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NUTRIENT STATUS AND ENZYME ACTIVITY ALTERATION IN CUCUMBER SEEDLINGS AS RESPONSED TO BORON DEFICIENCY

[33]

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ABSTRACT

Cucumber (Cucumus sativus L. var. Beit alpha) seedlings were grown in two groups on boron-deficient (traces of boron) and boron-sufficient (10.0 uM boron) hydroponic media for 30 days under controlled conditions. In harvest, concentrations of magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) in addition to boron (B) were determined in the dry tissues of roots and leaves. Concentrations of phenolic compounds in the roots were also determined. Peroxidase (POD) and catalase (CAT) enzyme activities were assayed in the fresh plant material. In addition, changes of peroxidase and catalase isozyme patterns were also identified. Results showed that vegetative growth of cucumber plants was negatively affected by boron deficiency. Decrease of biomass accumulation reached 24.3 % and 49.1 % shoots and roots respectviely. Nutrient concentrations in both leaves and roots of B-stressed plants were lower drastically. Phenolic compounds were accumulated in significant amounts in the roots of the deficient plants. Peroxidase and catalase enzyme activities increased significantly in the tissues of deficient plants and new isozymes were induced or activated. Irregular biochemical changes occurred in the B-deficient plants were explained as a plant physiological response to adapt with B-hunger conditions.

Key words: Boron deficiency, Nutrients, Peroxidase, Catalase, Cucumber

INTRODUCTION

Boron is an essential element for higher plants as it was established by **Warington (1923).** Recent studies, however, showed that most of boron localized in the cell wall especially in case of boron deficiency (**Hu and Brown, 1994, Matoh *et al* 1996).** Boron deficiency affects on most of nutrient concentrations, i.e. uptake and balance in the plant tissues. Boron effects on plasma membrane, **Cakmak *et al* (1995)** suggested that the primary effects of boron deficiency led to an increase in membrane permeability, which leads to nutrient leakage from the cells. **Muehling *et al* (1998)** observed that bound cellular calcium was low in the boron-deficient faba bean plants. Nitrate content in tobacco leaves decreased dramatically in the borom-deficient plants **(Camacho-Cristobal**

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and Gonzalez-Fontes, 1999). On the other hand, **Zude *et al* (1997)** found that boron foliar application led to increased concentrations of calcium, potassium and magnesium in apple leaves.

Boron plays a key role in carbohydrate transport **(Lewis, 1980)** and metabolism through controlling amylases, reductases and dehydrogenases in the plant tissues **(Goldbach, 1997).** It was also hypothesized that boron stimulates IAA-oxidase and reduces the auxin level to the limit, which allows the subsequent growth of the roots **(Jarvis *et al* 1984).** Plants deficient in boron were found to accumulate polyphenolic compounds as a result of phenolic oxidation and formation of free radicals **(Cakmak *et al* 1995).** However, increases of peroxidase **(Mittler *et al* 2001)** and catalase activities **(Willekens *et al* 1997)** were reported as an early response to stress that provides resistance against the formation of free radicals.

The present work aimed to study the behavior of some nutrients and the related enzyme activity in cucumber seedlings as a result to boron deficiency in hydroponic growth medium.

MATERIAL AND METHODS

Plant material and growth conditions

Seeds of cucumber (*Cucumus sativus* L. var. Beit alpha) were germinated on filter paper moistened with 0.2 mM $CaSO_4$. After 5 days incubation at 24°C in dark, seedlings were transferred into 2.0 L volume plastic aerated culture vessels filled with full strength nutrient solution as described by **Cesco (1994)** with the following composition: 0.7 mM K_2SO_4; 0.1 mM KCl; 2.0 Ca $(NO_3)_2$; 5.0 Mm $MgSO_4$; 0.1 mM KH_2PO_4; 0.5 uM $MnSO_4$; 0.5 uM $ZnSO_4$; 0.2 uM $CuSO_4$; 0.01 uM $(NH_4)_6$ Mo_7 O_{24}. with or without addition of 10.0 uM boron (B) as boric acid.

pH of the nutrient solution was adjusted at 6.2 using NaOH and the whole culture nutrient solution was renewed every three days.

The plants were maintained for 30 days under controlled conditions of 16/8 light/dark hours. Light intensity used was 200 µM. $m^2.s^{-1}$. Relative humidity was 65 %.

Sampling and sample preparation

At the 30th day growth, cucumber plants were harvested. Plant samples were divided into roots and shoots, washed with bidistilled water and oven dried at 65° C for 24 hours, then weighed and grounded. At the same time, fresh samples were taken to assay phenolic compounds and enzyme activity.

Determinations

1- Elements

One-gram sample was dry-ashed in a muffle furnace at 550° C for 6 hours using 3.0 N HNO_3. The residue was, then, suspended in 0.3 N HCl.

Magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were measured using Atomic Absorption Spectrophotometer (Zeiss PMQ3). Boron was extracted according to Wimmer and Goldbach (1999) and measured using UV-VIS-Spectrophotometer (Perkin-Elmer Lambda 2).

2-Phenolic compounds

Total phenols were extracted from root tissues according to Swain and

Hillis (1959) using methanol 80 %, and detected according to **Lam and Street** (1971). The developed blue color was measured at 725 nm using UV-VIS-Spectrophotometer

3-Enzyme activity

Extraction procedure and Enzymes assay

Extraction was carried out according to **Polar** (1976). The plant tissues were excised and homogenized in 250 mM sucrose, 0.2 mM DTT, 2 % polyvinyl polypyrolidine (w/v) and 0.1 mM EDTA. pH grinding buffer was adjusted at 7.2 and the homogenate was filtered through four layers of cheese cloth and centrifuged at 12,000 r.p.m. for 20 min.

Peroxidase (POD) activity was determined in the supernatant according to **Amako *et al* (1994).** The reaction mixture consisted of 1.5 ml (100 mM K. phosphate buffer pH 6.8); 1.0 ml (60 mM pyrogallol); 0.48 ml (0.6 mM H_2O_2 30%) and 20 µl of the crude enzyme extract. The increase in absorbance at 430 nm was recorded using UV-VIS-Spectrophotometer.

Catalase (CAT) activity was assayed at 240 nm according to **Chance and Maehly** (1955) in a total volume of 1.0 ml of 25.0 mM K-phosphate buffer (pH 6.8), 10 mM H_2O_2 (30%) and a diluted enzyme extract.

Isozyme electrophoresis

Isozyme electrophoresis was made according to **Davis (1964)** using 7.5 % polyacrylamid. Activity stain for enzymes was carried out as follows:

For peroxidase (POD), gels were stained with O-dianisidine as described by **Amako *et al* (1994).** The appearance of dark brown bands was due to peroxidase activity of the respective isozymes in the gel.

Catalase (CAT) isozymes were detected according to the method of **Woodbury *et al* (1971).** The gel was soaked in 5 mM phosphate buffer (pH 7.0), then transferred into 5.0 ml H_2O_2 30%. After 10 min. the gel was washed with water and stained with a reaction mixture containing 2% (w/v) ferric chloride and 2% (w/v) potassium-ferricyanide. The enzyme appeared in yellow bands on the dark green background. The reaction was then stopped by water and the gel was photographed.

Data analysis

Data were statistically analyzed using Costate Statistical Package **(Anonymous, 1989).**

RESULTS AND DISCUSSION

• Dry biomass accumulation

A considerable decrease in dry biomass accumulation of the untreated plant shoots and roots was lower and reached 24.3% and 49.1 % respectively in comparison to boron sufficient plants (Fig. 1) In this concern, Boron was assumed to play a role in carbohydrate transport **(Lewis, 1980)** and metabolism **(Goldbach, 1997).** Thus, boron deficiency was interpreted as less dry biomass accumulation. On the other hand, lower shoot/root ratio of B-deficient plants showed that roots are more severely affected than shoots. Similar findings were

found by **Dell and Huang (1997)** and **Camacho-Cristobal and Gonzale-Fontes (1999)** on different plants.

- **Tissue nutrient concentration**

Detected boron and other nutrients (Mg, Fe, Mn, Zn and Cu) concentrations in the plant tissues showed that boron deficiency led to low the amount of such elements in the untreated plants than the treated ones (Table, 1). It is also obvious that some nutrients like Mn are accumulated in the roots of boron-deficient plants. Amount of copper in the roots is the same, but its translocation to the leaves of boron-deficient plants was negatively affected. Low boron concentration in the hydroponics medium of the untreated cucumber plants led to its def - ciency in both roots and leaves. The Low concentrations determined for other nutrients (i.e. Mg, Fe, Mn, Zn and Cu), were considerd as reflections of boron insufficiency in root cell membrane, that caused a disturbance in membrane permeability for such nutrients, creating nutrient imbalance within the plant tissues. **Cakmak *et al* (1995)** even found nutrient leakage from sunflower tissues in case of severe boron deficiency.

- **Phenols and enzyme activity**

Significant amounts of phenolic compounds accumulated in the roots of boron-deficient cucumber plants (Fig. 2). Specific activities of peroxidase (POD) and catalase (CAT) in the leaves or roots of boron-deficient plants were significantly higher than that of the boron-sufficient plants (Table, 2). The highest activity of both POD and CAT was found in the roots of boron deficient plants. Boron deficiency was also postulated to increase oxidation reactions in the plant tissues (**Marschner, 1995**). Thus, increase in concentration of phenolic compounds in roots of B-deficient plants, proved that roots are suffering from boron deficiency, which led to oxidation of phenolics.

- **Isozyme expression**

- Peroxidase (POD)

Expression of POD was detected in water-soluble protein fraction of roots and leaves of cucumber plants (Fig. 3). Electrophoretic analysis showed that four distinct bands were appeared in roots of boron-deficient plants (lane 1), whereas in roots of boron-sufficient plants (lane 3), only two bands with different intensity were observed. Also four bands with different RF values were exhibited in boron-deficient leaves (lane 2), while two bands different in mobility were observed in the leaf tissue of the plants grown at sufficiency level (lane 4). It is also obvious that POD increased under boron deficiency and that stimulation was more expressed in roots than leaves.

- Catalase (CAT)

CAT isozyme patterns are shown in Fig.4. Only one band was exhibited for roots or leaves of B-deficient and B-sufficient plants. Boron deficient roots showed a faint band with a slow mobility (RF =0.18) (lane 1), while roots of boron-sufficient plants showed also a faint band (lane 3) but with different RF value (0.21). Bands of boron-deficient and boron-sufficient leaves (Lanes 2&4) were exhibited one band with same intensity but different in RF values (0.08 and 0.06).

Table 1. Boron and other nutrient concentration in leaves and roots of 30 days age cucumber as affected by boron level in the growth medium (Dw. basis)

Treatment	Boron (ppm)		Magnesium %		Iron (ppm)		Manganese (ppm)		Zinc (ppm)		Copper (ppm)	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
+ B	48.0	55	1.04	1.01	110	175	31.8	92.5	22.5	23.0	5.0	10.0
- B	13.0	16	0.75	0.81	70.0	75.0	60.0	23.0	12.0	14.0	5.0	6.0

Table 2. Peroxidase (POD) and catalase (CAT) specific activity in roots and leaves of 30 days age cucumber plants as affected by boron level in the growth medium

Treatment	Peroxidase activity (EU/mg protein/min.)		Catalase activity ($\mu M\ H_2O_2$ consumed/mg protein/min.)	
	Root	Leaf	Root	Leaf
+ B	272.7 a	118.55 a	311.4 a	98.9 a
- B	688.4 b	145.64 b	477.1 b	221.3 b
L.D.S (0.05)	63.8	11.33	90.96	12.62

Columns with the same letters are not significantly different

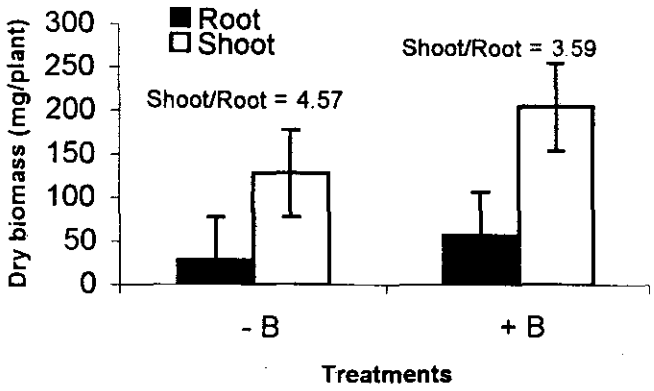

Fig. 1. Dry biomass accumulation in roots and shoots of 30 days age cucumber plants as affected by boron level in the growth medium

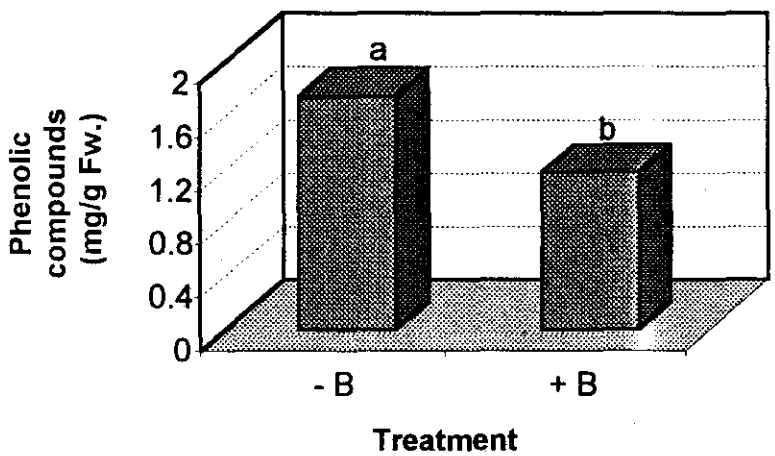

Fig. 2. Phenolic compounds (C_6H_5OH equiv. mg/g Fw.) content of the 30 days age cucumber's root as affected by boron level in the growth medium

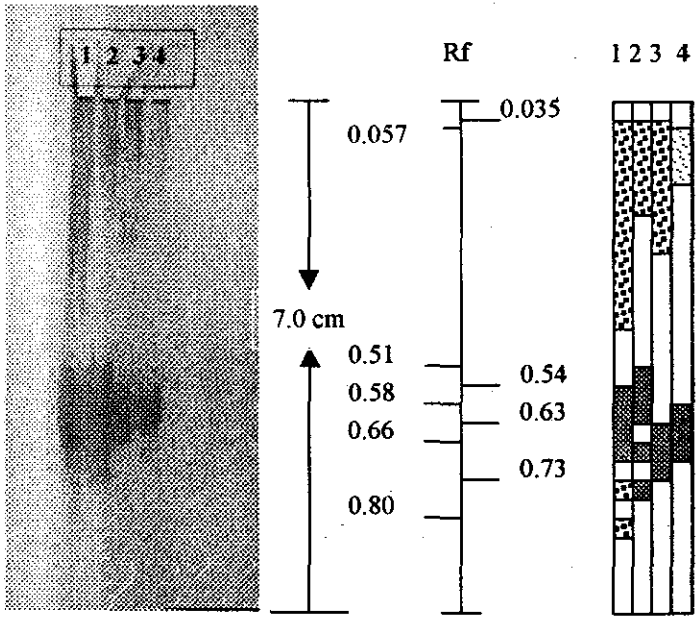

Fig. 3. Isozyme banding patterns of peroxidase (POD) in the 4 weeks age cucumber roots and leaves as affected by boron level in the growth medium
Lane 1 = Boron deficient root
Lane 2 = Boron deficient leaf
Lane 3 = Boron sufficient root
Lane 4 = Boron sufficient leaf

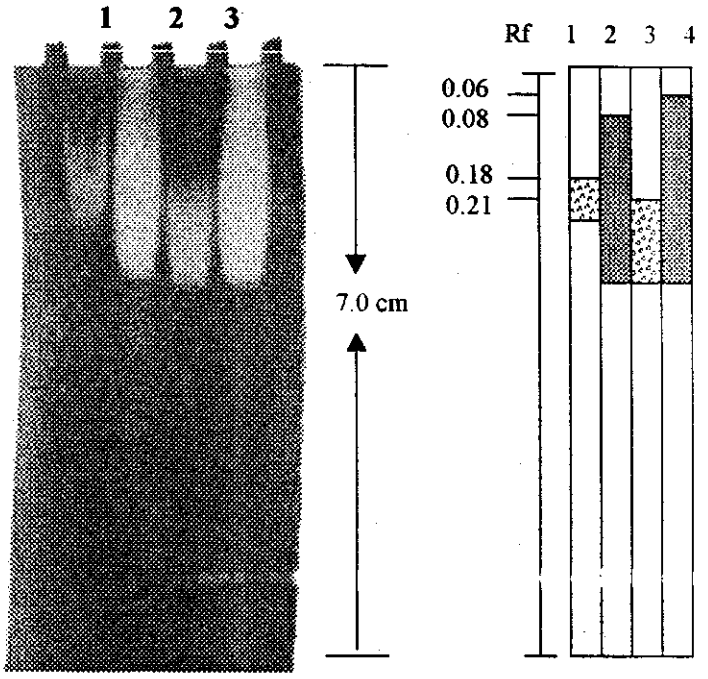

Fig. 4. Isozyme banding patterns of catalase in the 4 weeks age cucumber roots and leaves as affected by boron levels in the growth medium

Lane 1 = Boron deficient root
Lane 2 = Boron deficient leaf
Lane 3 = Boron sufficient root
Lane 4 = Boron sufficient leaf

Dark Faint

Different isoperoxidases in root and leaf cells were formed in B-deficient cucumber plants. Four distinct bands in different intensities, with RF values of 0.035, 0.54, 0.73 and 0.80 were present in the root cells of B deficient plant, while only two bands with the same intensity with RF values of 0.035 and 0.63 were present in the root cells of sufficient plants. In the B-deficient leaf cells, there were also four distinct bands differing in intensity with RF values 0.035, 0.51, 0.66, 0.73 versus two intensity different bands with RF values 0.057 and 0.58 in the leaf cells of B-sufficient plants. Catalase bands were completely different in numbers, intensity and mobility in B-deficient roots and leaves from B-sufficient plants. Increase in numbers and higher intensity bands were found in the B-deficient plants. This means that an induction or activation of different isozymes on the gene level took place as the plants responded to boron deficiency. This may also explain the additional amounts of peroxidase and catalase synthesized to protect the plants against the harmful effects of excess hydrogen peroxidase. Similar results were reported by **Palavan-Nsal *et al*** (2002).

In conclusion, boron deficiency caused reduction in dry biomass accumulation in shoots and roots of cucumber plants. Morever free radicals formed as a result of low boron level in the plant tissues, phenolic compounds are accumulated in the roots. As the plants responses to boron deficiency, peoxidase and catalase enzyme activities in roots and leaf tissues are increased and induction or activation of both peroxidase and catalase isozymes took place.

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التغير فى الحالة الغذائية والنظم الأنزيمية فى بادرات نبات الخيار كاستجابة لنقص عنصر البورون

[٣٣]

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١- قسم تغذية النبات - المركز القومى للبحوث - الدقى - القاهرة - مصر

نميت نباتات الخيار (صنف بيتا ألفا) لمدة ٣٠ يوم تحت ظروف محكمة فى مجموعتين منفصلتين على بيئتين مائيتين أحدهما تحتوى على تركيز ضئيل جدا لا يمكن تقديره من عنصر البورون ، والأخرى تحتوى على تركيز كاف منه (١٠ ميكرومولر)، وفور إنهاء التجربة تم تقدير الوزن الجاف للجذور والمجموع الخضري ، كما تم تقدير عناصر المغنيسيوم ، الحديد ، المنجنيز ، الزنك ، النحاس بالإضافة إلى عنصر البورون فى المادة الجافة للجذور والأوراق، كما تم أيضا تقدير تركيز المواد الفينولية فى الجذور ونشاط أنزيمى البيروكسديز والكتاليز والتغير فى أنماطهما فى الأنسجة الغضة للجذور والأوراق.

أوضحت النتائج أن نقص تركيز عنصر البورون فى بيئة الجذور أدى إلى انخفاض تراكم المادة الجافة بنسبة ٢٤,٣% فى المجموع الخضرى و ٤٩,١% فى المجموع الجذرى للنباتات، كما أن تركيز العناصر المعدنية فى النباتات التى تعرضت لنقص البورون كان منخفضا بدرجة كبيرة مقارنة بالنباتات التى تنمو على البيئة ذات المحتوى الكافى منه.

وجد أيضا أن المواد الفينولية قد تراكمت بكميات معنوية فى جذور النباتات التى تعرضت لنقص البورون ، كما زادت أنشطة إنزيمى البيروكسديز والكاتاليز بالإضافة إلى ظهور أنماط جديدة منهما فى أنسجة هذه النباتات مقارنة بالنباتات التى نمت على البيئة المحتوية على التركيز الكافى من العنصر ، ولقد فسرت هذه التغيرات الكيميائية الحيوية التى حدثت فى النباتات التى تعانى من نقص البورون بأنها رد فعل تكيفى للنظم الفسيولوجية فى مواجهة النقص فى هذا العنصر.

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