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Studies on the Manganese Excess of Muskmelon

VI. Manganese Distribution in the Plant Parts

Masao Masui, Akira Nukaya, Akira Ishida
and Takayasu Ogura

College of Agriculture, Shizuoka University, Ohya, Shizuoka 422

Summary

Muskmelons were grown in soil adjusted to 3 pH levels ; 6.4 (high), 5.5 (medium) and 4.5 (low), and in sand fertilized with a complete nutrient solution containing 0.5, 30 and 60 ppm Mn, and also 0, 0.5 and 60 ppm Mn to determine the effect of Mn excess on the growth and Mn distribution of the various parts of the organs. Plant growth was severely restricted at the low pH. Mn in the various parts of the organs was significantly increased by lowering soil pH, and by increasing Mn concentrations in the nutrient solution. Mn in the leaf blades and petioles tended to be higher in the lower part than in the middle and higher parts. More Mn accumulated in the external tissues, especially in the hairs. Mn in the fine roots was much higher than in the large ones. Mn in the fruit was increased in the inner flesh, outer flesh and rind in this order.

Introduction

In a previous manganese (Mn) study of muskmelon (7), excess Mn symptoms appeared to be severe in the lower leaves of the plant by visual observation. This may be caused by a different distribution of Mn in the various tissues or parts of the plants. However, the data of this distribution have not been obtained yet, because Mn analysis was made on the composite sample of each plant part. The objective of this investigation was to determine how Mn content varies with the different parts of leaf blades, petioles, stem, roots and fruit.

Materials and Methods

Experiment I.

Twenty-one uniform seedlings of muskmelon, cv. Fall No. 1 of Earl's Favourite grafted on Barnett Hill Favourite rootstock were used. Treatments consisted of 3 levels of soil pH (high, medium and low), and had 7 replications, with a total of 21 plots. On Sept. 19, 1972, seedlings with 4 leaves were planted in 40×40×20 cm boxes filled with the soil used for a previous muskmelon crop.

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This soil had a total Mn (water soluble+exchangeable+easily reducible) of 861 ppm. Before planting the soil was sterilized by steaming at 80℃ for 30 min. Thereafter the soil pH was adjusted with $Ca(OH)_2$ and dusting sulfur. At the end of the experiment mean values for high, medium and low pH were 6.4, 5.5 and 4.5, respectively. Rapeseed cake of 28 g was applied on Oct. 5, Oct. 28 and Nov. 14 to promote the growth of plants. All plants received uniform care except for experimental treatment. At the end of the experiment records were taken of top and root growth, fruit weight, and fruit soluble solids. For Mn analysis each plant was divided into leaf blades, petioles, stem, roots and fruit. The leaf blades, petioles and stem were further divided into 3 groups; lower (below 9 th node), middle (10 th to 15 th node) and higher (16 th to 23 rd node). The roots were divided into fine and large ones (below 1 mm and above 2.5 mm in diameter, respectively), and the fruit into 3 parts; inner flesh, outer flesh and rind. All plant and soil analyses were by the methods described in a previous paper (5).

Experiment II.

Twenty-one uniform seedlings of the same

cv. as described in Experiment I were used. Treatments consisted of 3 Mn levels (0.5, 30 and 60 ppm) in a nutrient solution, and had 7 replications, with a total of 21 plots. On Sept. 19, 1972, seedlings with 4 leaves were planted in $40\times40\times20$ cm boxes filled with Tenryu River sand. This sand was washed with deionized water. The differential Mn treatment was initiated when planted. The composition of nutrient solution was as follows: Mn from $MnSO_4$ (designated concentration), $Na_2HPO_4\cdot12H_2O$ (0.5 mM), K_2SO_4 (3 mM), $CaCl_2\cdot2H_2O$ (4 mM), $MgSO_4\cdot7H_2O$ (2 mM), Fe from $FeC_6H_5O_7\cdot5H_2O$ (1 ppm), B from H_3BO_3 (0.5 ppm), Zn from $ZnSO_4\cdot7H_2O$ (0.05 ppm), Cu from $CuSO_4\cdot5H_2O$ (0.02 ppm) and Mo from $Na_2MoO_4\cdot2H_2O$ (0.05 ppm). Nutrient solutions were adjusted to pH 5.0 and were applied by the same methods as decribed in a previous paper (6). Chemical analyses and records were accomplished with the same methods as described in Experiment I.

Experiment III.

Fifteen uniform seedlings of muskmelon, cv. Spring No. 3 of Earl's Favourite grafted on Barnett Hill Favourite rootstock were used. Treatments consisted of 3 Mn levels (0, 0.5 and 60 ppm) in a nutrient solution, and had 5 replications, with a total of 15 plots. On Apr. 22, 1973, seedlings with 4 leaves were planted in the boxes filled with Tenryu River sand, and were grown by the same methods as described in Experiment II. To determine the Mn content in further detailed tissues of leaf, stem, roots, rind and seeds, these tissue samples were carefully taken by an ophthalmologic knife under a binocular dissecting microscope, and analyzed for Mn.

Results

Experiment I.

1. Growth of plant and fruit (Table 1)

The growth of plants as expressed by the dry weight of leaf blades, petioles, stem and roots was markedly restricted at the low pH. Thus the fruit at the low pH hardly grew as shown by the fresh weight of 28 g. On the other hand, the growth of plant and fruit at the high and medium pH's was normal, and the fruit soluble solids in these treatments were as high as 16.0 to 16.6 for muskmelons at this growing season.

2. Mn content in leaf blades, petioles and stem (Table 2)

Mn in the various parts of leaf blades, petioles and stem was significantly increased by lowering soil pH. A decreasing order of the leaf blades Mn at each soil pH was generally found in the lower, middle and higher part, and the petiole Mn at each soil pH was higher in the lower part. No Mn difference was found among the stem

Table 1. Effect of soil pH on growth of plant and fruit of muskmelon.

pH	Leaf blades[x] dry wt (g)	Petioles dry wt (g)	Stem dry wt (g)	Roots dry wt (g)	Fruit	
					Fresh wt (g)	Soluble solids (%)
High	26.1^{A}	3.3^{A}	5.6^{A}	1.74^{A}	701^{A}	16.6^{A}
Medium	24.6^{A}	3.5^{A}	5.8^{A}	1.84^{A}	612^{A}	16.0^{A}
Low	10.3^{B}	0.8^{B}	2.7^{B}	0.82^{B}	28^{B}	6.5^{B}

x : Mean separation in columns by Duncan's multiple range test, 5% level.
The capital letters within columns in Tables 2 to 7 show the same as x in Table 1.

Table 2. Effect of soil pH on Mn content in various parts of leaf blades, petioles and stem of muskmelon (dry wt ppm).

pH	[z]Leaf blades[x]			Petioles			Stem		
	Lower	Middle	Higher	Lower	Middle	Higher	Lower	Middle	Higher
High	$^{a}1,415^{C}$	$^{b}976^{C}$	$^{c}767^{C}$	$^{a}1,114^{C}$	$^{ab}749^{C}$	$^{b}576^{C}$	$^{a}343^{C}$	$^{a}300^{C}$	$^{a}356^{C}$
Medium	$^{a}4,388^{B}$	$^{b}2,725^{B}$	$^{b}2,560^{B}$	$^{a}3,601^{B}$	$^{b}2,229^{B}$	$^{b}2,451^{B}$	$^{a}733^{B}$	$^{a}858^{B}$	$^{a}602^{B}$
Low	$^{a}11,863^{A}$	$^{ab}9,724^{A}$	$^{b}7,266^{A}$	$^{a}5,207^{A}$	$^{b}3,854^{A}$	$^{b}3,654^{A}$	$^{b}1,523^{A}$	$^{b}1,493^{A}$	$^{a}1,607^{A}$

z : Mean separation in rows of each plant part by Duncan's multiple range test, 5% level.
The small letters within rows of each plant part in Tables 3, 6 and 7 show the same as z in Table 2.

Table 3. Effect of soil pH on Mn content in various parts of roots and fruit of muskmelon (dry wt ppm).

pH	[z]Roots[x]		Fruit		
	Fine	Large	Inner flesh	Outer flesh	Rind
High	$^{a}1,227^{B}$	$^{b}257^{C}$	$^{b}0^{B}$	$^{b}0^{B}$	$^{a}211^{C}$
Medium	$^{a}3,053^{A}$	$^{b}555^{B}$	$^{b}0^{B}$	$^{b}41^{B}$	$^{a}761^{B}$
Low	—[w]	$1,154^{A}$	$^{c}78^{A}$	$^{b}520^{A}$	$^{a}1,889^{A}$

w : Samples were not taken due to poor growth.

parts at each soil pH. Excess Mn symptom of minute necrotic spots appeared on the leaf blades at the medium and low soil pH's, but not at the high soil pH, and the degree of the symptom was much lighter at the medium soil pH.

3. Mn content in roots and fruit (Table 3)

Mn in the large roots was significantly increased by lowering soil pH. A sample of fine roots at the low pH could not be obtained due to poor growth. If this sample could be obtained, Mn in the fine roots at the low pH would presumably by much higher than at the medium pH. Generally, Mn was greater in the fine roots. Mn in the various parts of the fruit was significantly increased by lowering soil pH though Mn was not detected in the inner flesh at the high and medium pH's, and in the outer flesh at the high pH (zero's in the Table show no detectable Mn with an atomic absorption spectrophotometer). An increasing order of the fruit Mn at each soil pH was generally found in the inner flesh, outer flesh and rind.

4. Mn content, pH and EC in soil (Table 4)

Mn of water soluble and exchangeable forms was significantly increased by lowering soil pH. Thus the content of water soluble and exchangeable Mn at the high pH was as low as 6 and 42 ppm, respectively, while that at the low pH was as high as 469 and 215 ppm, respectively. Total Mn was not different among the levels of soil pH. At the end of the experiment the high, medium and low pH values were 6.4, 5.5 and 4.5, respectively, and EC values were 2.88, 3.91 and 5.42 m℧/cm, respectively.

Experiment II.

1. Growth of plant and fruit (Table 5)

The dry weight of leaf blades, petioles and stem, and fruit fresh weight decreased at 60 ppm Mn though the degree of inhibited growth

Table 4. Mn content of various forms, pH and EC in soil at the end of the experiment.

pH	Mn in air dried soil (ppm)[x]					pH (H_2O)	EC (m℧/cm) (1 : 2)
	Water soluble (W)	Exchangeable (E)	Available (W)+(E)	Easily reducible(R)	Total (W)+(E)+(R)		
High	6^{C}	42^{B}	48^{C}	819^{A}	867^{A}	6.4^{A}	2.88^{C}
Medium	53^{B}	131^{AB}	184^{B}	679^{A}	863^{A}	5.5^{B}	3.91^{B}
Low	469^{A}	215^{A}	684^{A}	214^{B}	898^{A}	4.5^{C}	5.42^{A}

Table 5. Effect of Mn concentrations in nutrient solution on growth of plant and fruit of muskmelon.

Mn concn (ppm)	Leaf blades[x] dry wt (g)	Petioles dry wt (g)	Stem dry wt (g)	Roots dry wt (g)	Fruit	
					Fresh wt (g)	Soluble solids (%)
0.5	33.7^{A}	5.8^{A}	7.4^{A}	1.19^{A}	725^{A}	16.4^{A}
30	33.2^{A}	5.9^{A}	7.1^{A}	1.22^{A}	735^{A}	15.9^{A}
60	29.4^{B}	4.4^{B}	5.9^{B}	1.14^{A}	624^{B}	16.6^{A}

Table 6. Effect of Mn concentrations in nutrient solution on Mn content in various parts of leaf blades, petioles and stem of muskmelon (dry wt ppm).

Mn concn (ppm)	[z]Leaf blades[x]			Petioles			Stem		
	Lower	Middle	Higher	Lower	Middle	Higher	Lower	Middle	Higher
0.5	$^{a}799^{C}$	$^{b}561^{C}$	$^{b}528^{C}$	$^{a}527^{C}$	$^{b}429^{C}$	$^{b}462^{C}$	$^{a}233^{C}$	$^{b}147^{C}$	$^{b}140^{C}$
30	$^{a}6,208^{B}$	$^{b}5,584^{B}$	$^{b}5,488^{B}$	$^{a}3,836^{B}$	$^{b}3,226^{B}$	$^{b}3,208^{B}$	$^{a}1,637^{B}$	$^{a}1,476^{B}$	$^{a}1,319^{B}$
60	$^{a}12,269^{A}$	$^{ab}11,895^{A}$	$^{b}10,369^{A}$	$^{a}11,220^{A}$	$^{b}8,052^{A}$	$^{b}7,481^{A}$	$^{b}2,995^{A}$	$^{b}3,091^{A}$	$^{a}3,846^{A}$

Table 7. Effects of Mn concentrations in nutrient solution on Mn content in the various parts of roots and fruit of muskmelon (dry wt ppm).

Mn concn (ppm)	[z]Roots[x]		Fruit		
	Fine	Large	Inner flesh	Outer flesh	Rind
0.5	[a]606[C]	[b]353[C]	[b]0[A]	[b]23[B]	[a]141[C]
30	[a]3,553[B]	[b]1,902[B]	[b]0[A]	[b]25[B]	[a]1,492[B]
60	[a]5,771[A]	[b]2,942[A]	[b]0[A]	[b]31[A]	[a]2,545[A]

was not so marked as compared with the low pH in Experiment I. Fruit soluble solids were not different among the treatments.

2. Mn content in leaf blades, petioles and stem (Table 6)

Mn in the various parts of leaf blades, petioles and stem was significantly increased by increasing Mn concentrations in the nutrient solution. Generally, Mn in the leaf blades and petioles at each Mn concentration was higher in the lower part. Mn in the stem at 0.5 ppm Mn was higher in the lower part, while that at 60 ppm Mn was higher in the higher part.

3. Mn content in roots and fruit (Table 7)

Mn in the fine and large roots was significantly increased by increasing Mn concentrations, and Mn in the fine roots at each Mn concentration was markedly higher than in the large ones. Mn was not detected in the inner flesh at each Mn concentration, while it was found in large amounts in the rind, showing 141, 1,492 and 2,545 ppm at 0.5, 30 and 60 ppm Mn, respectively.

Experiment III.

Mn content in the various tissues of plant parts (Table 8)

Data at 0 ppm Mn were not shown because Mn at this treatment was scarcely detected. Generally Mn in the various tissues of plant parts was much higher at 60 ppm Mn though the content varied with the tissues. At 60 ppm Mn, (a) leaf: Mn in the leaf blade except for veins was much higher than that in the mesophyll. (b) petiole: Mn was most concentrated in the hairs, 26,413 ppm, less concentrated in the external tissues, and least concentrated in the vascular bundle or pith. (c) stem: Mn was as high as 8,293 ppm in the external tissues, and was markedly lower in the vascular bundle or pith. (d) roots: Mn in the fine roots (less than 1 mm in diameter) was much higher than that in the large ones (more than 2.5 mm in diameter), and Mn in the external tissues was much higher than in the internal ones. (e) rind: Mn in the rind's net was as high as 6,134 ppm, and Mn in the epidermal layers was 1,022 ppm. (f) seeds: No Mn was detected in the seed coat, while 398 ppm Mn was found in the kernel.

Discussion

As shown in Tables 2 and 6, Mn in the leaf blades and petioles of muskmelons tended to be higher in the lower part than in the middle and higher parts. In tobacco plants (9) Mn content was also higher in the lower leaves than in the higher ones. These seem to show that Mn is an element which is

Table 8. Effects of Mn concentrations on Mn content in the various tissues of plant parts of muskmelon (dry wt ppm).

Mn concn (ppm)	Leaf blade except for veins	Mesophyll (Tissues between lower and upper epidermis)	Hairs in petiole	External tissues to collenchyma in petiole	Vascular bundle in petiole	Pith in petiole	External tissues to collenchyma in stem	Vascular bundle in stem	Pith in stem
0.5	2,158	511	1,061	966	0	0	1,250	0	0
60	10,500	4,000	26,413	12,496	454	284	8,293	398	227

Mn concn (ppm)	External tissues to endodermis in fine roots	Internal tissues to endodermis in fine roots	External tissues to endodermis in large roots	Internal tissues to endodermis in large roots	Net in rind	Epidermal layers in rind (1 to 2 mm in thickness)	Seed coat	Kernel in seeds
0.5	158	0	57	0	45	0	0	0
60	2,499	398	994	170	6,134	1,022	0	398

relatively immobile within plant tissues. For this point Bukovac et al. (1) reported that Mn in the bean plants was intermediate in mobility between highly mobile phosphorus and relatively immobile calcium in an experiment using radioactive isotopes. Mn in the leaf blades and petioles accumulated in the external tissues, especially in the hairs as shown in Table 8. This accumulation in the hairs was also proved by the authors (8) using a sensitive potassium periodate-tetrabase test for Mn (2). The fact that large amounts of Mn was found in the external tissues seems to show that the plant has a potential to eliminate excess absorbed Mn through the hairs and water pores. A similar result was reported in cucumbers by Fukatsu et al. (4). Though Mn in the leaf blades and petioles was lower at the low pH in Experiment I than at 60 ppm Mn in Experiment II, the growth of plant and fruit was markedly inhibited at the low pH. This may be caused by higher EC resulting from the addition of dusting sulfur for adjusting pH.

In the soil culture in Experiment I no Mn difference was found among the stem parts at each soil pH. This result was probably due to the conductive function of the stem. In the nutrient solution culture in Experiment II Mn in the stem at 60 ppm Mn was higher in the higher part. The accumulation of Mn in the external tissues of the higher stem probably resulted from pinching at the 23 rd node. Actually Mn in the external tissues of the stem at 60 ppm Mn was as high as 8,293 ppm as indicated in Table 8. In general, Mn content was much lower in the stem than in the leaf blades as shown in Tables 2 and 6. This was probably due to smaller and fewer hairs on the stem in addition to the structural feature of the stem which consisted mainly of vascular bundles and pith.

Mn was much higher in the fine roots than in the large ones as shown in Tables 3 and 7. This may be caused by larger root surfaces and by greater oxidizing potential in the fine roots. That is, when the weight of large roots equals that of fine roots, total root surface should be larger in the fine roots. Therefore fine roots should have much more absorbing surface and root hairs which have a fairly high oxidizing potential. Thus much Mn was found in the external surface of the fine roots as a precipitate of MnO_2 as shown in Table 8 and in a previous paper (6).

Mn in the fruit increased in the inner flesh, outer flesh and rind in this order, especially higher in the rind as shown in Tables 3 and 7, and Mn in the rind was concentrated as high as 6,134 ppm in the net as indicated in Table 8. Actually much Mn was also detected in the net of the rind (8) showing darker blue color by the tetrabase test as described before. This seems to show that the fruit has a potential to eliminate excess Mn through the vascular bundles as in the leaves.

Recently the intake of heavy metals such as cadmium, copper, mercury and so on by human beings has become an object of public concern. When we consume foodstuffs containing high Mn, it might be a vital question for the health of human beings because Mn is one of the heavy metals. Fortunately we have never heard of Mn toxicity for human beings other than for persons who have worked at a Mn mine. On the otherhand, it is well known that Mn plays an important role in plants as well as animals as a cofactor in various enzymatic reactions. The Mn requirement for adults is estimated to be about 2.74 to 4.6 mg a day (3). When we consume 500 g of muskmelon fruit containing 10 ppm Mn on a fresh weight basis (100 ppm Mn on a dry weight basis), then Mn intake will be 5 mg. Thus Mn toxicity through muskmelon fruit will not be a serious problem as long as it does not contain extremely high Mn.

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メロンのマンガン過剰症に関する研究（第6報）

植物体各部におけるマンガン分布

増井正夫・糠谷　明・石田　明・小倉孝保
（静岡大学農学部）

摘　要

メロンを pH 3段階，6.4（高），5.5（中），4.5（低）に調節した土壌，及び Mn 0.5, 30, 60ppm と 0, 0.5, 60 ppm 含む培養液を施用して砂で栽培し，それらが生育，植物体各部のマンガン（Mn）分布に及ぼす影響について明らかにした．生育は，土壌 pH の低下で著しく抑制された．植物体各部の Mn は，土壌 pH が低下するにつれ，また，培養液中の Mn 濃度が高まるにつれて著しく増加した．葉身，葉柄の Mn は，植物体の中，上部よりも下部で高い傾向がみられた．Mn は外部組織に多く蓄積し，特に毛に多く蓄積した．細根の Mn は，太い根よりもかなり高かった．果実の Mn は，果肉内壁部，果肉外壁部，果皮の順で増加した．