



Article

Effects of Application Methods of Boron on Tomato Growth, Fruit Quality and Flavor

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Abstract: The effect of application methods with different boron levels on the growth, fruit quality and flavor of tomato (*Solanum lycopersicum* L., cv. 'Jinpeng No.1') were investigated under greenhouse conditions. Seven treatments used included two application methods (leaf and root application) with four boron levels (0, 1.9, 3.8 and 5.7 mg·L⁻¹ H₃BO₃). Experimental outcomes revealed that both application methods significantly increased net photosynthetic rate and chlorophyll content, and stabilized leaf structure of tomato. Leaf spray of 1.9 mg·L⁻¹ H₃BO₃ was more effective at improving plant growth and photosynthetic indices in tomato compared to other treatments. Additionally, root application of 3.8 mg·L⁻¹ H₃BO₃ resulted in better comprehensive attributes of fruit quality and flavor than other treatments in terms of amounts of lycopene, β-carotene, soluble protein, the sugar/acid ratio and characteristic aromatic compounds in fruit. The appropriate application of boron can effectively improve the growth and development of tomato, and change the quality and flavor of fruit, two application methods with four boron levels had different effects on tomato.

Keywords: boron; application methods; growth; fruit quality; tomato



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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops in the world [1,2]. Tomato fruit quality indices comprise appearance, taste, nutrient and characteristic aroma compounds [3]. Flavor is one of the most important factors of fruit quality, and levels of micronutrients are closely related to the flavor of the fruit [4,5].

Micronutrients can play direct or indirect roles in plant growth and quality [6,7]. Boron is one of the most important micronutrients, involved in carbohydrate metabolism, cell division and maintaining cell wall structure, flowering and fruit setting [8]. Boron has extremely low mobility compared to other micronutrients in plants [9], and it is transported in the xylem through the root pressure generated by leaf transpiration and in the phloem through other mechanisms [10,11]. Boron is absorbed by plants in the form of BO₃³⁻ [9]. Although boron is an essential micronutrient for plants [12], it has a narrow optimal concentration. In excess it causes biochemical, physiological and anatomical aberrations in plants [9,13,14]. When in boron-deficient conditions, plants have reduced photosynthetic capacity, thereby preventing cell division and development in chickpea plants [15]. Boron promotes the absorption of calcium and increases the content of vitamin C in the fruit by improving membrane integrity, slowing biosynthesis and reducing respiration in cherry tomato [16]. Davis et al. [17] found leaf sprays of boron increased shelf life of tomato fruit. Sotiropoulos et al. [18] reported that application of boron increased photosynthetic rate

(Pn), stomatal conductance (Cs) and intracellular carbon dioxide concentrations (Ci) in kiwifruit, and boron synergistically promoted the absorption of other plant nutrients and improved quality of kiwifruit [19].

Most studies have focused on the effect of boron stress on tomato growth, but research about different application methods with different boron levels on tomato fruit quality and flavor components is not well studied. The aim of this study was to explore effects of different application methods and boron levels on plant growth and fruit quality in tomato to select optimal application method.

2. Materials and Methods

2.1. Plant Material and Experimentation

This experiment was conducted in the Horticulture Research Greenhouse (College of Horticulture, Northwest A&F University, China). Tomato cultivar 'Jinpeng No.1' was used for experiments. At the five true leaf stage, seedlings were transplanted into 15 L pots containing perlite and vermiculite in a 3:1 *v/v* ratio. There were seven treatments in this study, and each treatment consisted of 20 pots placed randomly with a row spacing of 30 cm × 60 cm area. The plants were treated with 300 mL nutrient solution once every six days after the seedlings were transplanted until the first truss of fruit ripened. Then, 500 mL of nutrient solution was applied per plant every four days until to the second truss was harvested. The nutrient solution (control, CK) was modified slightly by Yamazaki nutrient solution formula without H₃BO₃. According to our previous results and other reports [17,20,21], three levels of boron were used in this experiment. The factorial experiment was performed in a completely random design using leaf and root application methods: CK; L1: control + leaf spray 1.9 mg·L⁻¹ H₃BO₃; L2: control + leaf spray 3.8 mg·L⁻¹ H₃BO₃; L3: control + leaf spray 5.7 mg·L⁻¹ H₃BO₃; W1: control + root application 1.9 mg·L⁻¹ H₃BO₃; W2: control + root application 3.8 mg·L⁻¹ H₃BO₃; W3: control + root application 5.7 mg·L⁻¹ H₃BO₃. To increase the absorption of nutrient elements, the pH of the solution was adjusted to 6.5.

2.2. Plant Growth Parameters and Photosynthetic Indices

In this experiment, three plants were randomly collected in each treatment for plant growth parameters and photosynthetic indices measurement.

Plant growth measurements: After the second fruit truss was harvested, each part of the plant was washed and the fresh weight (FW) was measured, and then plants were put into an oven to determine the dry weight (DW).

Leaf physiological indices: 2–3 leaves of each plant were collected at the fifth leaf below the growth point in the period of the first fruit truss ripening in each experiment. Chlorophyll content was measured using the method of Arnon [22]. Ascorbic acid content was measured using the method of Gao et al. [23]. Soluble protein content was measured using the method of Bradford et al. [24].

Photosynthetic parameters: On a sunny day in the morning at the stage of the first truss ripening stage, the fifth leaf below the growth point was selected to measure the net photosynthetic rate (Pn), intercellular carbon dioxide concentration (Ci), stomatal conductance (Cs), and transpiration rate (Tr) by Li-6400 platform (LI-COR, USA). The determination of each index was repeated four times in each experiment.

2.3. Tomato Fruit Quality Parameters

Two kilograms of fruit at similar ripening stage from the second truss (about 45 days after flowering) were collected from each treatment for fruit quality index determination. Soluble solid content was measured using a PAL-1 digital refractometer (Atago, Japan). Titratable acid content was measured by GMK-835f fruit acidity tester (G-WON, Korea). Sugar/acid rate was calculated by soluble solids/titratable acid. Vitamin C and soluble protein content were described as above. Nitrate nitrogen content was measured using the method of Gao et al. [19]. Lycopene, β-Carotene and Carotenoid content were measured

by the methods of De Nardo et al. [25] and Radu et al. [26], with slight modification. Single fruit weight measured from three plants with similar growth trends were selected in each treatment, and fruits of the second truss were weighed. The determination of each index was repeated four times.

2.4. Analysis of Characteristic Aromatic Compounds

Tomato fruit characteristic aromatic compounds analysis was measured as described by Tikunov et al. [27]. Five fruits at similar ripening stage were selected and homogenized in each treatment, 15 g of the finely powdered tomato sample from each treatment was transferred into a 40 mL Teflon cap vial (Thermo Fisher Scientific, USA) with 5 g of NaCl. The vials were sealed using a silicone/PTFE septum and a magnetic cap, and each sample was agitated (500 rpm) and sonicated for 10 min, then incubated at 50 °C for 10 min prior to HS-SPME-GC-MS analysis. Headspace volatiles were extracted by exposing each sample to a 75 µm carboxen-polydimethylsiloxane SPME fiber (Supelco, USA) for 30 min under continuous agitation (500 rpm) and heating at 40 °C. The fiber was then inserted into an ISQ GC-MS (Thermo Scientific instruments, USA) injection port and the volatiles were desorbed for 3 min at 250 °C. The chromatography was analyzed on an HP-INNOWAX column (60 m × 0.25 mm × 0.25 µm) with helium as the carrier gas at a constant flow of 1 mL·min⁻¹. The temperature of both the GC interface and MS source was 230 °C. The GC temperature program began at 40 °C (2.5 min), and then was raised to 160 °C (5 °C min⁻¹) and to 230 °C (10 °C min⁻¹) before being held at 230 °C for 5 min. The raw data of each sample were obtained from GC-MS with Xcalibur and AMDIS software and identified the volatile compounds on the basis of the NIST/EPA/NIH Mass Spectral Library (NIST, 2008) and Wiley Registry of Mass Spectral Data 8th edition. The characteristic aromatic compounds were measured according to the Baek and Cadwallader [28] and Wang et al. [29] modified slightly. The characteristic aromatic compounds (%) = (Peak area of particular compound/Total peak area) × 100%. Three independent reactions for each sample of each experiment were performed for the analysis of tomato fruits characteristic aromatic compounds.

2.5. Statistical Analysis

The statistical analysis was performed using SPSS software, version 16 (SPSS Inc., Chicago, IL, USA), and the means were compared using Duncan's multiple range test method at a significance level of 0.05.

3. Results

3.1. Effects of Boron on Plant Growth Parameters

There were different effects on growth parameters for different boron treatments (Table 1). Both leaf spray and root application methods significantly increased FW and DW of shoots. Root application of boron significantly increased the shoot weight (FW) compared to leaf spray, except for the level of 1.9 mg·L⁻¹ H₃BO₃. Leaf spray of boron decreased root FW with the increase of boron level, while the trend was opposite when boron was applied to the root. Boron treatment significantly increased the root DW in each treatment except L3, and W3 gave the largest root DW. The control and W1 had the highest (0.21) and lowest (0.11) root/shoot FW, respectively, and the root/shoot FW decreased after leaf spray treatment as boron levels increased. The root/shoot DW, except L1, it was better than W1 in the root/shoot DW, the root application boron had the higher rate of root/shoot DW than leaf spray with same boron level.

Table 1. Effects of boron on tomato growth parameters.

Treatment	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW(g)	R/S (FW)	R/S (DW)
CK	231.63 ± 1.09 e	34.43 ± 1.023 c	48.81 ± 1.26 c	5.45 ± 0.20 d	0.210 ± 0.006 a	0.160 ± 0.006 bc
L1	276.70 ± 6.07 d	41.55 ± 0.881 b	47.55 ± 2.35 c	6.79 ± 0.53 c	0.173 ± 0.009 b	0.160 ± 0.010 bc
L2	281.86 ± 0.70 cd	37.95 ± 0.731 c	45.66 ± 0.80 c	6.71 ± 0.55 c	0.163 ± 0.003 b	0.177 ± 0.012 b
L3	292.30 ± 0.60 c	34.64 ± 1.060 c	37.68 ± 1.15 d	4.77 ± 0.26 d	0.130 ± 0.006 c	0.140 ± 0.010 cd
W1	324.17 ± 7.42 b	43.58 ± 0.281 b	35.50 ± 1.62 d	5.50 ± 0.13 d	0.110 ± 0.006 d	0.127 ± 0.003 d
W2	326.50 ± 5.03 b	42.87 ± 0.925 b	64.52 ± 1.49 b	7.98 ± 0.36 b	0.197 ± 0.007 a	0.187 ± 0.003 b
W3	352.14 ± 4.50 a	48.88 ± 2.159 a	70.44 ± 1.50 a	10.63 ± 0.45 a	0.197 ± 0.003 a	0.220 ± 0.015 a

FW: Fresh weight; DW: Dry weight; Data are expressed as the mean ± standard error of three independent biological replicates. Different letters indicate significant differences at $p < 0.05$; CK: Yamazaki nutrient solution without H_3BO_3 ; L1: CK + leaf spray $1.9 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; L2: CK + leaf spray $3.8 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; L3: CK + leaf spray $5.7 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; W1: CK + root application $1.9 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; W2: CK + root application $3.8 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; W3: CK + root application $5.7 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$.

3.2. Effects of Boron on Soluble Protein and Ascorbic Acid Content in Leaves

Boron treatment significantly increased soluble protein and ascorbic acid content in leaves. Each application method with different boron levels had different effects (Figure 1). Soluble protein in leaves decreased with the increased level of boron by leaf spray, and there was no difference in root application with different boron levels. L1 and W2 treatments increased the soluble protein by 177.6% and 192.8%, respectively. Both leaf spray and root application of boron increased the content of ascorbic acid with increasing boron. Root application of boron was better than leaf spray at the same boron levels. W3 treatment resulted in the highest content of ascorbic acid in leaves, increasing ascorbic acid by 185.2% compared to the control.

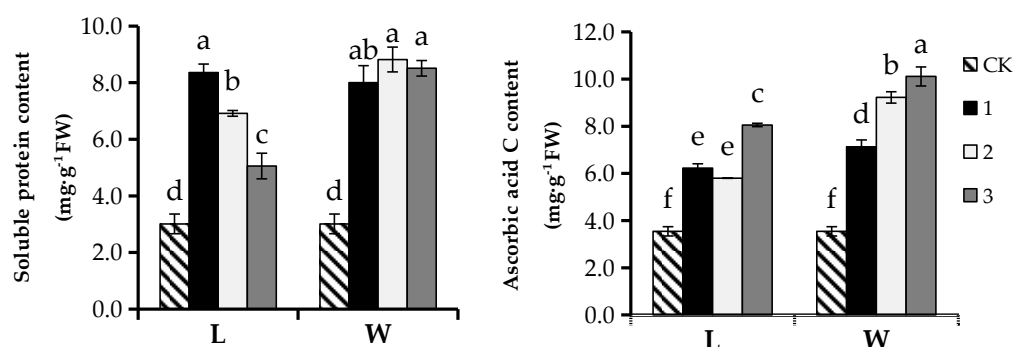


Figure 1. Effects of boron on soluble protein and ascorbic acid content in leaves. Different letters indicate significant differences at $p < 0.05$; L: Leaf spray; W: Root application; CK: Yamazaki nutrient solution without H_3BO_3 ; 1: $1.9 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; 2: $3.8 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; 3: $5.7 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$.

3.3. Effects of Boron on Chlorophyll and Carotenoid Contents and Photosynthetic Parameters

Boron treatment significantly increased Chl a, Chl b, total Chl and carotenoid contents and Chl a/b ratios in leaves (Table 2). Both treatment of leaf spray and root application boron had no significant difference in Chl a, Chl b, total Chl and carotenoid contents using same boron level. L2 treatment had the highest content of Chl a, Chl b, total Chl and carotenoid in the leaf spray group, while W3 had the highest Chl a, Chl b, total Chl and carotenoid content in root application group. Boron treatments had no obvious effects on Chl a/b, which ranged from 1.46 (W2) to 1.54 (L1), while the control had the lowest Chl a/b with only 1.35.

Table 2. Effects of boron on content of Chlorophyll a, Chlorophyll b, total Chlorophyll and Carotenoid in tomato leaves.

Treatment	Chl a ($\text{mg}\cdot\text{g}^{-1}$)	Chl b ($\text{mg}\cdot\text{g}^{-1}$)	Total Chl ($\text{mg}\cdot\text{g}^{-1}$)	Chl a/b	Carotenoid ($\text{mg}\cdot\text{g}^{-1}$)
CK	0.76 ± 0.07 c	0.56 ± 0.05 c	1.33 ± 0.12 c	1.35 ± 0.01 d	0.29 ± 0.03 c
L1	1.75 ± 0.06 b	1.14 ± 0.04 b	2.89 ± 0.10 b	1.54 ± 0.01 a	0.59 ± 0.02 b
L2	2.16 ± 0.03 a	1.42 ± 0.03 a	3.58 ± 0.07 a	1.53 ± 0.02 ab	0.74 ± 0.02 a
L3	2.07 ± 0.13 a	1.39 ± 0.10 a	3.46 ± 0.23 a	1.48 ± 0.01 c	0.68 ± 0.05 ab
W1	1.75 ± 0.06 b	1.17 ± 0.03 b	2.93 ± 0.09 b	1.49 ± 0.02 bc	0.58 ± 0.02 b
W2	2.07 ± 0.09 a	1.42 ± 0.07 a	3.49 ± 0.16 a	1.46 ± 0.01 c	0.69 ± 0.03 a
W3	2.13 ± 0.02 a	1.42 ± 0.01 a	3.56 ± 0.03 a	1.50 ± 0.01 bc	0.72 ± 0.01 a

Chl: Chlorophyll; Data are expressed as the mean \pm standard error of three independent biological replicates. Different letters indicate significant differences at $p < 0.05$; CK: Yamazaki nutrient solution without H_3BO_3 ; L1: CK + leaf spray $1.9 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; L2: CK + leaf spray $3.8 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; L3: CK + leaf spray $5.7 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; W1: CK + root application $1.9 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; W2: CK + root application $3.8 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; W3: CK + root application $5.7 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$.

Boron treatments significantly increased net photosynthetic rate (Pn), intercellular carbon dioxide concentration (Ci), stomatal conductance (Cs) and transpiration rate (Tr) in plants compared with the control (Figure 2). Different application methods showed no significant difference in Pn using the same boron level, except L1, Pn in L1 was significantly higher than other treatments. For Cs, L1 had the highest Cs in leaf spray boron group, and root application significantly increased Cs compared to leaf spray using the higher boron levels. W2 had the highest Cs, which increased by 27.7% compared with the control. The effects on Tr were similar to that in Pn by using different application methods of boron. L1 and W2 had the highest Tr in each treatment group, increasing significantly by 51.1% and 40.8%, respectively. There was no difference in Ci by leaf spray with different boron levels, while in root application treatments, W2 showed significantly increased Ci compared to others. W2 had the highest Ci increasing significantly by 72.6%.

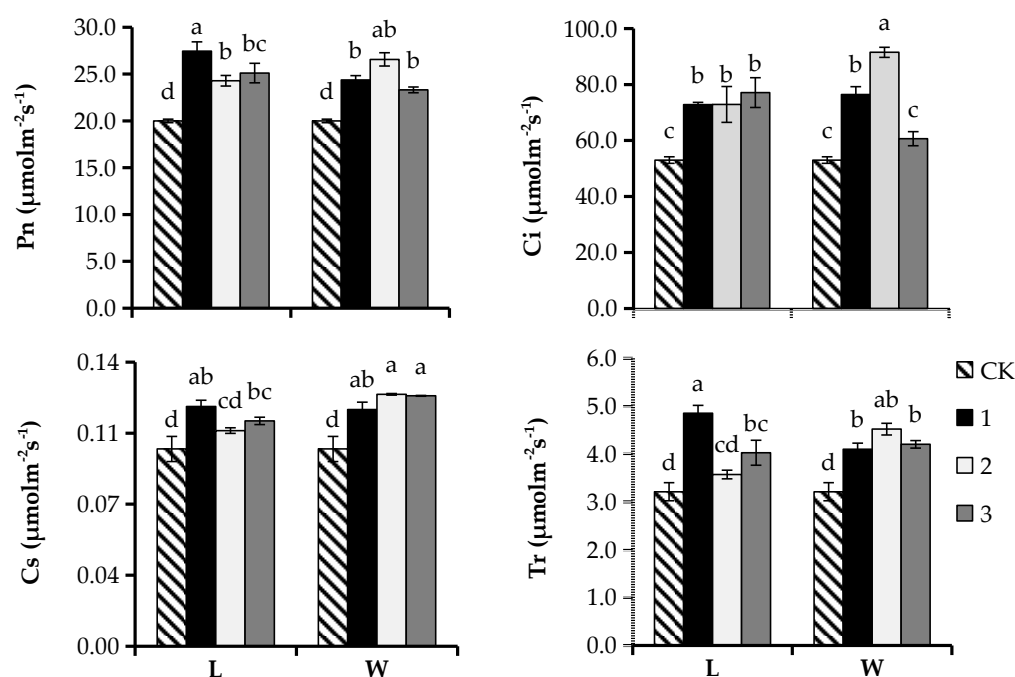


Figure 2. Effect of boron on net photosynthetic rate (Pn), intercellular carbon dioxide concentration (Ci), stomatal conductance (Cs) and transpiration rate (Tr) in tomato. Different letters indicate significant differences at $p < 0.05$; L: Leaf spray; W: Root application; CK: Yamazaki nutrient solution without H_3BO_3 ; 1: $1.9 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; 2: $3.8 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; 3: $5.7 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$.

3.4. Effects of Boron on Quality Indices of Tomato Fruit

In this experiment, two application methods with different boron levels had different effects on soluble solids, titratable acid and sugar/acid rate in fruit (Table 3). For soluble

solids, W3 treatment significantly increased the percentage of soluble solids, but there were no obvious differences in other treatments. Boron treatments significantly improved the percentage of titratable acid in fruit. Leaf spray boron gave a higher sugar/acid rate than root application using same boron level, except W3, it had the highest sugar/acid rate with 7.10 compared to other treatments. Additionally, both leaf spray and root application of boron significantly increased the content of soluble protein and vitamin C in fruit. Root application was better at improving the soluble protein and vitamin C content in fruit than leaf spray, except W2 treatment. Boron treatments significantly increased the nitrate content in fruit compared to the control, except L3 treatment. W3 had the highest nitrate nitrogen content with $403.02 \mu\text{g}\cdot\text{g}^{-1}$. Both leaf spray and root application increased the single fruit weight. Root application gave heavier single fruit than leaf spray treatments using same boron level. W1 had the heaviest single fruit with 193.81 g on average.

Table 3. Effects of boron on quality indices of tomato fruit.

Treatment	Soluble Solids (%)	Titratable Acid (%)	Sugar/Acid Rate	Soluble Protein ($\text{mg}\cdot\text{g}^{-1}$)	Vitamin C ($\text{mg}\cdot\text{kg}^{-1}$)	Nitrate Nitrogen ($\mu\text{g}\cdot\text{g}^{-1}$)	Single Fruit Weight (g)
CK	4.63 ± 0.20 bc	0.69 ± 0.02 c	6.75 ± 0.26 ab	3.01 ± 0.35 d	69.82 ± 0.52 e	177.58 ± 9.46 d	110.96 ± 1.11 e
L1	4.40 ± 0.15 c	0.76 ± 0.01 a	5.81 ± 0.17 d	8.36 ± 0.30 a	72.94 ± 1.50 de	213.19 ± 5.04 c	117.78 ± 1.72 e
L2	5.03 ± 0.09 ab	0.77 ± 0.01 a	6.57 ± 0.11 abc	6.92 ± 0.10 b	82.67 ± 0.90 b	231.98 ± 3.83 c	130.16 ± 3.94 d
L3	4.63 ± 0.19 bc	0.77 ± 0.01 a	6.05 ± 0.27 cd	5.06 ± 0.45 c	76.54 ± 0.44 cd	172.48 ± 4.26 d	130.78 ± 5.28 d
W1	4.47 ± 0.12 c	0.79 ± 0.01 a	5.68 ± 0.10 d	8.00 ± 0.60 ab	79.64 ± 1.98 bc	253.74 ± 7.10 b	193.81 ± 4.59 a
W2	4.53 ± 0.15 c	0.70 ± 0.01 bc	6.50 ± 0.15 bc	8.82 ± 0.44 a	75.58 ± 1.37 cde	220.11 ± 10.02 c	149.48 ± 2.17 c
W3	5.13 ± 0.03 a	0.72 ± 0.01 b	7.10 ± 0.05 a	8.51 ± 0.27 a	92.37 ± 4.00 a	403.02 ± 0.72 a	168.57 ± 3.41 b

Data are expressed as the mean \pm standard error of three independent biological replicates. Different letters indicate significant differences at $p < 0.05$; CK: Yamazaki nutrient solution without H_3BO_3 ; L1: CK + leaf spray $1.9 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; L2: CK + leaf spray $3.8 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; L3: CK + leaf spray $5.7 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; W1: CK + root application $1.9 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; W2: CK + root application $3.8 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$; W3: CK + root application $5.7 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$.

3.5. Effects of Boron on Lycopene, β -Carotene and Carotenoid Content in Fruit

Both leaf spray and root application had different effects on the content of lycopene, β -carotene and carotenoid in fruit (Figure 3). Leaf spray significantly increased the lycopene content with increasing boron, while in root application treatment, W1 treatment had the highest content of lycopene compared to other levels applied the same way. Lycopene content decreased with the increase of boron level. Both leaf spray and root application treatments significantly increased β -carotene content in fruit, and the effects on the content of β -carotene were similar to that of lycopene in both treatments. W1 had the highest β -carotene content with $5.6 \mu\text{g}\cdot\text{g}^{-1}$ compared to others. Moreover, boron treatments significantly increased the carotenoid content in fruit. Leaf spray and root application treatments with same boron level ($3.8 \text{ mg}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$) resulted in the highest carotenoid content in each group, respectively.

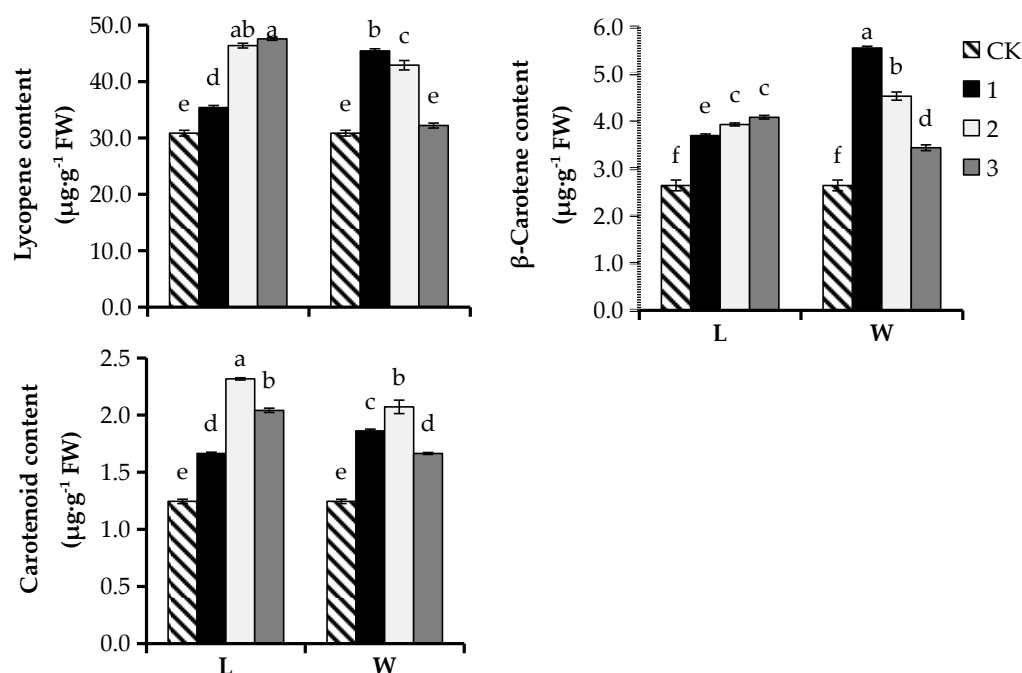


Figure 3. Effects of boron on lycopene, β -carotene and carotenoid in fruits. Different letters indicate significant differences at $p < 0.05$; L: Leaf spray; W: Root application; CK: Yamazaki nutrient solution without H_3BO_3 ; 1: $1.9 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; 2: $3.8 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; 3: $5.7 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$.

3.6. Changes in Volatile Substances and Characteristic Aromatic Compounds in Fruit

In this experiment, six kinds of volatile substances were measured by GC-MS: aldehydes, hydrocarbons, alcohols, ketones, esters and others. The total numbers of volatile substances in each treatment were 58 (control), 65 (L1), 64 (L2), 64 (L3), 66 (W1), 63 (W2) and 68 (W3) (Table 4). There were different degrees of decline in percentage of aldehydes, eaters, alcohols (except $1.9 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$ treatment) and ketones (except L2) in both leaf spray and root application treatments, and both application methods had higher percentage in hydrocarbons (except W1) and other volatile substances. Additionally, there were 14 characteristic aromatic compounds of tomato selected from the total volatile substances: 11 (control), 13 (L1), 12 (L2), 13 (L3), 12 (W1), 14 (W2) and 14 (W3) in each treatment (Table 5). Four categories of the tomato specific aroma were described: fruit aroma, fresh scent, floral fragrance and pungent smell (Table 5). Both leaf spray and root application treatments increased the numbers and the percentage of characteristic aromatic compounds compared with the control. In leaf spray treatments, the percentage of characteristic aromatic compounds increased with increase of boron level, while that in root application was variable. W2 had the highest percentage at 54.18%.

Table 4. Effects of boron on the quantity and the percentage of different volatile substances in fruits.

Treatment	CK		L1		L2		L3		W1		W2		W3	
	Qty.	Pct. (%)	Qty.	Pct. (%)	Qty.	Pct. (%)	Qty.	Pct. (%)	Qty.	Pct. (%)	Qty.	Pct. (%)	Qty.	Pct. (%)
Aldehydes	23	14.50	28	11.10	27	8.28	26	8.66	26	11.69	29	11.88	29	11.19
Hydrocarbons	5	49.00	7	54.05	6	59.39	6	59.52	7	46.38	4	64.37	8	60.38
Alcohols	13	24.58	13	24.61	13	20.15	14	17.34	14	30.21	12	13.32	13	17.93
Ketones	9	2.00	8	1.98	8	3.33	8	1.03	8	1.33	8	0.43	8	1.62
Esters	2	6.49	1	2.59	3	3.87	3	6.21	3	3.49	2	4.94	2	3.16
Others	6	3.43	8	5.67	7	4.98	7	7.24	8	6.90	8	5.06	8	5.71
Total	58	100	65	100	64	100	64	100	66	100	63	100	68	100

Qty.: quantity; Pct.: percentage; CK: Yamazaki nutrient solution without H_3BO_3 ; L1: CK + leaf spray $1.9 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; L2: CK + leaf spray $3.8 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; L3: CK + leaf spray $5.7 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; W1: CK + root application $1.9 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; W2: CK + root application $3.8 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$; W3: CK + root application $5.7 \text{ mg}\cdot\text{L}^{-1} H_3BO_3$.

Table 5. Effects of boron on the percentage of characteristic aroma in the total volatile substances in fruits.

Volatile Components	Flavor Description	Treatment (%)						
		CK	L1	L2	L3	W1	W2	W3
3-methyl-butanal	Fruit aroma	-	0.021%	-	-	-	0.037%	0.025%
3-methyl-1-butanol	Fruit aroma	0.136%	0.147%	0.108%	0.262%	0.135%	0.050%	0.197%
3-hexenal	Fruit aroma	0.063%	0.039%	0.036%	0.060%	0.028%	0.045%	0.012%
6-methyl-5-hepten-2-one	Fruit aroma	7.647%	5.862%	4.548%	5.148%	5.813%	7.004%	6.223%
(<i>E</i>)-2-hexenal	Fruit aroma	0.447%	-	0.472%	0.855%	0.440%	0.816%	0.548%
(<i>Z</i>)-3-hexen-1-ol	Fresh scent	4.590%	5.935%	5.681%	5.142%	4.782%	3.056%	3.725%
(<i>E</i>)-2-heptenal	Fresh scent	5.197%	5.120%	4.090%	3.339%	3.319%	3.288%	3.823%
Hexanal	Fresh scent	13.830%	23.093%	28.540%	26.790%	18.012%	30.590%	26.426%
2-isobutylthiazole	Fresh scent	-	3.324%	3.036%	5.886%	4.424%	3.266%	3.185%
Methylsalicylate	Fresh scent	6.428%	2.593%	3.653%	5.887%	3.392%	4.922%	3.110%
Benzeneacetaldehyde	Floral fragrance	-	0.166%	0.062%	0.225%	-	0.146%	0.182%
Phenylethyl Alcohol	Floral fragrance	0.347%	0.186%	-	0.449%	0.231%	0.220%	0.255%
Trans- α -ionone	Floral fragrance	0.355%	0.253%	0.193%	0.125%	0.202%	0.147%	0.155%
1-penten-3-one	Pungent smell	0.735%	0.646%	1.009%	0.808%	0.600%	0.590%	0.838%
Total		39.775%	47.385%	51.428%	54.975%	41.376%	54.176%	48.704%

'-' means Not detected; CK: Yamazaki nutrient solution without H₃BO₃; L1: CK + leaf spray 1.9 mg·L⁻¹ H₃BO₃; L2: CK + leaf spray 3.8 mg·L⁻¹ H₃BO₃; L3: CK + leaf spray 5.7 mg·L⁻¹ H₃BO₃; W1: CK + root application 1.9 mg·L⁻¹ H₃BO₃; W2: CK + root application 3.8 mg·L⁻¹ H₃BO₃; W3: CK + root application 5.7 mg·L⁻¹ H₃BO₃.

4. Discussion

Boron, as one of the essential micronutrients in plants, directly or indirectly participates in a variety of physiological and biochemical processes in plant growth and development [30]. Boron has very low mobility in tomato plants [8,31], and the optimum boron level varies with different tomato varieties, ranging from ~0.2 mg·L⁻¹ (1.2 mg·L⁻¹ H₃BO₃) to ~1 mg·L⁻¹ (6 mg·L⁻¹ H₃BO₃) [17,20,21]. Both deficiency and toxicity lead to irreversible effects on the growth and development in plants [32,33]. We compared the effects of two application methods with three boron levels on tomato plants. As expected, we found boron treatments significantly affected the biomass accumulation in tomato plants. Both leaf spray and root application of boron increased the FW and DW of shoots, and the root application resulted in higher ratios of root/shoot DW than leaf spray. Davis et al. [17] reported boron has an important function in cell wall metabolism and the stability of root structure, and Loomis and Durst [34] reported the appropriate boron level can promote the absorption of calcium (Ca) by tomato roots and increase the biomass accumulation of tomato plants, which is consistent with the results in this study.

Boron treatment significantly increased the soluble protein and ascorbic acid content compared to the control, and the root application boron had higher content than that of leaf spray using the same boron level. Chermisiri et al. [35] reported spraying boron significantly increased soluble protein and ascorbic acid in garlic. Ali et al. [36] reported boron application decreased the percentage of fruit drop and increased the total chlorophyll concentration in fruit peel of mango. We speculate that boron can improve the antioxidant capacity in tomato leaves. Our results were different between the two application methods with same boron level, possibly due to differences in absorption efficiency regulated by sink–source distribution of boron in leaves and roots.

Photosynthesis is one of the most important indices of plant growth and development, and chlorophyll content is a measure of photosynthetic efficiency [37]. Han et al. [38] reported photosynthesis was reduced under boron deficiency that damaged the structure of thylakoids, which affects electron transmission and decrease the optimal/maximal quantum yield of PSII (Fv/Fm). Excessive boron leads to the production of reactive oxygen species (ROS) in leaves, causing photo-oxidative damage to organic molecules and chloroplast structure and consequently decrease in the content of chlorophyll [39]. In this study, both methods significantly increased the content of chlorophyll a, chlorophyll b and carotenoids, and there were no obvious differences between the two application methods at the same boron level. We speculate boron could not the direct factor for photosynthetic efficiency of leaves that closely related to chlorophyll content and leaf structure affected by different boron levels. Kastori et al. [40] found boron deficiency led

to a decrease of chlorophyll content in sunflower leaves and the chloroplast in mesophyll cells, which damage the structure of chloroplast membrane, grana lamellae and grana, but the symptom was effectively alleviated by spraying boron, which is consistent with our results. Moreover, boron treatments effectively improved the ability of photosynthesis in terms of Pn, Ci, Cs and Tr in tomato plants, and two application methods had little effect with different boron levels, for example, L1 and W2 had the highest photosynthesis ability in each group, respectively (Figure 2). We think appropriate boron can stable leaf structure, which can effectively promote the synthesis of photosynthetic pigments that affect directly the photosynthetic capacity in tomato plants.

How to improve fruit flavor is an important area of research [41,42]. Micronutrients directly affect fruit quality. Boron plays an important role to improve fruit quality. For example, Niu et al. [43] found the flavor quality in konjac (*Amorphophallus rivieri*) was improved by spraying boron, which promoted the transportation of carbohydrates, increase glucomannan and soluble sugar content. Islam et al. [44] reported leaf spray of boron increased vitamin C and Ca in tomato fruit, and increased shelf life and single fruit weight [17]. We found that both leaf spray and root application boron treatments significantly increased the soluble protein and vitamin C content in fruit, and except L3, boron treatments significantly increased nitrate accumulation in fruit compared to the control, which could result from the absorption and transportation of boron always cooperate with other nutrients transportation in plants that lead to higher accumulation of nitrate, and the efficiency could be affected by different application methods with different boron levels [45]. We found boron-treated plants had a higher percentage of soluble solids and titratable acid in fruit, and the sugar-acid rate was affected by different boron levels (Table 3), which is consistent with Wójcik and Lewandowski [46], who found spraying Ca and boron on strawberry changed the ratio of soluble solids/titratable acids, inducing better flavor. Additionally, we found both application methods increased the lycopene, β -carotene and carotenoid content in fruit. Leaf spray of boron increased lycopene and β -carotene content with the increase of boron level, while root application of boron had a decreasing trend using same boron level (Figure 3). We speculate the efficiency of boron uptake by plants is affected by application methods and boron levels. Lycopene, β -Carotene and carotenoid are natural antioxidants in fruit, which is closely related to the quality of tomato fruit [47]. Lahoz et al. [48] reported lycopene is one of the most important quality indices of tomato, affecting flavor.

Over 400 volatile substances have been reported in tomato, including hydrocarbons, alcohols, aldehydes, ketones and esters [49], but only 30 volatile substances of levels of more than $1 \text{ nL}\cdot\text{L}^{-1}$ [50]. Buttery [51] reported there were just 29 volatile substances of concentration more than $1 \text{ nL}\cdot\text{L}^{-1}$ in tomato fruit, and among these substances, 16 substances with logarithmic threshold units more than 0 were identified. Baldwin et al. [52] claimed these 16 volatile substances are the main characteristic aroma compounds in tomato fruit. In this study, 68 volatile substances were detected by using GC-MS, including 14 characteristic aromatic compounds, but some characteristic aromatic compounds such as β -damascone and 1-nitro-2-ethylbenzene were not detected, which may be caused by different tomato varieties and cultivation conditions (Tables 4 and 5). Both application methods of boron increased the numbers of volatile substances in fruit, and leaf and root application of boron decreased the percentage of aldehydes and esters, but significantly increased the percentage of hydrocarbons (except W1) in fruit. We speculate that the efficiency of some substances synthesis pathways was affected by different application methods with different boron levels. Liu et al. [53,54] reported leaf spray and root application of melatonin with different concentrations had effects on the composition and quantity of tomato volatile substances, speculating that exogenous nutrients may directly or indirectly participate in synthesis of volatile substances in tomato fruit. Buttery et al. [51] reported the unique aroma of tomato is mainly composed of its characteristic aroma compounds. In this study, both leaf spray and root application boron treatment increased the percentage of characteristic aroma compounds in fruit, leaf spray boron resulted in a higher percentage in characteristic aroma compounds than root application using same boron level (Table 5). Krumbein and

Auerswald [55] and Lecomte et al. [56] reported the characteristic aroma of tomato can be divided into four categories: fruit aroma, fresh scent, floral fragrance and pungent smell, but it is still unknown that how the characteristic aroma compounds of tomato are directly regulated. In this study, L3 and W2 had the highest percentage of characteristic aromatic compounds in fruit in each application method group, which could have much richer tomato flavor.

5. Conclusions

The appropriate application of boron can effectively improve the growth and development of tomato, and change the quality and flavor of tomato fruit, two application methods with three boron levels had different effects on these indices of tomato. Based on the results of the tomato cultivar used in this study, leaf spray of $1.9 \text{ mg}\cdot\text{L}^{-1} \text{ H}_3\text{BO}_3$ and root application of $3.8 \text{ mg}\cdot\text{L}^{-1} \text{ H}_3\text{BO}_3$ gave better performance for each application method.

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