#### Effect of Boron on Growth and Some Physiological Activities of Tomato Plant

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**Abstract:** Boron is an essential micronutrient for normal growth of higher plants, when it absorbed in excess amounts, it can be toxic and induce a number of deleterious effects. Tomato is one of the crops which respond well to boron application. A germination and pot experiments were conducted in order to assess the possible effects of boron (0.0, 100, 200, 300 and 400 ppm) on the growth and some metabolic activities of tomato at 9 and 30 days of growth. Application of different concentrations of boron significantly increased fresh and dry weights at low boron concentrations (100 and 200 ppm) compared with control. In addition, the content of Ca and B were increased in shoot and root of tomato, K was increased in shoot and decreased in root, while Na increased in root and decreased in shoot in response to boron application. The activity of glutamate-oxoloacetate and glutamate-pyruvate transaminases enzymes was significantly increased with the applied boron concentrations. Moreover, the root and shoot soluble proteins were increased gradually with increasing boron concentrations. The protein profile of tomato seedlings revealed de novo synthesis of polypeptides with molecular weights 116, 97, 35, 33 and 11 KDa with the highest boron concentration (400 ppm) which may be associated with the tolerance of tomato to excess boron. Shaimaa Abd El-Hameed Abo-Hamad and Soad Soliman El-Feky. Effect of Boron on Growth and Some Physiological Activities of Tomato Plant. *Life Sci J* 2014;11(7):403-408]. (ISSN:1097-8135). http://www.lifesciencesite.com. 48

**Keywords:** growth parameters, mineral content, glutamate-oxoloacetate transaminase, glutamate-pyruvate transaminase, protein, protein pattern.

#### 1. Introduction

Tomato (*lycopersicon esculentum*) belongs to the family solanaceae and is one of the most popular, important and widely used vegetable crops. The yield potential and quality can be improved by maintaining proper fertilizer application. Tomato crop requires heavy manure and sufficient amount of fertilizers for heavy yield. For improving plant growth and development, use of organic and inorganic manure or fertilizers is essential. It is well established fact that chemical fertilizers improve plant growth directly *(Splittstoesser, 1990)*. Like other nutrients, boron has a pronounced effect on the production and quality of tomato.

Boron is one of the micronutrient; the primary function of B is in plant cell wall structural integrity. Under B deficiency, normal cell wall expansion is disrupted (*Havlin et al., 2006*). Boron is needed by the crop plants for cell division, nucleic acid synthesis, uptake of calcium and transport of carbohydrates (*Bose and Tripathi, 1996*). Boron also plays an important role in flowering and fruit formation (*Nonnecke, 1989*). Boron deficiency affects the growing points of roots and youngest leaves. The leaves become wrinkled and curled with light green colour. Its deficiency affects translocation of sugar, starches, nitrogen and phosphorus, synthesis of amino acids and proteins (*Stanley et al., 1995*).

For many plant species there is only a narrow range in critical tissue concentrations between boron

deficiency and born toxicity (Blamey et al., 1997). In spite of the obvious importance of boron, the mechanisms of boron tolerance and toxicity in plants are poorly understood (Cervilla et al., 2007 and Esim et al., 2012). Boron toxicity exerts different effects on very diverse processes in vascular plants, such as altered metabolism, reduced root cell division, lower photosynthetic rates, and decreased lignin and suberin levels (Nable et al., 1997). Accordingly a reduced growth of shoots and roots is typical of plants exposed to high B levels (Nable et al., 1990). Kava et al. (2009) showed that high B reduced dry matter of tomato plants compared to control. Blevins et al. (1993) reported that B has a major influence on the plasma membrane of plant cells and ion transport and that B amendments increased K, Ca and Mg levels in soybean leaves. Cervilla et al. (2008) found that, born toxicity significantly decreased soluble proteins in tomato plants.

The objective of this research was to study the response of some growth parameters and metabolic activities of tomato plant to the different boron concentrations.

#### 2. Materials and Methods

Seeds of tomato (*Lycopersicon esculentum*) were obtained from the Egyptian Ministry of Agriculture. Seeds were sterilized by 0.01% HgCl<sub>2</sub> for 1 minute and washed thoroughly with distilled water and divided into two groups. The first group of seeds was

germinated in a 9 cm diameter Petri dish supplemented with 20 ml of different born concentrations at 0.0, 100, 200, 300 and 400 ppm (as boric acid), 20 seeds in each dish and each treatment was replicated 5 times. After 9 days, the number of germinated seeds was recorded and the germination percentage was calculated. Fresh seedlings were used for protein gel electrophoresis. The second group of seeds was sown in plastic pots (5 seeds/pot) 16 cm height x 13 cm diameter containing 600 gram soil. The soil was composed of clay and sand 2:1 and received the same born concentrations. Each treatment was replicated 5 times and completely random design was used. After 30 days plants were collected for each treatment, shoot fresh weight was determined then dried in an oven at 60°C to a constant weight. Dry samples of shoots and roots were used for the determination of soluble proteins and mineral contents. Fresh leaves samples were used for assaying of GOT and GPT enzymes.

## **Mineral content**

Mineral concentrations in shoot and root were estimated as described by *Allen et al. (1974)*. Samples were analysed for sodium, potassium and calcium content in acid digested samples by using Atomic Absorption (Flame Emission Spectrophotometer Shimadzu Model A.A 640-12).

Born content was determined in plant samples according to *John et al. (1975)*. One ml of sample solution was transferred into a 10 ml polypropylene tube and 2 ml of buffer solution (prepared by dissolving 250 gm of ammonium acetate and 15 gm of EDTA disodium salt in 400 ml of distilled water then, 125 ml of glacial acetic acid were slowly added). Two ml of azomethine-H reagent (prepared by dissolving 0.45 gm of azomethine-H in 100 ml of 1% L-ascorbic acid solution) were added and the mixture was left for 30 min. Thereafter, the absorbance at 420 nm was read and the readings were referred to those of the standard curve.

### Estimation of transaminases activity

Glutamic-oxaloacetic and glutamic-pyruvic transaminases (GOT and GPT) activities were assayed spectrophotometrically in the cell-free extracts using the method described by **Bergmeyer (1974).** The number of units ( $\mu$ M keto acid / ml sample) was calculated using the standard curve of pyruvate.

# Estimation of total soluble protein

For extraction of soluble proteins, dry shoot and root samples (0.1g) were extracted for 24 hours with a borate buffer (28.63 g boric acid + 29.80 g potassium chloride + 3.50 g sodium hydroxide in one liter of distilled water). The buffer pH was adjusted at 8.0 and was kept standing for 24 hours at 4°C before use. The borate buffer plant extracts were centrifugated for 15 minutes at 3000 rpm. The residue left was washed several times with distilled water, dries at 80°C, and kept for estimating polysaccharides. The supernatants were collected, made up to known volumes and kept for estimating of soluble proteins. The total soluble proteins content was estimated quantitatively in the borate buffer extract using the method described by *Bradford (1976)*. The protein content was calculated as mg/gd.wt using a prepared calibration curve by Bovine Serum Albumin protein.

# Qualitative characterization of protein using SDS-PAGE

A sample of 0.5 g of tomato seedlings was homogenized with 1 ml of extraction buffer (25mM Na-acetate, pH 4.5 and 1mM phenyl methyl sulphonyl fluoride [PMSF]), vortexed and left for 2 hours at 4°C. The extract was centrifuged at 10.000 rpm at 0°C for 15 min. and the clear supernatant was used as the total protein extract.

Characterization and molecular mass determination of proteins were carried out using one dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by *Laemmli (1970)*.

## Statistical analysis

The obtained results were statistically analysed to determine the degree of significance between the treatments. Analysis of variance was determined according to the methods described by *Bishop (1983)*.

# 3. Results and Discussion

The seed germination percentages of tomato under different boron concentrations were represented in figure (1). Boron concentrations slightly affected germination percentages. Treatment of plant with 100 ppm B increased germination percentage by about 1% compared with the control. After that, by increasing born concentrations there was a gradual minor decrease in germination percentages. In contrast, treatment of tomato with different boron concentrations greatly affected shoot fresh and dry weights (Figure 2). There was a significant increase in both fresh and dry weights at 100 and 200 ppm B concentrations compared with control. In this connection, Bose and Tripathi (1996) indicated that boron is needed by the crop plants for cell division, nucleic acid synthesis, uptake of calcium and transport of carbohydrates. Plants not treated with B had lower shoot and root dry weights than plants treated with B. Boron was associated with increased tomato growth (Jeanine et al., 2003). Sathya et al., 2013 found that soil application of 20 kg B as borax ha can be recommended to farmers to get higher yeild. Our results indicated that 100 ppm B concentration induced the maximum growth for tomato. Data showed that 400 ppm B concentration reduced fresh and dry weights of tomato. These results are in agreement with those obtained by Eraslan et al. (2007a) on tomato and pepper plants, Koohkan et al. (2008) on rice, Kaya et al. (2009) on tomato and Esim and Atici (2013) on

maize plant. Boron at toxic levels may cause the cell membrane lipids to be damaged by excess of the free radicals induced by decreasing  $\alpha$ -tocopherol levels *(Keles et al., 2004)*.







Figure 2. Effect of different boron concentrations on shoot fresh and dry weights (mg) of tomato plant.



**Figure 3.** Effect of different boron concentrations on mineral contents (mg/g dry wt.) of tomato plant.



**Figure 4.** Effect of different boron concentrations on root and shoot boron contents (mg/g dry wt.) of tomato plant.



**Figure 5.** Effect of different boron concentrations on the glutamate- oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activity of tomato plant.



**Figure 6.** Effect of different boron concentrations on soluble proteins (mg/g dry wt.) of tomato plant.



**Figure 7.** SDS-PAGE of protein extracts of tomato seedlings under different boron concentrations.

Lane M: Marker	Lane 1: control
Lane 2: 100 ppm B	Lone 3: 200 ppm B
Larne 4: 300 ppm B	Lane 5: 400 ppmB

РК	Molecular	Percentage of protein in band					
	weight (KDa)	0	100	200	300	400	
1	126	1.73	-	-	-	-	
2	116	-	-	-	-	1.15	
3	97	-	-	-	-	2.74	
4	84	4.77	-	5.08	5.77	1.54	
5	83	-	5.69	-	-	-	
6	56	-	10.05	10.45	9.46	12.07	
7	55	11.57	-	-	-	-	
8	49	2.73	-	1.81	-	-	
9	44	2.78	5.72	4.19	7.00	6.18	
10	40	-	-	1.39	-	-	
11	35	-	-	6.56	-	6.01	
12	34	6.49	6.57	-	-	-	
13	33	-	2.58	2.62	-	2.03	
14	32	2.48	-	-	15.95	-	
15	31	2.45	-	3.81	-	3.73	
16	29	-	8.40	4.72	-	-	
17	28	3.63	-	-	-	3.28	
18	26	-	-	-	3.98	-	
19	24	10.72	8.95	9.53	10.91	11.33	
20	18	-	0.23	3.22	-	-	
21	17	-	-	1.85	9.15	-	
22	16	7.16	7.75	4.68	-	5.66	
23	15	-	2.78	11.40	8.08	-	
24	14	17.26	8.51	6.32	6.63	17.99	
25	12	10.59	21.77	10.70	11.50	7.93	
26	11	-	-	-	-	3.94	
27	10	3.07	6.62	5.73	5.73	-	
28	9	12.56	4.73	5.95	5.84	14.42	

Table 1. Effect of different boron concentrations on the protein pattern of tomato seedlings.

Different concentrations of boron improved the uptake of Ca into shoot and root. Potassium concentrations in tomato shoots responded positively to B application, while there was a negative response in tomato roots to B application. All the applied B concentrations increased Na in tomato root, while in the shoot there was a minor increase in Na at 100 ppm B and a decrease in Na at all the other concentrations (Figure 3). In this connection Blevins et al. (1993) reported that B has a major influence on the plasma membrane of plant cells and ion transport and that B amendments increased K, Ca and Mg levels in soybean leaves. Singh and Singh (1984) reported that boron increased the N, P, K, Na and B contents, but decreased the Ca and Mg contents of barley crop. On the other hand, the uptake of N, P, Na and B by grain and straw significantly increased, while K uptake remained unaffected with an increase in boron application. The data represented in Figure 4 showed that there was a gradual increase in root boron content and a minor increase in shoot boron content with increasing the applied boron concentrations. Our results suggested

that boron accumulation was higher in tomato roots than shoots. In this respect, *Eraslan et al. (2007a)* reported that, increasing levels of boron increased the boron content of tomato and pepper plants. *Papadakis et al. (2004)* showed that boron concentrations in all plant parts of orange increased by increasing boron concentration in nutrient solution.

The activity of glutamate-oxaloacetate transaminase (GOT) and glutamate- pyruvate transaminase (GPT) enzymes was sharply increased with increasing the applied B concentrations (Figure 5). It is a well known fact that the fluctuations in GOT and /or GPT reflect themselves on the biosynthesis of glutamate, oxaloacetate and pyruvate families of amino acids as well as the tricarboxylic acid cycle where oxaloacetic, a-ketoglutaric and/or pyruvic acids are required for completion of the cycle. GOT and GPT are from the key enzymes in nitrogen metabolism. Several reports have indicated that B may affect the expression level of genes related to nitrogen metabolism (Redondo-Nieto et al., 2001; Camacho-Cristobal and Gonzalez-Fontes, 2007).

*Cervilla et al. (2009)* reported that B significantly decreased nitrate reductase and nitrite reductase activities, while the activities of glutamine synthase, glutamate synthetase and glutamate dehydrogenase were increased.

There was a gradual increase in root and shoot soluble proteins with increasing B concentration. At 400 ppm B there was a decrease in shoot soluble proteins compared with control (Figures 6). Several reports have indicated that B may affect the expression level of genes related to nitrogen metabolism *(Redondo-Nieto et al., 2001; Camacho-Cristobal and Gonzalez-Fontes, 2007). Keles et al. (2004)* studied the effect of boron on orange plants irrigated with relatively high and low amounts of boron and they found that boron containing leaves have higher soluble protein.

The protein profile of tomato seedlings revealed the appearance of newly formed polypeptides with molecular weight of 56 KDa at all boron treatments and polypeptides with molecular weights 116, 97, 35, 33 and 11 KDa with the highest born concentration (400 ppm) compared with the control (Figure 7 and Table 1). Moreover, all the applied boron concentrations resulted in the disappearance of two polypeptides with molecular weight of 126 and 55KDa present in the control. Our results were in agreement with Demiray et al. (2011) who reported that born induced significant changes in the pattern of proteins and that six different proteins involved in plant defense system were identified in carrot plant. Mahboobi et al. (2000) also found the induction of two proteins (22.5 and 29 KDa) in barley leaves under high B supply.

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