GOÜ. Ziraat Fakültesi Dergisi 2001 18 (1), 13-16

The Effects of High and Low Zinc Doses On Plant Fresh Weight, Chlorophyll Content, and Calcium, Phosphorus and Iron Nutrition in Hydroponically Grown Tomato Cultivars

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ABSTRACT: A short-term experiment was conducted with two tomato cvs, 'Blizzard' and 'Liberto' in a Controlled Temperature (C.T.) room to test the effects of both low and high zinc concentrations on plant growth, chlorophyll and some water-soluble macro and micro nutrient contents of the plant. Zn was added into nutrient solution at 0.154, 7.70 and 77.0 µmol/l. Plant fresh weight, chlorophyll and macro and micro nutrient concentrations were within optimal range in 7.70 µmol/l Zn treatment. The plants grown at 0.154 µmol/l Zn exhibited P toxicity and those at 77.0 µmol/l showed Fe deficiency. There were some cultivar differences in response to both low and high zinc, but no consistent relationship between cultivars and nutrient concentration within the plant.

Yüksek ve Düşük Dozlardaki Çinkonun, Hidroponik Olarak Yetiştirilen Domates Çeşitlerinde Bitki Yaş Ağırlığı, Klorofil İçeriği ile Kalsiyum, Fosfor ve Demir Beslenmesine Etkileri

ÖZET: Düşük ve yüksek dozdaki çinkonun, bitki gelişmesi, klorofil içeriği ve bazı suda çözülebilir makro ve mikro besin elementlerine olan etkilerini test etmek için, Blizzard ve Liberto domates çeşitleriyle kontrollu ısıtmalı odalarda kısa-dönemli bir deneme yürütülmüştür. Çinko besin çözeltilerine 0.154, 7.70 ve 77.0 µmol/l olarak katılmıştır. Bitki yaş ağırlığı, klorofil içeriği, makro ve mikro elementlerin konsantrasyonları 7.70 µmol/l Zn uygulanmasında optimum düzeyde tesbit edilmiştir. 0.154 µmol/l Zn' da gelişen bitkiler P toksisitesi göstermişlerdir ve 77.0 µmol/l Zn'da yetiştirilenler ise Fe noksanlığı göstermişlerdir. Hem düşük ve hem de yüksek çinkoya karşı çeşitler arasında farklılık bulunmuş fakat bu farklılıkla bitkideki besin konsantrasyonları arasında düzenli bir ilişki saptanmamıştır.

INTRODUCTION

Zinc plays an important role in the production of biomass. The biomass production was suppressed under higher levels of zinc (0.75 mg/l) in bush bean plants grown hydroponically (1, 2). Zinc also has an important role either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes (3). Furthermore, zinc may be required for chlorophyll synthesis. Chlorophyll a levels of *Halimione portulacoides* (L) Aellen plants treated with 50 mg/l zinc in nutrient solution increased throughout the experiment, while the chlorophyll b levels remained approximately constant (4). Hu and Sparks (5) showed that leaf chlorophyll content of Stuart pecan plants were adversely affected by zinc deficiency.

Zinc deficiency is a major problem globally and particularly in some parts of Turkey. Although the Turkish soils are rich in total zinc, plant available Zn is extremely low due to the high pH. Zinc deficiency in soil leads to depression in plant growth and yield (6).

Zinc toxicity is also a significant problem in soil contaminated with zinc by mining and manufacturing activities or where sewage sludge has been applied long-term for agricultural purposes (7).

Current knowledge, relating to the influence of zinc at both low and high levels on plant growth, leaf chlorophyll content and other macro and micro-nutrients in tomato plant, is rather limited. Some research has been done on monocotyledons (8, 9, 10) but there seems to be much less information for dicotyledons and in particular for tomato. This study investigates the effects of both low and high zinc on the plant growth and chlorophyll content and the relationships between zinc and some macro (P and Ca) and micro-nutrients (Fe).

MATERIAL AND METHODS

This Experiment was conducted in Field Station of University of Hertfordshire, UK in 1997. A short-term experiment with tomato cvs. "Blizzard" and "Liberto" was carried out in a growth room with relative humidity at 65-70% and the temperature at 23±2°C. The total photoperiod was 16 h/day and photosynthetic photon flux density (PPFD) on top of the plant was between 355-390 µmol m^{-2} s^{-1} depending on the exact height of the plants. Seeds were germinated in glass beads moistened with 10% strength complete nutrient solution for a week. After germination seedlings were transplanted to a bowl filled with 10 litres of continuously- aerated nutrient solution containing 0.77 µmol/l zinc and grown on for a further week. Seedlings were then transplanted into 4 litre black polyethylene containers (three plants per container) and at the second true leaf stage (4 days after transplanting), seedlings started to receive zinc at concentrations of 0.154, 7.70 or 77.0 µmol/l in nutrient solution. This experiment was replicated three times and all plants were grown on in the 4 l containers for five weeks.

Composition of nutrient solution was (mg/l): 270 N, 31 P, 234 K, 200 Ca, 48 Mg, 2.8 Fe, 0.5 Mn, 0.5 B, 0.02 Cu, 0.05 Zn and 0.01 Mo. Fe was added in organic form (Fe-chelate) in order to prevent precipitation. All chemicals used in these experiments were "Analar" grade. Stock solutions were prepared with deionised water and stored in 1 L-polyethylene containers separately except for Mn, Cu, Mo and B which were stored in the same container. The pH was adjusted to 5.6±0.2 immediately prior to use with minimum of 0.1M KOH.

The leaf samples were washed with deionised water to remove any contamination on leaf surface prior to extraction. Chlorophyll extraction was carried out on fresh leaf material (sample was taken from fifth leaf of top of plant and 1 g leaf sample was ground, using a pestle and mortar, in 90% acetone). Absorbance was measured with Pye Unicam SP6-550 UV/Vis. Spectrophotometer and chlorophyll concentrations were calculated using the formulae from Strain and Svec (11). All other chemical analyses were carried out on plant material dried at 70°C for 48 hours, ground to powder using a pestle and mortar and stored in polyethylene bottles.

Water-soluble Zn, Fe, Ca and P were based on method explained by some researchers (12, 13); 0.10 g ground sample was extracted with 10 mM 2-(4-morpholino)-ethanosulphonate buffer (MES) at pH 6.0, 5 h after shaking. Extracted samples were then filtered through a 0.45 µM membrane filter. Samples were analysed for Zn, Fe and Ca using (Unicam Solar 929 A.A.S.) with an air acetylene flame (with the addition of lanthanum to samples analysed for Ca). P was analysed by a vanadate molybdate method using a Pye Unicam SP6-550 UV/Vis. Spectrophotometer (14).

All data were statistically analysed using an Statview-ANOVA test. Statistically different groups were determined by LSD test ($P<0.05$).

RESULTS AND DISCUSSION

Visual symptoms

The tomato plants in the low (0.154 µmol/l) zinc treatment showed typical zinc deficiency symptoms such as little leaf- rosette and interveinal chlorosis and shoot and root growth were visibly suppressed. These symptoms appeared within two weeks of first treatment and are similar to those reported for tomato plant by researchers (15, 16).e

High (77.0 µmol/l) zinc also inhibited normal root and shoot growth; roots became brown in colour and the growth of the root was limited, the size of leaves was smaller than normal and they were chlorotic. Again these symptoms appeared within two weeks of beginning the high zinc treatment.

The production of biomass

The fresh weights of all parts of both cultivars of tomato plants receiving zinc at the high (77.0 µmol/l) and low (0.154 µmol/l) levels were significantly less than those of the plants grown at intermediate (7.70 µmol/l) zinc concentration (Table 1). The low zinc treatment reduced fresh biomass most in both cultivars, but to a greater extent in Liberto. Generally, fresh weights were higher in Liberto cultivar than Blizzard, except in the low zinc treatment. The plants grown at low zinc concentration were found to be zinc-deficient as evidenced by water-soluble zinc concentration of less than 5.2 mg/kg dry matter in their leaves and by a depression in fresh weight and chlorophyll concentration of plants. A value, less than 5.2mg/kg is considered to be an inadequate level for plants (12). The biomass was well-correlated with the leaf zinc concentration in wheat plants; an increase in zinc level of leaf from 6 to 78 mg/kg dry matter resulted in significant increases in biomass (8). Similarly, Cakmak and Marschner (17) reported that increasing leaf zinc from 13 to 55 mg/kg led to increased biomass of 19- day wheat plants from 0.74 to 0.89 g/plant. Marschner et al. (18) noted that with increasing zinc levels from 0.00975 to 0.06500 mg/l in nutrient solution, increases in dry matter and zinc concentration in leaves of cucumber plants were observed

Table 1. Fresh weight (g/plant) * of two cultivars of tomato grown under various levels of zinc

	Blizzard			**Liberto**		
Zn Conc. µmol/l	Shoot	Root	Whole Plant	Shoot	Root	Whole Plant
0.154	22 a (38)	2.3 a (35)	24.3 a (38)	20 a (26)	2.4 a (28)	22.4 a (26)
7.70	58 b (100)	6.5 c (100)	64.5 b (100)	78 c (100)	8.7 c (100)	86.7 c (100)
77.0	24 a (41)	4.8 b (74)	28.8 a (45)	29 b (37)	5.2 b (60)	34.2 b (39)

*Means of three replicates and each replicate includes two plants (3x2= 6 plant/treatment).
Within each column, same letter indicates no significant difference ($P< 0.05$)
Values in parentheses show straight percentage over 7.70 µmol/l Zn treatment

Chlorophyll concentration

There were significant reductions in the chlorophyll a, chlorophyll b and total chlorophyll concentrations in the leaves of plants grown at both low (0.154) and high (77.0) µmol/l zinc compared with those at 7.70 µmol/l. (Table 2). Similar results were observed by Zhang and Wu (19) who noted that zinc deficiency decreased the chlorophyll a and the chlorophyll b contents and increased the chlorophyll a/b ratio in tomato plants grown in culture solution.

The reduction in chl. a was relatively less than in the chlorophyll b with low and high zinc. The chl.a, chl. b and the total chlorophyll were slightly higher in Blizzard cultivar than Liberto in all treatments, but the ratios of the chlorophyll a to the chlorophyll b were almost the same for both cultivars with the exception of being higher in Liberto grown at high zinc. Similarly it has been showed in previously by Kaya et al., (20, 21) that high zinc reduced chlorophyll content.

Table 2. The chlorophyll concentrations (mg/kg Fresh Weight) * of two tomato cultivars grown under various levels of zinc

Zn Conc. µmol/l	Chl. a	Chl. b	Chl.a+b	%	Chl. a	Chl.b	Chl. a+b	%
	Blizzard				Liberto			
0.154	764 a	371 a	1135 a	79	745 b	358 b	1103 b	85
7.70	916 b	513 b	1429 b	100	826 c	476 c	1302 d	100
77.0	740 a	369 a	1109 a	78	658a	286 a	944 b	73

*Means of three replicates and each replicate includes one plant (3x1=3 plant/treatment).
Within each column, same letter indicates no significant difference (P< 0.05).
%: Straight percentages for Chl. a+b over 7.70 µmol/l Zn treatment

Macro-elements

In the low (0.154 µmol/l) zinc treatment, concentrations of both P and Ca were significantly increased in the leaves, but significantly decreased in the root of both cultivars compared with 7.70 µmol/l zinc treatment (Table 3). In the high (77.0 µmol/l) zinc treatment both P and Ca showed significant reductions in both leaves and roots in all cases except for roots of Liberto. The P concentrations in the leaves of both cultivars were at toxic levels (reported to be greater than 1% for tomato leaves by Adams (22)). Marschner et al. (18) reported that the concentration of P exceeded critical toxicity levels (greater than 1.5% P in leaf dry matter) in leaves of cucumber plants grown in nutrient solution containing zinc at low level (0.00975 mg/l). Zinc deficiency enhanced concentration of phosphorus in leaves or shoots of many species to levels known to be detrimental to plants e. g. in okra (23), cotton (12) and wheat (8). In the present experiment, the reduction in the fresh matter of the plants grown at low zinc concentration may be due to the combination of zinc deficiency and P toxicity. Low zinc also resulted in significant increases in the Ca in the leaves of both cultivars. The Ca concentration, however, was still at optimum level, 2.4 - 7.2 % , a value reported for tomato plants by Adams (22). Our data are in agreement with Norvell and Welch (24) who found that in barley shoot. Ca levels increased with low zinc but not in agreement with Graham et al. (25) who noted that low zinc depressed the Ca concentration in barley.

Table 3. Water- soluble P and Ca concentrations (%)* in leaves and roots of two tomato cultivars grown at different zinc supplies

	Phosphorus				Calcium			
	Blizzard		Liberto		Blizzard		Liberto	
Zn Conc.µmol/l	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
0.154	1.30 c	1.08 a	1.02 c	1.09 a	3.35 c	0.65 a	2.79 c	0.68 a
7.70	0.77 b	1.50 c	0.74 b	1.52 b	2.54 b	1.15 b	2.25 b	1.24 b
77.0	0.20 a	1.32 b	0.19 a	2.04 c	1.71 a	0.49 a	1.88 a	0.59 a

*Means of three replicates and each replicate includes two plants
(3X2=6 plant/treatment)
Within each column, same letter indicates no significant difference (P< 0.05)

Micro-elements

In the leaves of both cultivars grown at low (0.154 µmol/l) Zn in nutrient solution, water-soluble zinc concentration was below the 5.2mg/kg, a value reported to be adequate for normal plant growth by Çakmak and Marschner (12), but there were significant increases in Fe in the leaves of both cultivars in this treatment. However, Fe concentration still seemed to be within adequate range. Similar results were obtained by Ruano et al. (1) who found higher concentration of iron in leaves and lower concentration in roots of bush bean plants grown at 0.13 mg/l zinc in a complete nutrient solution (a deficient level for bush bean plants). Parker (26) reported that the shoot concentration of iron was increased in zinc-deficient tomato plants. Cakmak and Marschner (17) suggested that in zinc- deficient cotton and sunflower plants, possible mechanisms may be acidification in the rhizophere as a result of enhanced net excretion of H^+.

In the high (77.0 µmol/l) zinc treatment, Fe in the leaves was deficient in both cultivars but massively elevated in the roots (Table 5). This is almost certainly due to reduced iron translocation from root to shoot. Marschner (27) reported that zinc toxicity led to a deficiency of iron in shoots. Ambler *et al.* (28) noted that zinc at 1mg/l in nutrient solution suppressed the iron concentration in stem exudate of soybean plants.

Table 4. Water- soluble Zn and Fe concentrations (µg/g dry matter)* in leaves and roots of two tomato cultivars grown at different zinc supplies

	Zinc				Iron			
	Blizzard		Liberto		Blizzard		Liberto	
Zn Conc. µmol/l	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
0.154	4 a	16 a	4 a	17 a	28 c	206 a	57 c	301 a
7.70	28 a	123 a	22 a	134 a	22 b	329 b	27 b	472 b
77.0	328 b	3567 c	216 b	4563 c	7 a	530 c	6 a	890 c

* Means of three replicates and each replicate includes two plants
Within each column, same letter indicates no significant difference (P< 0.05)

CONCLUSION

In the light of the results obtained from this experiment, it can be inferred that in two tomato cultivars investigated:

- Both low and high zinc in nutrient solution suppress the plant growth and chloropohyll production.
- Low zinc produces P toxicity and high zinc produces Fe deficiency
- There are no clear or consistent differences in response between the two cultivars.

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