THE EFFECT OF INTERACTION BETWEEN BORON TOXICITY AND CALCIUM ON GROWTH, SOLUBLE CARBOHYDRATES IN WHEAT PLANTS

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Abstract. This study aimed to explain the effects of different concentrations of calcium (0, 40, 80 and 120 ppm Ca), which showed an antagonistic effect on toxic levels of boron (0, 15, 30 and 45 ppm B), on growth and soluble carbohydrate levels in the seedlings of *Triticum durum* Desf. cv Kızıltan-91, which was sensitive to B toxicity, and *Triticum aestivum* L. cv Gün-91, which was tolerant to B toxicity. Compared to the control, all B concentrations treated alone caused a decrease in root length in Gün-91, all of them except for B_{45} resulted in increased root length in Kızıltan-91and B_{30} and B_{45} treatments reduced shoot length and shoot fresh weight in both cultivars. The longest shoot length was determined in Ca_{80} treatment in both cultivars. Among the B+Ca treatments, B_{15} + Ca_{120} and B_{45} + Ca_{120} increased shoot length in Gün-91, while B_{30} + Ca_{80} treatment increased shoot length in Kızıltan-91 when compared to all B concentrations treated alone. In both cultivars, the highest root fresh weight was found in Gün-91 treated with B_{15} + Ca_{120}. B_{45} treatment decreased shoot dry weight and relative water content (RWC) in both cultivars and root dry weight in Kızıltan-91. The inhibitory effect of 45 ppm B on RWC was reduced by the presence of Ca in both cultivars. Compared to all B treatments, B_{45} + Ca_{40} was determined as the common treatment that increases RWC in both cultivars. The highest RWC value was determined in Ca_{40} treatment in Kızıltan-91. The lowest glucose level was determined in the B_{45} treatment in Gün-91 when compared to the control, and the same treatment led to decreased fructose amounts in both cultivars. The toxic effect on glucose of 30 and 45 ppm B was observed to be alleviated by calcium application, particularly at 120 ppm in both cultivars. Compared to all B treatments, B_{30} + Ca_{120} was determined as the common treatment that increases both glucose and fructose amounts in both cultivars. Findings show that the treatment of appropriate Ca concentrations might be useful in reducing B toxicity.

Keywords: boron toxicity; calcium; glucose; fructose; wheat cultivars.

INTRODUCTION

It has been determined that boron (B) acts as a major micronutrient for the optimal growth of vascular plants. However, B contamination is a significant environmental problem, which affects both ecosystems and human activities. Factors such as irrigation and salt deposit due to dry regional climate, overfertilisation, industrial wastewater and mining cause B accumulation on the soil surface and deep soil. Additionally, irrigation with water containing a high level of B is another reason for soils rich in B [30, 49]. Boron contamination caused by these factors reduces vegetative quality and decreases the product yield for humans. When soil or underground water contains B above the permissible levels, plant growth and production are affected, and the crop yield is restricted. Boron toxicity is commonly seen in the agricultural fields of Australia, North Africa, Turkey, West Asia, Italy, USA, Chile, Argentina [33, 41]. Turkey and the USA, which have the highest B reserves in the world [6], are also the countries facing the most severe B contamination problems [6, 47].

Boron has a narrow concentration range for optimum plant growth. Abnormal B levels can be toxic. The optimal B level for species can be toxic or inadequate for other species [12]. It is thought that crops are sensitive to medium-to-high B levels in general [11]. High values may frequently result in toxicity symptoms such as chlorosis, necrosis and reduction in wheat plants.

Despite its considerable agricultural importance, our knowledge and understanding of B toxicity is highly limited. As a result of the increases in arid and semi-arid areas and B toxicity in wheat-growing lands, studies for determining species tolerant to B toxicity and determining whether there is a relationship between B and other nutrients in wheat have gained impetus. However, some nutrients are applied into the growth medium to reduce the negative effects of B toxicity. For instance, it has been reported that adding minerals such as calcium (Ca) and phosphorus (P) into the irrigation water and soil might decrease B accumulation in plants [32, 54].

In soil-plant systems, the relationship between Ca and B has attracted the attention of many researchers. On this topic, Brenchley and Warington [7] were the first researchers suggesting this relationship. In the later years, this relationship came to be expressed in terms of the ratio of these two elements. The results of these researchers show that plants grow normally if there is a certain balance in both intake and tissue concentration of Ca and B. Furthermore, numerous reports show that the Ca/B ratio can be effectively used to detect B deficiency, adequacy and toxicity in plants since Ca/B ratios are inversely correlated with B concentrations in the substrate. Also, it has been emphasized that the toxic effects of B can be reduced or prevented with the addition of Ca into the soil [29, 47].This has been realized by applying it both to reactions in the soil and metabolic processes in plants. The Ca/B ratio demonstrates the situation or balance between these nutrients in the plant or soil and shows how indispensable they are for each other [43, 47, 48]. Even minor changes in the Ca/B ratio can affect the growth of a plant. The limited number of studies on B and Ca combination in wheat shows that appropriate concentrations of Ca can play a key role in reducing the negative effects of B [47, 48].

No study examining the effect of the interaction between B toxicity and Ca (B in combination with Ca) on soluble carbohydrates and relative water content in wheat was found in the literature. The purpose of this study was to examine the effects of different Ca concentrations, which show the antagonistic effect on B element at the toxic level, on growth and soluble carbohydrates in the seedlings of durum wheat (*Triticum durum* Desf. cv Kızıltan-91), which is known to be sensitive to B toxicity, and bread wheat (*Triticum aestivum* L. cv Gün-91), which is known to be tolerant to B toxicity. Reactions of wheat seedlings were evaluated with the predetermined physiological and bio-chemical parameters depending on the effects resulting from the application of different B and Ca concentrations.

MATERIAL AND METHODS

Plant material and growing plants

In the study, durum wheat (*Triticum durum* Desf. cv Kızıltan-91), which is known to be sensitive to B toxicity, and bread wheat (*Triticum aestivum* L. cv Gün-91), which is known to be tolerant to B toxicity, were used as plant material. *Triticum durum* Desf. cv Kızıltan-91 and *Triticum aestivum* L. cv Gün-91 seeds were supplied from the Field Crops Central Research Institute (TARM-Ankara, Turkey). The soil used in the study was air-dried in a greenhouse before it was putting the pots. Air-dried soil was scattered to 128 pots, each of which contained 1000 g soil, in polyethylene bags. 0.72 g ammonium sulphate ($(NH_4)_2SO_4$), 0.22 g mono potassium phosphate (KH_2PO_4) and 0.08 g potassium sulphate (K_2SO_4) were treated into the soil used in the study to represent a basal fertilizer. This treatment aimed to ensure that the soil contains adequate levels of nitrogen (N), P and potassium (K). The air-dried soil was treated with 0, 15, 30, 45 ppm B and 0, 40, 80, 120 ppm Ca, and the simultaneous applications of these treatments were performed homogenously. Control groups were not treated with B and Ca. B is not found free in nature, and its form that can use by the plants is boric acid (H_3BO_3) [5]. Therefore, H_3BO_3 was used for B treatments during the study. In Ca treatments, $Ca(NO_3)_2 \cdot 4H_2O$ was used. Each trial group was prepared to form four parallels, and 128 pots were used in total. Maximum effort was exerted to keep the soil used in the trials at a point close to field capacity (% FC= % 25.78). The seeds of both wheat species were kept in 2% sodium hypochlorite for 20 minutes and then washed with sterile water. 30 seeds were sown into each pot, and seedlings were grown for seven weeks under greenhouse conditions at 24-33 °C with 51% relative humidity at 14 hours daylight and 10 hours dark. Pots used in the trial were watered every other day. The locations of the pots were randomly determined, and by rotating the pots, it was ensured that each pot received enough daylight. Seven weeks after sowing, the plants were harvested from the soil surface.

Plant growth measurements

The harvested seedlings were washed and cleaned, root length and shoot length were measured in cm in randomly selected ten seedlings for each treatment. The weight of roots and shoots was measured separately to determine the fresh weight and dried in an oven of 110 ºC for 24 hours, and dry weights were determined.

Relative water content (RWC)

Four mm discs were randomly received from ten seedlings selected from the control group, and their fresh weights (FW) were measured. The discs were kept in a petri dish containing distilled water at 25 ºC for two hours, and turgor weights (TW) were determined. After the same discs were dried at 110 ºC for 24 hours, dry weights measured and the relative water content (RWC) calculated [23]. The RWC value was calculated using this method refers to water in the original sample as the percentage of water in the fully hydrated tissue.

Soluble carbohydrate analysis

The determination of soluble carbohydrate was conducted in accordance with Halhoul and Kleinberg [19]. Samples were shaken with 80 % ethyl alcohol, were taken to erlenmeyer after being centrifuged, and the supernatant was obtained after 80 % ethanol was put on the residue and centrifuged at 5000g for 10 minutes. The supernatant was transferred into an erlenmeyer, completed to 100 mL with distilled water after the alcohol evaporated, and stored in a cold environment glucose and fructose were analyzed by reacting 1 mL of the extract with 2 mL of cold freshly prepared anthrone solution and placed in 95 ºC water bath for 15 minutes for detection of glucose or in a water bath at in 40 ºC for 30 min dor detection of fructose, they were transferred into ice bath. Five minutes later, they were read at 620 nm in a Cecil 5000 spectrophotometer.

Statistical analysis

In the variance analyses, cultivars factor was evaluated with two levels as Kızıltan-91 and Gün-91; B factor with four levels as 0, 15, 30 and 45; and Ca factor with four levels as 0, 40, 80 and 120. The obtained data were evaluated through the variance analysis technique on the factorial design. Duncan multiple comparison method was used in the determination of different groups. Statistica 7 package program was used for conducting the analyses. Data presented are mean values ± standard deviation of measurement.

RESULTS

Effects of B, Ca and B + Ca treatments on root and shoot length in wheat seedlings

In Gün-91, the longest root length was detected in the control group to which no treatment was performed. Compared to the control group, in general, B, Ca and B+Ca treatments alone caused a decrease in the root length ($p < 0.05$). However, $B_{30} + Ca_{120}$ and $B_{45} + Ca_{80}$ treatments were found to be the most effective treatments on the root length when compared to other treatments apart from the control group ($p<0.05$) (Table 1). In Kızıltan-91, the longest root length was determined in Ca_{40} treatment ($p<0.01$). In B+Ca treatments, the longest root length was detected in $B_{30} + Ca_{120}$ treatment while the same treatment caused an increase when compared to the control group and all B treatments (Table 1).

In Gün-91, the longest shoot length was determined in Ca_{80}, $B_{15} + Ca_{120}$ and $B_{45} + Ca_{120}$ treatments when compared to all other treatments ($p<0.01$) (Table 1). In comparison to the control group, all B concentrations applied alone decreased shoot length in Gün-91 ($p<0.01$) (Table 1). As for Kızıltan-91, all Ca concentrations applied alone were found to be more effective than all other treatments, and the longest shoot length was determined in 40 and 80 ppm Ca treatments. Compared to B_{30} and B_{45} treatments, shoot length was found to be higher in all B+Ca treatments while the longest shoot length was found in $B_{30} + Ca_{80}$ treatment ($p<0.01$) (Table 1).

Effects of B, Ca and B + Ca treatments on shoot and root fresh weight in wheat seedlings

In Gün-91, when all treatments were compared, the highest root fresh weight was determined in $B_{15} + Ca_{120}$ treatment ($p<0.01$). Compared to the control group, all B treatments resulted in a decrease in root fresh weight in Gün-91 ($p<0.01$) (Table 2). In Kızıltan-91, the highest root fresh weight was determined in $B_{15} + Ca_{40}$ treatment. When both cultivars were compared in terms of root fresh weight, the highest root fresh weight was found in the Gün-91 treated with $B_{15} + Ca_{120.}$ These two treatments led to increases when compared to all B treatments, and the difference was found to be significant ($p<0.01$) (Table 2).

In Gün-91, the highest shoot fresh weight was determined in the control group. Compared to the control group, decreases in the shoot fresh weight Gün-91 in all B treatments were found significant ($p<0.01$). In Ca treatments, the highest shoot fresh weight was found in Ca_{80} treatment. In B+Ca treatments, the highest shoot fresh weight was determined in $B_{15} + Ca_{120}$ and $B_{30} + Ca_{120}$ treatments. In Kızıltan-91, when all treatments were compared, the highest shoot fresh weight was determined in the B_{15} treatment. Compared

Table 1. Changes in root and shoot length of wheat seedlings grown in different B, Ca and B + Ca concentrations (n = 10)

Treatments	Root length (cm)		Shoot length (cm)	
	Gün-91	Kızıltan-91	Gün-91	Kızıltan-91
Control	15.8±0.482*	13.8±1.190	33.4±0.540	44.5±1.14
B_{15}	14.3±0.932	14.7±1.180	32.3±0.746	44.2±1.20
B_{30}	11.8±0.646	14.3±0.883	31.5±0.764	42.4±1.45
B_{45}	14.1±1.000	13.9±1.300	31.9±1.340	40.9±1.32
Ca_{40}	13.3±0.955	15.8±0.69*	32.3±0.989	46.1±1.22*
Ca_{80}	13.8±0.742	11.6±0.819	34.4±1.000*	46.4±1.63*
Ca_{120}	11.7±0.972	13.9±0.948	32.2±1.110	45.5±1.10
$B_{15} + Ca_{40}$	12.7±0.775	14.6±1.090	33.7±0.817	43.7±1.37
$B_{15} + Ca_{80}$	13.8±0.952	11.6±0.846	33.6±0.653	43.7±1.86
$B_{15} + Ca_{120}$	11.9±0.983	11.9±1.060	34.4±0.945*	44.9±1.50
$B_{30} + Ca_{40}$	13.1±1.048	11.6±0.884	31.9±0.767	44.6±0.968
$B_{30} + Ca_{80}$	11.4±0.991	14.1±0.795	31.4±0.748	45.3±1.550*
$B_{30} + Ca_{120}$	13.9±0.629*	14.8±0.772*	32.3±0.653	44.4±0.897
$B_{45} + Ca_{40}$	13.8±0.879	13.8±1.280	32.1±1.120	43.3±1.190
$B_{45} + Ca_{80}$	14.4±0.731*	12.9±0.849	30.9±0.781	42.3±0.895
$B_{45} + Ca_{120}$	11.1±0.849	11.9±0.674	33.8±1.130*	42.5±1.670

Table 2. Changes in root and shoot fresh weight of wheat seedlings grown in different B, Ca and B + Ca concentrations (n = 10)

Treatments	Root fresh weight (g)		Shoot fresh weight (g)	
	Gün-91	Kızıltan-91	Gün-91	Kızıltan-91
Control	0.04±0.005	0.03±0.002	0.34±0.028*	0.36±0,021
B_{15}	0.03±0.004	0.03±0.005	0.33±0.027	0.41±0.031*
B_{30}	0.03±0.009	0.03±0.010	0.21±0.084	0.26±0.007
B_{45}	0.03±0.002	0.03±0.004	0.24±0.027	0.28±0.026
Ca_{40}	0.05±0.011*	0.03±0.001	0.2±0.0230	0.37±0.028*
Ca_{80}	0.03±0.003	0.02±0.002	0.25±0.015	0.36±0.014*
Ca_{120}	0.05±0.005*	0.03±0.004	0.24±0.017	0.32±0.026
$B_{15} + Ca_{40}$	0.03±0.003	0.05±0.005*	0.22±0.017	0.33±0.031
$B_{15} + Ca_{80}$	0.04±0.003	0.02±0.002	0.20±0.018	0.37±0.025*
$B_{15} + Ca_{120}$	0.13±0.040*	0.03±0.003	0.25±0.024*	0.37±0.044*
$B_{30} + Ca_{40}$	0.04±0.015	0.03±0.007	0.17±0.079	0.35±0.012
$B_{30} + Ca_{80}$	0.04±0.007	0.03±0.006	0.14±0.037	0.34±0.026
$B_{30} + Ca_{120}$	0.03±0.012	0.03±0.014	0.27±0.066*	0.29±0.025
$B_{45} + Ca_{40}$	0.04±0.002	0.03±0.003	0.17±0.010	0.33±0.016
$B_{45} + Ca_{80}$	0.03±0.003	0.03±0.002	0.14±0.011	0.29±0.029
$B_{45} + Ca_{120}$	0.03±0.003	0.02±0.003	0.22±0.021	0.25±0.020

to the control group, B_{30} and B_{45} led to decreases in shoot fresh weight (p<0.01). In Ca treatments, the highest shoot fresh weight was found in Ca_{40} and Ca_{80} treatments. In B+Ca treatments, the highest shoot fresh weight was determined in B_{15} + Ca_{80} and B_{15} + Ca_{120} treatments (p<0.01) (Table 2).

Effects of B, Ca and B + Ca treatments on shoot and root dry weight in wheat seedlings

In Gün-91, when compared to the control group, the highest root dry weight was determined in B_{15} treatment (p< 0.01). Also, in comparison to B_{30} and B_{45} treatments, the increase observed in the root dry weight in Ca_{120} treatment was found to be significant. In Kızıltan-91, compared to B_{45}, increases were observed in Ca and B+Ca treatments (p<0.01).The highest root dry weight was determined in the Gün-91 treated with B_{15} (p<0.01) (Table 3).

In Gün-91, the highest shoot dry weight was found in the control group and B_{30} treatment (p<0.01). Compared to the control group, B and Ca treatments decreased shoot dry weight in the Gün-91 (Table 3). In B+Ca treatments, B_{45} was compared to B_{15} + Ca_{80}, B_{30} + Ca_{80}, B_{15} + Ca_{120}, B_{45} + Ca_{40} and B_{45} + $Ca_{120,}$ the difference among the shoot dry weights was found to be statistically significant (p<0.01) (Table 3). In Kızıltan-91, the highest shoot dry weight was determined in B_{15} treatment. Compared to the control group, B_{30} and B_{45} treatments led to decreases in the shoot dry weight (p<0.01). When B_{30} and B_{45} were compared to Ca_{40}, B_{15} + Ca_{80} and B_{30} + $Ca_{80,}$ the difference among the shoot dry weights was found to be significant (p<0.01). The highest shoot dry weight was determined in the Kızıltan-91 treated with B_{15} (Table 3).

Effects of B, Ca and B + Ca treatments on glucose and fructose amount, RWC in wheat seedlings

In Gün-91, compared to the control group, all treatments except for high concentrations of 45 ppm B and 120 ppm Ca resulted increased in RWC (p<0.05). Among all treatments, the highest RWC values were determined in the B_{15}, B_{45} + $Ca_{40,}$ and B_{45} + Ca_{120} treatments (p<0.05) (Table 4). In Kızıltan-91, when B_{45} was compared to the other treatments, the difference among the increases observed in RWC was found to be significant (p<0.05). The highest RWC was determined in the Kızıltan-91 treated with Ca_{40} treatment (p<0.01) (Table 4).

In Gün-91, compared to the control group, the lowest amount of glucose was determined in B_{45} treatment (p<0.01) (Table 4). When B_{45} was compared to all treatments, the increase observed in the amount of glucose was found to be significant. Among all

Table 3. Changes in root and shoot dry weight of wheat seedlings grown in different B, Ca and B + Ca concentrations (n = 10)

Treatments	Root dry weight (g)		Shoot dry weight (g)	
	Gün-91	Kızıltan-91	Gün-91	Kızıltan-91
Control	0.01±0.000	0.02±0.001	0.08±0.002*	0.09±0,004
B_{15}	0.06±0.011*	0.01±0.001	0.06±0.003	0.10±0.009*
B_{30}	0.02±0.001	0.02±0.001	0.07±0.005*	0.07±0.003
B_{45}	0.02±0.007	0.01±0.001	0.05±0.005	0.07±0.004
Ca_{40}	0.01±0.001	0.02±0.002*	0.05±0,003	0.09±0.005*
Ca_{80}	0.01±0.001	0.01±0.001	0.06±0.002	0.08±0.003
Ca_{120}	0.03±0.002*	0.02±0.002*	0.06±0.001	0.07±0.006
B_{15} + Ca_{40}	0.02±0.007	0.02±0.003*	0.05±0.003	0.08±0.006
B_{15} + Ca_{80}	0.02±0.001	0.01±0.002	0.06±0.003*	0.09±0.008*
B_{15} + Ca_{120}	0.02±0.001	0.01±0.001	0.06±0.005*	0.08±0.002
B_{30} + Ca_{40}	0.02±0.001	0.01±0.001	0.05±0.001	0.07±0.005
B_{30} + Ca_{80}	0.01±0.001	0.02±0.002*	0.06±0.004*	0.09±0.005*
B_{30} + Ca_{120}	0.02±0.001	0.01±0.001	0.05±0.004	0.08±0.005
B_{45} + Ca_{40}	0.01±0.000	0.01±0.001	0.06±0.001*	0.07±0.003
B_{45} + Ca_{80}	0.01±0.001	0.02±0.002*	0.04±0.002	0.08±0.006
B_{45} + Ca_{120}	0.01±0.001	0.01±0.001	0.06±0.004*	0.06±0.003

Table 4. Changes in RWC, glucose and fructose amount of wheat seedlings grown in different B, Ca and B + Ca concentrations (n = 10)

Treatments	RWC		Glucose (mg g^{-1} DW)		Fructose (mg g^{-1} DW)	
	Gün-91	Kızıltan-91	Gün-91	Kızıltan-91	Gün-91	Kızıltan-91
Control	58.44±7.436	76.89±6.466	1.78±1.35	1.17±0.123	1.65±0.059	2.53±0.022*
B_{15}	73.62±7.545	77.78±7.871	1.63±0.145	1.37±0.128	1.54±0.196	2.07±0.036
B_{30}	67.56±4.912	77.61±7.802	1.75±0.307	1.21±0.031	1.65±0.057	1.92±0.026
B_{45}	48.16±3.407	53.33±2.899	1.26±0.132	1.24±0.109	1.38±0.082	1.61±0.042
Ca_{40}	58.23±10.23	83.92±5.602*	1.80±0.143*	1.06±0.334	1.75±0.125*	1.43±0.053
Ca_{80}	69.14±12.62	63.99±6.131	1.60±0.131	1.93±0.046	1.58±0.141	1.56±0.092
Ca_{120}	49.13±4.462	52.91±3.797	1.35±0.248	1.99±0.133	1.36±0.264	1.92±0.157
B_{15} + Ca_{40}	52.53±5.932	78.20±2.377	1.55±0.234	1.02±0.037	1.28±0.203	1.52±0.038
B_{15} + Ca_{80}	57.39±14.40	73.13±0.080	1.33±0.015	1.96±0.064	1.06±0.138	1.95±0.221
B_{15} + Ca_{120}	67.22±6.289	69.65±5.471	1.56±0.263	2.15±0.101*	1.48±0.231	1.66±0.044
B_{30} + Ca_{40}	64.91±7.926	76.17±6.611	1.50±0.11	1.43±0.31	1.39±0.195	1.22±0.022
B_{30} + Ca_{80}	59.01±8.016	80.45±0.130*	1.36±0.243	2.00±0.061*	1.30±0.228	1.68±0.144
B_{30} + Ca_{120}	67.29±3.522	64.18±1.780	1.9±0.107*	2.17±0.074*	1.68±0.249*	2.10±0.074*
B_{45} + Ca_{40}	73.95±4.289*	78.29±7.466	1.48±0.105	1.30±0.053	1.28±0.097	1.53±0.046
B_{45} + Ca_{80}	57.56±1.695	69.68±2.660	1.44±0.068	1.96±0.095	1.34±0.057	1.78±0.069
B_{45} + Ca_{120}	73.00±4.063*	48.12±1.957	1.56±0.079	2.10±0.068*	1.49±0.077	1.44±0.043

treatments, the highest amount of glucose was determined in B_{30} + Ca_{120} treatment, and the same treatment led to increase in comparison to different B concentrations. In Kızıltan-91, compared to the control group, the lowest amount of glucose was determined in B_{45} treatment (p<0.01) (Table 4). When all treatments were compared, the highest increases were determined with B_{15} + Ca_{120}, B_{30} + Ca_{80}, B_{30} + Ca_{120} and B_{45} + Ca_{120} treatments, and the difference was found to be significant (p<0.01) (Table 4).

In Gün-91, the decrease observed in the amount of fructose when B was treated alone was found to be statistically significant in comparison to the control group (p<0.01). The highest amount of fructose was determined in Ca_{40} treatment. Compared to B_{45} and Ca_{40} treatments, the increases observed in the amount of fructose in B_{15} + Ca_{120}, B_{30} + Ca_{120} and B_{45} + Ca_{120} treatments were found to be significant (p<0.05) (Table 4). In Kızıltan-91, all treatments decreased the amount of fructose in comparison to the control (p<0.05). In B+Ca treatments, the highest amounts of fructose were determined in B_{30} + Ca_{120} and B_{15} + Ca_{80}, respectively (p<0.05), and when these treatments were compared to B_{30} and B_{45} concentrations, the difference between the increases was found to be significant (Table 4).

DISCUSSION

Both Ca and B are basic elements for the growth and development of plants [43]. Calcium treatments promote plant growth and fruit development. Studies conducted so far have reported that B treatments increase yield and quality [17, 53]. In the present study, Ca and B treatments and Ca-B interactions led to different effects on the growth parameters of wheat. While all B concentrations treated alone increased root length in Kızıltan-91, it decreased shoot fresh weight, shoot length in both cultivars (especially 30, 45 ppm). Our results is in accordance with findings of Koohkan and Maftoun [24] who reported high B concentration was found to cause a reduction in the shoot length. In our study, the increase in the root length might be attributed to the promotion of cell division and differentiation by B, which in turn is associated with the preservation of meristematic activity and vegetative growth, while it is considered that the most important reason for the decrease in the seedling development might be the toxic effect caused by increasing B concentrations. In this experiment, it was also determined that all boron concentrations caused a decrease in root length in Gün-91 cultivars. Boron plays a key role in the activation of cell division and cell elongation [6, 40]. The reduction in root growth and length observed in wheat plants could be dependent to an lignification of the cell wall, which is a known phenomenon induced by B excess in some plants [9,10]. Also, these different reactions can be associated with different genotypes. These results are compatible with the findings and conclusions of studies by Princi *et al.* [39] and Turan *et al.* [47] who observed different responses to toxic B concentrations in different species of some plants. Similarly, Chantachume *et al.* [8] reported that changes observed in the root length of wheat cultivars in the treatments of high B concentrations might have resulted from genetic differences. It was reported in some studies that the decrease in the roots of the plants exposed to B toxicity was associated with the decrease in the transport of carbohydrates from leaves to roots and thus, root growth might have been inhibited [42]. For many cultivars, there is a narrow range at the critical concentrations between B deficiency and B toxicity.

The effect of Ca on root elongation has demonstrated that different Ca^{2+} concentrations both inhibits [29] and stimulates vegetative reproduction or shoot growth of the plant [18, 20, 52]. Liu *et al.* [26] reported that B and Ca deficiencies caused reduced plant biomass and root growth. In this study, the longest root length was determined in Kızıltan-91cultivar treated with Ca_{40}. This result can be associated with the activation of the enzymes in the mitosis and cytokinesis by Ca [1, 14]. It was proven that Ca+B combination was more effective in the production of longer plants having higher numbers of branches and leaves [18]. The inhibitory effect of 45 ppm B on the shoot length was reduced in Kızıltan-91 by the presence of Ca. The highest shoot length in both cultivars was determined in the treatment of 80 ppm Ca. This increase observed depending on the treatment can be attributed to the fact that Ca is a major component of the plant cell wall and plays a key role in cell division, cell elongation and expansion as well as the carbohydrate metabolism [22]. Ca can regulate the transcription and translation of genes that encode chloroplast proteins and enzymes, which are involved in the reactions of photosynthesis. Ca involved in regulating photosynthetic enzyme activity of carbon assimilation [51]. Sotiropoulos *et al.* [44] and Turan *et al.* [47] stated that tolerance increased depending on the increase in Ca concentration, and high Ca concentration reduced B absorption. This result can be explained by the localisation of Ca in the cell wall. Therefore, the localisation of Ca in the cell wall reduces B permeability of the cell wall and also inhibits B uptake. Thus, it is important to apply these two elements to plants at the most appropriate Ca/B ratio combination.

Primary phenotypic effects of B toxicity are generally reduced plant dry weight and inhibition of root growth along with increased B level in the root tissue [47]. Although reactions of wheat cultivars to B has been different, B treatment has resulted in reduced dry weight in most of the cultivars. In our study, especially B_{45} treatment caused significant decreases in shoot dry weight in both cultivars and root dry weight in Kızıltan-91, and thus made an inhibiting effect. The same concentration caused an increase in the root dry weight in Gün-91. As in the case of different plant varieties, different species of the same plant have significant differences in terms of B sensitivity, and the

main reason of these differences is that plants are not affected by B toxicity physiologically at the same rates [31]. Similar results were reported by Huang *et al.* [21]. It has been determined that dry matter and product amount has not changed obviously in cultivars resistant to B, decreases have been observed in sensitive cultivars [19]. Güneş *et al.* [16] reported that the treatment of increasing concentrations of B increased stem dry weight in both bread (*Triticum aestivum* L. cv. Bezostaya) and durum (*Triticum durum* L. cv. Kızıltan-91) wheat cultivars while Taban and Erdal [45] reported that durum wheat cultivars were affected by B more than bread wheat cultivars and growth regressed, and dry weight increased in some cultivars of the bread wheat. A significant decrease was not observed in the product when wheat cultivars resistant to B toxicity were treated with B at high concentrations, but significant product and yield losses were determined in the sensitive cultivars grown at the same and lower concentrations [36].

Reduced water content and chlorosis are among the known symptoms of B toxicity. It has been reported that B treatments have significantly reduced relative water content in the shoot and root along with growth [18]. Studies have demonstrated that there is a strong negative correlation between water content and B concentration in the shoot and root, and high B levels reduce water uptake and causes water stress [35]. When compared to B-stressed plants, Ca treatment targeted B toxicity by increasing shoot and root length, shoot and root dry weight and root water content. Similarly, it was reported that $CaCl_2$ treatments to different plant varieties under B toxicity such as barley and turnip alleviated the toxic effect of B [43]. Metwally *et al.* [29] reported that water uptake was reduced considerably due to B stress in canola plant, Ca treatment decreased the negative effect of B on water uptake and Ca treatment also increased yield. In our study, 45 ppm B treatment decreased RWC in both cultivars. The inhibitory effect of 45 ppm B on the RWC was reduced by the presence of Ca. The findings of the present study show that Ca alleviates B toxicity by improving the plant-water situation. Localization of Ca in the cell wall may have caused a decrease in cell wall boron permeability. Also, Ca controls the transition of B from root to leaves. Weak and strong negative correlations between water content in shoots and root and B concentration indicate that preserving the critical Ca levels might be essential for keeping the water channels open through signal processes such as phosphorylation [27].

Boron is critical for sugar transport and carbohydrate metabolism. Metwally *et al.* [28] reported that B treatment caused different effects on two different wheat cultivars and that B toxicity increased the soluble carbohydrate level in the cultivar sensitive to B while an apparent change was not observed in the soluble carbohydrate level in the cultivar tolerant to B. Our results demonstrate that B treatments in increased glucose amount in the Kızıltan-91 cultivar sensitive to B toxicity. Increased levels of glucose in the Kızıltan-91 may help in turgor upkeeping and cellular membrane stabilization. Metwally *et al.* [28] associated this carbohydrate accumulation with the restriction of its use rather than the increase in its synthesis. Differently to fructose, a huge body of reports show that glucose acts as an osmolyte in plants subjected to stress factors [15]. Pérez-López *et al.* [37] reported that sugar accumulation in plants under stress conditions could be related to osmotic adjustment. Sugars, which play a key role in the regulation of the osmotic density of cellulary juice and are defined as protective soluble molecules, accumulated under stress conditions in particular. Also, it was reported that B was likely to change the transmembrane movement of soluble substances such as sugar and acted as a shuttle for compounds along the plasma membrane [3, 38]. In the same B treatments there was in decrease change in glucose in the Gün-91 cultivar, which was tolerant to B toxicity; the lowest amount of glucose was determined in B_{45} treatment. Many researchers have indicated that B treatment can alter carbohydrate metabolism Bonilla *et al.* [4] and Perica *et al.* [38]. In this context, our results corroborate the findings of Bonilla *et al.* [4] and Perica *et al.* [38], who reported high B concentration was found to cause a reduction in the amount of soluble carbohydrates such as glucose. This decrease can be associated with the increase in proline amount for the acquisition of resistance [2]. The possible multiple roles of proline as an osmolyte (or as an energy source or an ROS scavenger) and proline as a signal molecule, were reminiscent of the multiple roles of glucose serving as a carbon and energy source [46]. The B_{45} treatment resulted in decreased amount of fructose in both cultivars. Fructose is required for the biosynthesis of many defense compounds and showed an increase in the amount of defense compounds such as phenolic compounds anthocyanin, ascorbate etc. in plants exposed to B toxicity [25]. Perhaps fructose may have been used in the synthesis of these defense compounds. On the other hand, most of the available reports emphasize that in some cases B is directly or indirectly responsible for stomatal closures. B toxicity reduced CO_2 assimilation thus impairment of diffusion of CO_2 has been attributed to stomatal limitation [34].Thus, this stomal limitation may have led to a reduction in photosynthesis and hence the amount of glucose and fructose.

The accumulation of soluble sugars stimulated by Ca shows the positive effect of Ca for alleviating the negative effect of B stress. Ca acts as second messenger in the highly diverse signal transduction pathways of the plants. Our results demonstrate that toxic effect on glucose of 30 and 45 ppm B was observed to be alleviated by calcium application, particularly at 120 ppm in both cultivars. B_{30} + Ca_{120} treatment increased both glucose and fructose amounts in both cultivars. The reason of the sugar accumulation due to Ca treatment might be associated with such functioning of Ca as a signal molecule as well as the

stimulation of genes responsible for the biosynthesis of carbohydrates by Ca. Similar results were reported by Furuichi *et al.* [13] on *Arabidopsis thaliana*, it was reported that Ca resulted in sugar accumulation as a signal molecule. Accumulation of osmotic compounds such as soluble sugars helps plants maintain their cellular functions by preserving the water content in the tissue. It is assumed that sugars, which act as an osmolyte, preserve specific macromolecules and contribute to the stabilization of membrane structures and protect cells at times of stress, and phospholipids in the cell membrane interact with polar head groups to prevent membrane fusion [3]. An appropriate B and Ca ratio is highly important for plant growth. The present study demonstrates that an excessively low or high Ca/B ratio can considerably change the growth response of plants. The decreases observed in growth parameters and carbohydrate levels as a result of B+Ca combination treatments show that Ca losses its supportive effect when combined with B, and a reverse effect can occur between Ca and B at the concentrations evaluated. It was also reported that B affected Ca metabolism in plant species with low adaptation to high B ratios [50]. Probably, the decreases in the product yield due to the interaction of these two elements result from increases or decreases in the other nutrient elements. This is because of the fact that the maximum growth of a product occurs when all basic elements exist at an optimal concentration and an appropriate balance is ensured among them.

In conclusion, findings showed that root and shoot length, shoot dry and fresh weight, relative water content and fructose and glucose amounts decreased in the treatments containing high B concentrations. Therefore, it can be concluded that excessive or toxic levels of B might be responsible for decreased plant growth and changes in the physiology and biochemistry of plants. It was also determined that the treatment of Ca with low levels of B in general, had a significant effect on plant growth. The present study suggests that the treatment of appropriate concentrations of Ca might be effective for reducing B toxicity. This interaction between Ca and B can be used for eliminating B toxicity in wheat breeding, but further cellular and biochemical studies are needed on this interaction between Ca and B.

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REFERENCES

[1] Atilla-Gokcumen, G.E., Castoreno, A.B., Sasse, S., Eggert, U.S., (2010): Making the cut: the chemical biology of cytokinesis. ACS Chemical Biology, 5(1): 79-90.

[2] Başalp, A., Öncel, I., Koç, E., (2011): Determination of some physiological and biochemical changes in tolerant and sensitive wheat seedlings to boron (B) toxicity. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 15(3): 135-141.

[3] Bellaloui, N., Hu, Y., Mengistu, A., Kassem, M.A., Abel, C.A., (2013): Effects of foliar boron application on seed composition, cell wall boron, and seed $15^{\delta}N$ and $13^{\delta}C$ isotopes in water-stressed soybean plants. Frontiers in Plant Science, 4: 1-13.

[4] Bonilla, I., Cadahia, C., Carpena, O., Hernando, V., (1980): Effects of boron on nitrogen metabolism and sugar levels of sugar beet. Plant and Soil, 57(1): 3-9.

[5] Boşgelmez, A., Boşgelmez, İ.İ., Savaşçı, S., Paslı, N., (2001): Ekoloji II Toprak. Başkent Klişe Matbaacılık, Ankara, pp. 675.

[6] Brdar Jokanovic, M., (2020): Boron toxicity and deficiency in agricultural plants. International Journal of Molecular Sciences, 21: 1-20.

[7] Brenchley, W.E., Warington K., (1927): The role of boron in the growth of plants. Annals of Botany, 41: 167-187.

[8] Chantachume, Y., Smith, D., Hollamby, G.J., Paull, J.G., Rathjen, A.J., (1995): Screening for boron tolerance in wheat (*T. Aestivum*) by solution culture in fitler paper. Plant and Soil, 177: 249-254.

[9] Chatsizsavvidis, C., Antonopoulou, C., (2019): Fruit Crops: diagnosis and management of nutrient constraints. pp. 211-221. In: Srivastava, A.K., Hu, C. (eds.): Boron toxicity in fruit crops: agronomic and physiological implication. Elsevier.

[10] Chen, L.S., Han, S., Qi Y.P., Yang, LT., (2012): Boron stresses and tolerance in citrus. African Journal of Biotechnology, 11: 5961-5969.

[11] Eaton, F.M., (1944): Deficiency, toxicity and accumulation of boron in plants. Journal of Agricultural Research, 69: 237-277.

[12] Fang, K., Zhang, W., Xing, Y., Zhang, Q., Yang, L., Cao, Q., Qin L., (2016): Boron toxicity causes multiple effects on *Malus domestica* pollen tube growth. Frontiers Plant Science, 7: 1-13.

[13] Furuichi, T., Mori, I.C., Takahashi, K., Muto, S., (2001): Sugar-induced increasein cytosolics Ca in Arbidopsis thaliana whole plants. Plant and Cell Physiology, 42(10): 1149-1155.

[14] Gislerød, H.R., (1999): The role of calcium on several aspects of plant and flower quality from a floricultural perspective. Acta Horticulturae, 481: 345-351.

[15] Gupta, A.K., Kaur, N., (2005): Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. Journal of Bioscences, 30: 761-776.

[16] Güneş, A., Alpaslan, M., İnal, A., Adak, M.S., Eraslan, F., Çiçek, N., (2003): Effects of boron fertilization on the yield and some yield components of bread and durum wheat. Turkish Journal of Agriculture Forestry, 27: 329-335.

[17] Güneş, A., Ataoğlu, N., Esringü, A., Uzun., O., Ata, S., Turan, M., (2011): Yield and chemical composition of corn (*Zea ma*ys L.) as affected by boron management. International Journal of Plant, Animal and Environmental Sciences, 1(1): 42-53.

[18] Haleema, B., Rab, A., Hussain, S.A., (2018): Effect of calcium, boron and zinc foliar application on growth and fruit production of tomato. Sarhad Journal of Agriculture, 34(1): 19-30.

[19] Halhoul, M.N., Kleinberg, I., (1972): Differential determination of glucose and fructose yielding substances with anthrone. Analytical Biochemistry, 50: 337-343.

[20] Han, S., Fang, L., Ren, X., Wang, W., Jiang, J., (2015): MPK6 controls H_2O_2-induced root elongation by mediating Ca^{2+} influx across the plasma membrane of root cells in Arabidopsis seedlings. New Phytologist, 205(2): 695-706.

[21] Huang, C., Graham, R.D., (1990): Resistance of wheat genotypes to boron toxicity is expressed at cellular level. Plant and Soil, 26: 295-300.

[22] Ilyas, M., Ayub, G., Hussain, Z., Ahmad, M., Bibi, B., Rashid, A., Luqman, A., (2014): Response of tomato to

different levels of calcium and magnesium concentration. World Applied Sciences Journal, 31(9): 1560-1564.
[23] Kastori, R., Petrovic, M., Petrovic, N., Loomis, W.D., Durts, R.W., (1992): Chemistry and biology of boron. Biofactors, 3: 229-239.
[24] Koohkan, H., Maftoun, M., (2016): Effect of nitrogen-boron interaction on plant growth and tissue nutrient concentration of canola (*Brassica napus* L.). Journal of Plant Nutrition, 39: 922-931.
[25] Landi, M., Pardossi, A., Remorini, D., Guidi, L., (2013): Antioxidant and photosynthetic response of a purple-leaved and a green-leaved cultivar of sweet basil (*Ocimum basilicum*) to boron excess. Environmental and Experimental Botany, 85: 64-75.
[26] Liu, Y., Riaz, M., Yan, L., Zeng, Y., Cuncang, J., (2019): Boron and calcium deficiency disturbing the growth of trifoliate rootstock seedlings (*Poncirus trifoliate* L.) by changing root architecture and cell Wall. Journal of Plant Biochemistry and Physiology, 144: 345-354.
[27] Martínez-Ballesta, M.C., Cabañero, F., Olmos, E., Periago, P.M., Maurel, C., Carvajal, M., (2008): Two different effects of calcium on aquaporins in salinity-stressed pepper plants. Planta, 228: 15-25.
[28] Metwally, A.M., El-Shazoly, R.M., Hamada, A.M., (2017): Physiological responses to excess boron in wheat cultivars. European Journal of Biological Research, 7: 1-8.
[29] Metwally, A.M., Radi, A.A., El-Shazoly, R.M., Hamada, A.M., (2018): The role of calcium, silicon and salicylic acid treatment in protection of canola plants against boron toxicity stress. Journal of Plant Research, 131(6): 1015-1028.
[30]Musvoto, C., De Lange, W., (2019): A framework for selecting crops for irrigation using mining contaminated water: an example from the Olifants basin of South Africa. Journal of Environmental Management, 31: 49-58.
[31] Nable, R.O., (1991): Distribution of boron within barley genotypes with differing susceptibilities to boron toxicity. Journal of Plant Nutrition, 14(5): 453-461.
[32] Nable, R.O., Banuelos, G.S., Paull, J.G., (1997): Boron toxicity. Plant and Soil, 193: 181-198.
[33] Nejad, S.A.G, Savaghebi, G.R., Farahbakhsh, M., Amiri, E.M., Rezaei, H., (2015): Tolerance of some wheat varieties to boron toxicity. Cereal Research Communications, 43(3): 384-393.
[34] Oikonomou, A., Ladikou, E.V., Chatziperou, G., Margaritopoulou, T., Landi, M., Sotiropoulos, T., Araniti, F., Papadakis, I.E., (2019): Boron excess imbalances root/shoot allometry, photosynthetic and chlorophyll fluorescence parameters and sugar metabolism in apple plants. Agronomy, 9(11): 731; doi: 10.3390/agronomy9110731.
[35] Pandey, N.A., (2013). Antioxidant responses and water status in brassica seedlings subjected to boron stress. Acta Physiologia Plantarum, 35: 697-706.
[36] Paull, J.G., Cartwright, B., Rathjen, A.J., (1988): Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica, 39: 137-144.
[37] Pérez-López, U., Robredo, A., Lacuesta, M., Muñoz-Rueda, A., Mena-Petite, A., (2010): Atmospheric CO_2 concentration influences the contributions of osmolyte accumulation and cell wall elasticity to salt tolerance in barley cultivars. Journal of Plant Physiology, 167: 15-22.
[38] Perica, S., Bellaloui, N., Greve, C., Hu, H., Brown, P.H., (2001): Boron transport and soluble carbohydrate concentrations in olive. Journal of the American Society for Horticultural Science, 126(3): 291-296.
[39] Princi, M.P, Lupini, A., Araniti, F., Sunseri, F., Abenavoli, M.R., (2013): Short-term effects of boron excess on root morphological and functional traits in tomato. pp. 1150-1151. In: XVII International Plant Nutrition Colloquium-Boron Satellite Meeting, 17-18 August, Proceedings Book, İstanbul.
[40] Princi, M.P., Lupini, A., Araniti, F., Longo, C., Mauceri, A., Sunseri, F., Abenavoli, M.R., (2016): Boron toxicity and tolerance in plants: Recent advances and future perspectives. pp. 115-147. In: Ahmed, P. (eds.): Plant metal interaction. Elsevier.
[41] Reid, R., (2007): Update on boron toxicity and tolerance in plants. pp. 83-90. In: Xu, F., Goldbach, H.E., Brown, P.H., Bell, R.W., Fujiwara, T., Hunt, C.D., Goldberg, S., Shi, L. (eds.): Advances in plant and animal boron nutrition. Springer, Dordrecht.
[42] Sheng, O., Zhou, G., Wei, Q., Peng, S., Deng, X., (2010): Effects of excess boron on growth, gas exchange, and boron status of four orange scion-rootstock combinations. Journal of Soil Science and Plant Nutrition, 173: 469-476.
[43] Siddiqui, M.H., Al-Whaibi, M.H., Sakran, A.M., Ali, H.M., Basalah, M.O., Faisal, M., Alatar, A., Al-Amri, A.A., (2013): Calcium-induced amelioration of boron toxicity in radish. Journal of Plant Growth Regulation, 32: 61-71.
[44] Sotiropoulos, T.E., Therios, I.N., Daimassi, K.N., Bosabalidis, A.M., Kofidis, G., (2002): Nutritional status, growth, CO_2 assimilation and leaf anatomical responses in two kiwi fruit species under boron toxicity. Journal of Plant Nutrition, 25(6): 1249-1261.
[45] Taban, S., Erdal, İ., (2000): Bor uygulamasının değişik buğday çeşitlerinde gelişme ve toprak üstü aksamda bor dağılımı üzerine etkisi. Turkish Journal of Agriculture Forestry, 24: 255-262.
[46] Trovato, M., Mattioli, R., Costantino, P., (2008): Multiple roles of proline in plant stress tolerance and development. Rendiconti Lincei, 19(4): 325-346.
[47] Turan, M.A., Taban, N., Taban, S., (2009): Effect of calcium on the alleviation of boron toxicity and localization of boron and calcium in cell wall of wheat. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2): 99-103.
[48] Turan, M.A., Taban, S., Kayin, G.B., Taban, N., (2018): Effect of boron application on calcium and boron concentrations in cell wall of durum (*Triticum durum*) and bread (*Triticum aestivum*) wheat. Journal of Plant Nutrition, 41(11): 1351-1357.
[49] Türker, O.C., Yakar, A., Türe, C., Saz, Ç., (2019): Cost-effectiveness of boron (B) removal from irrigation water: an economic water treatment model (EWTM) for farmers to prevent boron toxicity. Environmental Science and Pollution Research, 26: 18777-18789.
[50] Wang, H., Wang, Y., Du, C., Xu, F., Yang, Y., (2003): Effects of boron and calcium supply on calcium fractionation in plants and suspension cells of rape cultivars with different boron efficiency. Journal of Plant Nutrition, 26: 789-800.
[51] Wang, Q., Yang, S., Wan, S., Li, X., (2019): The significance of calcium in photosynthesis. International Journal of Molecular Sciences, 20(6): 1-14.
[52] Watanabe, T., Okada, K., (2005). Interactive effects of Al, Ca and other cations on root elongation of rice cultivars under low pH. Annals of Botany, 95(2): 379-385.
[53] Wrobel, S., (2009): Response of spring wheat to foliar fertilization with boron under reduced boron availability. Journal of Elementology, 14(2): 395-404.
[54] Zhen, M., Cui, M., Xia, J., Ma, C., Liu, C., (2019): Effect of nitrogen and phosphorus on alleviation of boron toxicity in *Puccinellia tenuiflora* under the combined stresses of salt and drought. Journal of Plant Nutrition, 42(14): 1594-1604.

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