Introduction

Manganese (Mn) affects numerous physiological and biochemical processes of plants. In general, the nutritional status of plants influences considerably the allocation of carbohydrates and thus partitioning of the dry mass between the shoots and the roots, with a result the change of shoot/root ratio (Marschner, 1995). However, it is not known if Mn supply affects partitioning of carbohydrates between shoots and roots of citrus plants.

The existence of many enzymes in the plant cells for protective purposes has been recognized for both deficiency and toxicity of Mn. These enzymes include superoxide dismutase, catalase and ascorbate peroxidase (Candan and Tarhan, 2003). However, little data have been published concerning the effects of Mn on various non-enzymatic molecules, such as proline, that contributes in the antioxidant protection of plants (Heuer, 2003). In this experiment, the Mn-induced changes in growth of *Citrus volkameriana* plants in relation to carbohydrate production and partitioning as well as to total nutrient uptake were investigated. The concentrations of proline were also determined in both roots and leaves.

Materials and Methods

Seedlings of *Citrus volkameriana* (L.) were grown in plastic pots containing 1.2 L of modified Hoagland (No. 2) nutrient solution (Hoagland and Arnon, 1950). All nutrients, except Mn, were supplied at half strength. The Mn was supplied at five concentrations (treatments), i.e., 0, 2, 14, 98 and 686 μM. For each treatment, 5 pots were used. In each pot, 5 seedlings were grown. The seedlings were grown in a growth chamber (temperature, 25±1 °C; light intensity, 270-300 μmol m^{-2} s^{-1}; light:dark, 16:8 h).

Table 1: Growth parameters of *C. volkameriana* plants grown in nutrient solutions with different Mn concentrations.

Parameter	Plant part	Mn concentration in the nutrient solution (μM)					Correlation coefficient (r)
		0	2	14	98	686	
Fresh weight (g)	Shoot	0.78bc	1.18d	0.87c	0.60ab	0.39a	-0.569***
	Root	0.51b	0.67c	0.57bc	0.45ab	0.35a	-0.438**
	Total	1.29bc	1.85d	1.44c	1.05ab	0.74a	-0.539***
Dry weight (g)	Shoot	0.24bc	0.38d	0.28c	0.18ab	0.14a	-0.504***
	Root	0.12ab	0.19c	0.13b	0.10ab	0.08a	-0.408**
	Total	0.36bc	0.57d	0.41c	0.28ab	0.22a	-0.482***
Length (cm)	Shoot	9.5b	11.8c	10.4b	9.7b	8.2a	-0.545***

In all tables: N=5, each replication represented the mean value of 3 plants. For each line, the means marked with the same letter(s) did not differ significantly from each other for P≤0.05 (Duncan's multiple range test). For correlation coefficient analysis, N=75 (5 replications of 3 plants x 5 Mn treatments). ***P≤0.001, **P≤0.01, *P≤0.05.

The experiment was terminated after 43 days, when visible symptoms of both Mn deficiency and toxicity had developed in the leaves of plants treated with 0 and 686 μM Mn, respectively. Fifteen plants per Mn treatment were initially used for the measurement of various plant growth parameters. Each plant was divided into shoot

and root, weighed (fresh weight), washed with tap water and then twice with distilled water, oven-dried at 75 °C for 3 days, and weighed again (dry weight).

To determine the concentrations of various nutrients in the shoot and root, 5 oven-dried (75 °C, 3 days) samples per Mn treatment, were wet digested in a HNO_3:$HClO_4$ (4:1, v/v) solution for 5.5 h (1 h at 50 °C, 4.5 h at 130 °C). Afterwards, the concentrations of Mn, K, Ca, Mg, Fe, and Zn were measured by atomic absorption spectroscopy. The content (absolute quantity) of each nutrient existed in the shoots and roots at the end of this experiment was calculated as well as the total nutrient content (μg) per plant, and thus the total nutrient uptake per plant, was computed. The concentrations (μmol g^{-1} f.w.) of free proline and carbohydrates in the shoot and the root of each plant were determined following the acid ninhydrin reagent and the anthrone methods, respectively (Khan et al., 2000).

Results and Discussion

The highest growth of *C. volkameriana* plants was recorded under 2 μM Mn (Table 1). In general, the increase of Mn concentration in the nutrient solution resulted in a linear decrease not only of total fresh (r=-0.539, P≤0.001) and dry (r=-0.482, P≤0.001) weights of plants but also of their shoot length (r=-0.545, P≤0.001). Plants treated with 686 μM Mn weighed significantly less than those grown under 0-14 μM Mn (Table 1) and presented intensive visible symptoms in their leaves, especially in the youngest ones which were very chlorotic, small and crinkled. On the other hand, the total weight of plants grown under 0 μM Mn was also significantly lower than this of plants treated with 2 μM Mn. The absence of Mn from the nutrient solution resulted in the appearance of a mild interveinal chlorosis in the leaves. The decreased growth of *C. volkameriana* plants treated with 0, 98 and 686 μM Mn, compared to those grown under 2-14 μM Mn, could be ascribed to the significantly lower concentrations of carbohydrates found in the leaves under 0 and 686 μM Mn, and in the roots under 0, 98 and 686 μM Mn, compared to the treatments containing 2 or 14 μM Mn (Table 2). Multiplying the corresponding data of the Tables 1 (fresh weight of root) and 2 (carbohydrates concentration in root), the mean carbohydrates content (μmol) existed in the roots at the end of the experiment is computed. This was: 19.64, 41.16, 39.84, 18.64, and 13.72 μmol of carbohydrates in the root of plants grown for 43 days under 0, 2, 14, 98, and 686 μM Mn, respectively.

Table 2: Concentrations of carbohydrates (leaves, root), and proline (leaves, root), as affected Mn concentration in the nutrient solution.

Parameter	Plant part	Mn concentration in the nutrient solution (μM)					Correlation coefficient (r)
		0	2	14	98	686	
Carbohydrates (μmol g^{-1} f.w.)	Leaves	75.54a	111.63b	120.76b	122.47b	84.43a	-0.250
	Root	38.51a	61.44b	69.90b	41.43a	39.19a	-0.417
Proline (μmol g^{-1} f.w.)	Leaves	13.83a	19.27b	19.86b	18.68b	23.03c	0.406
	Root	18.03a	21.64b	23.94b	22.16b	27.94c	0.735**

Proline contributes to the antioxidant protection of plants (Heuer, 2003). Table 2 shows that as Mn concentration in the nutrient solution was increased, proline concentration in leaves and roots of *C. volkameriana* plants was also enhanced. This

increase was more pronounced in roots than in leaves. The significantly lower concentrations of proline in leaves and roots of plants grown under 0 μM Mn, compared to the other Mn treatments, could be probably ascribed to the lower rates of anabolic procedures occurred under 0 μM Mn due to Mn deficiency (decreased PSII activity, increased photooxidative damage, decreased production of photosynthates) (Papadakis et al., 2007).

Table 3: Total contents (μg) of K, Ca, Mg, Mn, Fe and Zn per *C. volkameriana* plant as affected by Mn concentration in the nutrient solution.

Total nutrient content per plant (μg)	Mn concentration in the nutrient solution (μM)				
	0	2	14	98	686
K	4422.8c	6307.6d	3928.0bc	3575.9b	2570.2a
Ca	3011.0bc	3718.7c	2697.1b	2153.2ab	1897.9a
Mg	306.3b	407.6c	239.2a	245.7a	228.7a
Mn	6.4a	14.1b	27.5c	103.9d	423.4e
Fe	50.1c	51.5c	42.9b	35.9a	39.6ab
Zn	22.9b	24.4b	14.9a	14.9a	16.6a

The uptake of all the determined nutrients was significantly affected by Mn concentration in the nutrient solution. Precisely, the total uptake of Fe and Zn was decreased significantly when the nutrient solution contained ≥14 μM Mn. The same was found for K and Ca but only in the treatments 98 and 686 μM Mn (Table 3). In rice plants grown in solutions containing high Mn concentrations, the uptake of Ca, Mg, Cu, P and Fe was increased ,while this of K and Zn was decreased (Lidon, 2001).

References

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