0.1 μM $Na_2MoO_4.2H_2O$ was prepared (Nambiar *et al.*, 1983). Each day, 250 ml of this solution was supplied to each pot and the solution that drained through the pot was retained in a base tray (18 cm diam.) in which the pot was placed. On the following day, the drained solution was removed from the base tray and fresh nutrient solution (250 ml) added to the pot. Additional manganese was added to the nutrient solution as the sulphate at the concentrations required for particular treatments in individual experiments. Each week the pots were flushed with 1 litre of deionised sterilised water to prevent the accumulation of nutrient salts. Other details of the pot culture were as described previously (Nambiar *et al.*, 1983).

Three experiments were carried out. Expt 1 was carried out during February-March 1985. It tested the Mn-toxicity responses of the genotype ICG 5394 at two levels of pH, 4.0 and 7.0. Two concentrations of Mn in the nutrient solutions were used, 7.5 and 15 μg ml^{-1} and the experimental plants were harvested 44 days after sowing.

Expt 2 was carried out during July-August 1985 and tested the Mn-toxicity responses of the same genotype (ICG 5394) to a range of concentrations of Mn in the nutrient solution (0, 5, 10, 15 and 20 μg ml^{-1}, all at pH 4.0). This experiment was harvested 60 days after sowing. In Expts 1 and 2, two sources of nitrogen were used. Plants were either: a) inoculated with *Bradyrhizobium* strain (NC 43.3) and supplied with N-free nutrient solution so that they were dependent on symbiotically-fixed gaseous N_2, or b) not inoculated with *Bradyrhizobium* but regularly supplied with 300 μg N ml^{-1} as ammonium nitrate in the nutrient solution so that they were dependent on inorganic N.

Expt 3 was carried out during January-February 1986 and tested the Mn-toxicity responses of 10 genotypes to 15 μg Mn ml^{-1} in the nutrient solution at pH 4.0. All plants in this experiment were inoculated with *Bradyrhizobium* strain (NC 43.3) and supplied with N-free nutrient solution. These were harvested 60 days after sowing.

The factorial combinations of treatments were arranged in four randomised blocks. At harvest, total dry matter per plant, the number of nodules and their nitrogenase activity, were measured (Nambiar & Dart, 1983). The total contents of nitrogen and manganese were measured as described by Nambiar *et al.* (1983) and Sahrawat (1987), respectively.

Results and Discussion

Although hydroponics is an ideal system for studying nutrient deficiencies and toxicities in plants, it is not especially suited to legumes because nodulation and the fixation of gaseous N_2 are usually extremely poor. The quartz-sand/nutrient solution system used in the present experiments overcomes some of these problems but was not ideal because localised pH changes occurred near root surfaces which might have influenced Mn-uptake. Preliminary experiments had shown that the pH of the nutrient solution could change from 4.0 to 3.5 during a 24 h period (P. T. C. Nambiar and V. Anjaiah, unpublished data) and for this reason solutions were drained daily.

When grown in the standard nutrient solution in Expt 1, the groundnut genotype ICG 5394 tolerated low pH fairly well when measured in terms of dry matter production. The 15 μg ml^{-1} concentration of Mn decreased plant dry matter and N uptake in plants that depended

on the inorganic source of nitrogen at pH 7.0, but not in plants that symbiotically fixed gaseous N_2. High concentrations of Mn in the nutrient solution decreased the amount of inorganic N assimilated by the plants at pH 7.0 but not at pH 4.0. Considerable amounts of Mn between 1-3 mg g^{-1} dry weight were accumulated in plants at both pH 4.0 and pH 7.0 (Table 1).

Table 1. *Effect of increased manganese supply on dry matter (DM), total nitrogen and Mn contents of the groundnut genotype ICG 5394, at 44 days after sowing*

Treatment	pH 7.0		pH 4.0	
Nitrogen Source	N*	N_2	N	N_2
Dry matter (g per plant)				
NS†	4.9	3.7	4.2	3.3
NS + 7.5 μg Mn ml^{-1}	3.3	3.8	3.8	3.6
NS + 15 μg Mn ml^{-1}	2.9	3.5	3.2	2.6
S.E.[a]	0.45		0.36	
S.E.[b]	0.40			
Total nitrogen (mg per plant)				
NS	221	99	179	96
NS + 7.5 μg Mn ml^{-1}	152	115	184	107
NS + 15 μg Mn ml^{-1}	129	118	153	90
S.E.[a]	19.60		16.90	
S.E.[b]	17.9			
Mn concentration (μg g^{-1} DM)				
NS	58	69	60	95
NS + 7.5 μg Mn ml^{-1}	1844	1042	1939	1077
NS + 15 μg Mn ml^{-1}	2929	2085	3074	1994
S.E.[a]	56.70		69.60	
S.E.[b]	62.8			

N*, inorganic nitrogen; N_2, symbiotically-fixed gaseous N_2.

NS† = standard nutrient solution.

a, S.E. for comparisons within each pH level; *b*, S.E. for comparisons across pH levels (9 D.F.).

In Expt 2, which had a longer growth period (60 cf. 44 days), the dry weights of the plants were greatly decreased by increasing Mn concentration irrespective of whether they depended on inorganic N or symbiotically-fixed gaseous N_2 for their nitrogen (Table 2). Plants grown with inorganic N in the nutrient solution produced more dry matter than those relied on symbiotic fixation of N_2, but the decrease in growth at high concentrations of Mn was much greater in the former. Mn-toxicity did not significantly influence the number of nodules, but nitrogenase activity was decreased by high concentration of Mn (Table 2). Mn is therefore toxic to the groundnut plant but is not altogether clear to what extent Mn decreased symbiotic fixation of gaseous N_2 through effects on plant growth or through the effects on the fixation process.

The range groundnut genotypes tested in Expt 3 differed considerably in the concentration of Mn they accumulated in their dry matter (Table 3). The highest concentrations of Mn were accumulated by the genotypes Gangapuri and TMV 2 (*c.* 1.4 mg g^{-1} dry matter) and the lowest by genotype NC Ac 2821 (1.0 mg Mn g^{-1} dry matter). High internal concentration of Mn did not significantly affect the growth of some genotypes, e.g. JL 24, J 11, PI 259747 and MH 2, slightly affected the growth of others, e.g. NC Ac 2821 and Argentine, and severely restricted the growth of Gangapuri (Table 3).

Table 2. *Effect of increased manganese supply on the dry matter (DM), nodule number, and nitrogenase activity of groundnut genotype ICG 5394, at 60 days of sowing*

Nitrogen Source	N	N_2*
Dry matter (g per plant)		
NS†	11.07	5.99
NS + 5 μg Mn ml^{-1}	11.09	5.90
NS + 10 μg Mn ml^{-1}	10.56	5.62
NS + 15 μg Mn ml^{-1}	7.17	4.38
NS + 20 μg Mn ml^{-1}	5.41	3.23
S.E.	0.84	
Nodule number per plant		
NS	n.d.	84
NS + 5 μg Mn ml^{-1}	n.d.	73
NS + 10 μg Mn ml^{-1}	n.d.	97
NS + 15 μg Mn ml^{-1}	n.d.	90
NS + 20 μg Mn ml^{-1}	n.d.	72
S.E.		9.4
Nitrogenase activity (μ moles per plant per hour)		
NS	n.d.	15.5
NS + 5 μg Mn ml^{-1}	n.d.	12.7
NS + 10 μg Mn ml^{-1}	n.d.	15.9
NS + 15 μg Mn ml^{-1}	n.d.	8.4
NS + 20 μg Mn ml^{-1}	n.d.	5.8
S.E.		1.91

N*, inorganic nitrogen; N_2, Symbiotically-fixed gaseous N_2; n.d., not detected.
NS† = standard nutrient solution

Table 3. *Effect of increased manganese supply on the dry matter (DM) and manganese content of different groundnut genotypes grown at pH 4.0 and dependent on symbiotic N_2 fixation; 60 days after sowing*

Genotype	Dry matter (g per plant)		Mn concentration (μg g^{-1} DM)		Mn content (mg per plant)	
	*NS	NS + 15 μg Mn ml^{-1}	NS	NS + 15 μg Mn ml^{-1}	NS	NS + 15 μg Mn ml^{-1}
JL 24	7.2	7.4	100	1319	0.75	9.65
J 11	7.4	7.8	102	1209	0.76	9.38
NC Ac 2821	10.0	9.1	85	1036	0.84	9.44
Argentine	8.6	6.7	121	1161	1.03	7.91
Robut 33-1	8.8	8.0	99	1141	0.87	9.05
Kadiri 71-1	8.5	7.8	103	1175	0.87	9.09
PI 259747	8.0	7.9	106	1139	0.84	9.01
TMV 2	8.4	7.6	98	1398	0.83	10.67
MH 2	6.2	6.4	97	1338	0.60	8.58
Gangapuri	9.8	7.7	89	1418	0.87	10.75
S.E.	0.52		45.1		0.495	
Mean	8.3	7.6	100	1233	0.83	9.35
S.E.	0.17		14.3		0.156	

*, NS = standard nutrient solution

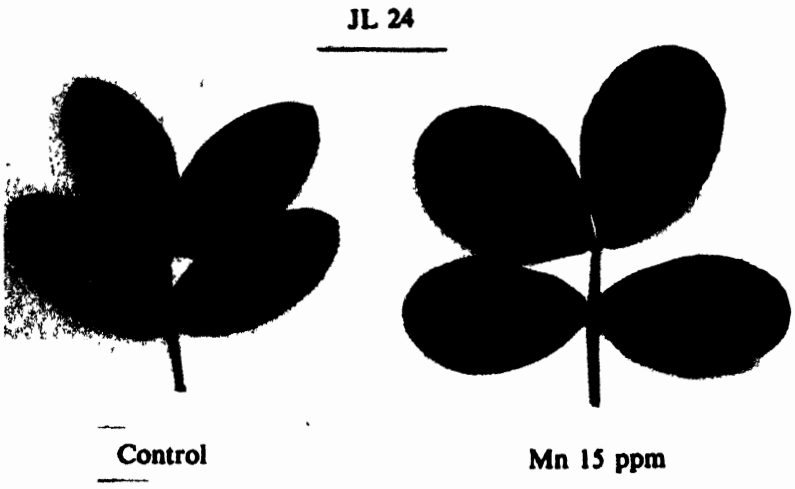

Fig. 1 (a)

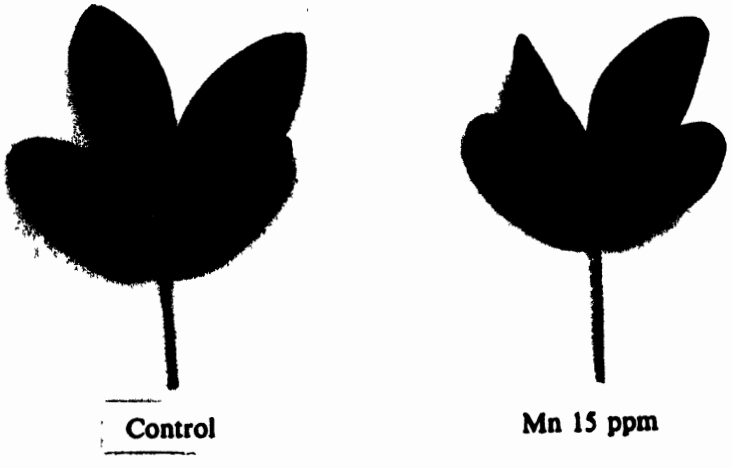

Fig. 1 (b)

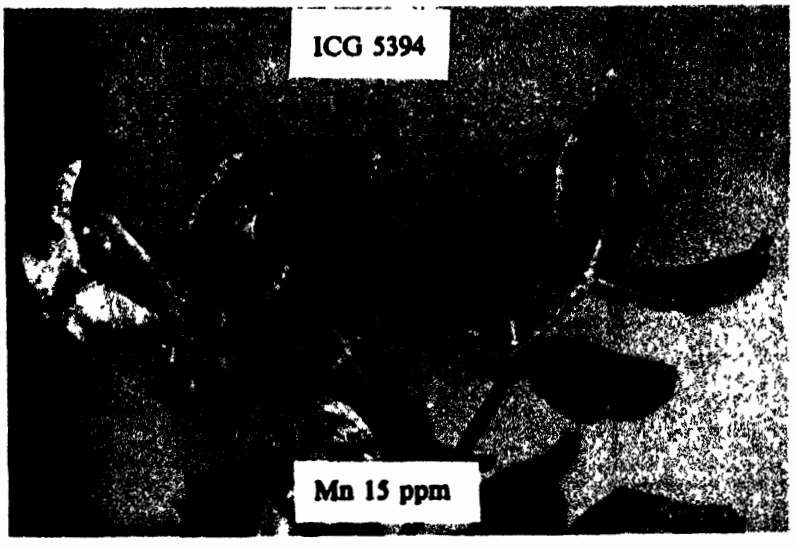

Fig. 1. Genotype differences in Mn-toxicity in groundnut a) marginal leaf spotting in genotype JL 24, and genotype PI 259747 without any clearly visible symptoms; b) inward rolling of young leaves of genotype ICG 5394.

Different visual symptoms of Mn-toxicity developed on leaves of the various genotypes. Mn-toxicity symptoms started to appear around days 35 from sowing. There were no visible symptoms in some genotypes (e.g. PI 259747, Kadiri 71-1 and NC Ac 2821) despite large accumulations of Mn in plant dry matter. In others (e.g. Gangapuri, JL 24, TMV 2 and J 11), necrotic spots developed on the margins of leaves. In ICG 5394, this marginal leaf spotting was accompanied by an inward rolling of the young leaves (Fig. 1*a*,1*b*). Chlorotic leaf margins such as those described by Morris & Pierre (1949) were not observed in these experiments. Leaf-spot symptoms, such as those described here, have been observed in crops grown on soils of neutral pH in Andhra Pradesh with more than 50 μg extractable Mn g^{-1} dry soil (Anon., 1983; R. Rajeswari, personal communication). A rapid acidification of soil at the root: soil interface is the probable cause of Mn-toxicity in these neutral soils.

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