

Influence of Zn and Mn Levels on Growth and Micronutrient Acquisition of Apple Microculture

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Abstract: Growth and micronutrient acquisition of two apple rootstock (MM106 and M 26) and one cultivar (Galla) in response to different level of Zn (0.8, 1.6, 4.8, or 6.4 mg L⁻¹) and Mn (2.7, 5.4, 16.2 or 21.6 mg L⁻¹) concentration were studied *in vitro*. Microshoot fresh weight and dry weight were increased with increasing Zn level in the medium in all apple varieties. Similar response was shown for leaf number, number of shoot and microshoot length with increase Zn up to 4.8 mg L⁻¹ and decreased at higher level. Zn acquisition in all varieties was elevated with increasing Zn level in the medium, while Zn had an antagonistic effect on Mn and Cu concentration at high Zn level (6.4 mg L⁻¹). Antagonistic effect of Zn was observed on Fe concentration on Galla and M26 and a synergistic effect was obtained in MM106 tissues. In Mn experiment, microshoot fresh weight, dry weight and length in all apple varieties increased with increasing Mn level (up to 16.2 mg L⁻¹) in the medium. Meanwhile, leaf number and number of shoots decreased with increasing Mn level in MM 106 and M 26. Increasing Mn level in the medium had a positive effect on Zn uptake in MM 106 and Galla tissues and Zn uptake decreased in M 26. Cu acquisition in Galla was decreased with elevated Mn level in the medium. In Galla and M 26 varieties Mn had a positive effect on Fe concentration.

Key words: Acquisition • apple • micronutrient • microculture

INTRODUCTION

Micronutrients availability and utilization by plants are highly dependent on soil and climatic factors, such as, soil type, aeration, water and mineral interaction [1-3]. Basic soil reaction and CaCO₃ content in the soil significantly limit the availability of Zn and Mn. Under these soil conditions, plant genotype that genetically controls the use efficiency of soil nutrients, can play a key role in enhancing Zn and Mn acquisition under unfavorable soil conditions. Moreover, varieties within each genotype may have differential response to low or toxic levels of soil micronutrients [4, 5].

In tissue culture, growth medium was designed to provide the plant tissues with mineral nutrients (macro and micronutrients) that are necessary for *in vitro* and development. Ratios of nutrients in the medium were stated to mimic those found in plant tissues after ashing

[6] which represent the actual plant needs of minerals *in vitro*. So using tissue culture techniques have made it possible to estimate plant needs in different growth stages of micronutrients that might taken as a guide in many fertilization programs [7, 8].

Tissue culture has been considered a relevant system for selection of plant tolerance to the imposed treatment in the medium [9]. *In vitro* cultures offer greater control and precise measurement for growth and development of plant tissues [9-11]. The use of tissue culture can help to focus on the physiological and biochemical mechanisms, which help plant tolerance to medium-included stress [10-13]. It has been reported that the whole plant response to stress conditions at the cellular level and those of the tissue culture are similar [11, 12]. In addition, tissue culture been used to assess plant reaction to salinity [9, 13] or drought [14] in many plant species. Although several studies [14-16] have been conducted *in vitro* to

monitor plant genotypes responses to low, normal and even toxic levels of a certain micronutrient under controlled environment. More research is needed to investigate more genotypes and to study the effect of different levels of Zn and Mn in the medium on growth and acquisition of other micronutrients. This would also help in screening varieties that are resistant to either micronutrient deficiency or toxicity. In the study, growth and micronutrient acquisition of apple rootstocks (MM 106, M 26) and one cultivar (Galla) in response to different levels of Zn and Mn were evaluated *in vitro*.

MATERIALS AND METHODS

Two apple rootstocks (MM 106 and M 26) and one cultivar (Galla) were established *in vitro* according to Shibli *et al.* [11]. Mature dormant wood (one year) was collected in late winter and cut into section (10 cm long). The basal end of each section was immersed in tap water at room temperature $24\pm 2^\circ\text{C}$ day and $18\pm 2^\circ\text{C}$ night for four weeks until buds broke their dormancy. Buds were excised and surface sterilized in 5.52% sodium hypochlorite and rinsed aseptically with sterile distilled water. Shoot tips (0.5-0.7 mm) were excised and inoculated on MS medium [17] supplemented with 30 g L^{-1} sucrose, 1.0 mg L^{-1} N^6 -Benzyl adenine (BA), 0.1 mg L^{-1} gibberellin (GA_3) and 8.0 g L^{-1} Bacto ager. Cultures were transferred to the growth room and maintained under daily regime of 16 h light (photosynthetic photon Flux Density; PPF = $40\text{-}45\ \mu\text{mol m}^{-2}\text{ s}^{-1}$)/8 h dark and incubated at $22\pm 2^\circ\text{C}$ and subculturing was performed every four weeks.

For Zn experiment, microshoots (1.0 cm) were transferred into a full strength MS medium supplemented with different Zn concentration (0.8, 1.6, 4.8 and 6.4 mg L^{-1}) added from $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ (1.6 mg L^{-1} = regular Zn concentration described in MS medium). For Mn experiment, other microshoot were subculture into MS medium supplemented with different Mn level (2.7, 5.4, 16.2 and 21.6 mg L^{-1}) added from $\text{MnSO}_4\cdot 4\text{H}_2\text{O}$ (5.4 mg L^{-1} = regular Mn concentration described in MS medium). The medium was also supplemented with 0.5 mg L^{-1} 6-Benzyladenine, 2.0 mg L^{-1} gibberelline, 0.1 mg L^{-1} Indolebutyric acid (IBA), 30 g L^{-1} sucrose and 8 g L^{-1} Bacto agar and pH was adjusted to 5.7. The cultures were maintained under similar growth room conditions described above. Each treatment consisted of ten replicates arranged in a Complete Randomized Designed. Each experiment was repeated 3 times.

The experiment duration was 4 weeks and at the end of each experiment; data were collected on fresh weight,

leaf number, number of proliferated shoots, number of dead leaves and microshoots length. Microshoots were then oven dried at 70°C and dry weight was recorded. Dry microshoots were milled and allowed to pass 1.0 mm sieve size before dry ashing at 55°C . Micronutrient (Zn, Mn, Cu and Fe) were analyzed using an atomic absorption spectrophotometer (Pye Unicam Sp9). Data collected for growth were analyzed statistically according to analysis of variance ANOVA and mean were separated using Least Significant Difference (LSD) test at probability of (0.05). For micronutrient data, the Standard Deviation (SD) between two concentrations of each micronutrient was calculated.

RESULTS AND DISCUSSION

All apple varieties showed some increased in microshoot fresh and dry weight in response to increasing Zn acquisition in the medium (Table 1). Similar results were obtained in tomato and cucumber shoot grown on solution medium supplemented with elevated levels of Zn [4, 15]. Leaf number and number of shoots, however, were highest when Zn concentration was 4.8 mg L^{-1} in the medium. These trends indicated no varieties differences in response to Zn level used in this study (Table 1). Number of dead leaves was decreased in the MM 106 variety when Zn level was lowered below 1.6 mg L^{-1} . Highest number of dead leaves of Galla was at Zn level of 1.6 mg L^{-1} , while with M 26 dead leaves were increased as Zn level increased in the medium. This may suggest that M 26 was the most sensitive to the increased level of Zn. Microshoot length increased in MM 106 and Galla along with the increased Zn level (up to 4.8 mg L^{-1}). However, it was reported that Zn has a role in activation of enzyme responsible of auxin synthesis [18] that intern might be toxic enough to inhibit microshoot elongation. Although M 26 had higher number of dead leaves as Zn increased in the medium, the length of the microshoot was highest.

Increasing Zn concentration in the medium was accompanied with continuous increase in Zn acquisition in an all apple varieties (Table 2). Similar results were reported in tomato and cucumber [4, 11]. However, MM 106 was more efficient in Zn acquisition from the medium at all Zn levels. Similar trend was observed in Mn acquisition in M 106 and Galla as Zn level increased to 4.8 mg L^{-1} (Table 2). Higher levels of Zn (6.4 mg L^{-1}) resulted in lower Mn concentration in the tissues suggesting a negative effect on Mn at the highest Zn level in the medium for all varieties. An obvious

Table 1: Effect of medium Zn concentration on microshoot growth of different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg L⁻¹ BAp, 2.0 mg L⁻¹ GA₃ and 0.1 mg L⁻¹ IBA

Apple variety	Zn conc. (mg L ⁻¹)	Fresh weight (g)	Dry weight (g)	Leaf number	Number of shoots	Number of dead leaves	Microshoot length (cm)
MM 106	0.8	0.320	0.032	11.60	1.8	3.8	1.86
	1.6 ^a	0.322	0.037	18.00	2.2	2.2	1.98
	4.8	0.341	0.066	12.20	1.4	1.4	4.16
	6.4	0.375	0.088	11.60	1.2	1.0	2.16
Galla	0.8	0.237	0.017	6.40	1.0	2.0	1.12
	1.6	0.296	0.033	24.60	1.8	3.2	2.74
	4.8	0.321	0.057	13.40	1.4	2.6	3.20
	6.4	0.374	0.068	9.40	1.2	1.8	2.28
M 26	0.8	0.271	0.035	14.40	1.0	1.8	1.04
	1.6	0.320	0.040	16.21	1.4	2.0	1.48
	4.8	0.327	0.051	14.20	1.0	3.2	2.04
	6.4	0.341	0.054	6.80	1.0	4.2	2.36
LSD		0.019	0.004	0.947	0.167	0.253	0.200

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

Table 2: Effect of medium Zn concentration on micronutrient acquisition in different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg L⁻¹ BAp, 2.0 mg L⁻¹ GA₃ and 0.1 mg L⁻¹ IBA

Apple variety	Zn conc. (mg L ⁻¹)	Mineral content (µg g ⁻¹)			
		Zn	Mn	Cu	Fe
MM 106	0.8	240.31(0.9)	110.00(0.0)	46.25(3.7)	31.25(0.0)
	1.6 ^a	342.27(0.8)	200.63(1.2)	36.20(5.6)	116.66(0.3)
	4.8	417.29(0.9)	232.46(0.4)	26.11(2.8)	246.51(0.5)
	6.4	594.47(0.3)	228.40(0.8)	15.50(0.8)	250.23(0.3)
Galla	0.8	81.13(0.0)	83.77(1.9)	20.29(6.2)	162.90(2.0)
	1.6	130.70(1.5)	160.00(0.5)	21.25(7.8)	174.10(4.0)
	4.8	391.18(0.0)	178.07(1.1)	35.83(2.4)	241.29(2.0)
	6.4	489.44(3.6)	123.15(1.8)	21.80(4.7)	210.60(2.0)
M 26	0.8	283.16(0.3)	217.18(2.4)	70.00(1.6)	731.25(2.0)
	1.6	326.87(0.8)	216.32(0.5)	68.43(2.6)	565.70(0.0)
	4.8	356.88(0.9)	199.71(1.4)	59.14(4.8)	526.32(0.0)
	6.4	478.23(0.3)	171.70(1.5)	34.41(0.9)	308.85(0.6)

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

antagonistic effect of Zn concentration on Cu acquisition was recorded in MM 106 and M 26, while Cu acquisition declined in Galla and M 26 varieties was similar to that of Zn and Mn. The MM 106, however continued to take up more Fe even at highest Zn concentration in the medium (Table 2).

In Mn experiment, fresh and dry weight of microshoots increased with increasing Mn level in the medium (up to 16.2 mg L⁻¹) and any increase beyond this concentration caused a decrease in both weight (Table 3). Increasing Mn concentration in the

medium decreased leaf number of shoots in MM 106 and M 26 varieties but this trend was the opposite in Galla (Table 3). Microshoots length showed some increase with increasing Mn level to 16.2 mg L⁻¹ in all varieties then decline at higher Mn levels (Table 3).

Increasing Mn level in the medium resulted in increased level of Zn in tissue by MM 106 and Galla. M 26 also took up more Zn with increasing Mn level until Mn reached 16.2 mg L⁻¹ (Table 4), where negative interaction between Zn and Mn was observed [8]. Mn acquisition

Table 3: Effect of medium Mn concentration on microshoot growth of different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg L⁻¹ BAp, 2.0 mg L⁻¹ GA₃ and 0.1 mg L⁻¹ IBA

Apple variety	Zn conc. (mg L ⁻¹)	Fresh weight (g)	Dry weight (g)	Leaf number	Number of shoots	Number of dead leaves	Microshoot length (cm)
MM 106	2.7	0.327	0.024	18.20	1.60	1.00	3.40
	5.4 ^a	0.507	0.056	16.80	1.60	0.60	3.90
	16.2	0.602	0.102	16.00	1.00	0.20	4.30
	21.6	0.543	0.086	9.20	1.00	2.00	1.84
Galla	2.7	0.330	0.059	15.60	2.40	0.80	3.20
	5.4	0.430	0.066	16.40	2.40	0.40	3.34
	16.2	0.469	0.088	18.80	3.00	1.00	4.12
	21.6	0.38	0.082	20.40	1.20	2.00	3.40
M 26	2.7	0.433	0.068	28.00	4.80	1.60	1.60
	5.4	0.498	0.078	26.40	3.40	2.00	2.70
	16.2	0.591	0.154	18.60	2.20	1.00	3.88
	21.6	0.568	0.107	14.80	1.60	1.60	2.62
LSD		0.027	0.001	1.29	0.20	0.22	0.35

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

Table 4: Effect of medium Mn concentration on micronutrient acquisition in different apple varieties grown *in vitro*. Medium supplemented with 0.5 mg L⁻¹ BAp, 2.0 mg L⁻¹ GA₃ and 0.1 mg L⁻¹ IBA

Apple variety	Zn conc. (mg L ⁻¹)	Mineral content (µg g ⁻¹)			
		Zn	Mn	Cu	Fe
MM 106	2.7	80.78(0.3)	42.82(1.6)	10.86(0.4)	149.13(0.6)
	5.4 ^a	148.66(0.0)	101.40(0.9)	20.00(0.5)	144.77(1.0)
	16.2	239.99(0.0)	116.31(0.2)	15.69(0.7)	100.43(0.8)
	21.6	245.82(0.3)	138.53(0.1)	13.29(0.1.7)	93.90(2.0)
Galla	2.7	41.00(1.1)	40.90(0.8)	10.13(1.5)	41.25(1.0)
	5.4	184.70(0.4)	105.00(0.7)	10.50(2.0)	97.70(1.4)
	16.2	239.36(0.3)	131.37(1.1)	19.84(2.4)	176.20(0.6)
	21.6	283.78(0.1)	168.88(0.2)	15.33(1.1)	207.14(0.7)
M 26	2.7	126.25(0.3)	50.00(0.3)	11.38(1.2)	83.30(0.7)
	5.4	238.75(0.1)	90.50(0.4)	18.39(1.4)	135.00(1.0)
	16.2	277.75(0.2)	150.75(0.4)	16.37(3.7)	137.1(0.1)
	21.6	110.00(0.6)	165.10(0.1)	8.90(0.1)	148.44(1.0)

a = Same as the regular Zn level in the MS medium. Means were separated using Least Significant Differences (LSD) test at p = 0.05. Means±standard deviation, n = 3. Approximately 10-12 microshoots were tested for each replicate

increased along with increasing along with increasing Mn level in the medium in all apple varieties (Table 4). In MM 106 and M 26, Cu concentration in tissue was the highest for the control treatment and continued to decrease with increasing Mn. This suggests an antagonistic effect of Mn on Cu in these two varieties. Meanwhile, Cu concentration in Galla continued to increase with increasing Mn level (up to 16.2 mg L⁻¹) in the medium (Table 4). Other researcher found a

negative interaction between Mn and Fe but not with Cu [19, 20, 22]. Mn is suggested to interfere with Fe transport in annual crop [15] Fe concentration decreased with increasing Mn level in the medium in MM 106 tissues but this trend was the opposite in Galla and M 26 varieties (Table 4).

Antagonistic interaction between Mn and Fe was observed in avocado trees but not in apple tree [15, 21, 22]. This suggests variations among plant species

in response to differential level of Mn and Fe. Our results suggest, furthermore, varieties variations in their response to Mn and Fe levels. This indicates a positive Mn/Fe relationship in Galla and M 26 varieties while an antagonistic on Fe was observed with MM 106. Antagonistic interaction has been reported among micronutrients [23]. However, these results indicate a significant difference among varieties in their response to different level of micronutrient and to the deficient and/or toxic levels of micronutrient.

CONCLUSIONS

Growth and micronutrient acquisition of the three tested apple varieties were influenced by increasing Zn or Mn level in the medium. Generally, most growth parameters and acquisition of some micronutrients in all apple varieties increased with increasing the Zn or Mn level until the regular micronutrient concentration was tripled as they decline at such high level. Also apple varieties varied in their response to the experimental treatments. This might refer to the varietal sensitivity that varies from one variety to another in micronutrient acquisition. Therefore, this can be suggested as a tool to screen varieties for their responses to deficient and/or toxic level of micronutrient and to their interaction.

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