Moderate Boron Concentration Beneficial for Flue-cured Tobacco Seedlings Growth and Development

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Abstract

Boron (B) is a micronutrient tobacco needs in minute amounts, and Boron insufficient supply can causes significant tobacco yield loss, however, the appropriate concentration for flue-cured tobacco seedlings to growth remains unknown. In this sense, a hydroponic experiment was conduct to measure the agronomic traits, dry matter mass, chlorophyll content, photosynthetic performance, antioxidant enzymes, boron ion and nicotine content of flue-cured tobacco seedlings K326 under different boron concentrations of 0.000mmol/L (B1, CK), 0.125mmol/L (B2), 0.250mmol/L (B3), 0.750mmol/L (B4), 5.000mmol/L (B5), 10.000mmol/L (B6), 20.000mmol/L (B7), 40.000mmol/L (B8) after 30 days. B significantly influenced flue-tobacco seedlings growth on agronomic traits, photosynthetic performance, the activities of antioxidant enzymes, boron ion and nicotine content aspects. B linearly enhanced the accumulation of boron ion by 24.00%~96.44%, and decreased nicotine content by 21.60%~82.03% in tobacco seedlings. Solution B concentration at 0.750 and 5.000mmol/L markedly improved tobacco seedlings maximum leaf length by 4.83%~82.03% and leaf width by 0.77%~24.36%, root weight by 13.64%~56.82%, stem weight by 12.26%~52.36%, leaf weight by 9.68%~36.56%, dry matter mass by 10.65%~38.92%, the Pn parameter by 1.22%~80.28%, the Cond paramete by 33.40%~75.86%, while decreased the activities of SOD by 10.44%~91.67%, POD by 21.32%~65.62% and CAT by 50.05%~96.44%, and MDA by 16.23%~75.16%. The B concentration concluded in this study enhanced the agronomy traits, photosynthetic and biochemical characteristics of flue-cured tobacco seedlings, which lays a scientific theoretical foundation for rational application of B in tobacco production and improve the internal quality of flue-cured tobacco.

Introduction

Boron is an indispensable nutrients for the growth of plant, it has been relevant to various functions including synthesis of plant uracil nucleochlorophyll, root development, cytokinins, auxin, cell wall pectin formation[1, 2], protein metabolism, alkaloid production, etc., then affects the growth and development of plants[3]. Tobacco (*Nicotiana tabacum* L.) is an important cash crop and pillar industry in China, the total tax and profit of the tobacco industry reached 1.2803 trillion yuan in 2020, which plays an important role in supporting the national fiscal revenue. Tobacco is sensitive to B and the response curve is reported to have a very narrow sufficiency range, which means even in boron-deficient tobacco planting areas and excessive boron supplementation is likely to cause malnutrition of tobacco plants [4]. Appropriate concentration of boron increases root system, leaf area, photosynthetic capacity, and dry matter accumulation of tobacco plants, and improve its stress resistance, then lays a good substance foundation for the growth of flue-cured tobacco in the field. Therefore, exploring an optimum boron application rate for major cultivar of flue-cured tobacco seedlings is crucial for achieving sustainable development of tobacco.

Boric acid (BO_3^{3-}) is the main form of absorption of B by plants. Recent research showed that the appropriate B concentration range of flue-cured tobacco in flied stage has been listed between 25.700-

107.250mmol L^{-1}[5], however in the hydroponic experiment, it was found that of 0.500-200mol L^{-1}[6]. Plants under B deficiency condition generally show growth retardation, limitation of the elongation and expansion of leaves, deformation of the leaves, and cessation of the bud [7, 8]. Additional, B deficiency leads to a large amount of auxin production, promote the growth of roots, increasing nicotine content [9], disturb the plant physiological function and metabolism [10], increase cell membrane permeability, induce the accumulation of reactive oxygen species, MDA and other harmful substances, and thus produce lipid membrane peroxidation, which affects the normal structure and function of cells [11].

Boron toxicity, on the other hand, causes a great loss of crop yield worldwide. Previous studies have shown that when solution B concentration was at or above 200mol L^{-1}, the tobacco plant showed B poisoning [6]. Plants with boron toxicity usually characterized by leaf vein loss of greenness, leaf tip burning, leaf tip curling, the margins of older leaf necrotic, in severe cases, complete leaf necrosis [12]. As for tobacco, the B poisoning symptoms features brown spot at the tip or margins of older leaf, burning of the lamina, midrib and based in patches, in extreme cases, browning and brittleness of the lamina, dried and rapidly defoliation [13]. Barley leaf length, width and area do not show any differences between excessive and normal boron application. However, root weakness and death of lateral root were noted in cultures of excessive solution B concentration [14, 15]. Besides that, oxidative stress become more severe under B poisoning [16, 17]. Cervilla [18] found that SOD, POD, APX and CAT activities are relatively higher at B excess conditions.

This study investigates the effects of boron deficiency on yield, quality, physiological and biochemical characteristics of flue-cured tobacco in the field stage [9, 19, 20], while there were few reports on boron on photosynthesis characteristics, antioxidant enzymes and nicotine content in the seedling stage. The objectives of this study were to identify the critical values of boron deficiency and toxicity in tobacco seedlings, to provide a basis for the regulation of boron nutrition in the production and further to enrich the growth mechanism of boron on flue-cured tobacco, which is of great significance for the yield and quality of flue-cured tobacco.

Results

Effect of B solution concentration on seedling growth and shoot biomass formation

30 days after transplanting, B deficiency was observed at B1 treatment, while B toxicity was observed at B5, B6, B7 and B8 treatments (Fig. 1). B1 showed B deficiency symptoms with light green, corking of leaf veins, and leaf marginal curling. Toxicity symptoms were: with B concentration increased form B5 to B8, the area of scorched leaf surface gradually expanded from leaf tip to whole leaf. At B8 treatment, the lower leaves of flue-cured tobacco were completely withered and general deformation.

The B concentration in solution did not affect the plant height and stalk width (Fig. 2A and 2B), but it significantly ($P<0.05$) affect the leaf number (Fig. 2C), maximum leaf length (Fig. 2D) and leaf width (Fig. 2E). When B concentration was at B4 treatment, leaf length was increased 4.83%~20.175. At B5 treatment, the leaf width was higher than other treatments by 0.77%~24.36%. The leaf number peaked at B7 treatment, and significant higher than B2 and B8, compared with other treatments which increased by 2.38% ~19.05%.

All plant growth parameters were significantly different between the eight treatments ($P < 0.05$) (Fig. 3 A to D). with the increase of B concentration, tobacco seedlings leaf, stem, root and total dry weight were showed a downward parabolic trend, and peaked at B concentrations at B5 treatment, which increased by 9.68%~36.56% (leaf), 12.26%~52.36% (stem), 13.64% ~56.82% (root) and 10.65%~38.92% (whole plant) compared with other treatments, respectively.

Effect of B solution concentration on SPAD value

B concentrations could significantly impact the SPAD content of flue-cured tobacco seedlings leaves (Figure 4). SPAD content reached its maximum at B7, which increased by 9.16% (B1), 15.04% (B2), 13.71% (B3), 7.88% (B4), 4.84% (B5), 3.46% (B6) and 12.00% (B8), respectively.

Effects of B solution concentration on photosynthetic rate

Photosynthetic properties of tobacco seedlings were markedly influenced by solution B concentration ($P < 0.05$; Table 1). Photosynthetic properties parameters showed a parabolic trend with the increase of boron concentration. The Pn parameter peaked at B4 treatment, which increased by 1.22%~80.28%, and significant different with B1 by 63.96%, B7 by 46.83% and B8 by 80.28%. The Cond parameter at B4 increased most obviously, by 33.40%~75.86% compared to other treatments. When solution B concentration was at B3 treatment, the Ci parameters reached the maximum, by 0.83%~57.95%. The Tr parameters reached the highest at B3 treatment, by 5.51%~62.05%, and significant different with B1 by 49.02%, B2 by 30.83%, B7 by 62.05% and B8 by 61.55%.

Effect of B solution concentration on Antioxidant enzymes and MDA content

Antioxidant enzymes in tobacco seedlings were significantly influenced by boron ($P < 0.05$, Figure 5). As solution B concentration increased, the SOD, POD and CAT activities showed a "down-up" trend. SOD activities reached a maximum at B7, by 10.44%~91.67% compared to other treatments. POD activity was increased more severely at B6 treatment, by 21.32%~65.62% compared to other treatments. When B concentration was at B7 treatment, the CAT activities were significantly higher than other treatments, by 50.05%~96.44%. Concentrations at B4 had minimal MDA content with significant variability between each treatment ($P < 0.05$), which decreased by 46.65%~302.57%.

Effect of B solution concentration on Boron ion content in plants tissue

Boron ion content in tobacco seedlings root, stem and leaf were significantly affected by solution B concentration ($P < 0.05$, Figure 6). With the increased of solution B concentration, there were sharp increases in the root, stem and leaf tissue. All parameters were significantly different and reached the maximum at B8 treatment. Compared with B8 treatment, the concentration of B ion accumulation increased by 24.43%~86.76% (roots), 26.97%~96.44% (stems) and 24.00%~67.01% (leaves), respectively.

The correlation analysis shows that the accumulation of B ion in each part of tobacco seedlings was significantly positively correlated with the B solution concentration ($P < 0.01$), and the correlation coefficients were 0.976 (roots), 0.943 (stems) and 0.985 (leaves), respectively (Table 2).

Effect of B solution concentration on nicotine content

The content of nicotine in roots and leaves of tobacco seedlings was strongly affected by boron in solution (P<0. 05, Figure 7). Nicotine content in root and leaves declined significantly when B concentration at or above B1 treatment, while within the parameter in stems did not show significant difference. Compared with other treatments, B1 was increased by 33.58% ~82.03% (roots), 43.83% ~72.93% (stems), 21.60%~70.34% (leaves), respectively.

The correlation analysis shows that the nicotine content in roots and leaves of flue-cured tobacco seedlings was significantly negatively correlated with the B solution concentration ($P < 0.01$), and the correlation coefficients were -0.783 (roots) and -0.622 (leaves). While in stem there was negatively correlated with -0.490 correlation coefficients (Table 3).

Discussion

B plays a vital role in the growth and develop of crops [21], it reduce the content of phenolic compounds, regulate the activity of indole acetic acid oxidase by preventing excessive accumulation of auxin [22]. In this study, during the period of seedling stage, maximum leaf length peaked at 0.750mmol L^{-1} and leaf width of tobacco peaked at 5.000mmol L^{-1}. Leaf number reached the maximum at 20.000mmol L^{-1}, but there was only a significant difference from 0.125mmol L^{-1} B concentration in solution. These results were consistent with the found in Zhang [9]and Qu [20]. With the increase of B concentration, flue-cured tobacco seedlings grew vigorously, agronomic traits have been improved.

This finding that with the increase of B concentration, the leaf, root, stem and total dry matter mass of tobacco seedlings showed a unimodal curve change, when the concentration was 5.000mmol L^{-1}, all parameters reached the maximum. Evidence suggests that B deficiency decreased the activities of plasmalemma-bound enzymes, limited the change of membrane potential and ion fluxes across membranes [23], hindered the accumulation of phenolics and polyamines [24], and ultimately affects the accumulation of plant matter. On the other hand, B toxicity altered metabolism, reduced root cell division and destroy the stability of root cell membrane, hinder the ability of absorb nutrient in soil. Accordingly a reduced growth of shoots and roots is typical of plants exposed to high B levels [15].

Tobacco leaves is the mainly harvests objects, and leaf photosynthesis is the basis of yield and quality formation. The SPAD content is a standard to elevate the ability of leaves photosynthesis [25, 26]. In this study, when solution B concentration at 20.00mmol L^{-1}, SPAD content reached the highest, this can be explained by chloroplast structure disruption under high B concentration leading to lower CO_2 assimilation and Chl content [27]. Excessive B decreases the activities of rubisco enzyme, thylakoid photosynthetic proteins and photorespiration enzyme, finally decreased photosynthetic capacity [28].

Previous researches have proofed that moderate B concentration improve plant growth and photosynthesis ability, and effectively alleviate the damage to plants caused by other stresses [29]. Boron insufficiency significantly decrease photosynthetic rate. B deficiency mainly decreases the photosynthetic area, transpiration rate and stomatal conductance, while B toxicity results in leaf yellowing, necrosis and reduced chlorophyll, leading to impaired synthesis of photosynthetic [30]. In the current study, when B concentration was at 0.750mmol L^{-1}, Pn and Cond reached a maximum, while Ci and Tr were at 0.250mmolL^{-1}. B concentration significantly affects the photosynthesis of plants. Previous studies indicate that if Ci is consistent with change in Pn and Cond, the decrease of Pn can be considered to be restricted by stomatal factors; otherwise, it is restricted by non-stomatal factors [31]. The results of this study showed that under different B treatments, Ci, Pn and Cond showed a consistent trend, indicating that stomatal factors might be the main reason for the decrease of photosynthetic rate.

Antioxidant metabolic enzymes are important protective enzymes for plant to against various environmental stresses [32, 33], such as scavenge reactive oxygen species (ROS) and inhibit the synthesis of oxidation of phenolic compounds [34, 35]. Based on this theory, the degree of damage to plants caused by stress can be accessed through various antioxidant enzymes activities. SOD, POD and CAT are important protective enzymes for plants to resist biotic and abiotic stresses, and they mainly eliminate reactive oxygen species in cells through their coordination and cooperation [36]. The content of membrane lipid peroxidation products of MDA crops can represent the stress degree of plant cells and the stress resistance of tobacco plants [37]. In this case, B concentration significantly affects the antioxidant enzymes activities, the SOD, POD and CAT activities peaked at 20.000mmol L^{-1}, 10.000mmol L^{-1} and 20.000mmol L^{-1}, respectively. It is indicated that B toxicity is damage to tobacco seedlings. Moreover, there was a significant difference in CAT among all treatments, indicating that CAT was more sensitive to B deficiency or toxicity stress. In this study, the tendency for 0.750mmolL^{-1} B treatment, significantly reduced MDA content, shows a certain concentration of B can protect the blade from active oxygen damage, and low or relatively higher B concentration damage to the antioxidant system of blade, the concentration of reactive oxygen species is beyond the scope of system to withstand, causing the structure and function of the biofilm.

Boron is phloem immobile in plants, hence, excessive amount of B accumulates at the end of the transpiration stream, and B poisoning often occurs in leaves [38]. This study found that B toxicity symptoms started at the lower leaves and gradually spread to the upper leaves, similar results have been obtained in bean [39], pepper [40], wheat [38] and barley [41]. B is an essential micronutrient for the growth of plants, the dramatically differences sensitive of different plant to boron concentrations may be explained by plant species (boron mobility) [42], stage of life (older plants contain more boron) [43] and genotype [44]. It was found that the normal B solution concentration of citrus at seedling stage was 10μmol/L and that of Arabidopsis thaliana was 30μmol L^{-1}[45]. In current results, the agronomic traits and dry biomass weigh showed low promotion and high inhibition with the increase of B iron concentration. When B solution concentration was at 0.750 and 5.000mmolL^{-1}, the agronomic traits and dry biomass weigh were in best situation, which conducive to the growth of flue-cured tobacco seedlings. This is consistent with the research results of Jin [46] and Luo [6].

Total nitrogen content contributes to the smoking intensity and irritation of cigarettes [47], and affects the consumption experience of the final cigarette product. Boron is a structure and component of cell wall and cell membrane. It is involved in physiological processes such as protein synthesis, affects root activity, transport and metabolism of nitrogen-containing compounds. Moderate B concentration beneficial to promote the coordination of chemical properties in tobacco leaves and improve the quality of tobacco leaves [48]. In this experiment, when the B concentration was at 0.00mmol L^{-1}, the content of nicotine in each part of flue- cured tobacco seedlings was significantly higher than that of other treatments. This can be explained by B deficiency would cause strong branching of tobacco root, increase the number of fine roots, and increase the nicotine production in roots. For the tobacco with B deficiency, the content of nicotine in the tobacco leaves decreased after applying a certain amount of B fertilizer. However, excessive supply of boron will cause the decomposition of nucleic acid in plants and the inhibition of protein synthesis, thus reducing the content of nicotine, with the increasing of B fertilizer, the content of nicotine in the tobacco leaves showed a trend of gradually increasing first and then slowly decreasing. This is similar to the research conclusions of Wang [49] and Feng [50].

In conclusion, B nutrient significantly increased boron ion accumulation but decreased nicotine content in tobacco seedlings root, stem and leaves. However in the terms of agronomic traits, photosynthetic properties and the activity of antioxidant enzymes did not increase continuously as solution B concentration increased, showing a unimodal distribution B nutrient response curve and provide a referential B concentration for growth and development. With increasing of B concentration in solution, the growth parameters and SPAD content of flue-cured tobacco seedlings initially increased and then decreased, and peaked at 0.750 and 5.000mmol L^{-1} B in the nutrient solution. When the B concentration was at 0.750 and 5.000mmol L^{-1}, the activities of SOD, POD and CAT and the content of MDA in flue-cured tobacco seedlings were reached minimum. Moreover, B deficiency was evident by 0.000mmol L^{-1}, and B toxicity symptoms were observed at or above 10.000mmol L^{-1}, the growth and development of flue-cured tobacco seedlings reduced and the passive to the quality increased. Therefore, solution B concentration of 0.75 0and 5.000mmol L^{-1} is benefit for tobacco seedlings growth and further yield and quality.

Materials And Methods

Experiment design

This study was carried out at the greenhouse of Yunnan Academy of Tobacco Agricultural Science, Yuxi, China (24°14'N, 102°30'W; altitude 1680 m) from August to December 2020. On 18th September (6 weeks after germination), uniform tobacco seedlings (varieties K326) with a single stem were selected, transplanted into 500mL pots containing 1/2 Hoagland's solution with no added boron [51]. The pH was adjusted to 6.0 with 0.1 mol/L NaOH and HCl. The plants were kept at 25°C under 16 h light period, humidity about 60%, average irradiation 650μM m^{-2} s^{-1}. The roots were covered with tin foil to protect them from light to promote root growth and were ventilated for 2h a day, and the nutrient solution was replaced once every 3 days (including the measurement time). After 7 days of pre-culture, eight different B concentrations (H_3BO_3) were used (0.00 (B1), 0.125 (B2), 0.250 (B3), 0.750 (B4), 5.000 (B5), 10.000 (B6), 20.000 (B7), 40.000 (B8) mmol L^{-1}). The nutrient solution without BO_3^{3-} (0.00mmol L^{-1}) was used as the control. Nine tobacco plants were planted in each treatment for three replicates. Relevant physiological indexes were sampled and measured at the 30th day of treatment.

Analyses methods

Agronomictraits. 30 days after transplanting, the height of tobacco seedlings was determined from epicotyls along the stem to the growing tip, and three replicates were determined. The tobacco seedlings leaf length and width were determined by the sixth leaf from the epicotyls to the growing tip.

Biomass of tobacco seedlings. The tobacco seedlings root, stem and leaf were harvested and weighed separately. After being killed out at 105°C for 10min and dried at 55°C to constant weight, the root, stem and leaf dry weight were measured and calculated to obtain the total weight.

Soil and plant analysis development (SPAD) value. 30 days after transplanting, chlorophyll content in tobacco seedlings leaves were measured using a SPAD-502 (Konica Minolta Inc, Japan) portable chlorophyll detector. The middle part of sixth leaf from the epicotyls to the growing tip was measured for SPAD value.

Photosynthetic performance. Each treatment selects 3 uniform seedlings, fixed-point selection of a piece of the same toward the blade, with a soft hair brush to dust on the surface of the central lobe, and with Li-6400 portable photosynthetic apparatus (Li-COR Inc, USA) and application of Li-6400-02B blue and red light detector for the determination of photosynthetic response curves, set the photosynthetic active radiation intensity (PAR, μmol /(m^2·s)) for 2000, 1500, 1000, 500, 250, 120, 15, 0, 30, 60. The set value of the CO_2 injection system was 400μmol/mol, the gas flow rate was 500mmol/s, and the leaf temperature was 25°C. Before the measurement, the leaf chamber PAR was set to 1800μmol/ (m^2·s) to induce the leaves, and the automatic measurement procedure was started when the photosynthesiometer readings were stable. Then, the average value of the three measurement results was taken to facilitate the analysis.

Antioxidant enzyme activity. After measuring the agronomic traits, 0.5g tobacco seedlings leaves in each treatment were taken to determine the activity of antioxidant enzymes. SOD activities was evaluated by

SOD kit (NBT method), POD activities was accessed by POD kit, CAT activities was checked by CAT kit (UV absorption method), and MDA content was determined by MDA kit. These applying kits were provided by Keming Biotechnology Co., Ltd, Suzhou, China.

Boron ion content in tobacco seedlings. The wet ashing technique (nitric and perchloric acid digestion method) and the atomic absorption spectrophotometry were used in the determination [52].

Nicotine content. Refer to "Determination of nicotine in tobacco and tobacco products by gas chromatography" YC/T 246-2008.

Statistical Analysis

Data were statistically submitted to single factor analysis of variance (One-way ANOVA) and Spearman correlation analysis design in SPSS 20.0 (IBM SPSS Statistics Inc., Chicago, IL, USASPSS Inc., Chicago, IL, USA). Duplicate measurements on tobacco plants samples (n=3) were averaged for statistical analysis of treatments effects. The analysis considered replicate effects to be random and the effects of treatment to be fixed. Treatment effects were considered significant at alpha equals 0.5 level of probability. Means separation was done using the Duncan multiple tests. Figures were created with GraphPad Prism 8 Software (GraphPad Software, Inc., CA, USA).

Declarations

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Author contributions

Mengxia Li: writing- Original Draft, Writing - Review & Editing. Xiaopeng Deng: Validation. Ke Ren and Rui Liu: Formal analysis. Tao Wang and Xinwei Ji: Investigation. Chenggang He: Data Curation. Yongzhong Li and Congming Zou: Conceptualization, Methodology, Resources.

Competing interests:

The authors declare that they have no competing interests. All authors approved the final manuscript.

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Tables

Table 1: Photosynthetic rate of tobacco under different boron application rates

Treatments	Pn/[μmol/(m^2·s)]	Cond/ [mol/(m^2·s)]	Ci/[μmol/mol]	Tr/ [mmol/((m^2·s)]
B1(CK)	4.29±1.43cd	0.064±0.011cd	397.77±6.26b	2.56±1.15cd
B2	10.25±1.12a	0.080±0.025cd	497.29±4.83a	3.48±0.68bc
B3	10.50±1.50a	0.108±0.025bc	501.48±5.95a	5.02±0.82a
B4	11.90±1.59a	0.205±0.038a	410.79±8.05b	4.75±0.70ab
B5	11.75±1.62a	0.129±0.027b	267.78±4.46c	3.70±0.88abc
B6	9.61±1.32ab	0.136±0.021b	381.16±4.5b	3.79±0.82abc
B7	6.33±1.59bc	0.071±0.020cd	254.11±7.04c	1.91±0.64d
B8	2.35±0.74d	0.049±0.017d	210.85±6.29c	1.93±0.78d

Data are means ± standard errors (n=3). Different letters denote significant differences ($P < 0.05$, Duncan multiple-range test).

Table 2: Correlation analysis of boron ion content and boron ion concentration in different parts of flue-cured tobacco seedlings

	Roots	Stems	Leaves
Boron concentration	0.976^{**}	0.943^{**}	0.985^{**}

** was extremely significant level (P＜0.01)，* was significant level (P＜0.05)。

Table 3: Correlation analysis of boron ion content and boron ion concentration in different parts of flue-cured tobacco seedlings

	Roots	Stems	Leaves
Boron concentration	-0.783^{**}	-0.490^{*}	-0.622^{**}

** was extremely significant level (P＜0.01)，* was significant level (P＜0.05)。

Figures

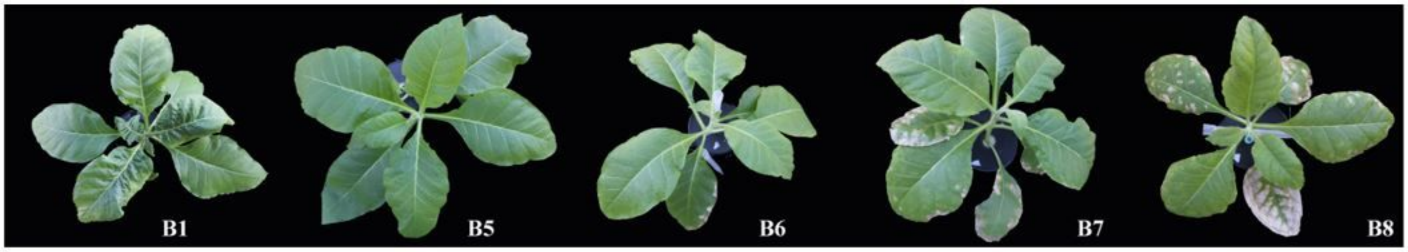

Figure 1

Boron deficiency and toxicity symptoms

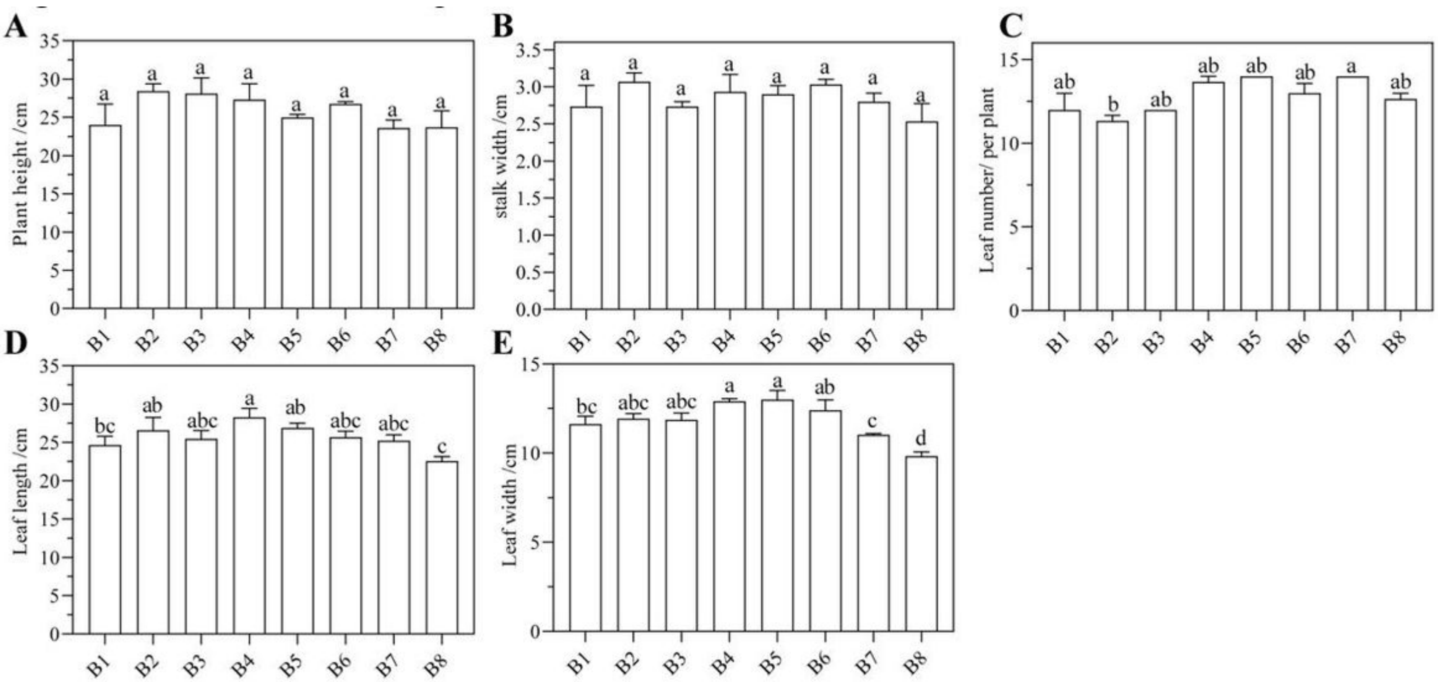

Figure 2

Basic characteristics of agronomic characters of flue-cured tobacco under different boron levels

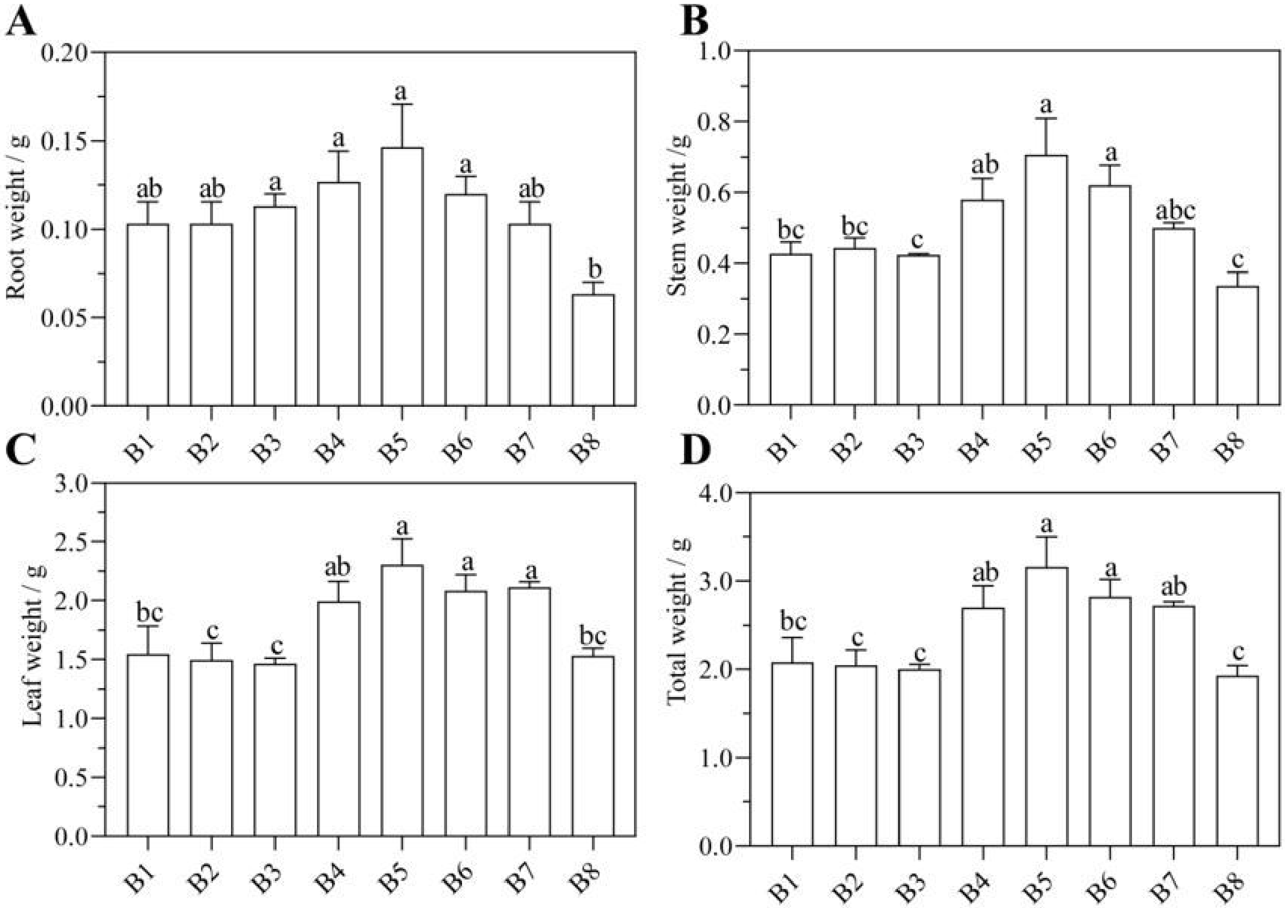

Figure 3

Accumulation of flue-cured tobacco dry matter weight under different boron levels

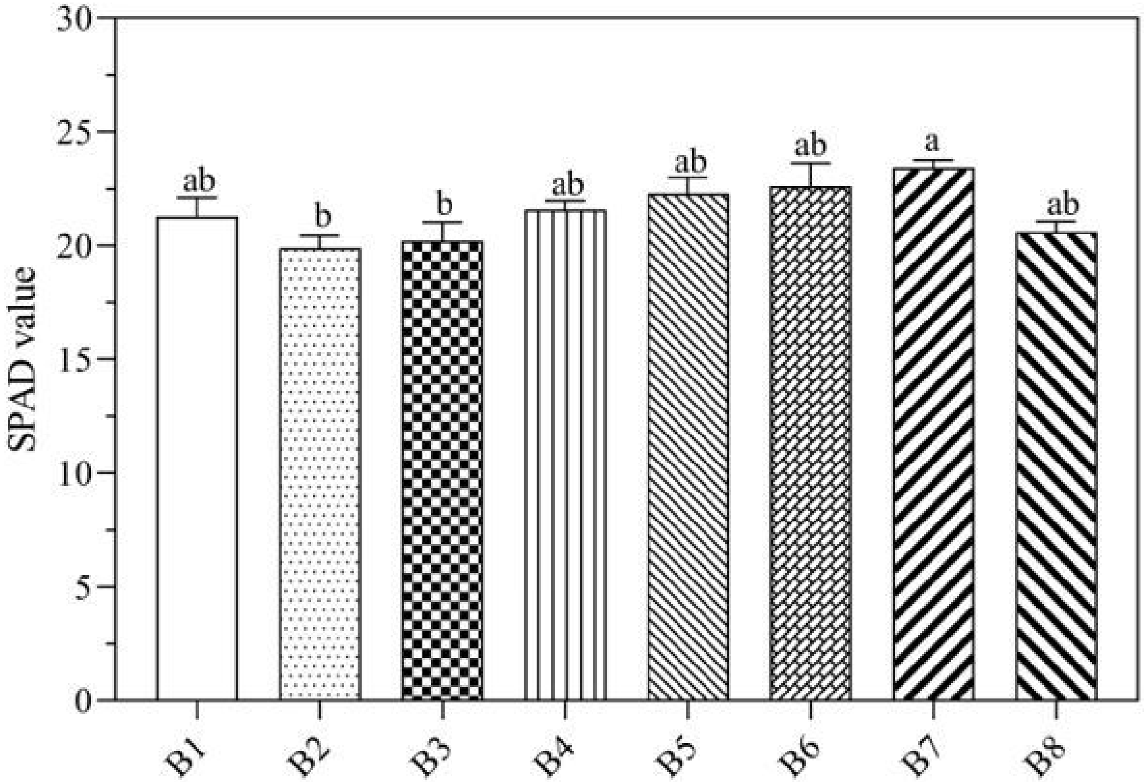

Figure 4

The value of SPAD of flue-cured tobacco leaves under different boron levels

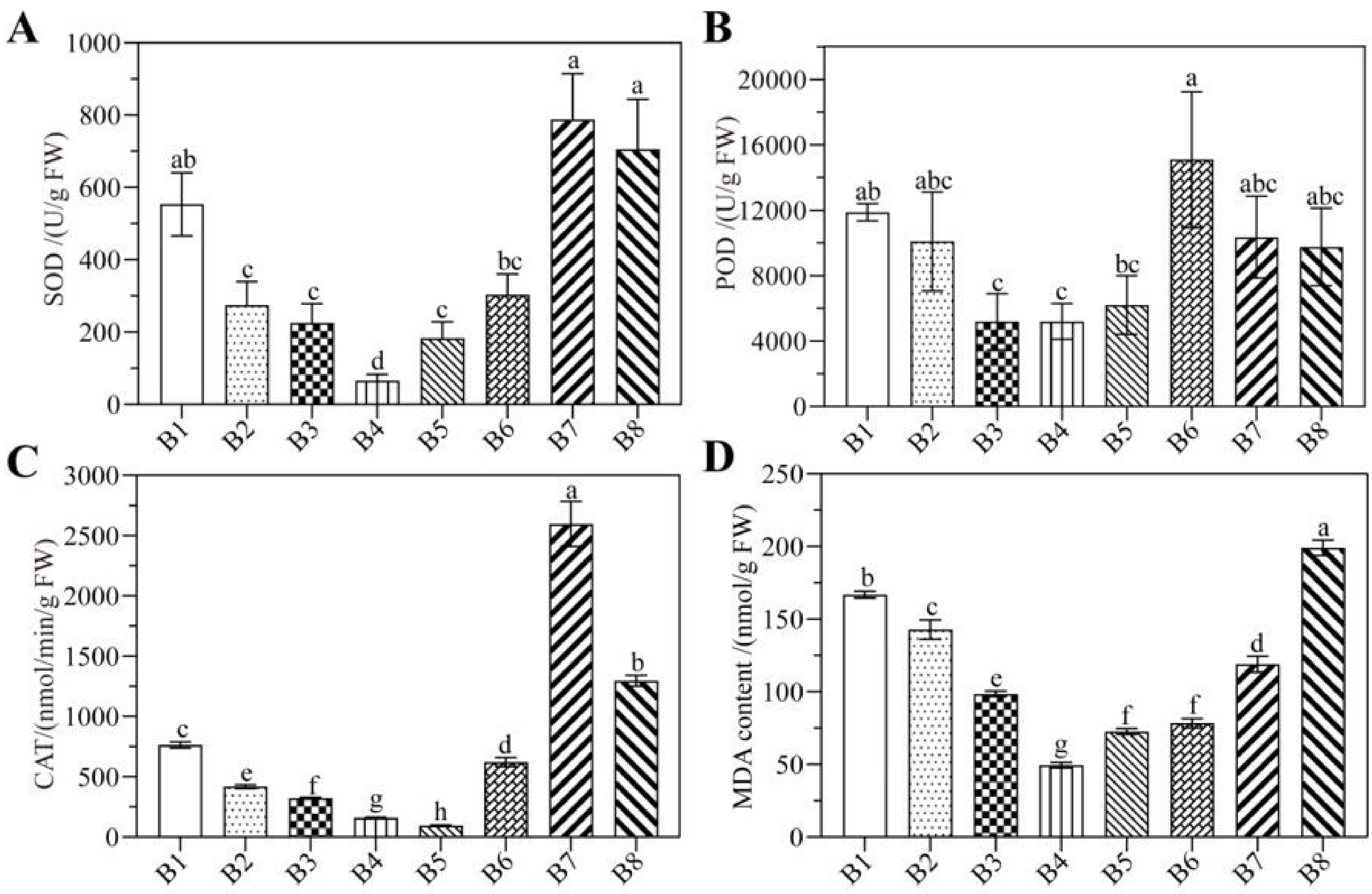

Figure 5

Antioxidase activity and MAD content of flue-tobacco seedlings under different boron levels

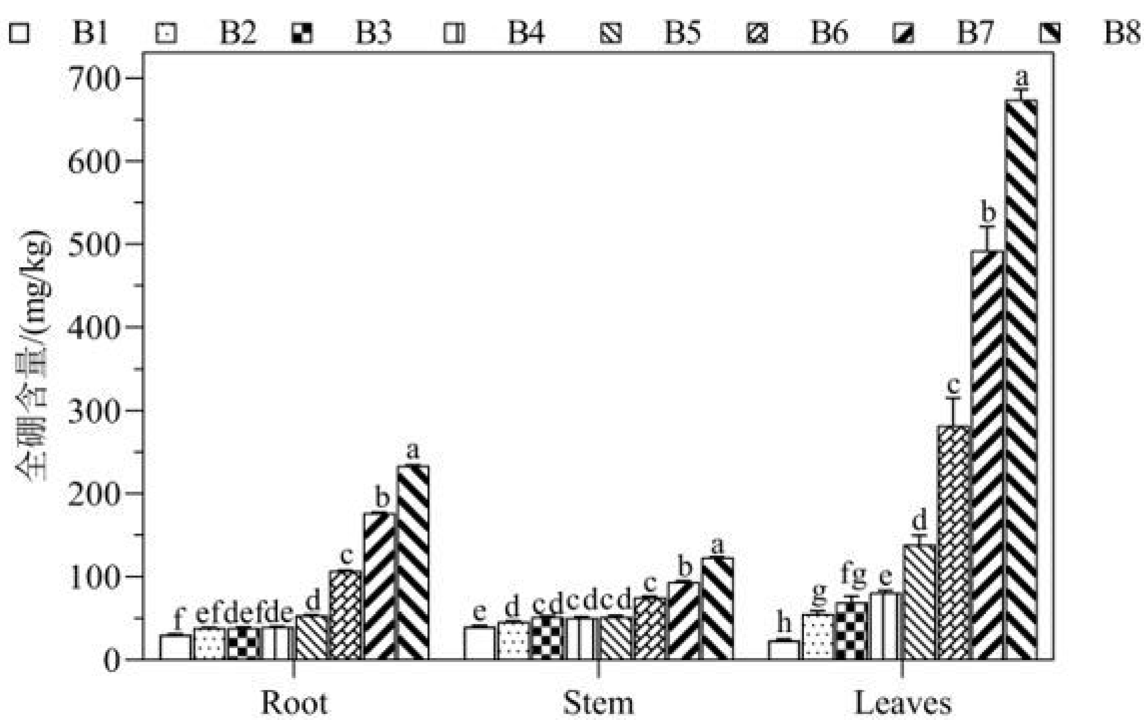

Figure 6

Changes of boron content in different parts of flue-cured tobacco

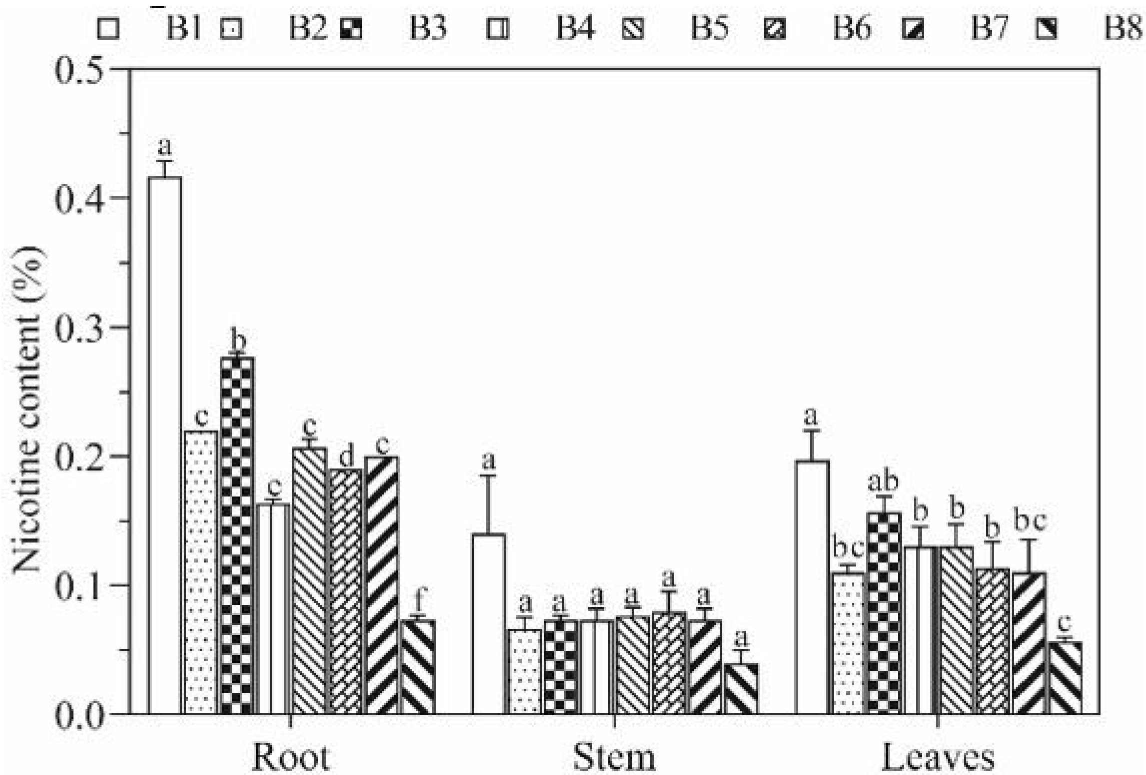

Figure 7

nicotine content in different parts of flue-cured tobacco under different boron ion concentration