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SCREENING OF THREE SAFFLOWER (*Carthamus tinctorius* L.) CULTIVARS UNDER BORON STRESS

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ABSTRACT

Excess of boron in soil and irrigation water is a serious constrain to crop production in many areas of the World as well as in Turkey. A pot experiment was carried out with to screen safflower cultivars in order to investigate the effects of boron toxicity stress on early growth and ions composition. Three safflower cultivars (*Carthamus tinctorius* cv. Balci, Yenice, Remzi Bey) were grown in pots containing alkaline and potassium rich soil, additionally supplemented with 0, 4, 8, 16, 32, 64 and 128 mg kg^{-1} boron. Chlorophyll content of all cultivars decreased with excessive boron levels. Plant height, shoot fresh and dry weight significantly increased at 4 mg kg^{-1} boron level followed by sharp decline with the other treatments. Boron content of cultivars increased and the highest amount was observed at 128 mg kg^{-1} boron level. Sodium content of all cultivars gradually increased with increase in B concentration. Whereas, potassium and calcium content reduced with increased B. Phosphorus content of all cultivars were least at 128 mg kg boron level. Results revealed that cv. balci appeared to tolerant to boron in soil up to 32 mg kg^{-1} and can be recommended for growing and breeding material for boron rich soils of Central Anatolia.

Key words: boron, toxicity, chlorophyll content index, safflower

INTRODUCTION

Boron (B) is an important micronutrient that is found in very low quantity mainly in the cell walls and reproductive organs and regulate the plant growth. Availability of B is essential for plants at all stages of growth. Boron has important role in sugar transport [Tariq et al. 1993] and provides integrity to cell wall structure and their functions including lignification and cell wall synthesis [Brown et al. 2002]. It also plays an important role in carbohydrate formation, respiration, phenol, indole acetic acid (IAA) and ribose nucleic acid (RNA) metabolism [Ayvaz et al. 2012]. However, relatively more quantity of B is required for reproductive organs and also for flowering and seed set.

Excessive B can inhibit plant growth and productivity in arid and semi arid environment [Metwally et al. 2012]. B toxicity is a chronic problem of soils of Central Anatolian region where under ground water, water from streams or rivers near geothermal areas and earthquake faults (Aksaray, Kızılcahamam, Haymana etc. and its surrounding areas etc.) contain B in toxic amounts with more than 10 mg kg^{-1} B concentration [Gezgin et al. 2002]. A report by Gezgin et al. [2002] revealed the availability of

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0.01–63.90 mg kg^{-1} and 0.012–13.70 mg l^{-1} B in soil and water, respectively, in the samples collected from seven different provinces of Central Anatolia. Furthermore, continuous application of fertilizers containing B, fly ash released by electric power plants, surface mining and industrial application have also increased B levels in the soils [Kabata-Pendias 2001].

Boron is relatively immobile in plant, and its excess cause malfunction in growth. Boron toxicity can affect nearly all crops that have different range of tolerance. Excessive amount of B exert negative effect on number of plant species like reduced photosynthetic efficiency in sunflower leaves [El-Shintinawy 1999], reduced chlorophyll content in cotton older leaves [Ahmed et al. 2008], decreased fresh and dry matter and increased B concentration of sunflower shoots [Day 2016a, 2016b], decreased height and shoot growth [Paull et al. 1990] and delay in development of wheat [Paull et al. 1988], decreased yield of radish [Tariq and Mott 2006], tomato [Eraslan et al. 2007], rice [Koohkan et al. 2008] and barley [El-Feky et al. 2012]. Boron toxicity also has an impact on uptake of other micro or macro elements such as K, Ca, Mg and Na concentration [Tariq and Mott 2006] in plants.

Safflower (*Carthamus tinctorius* L.) is an important oil seed crop of Central Anatolia that can tolerate drought, which is the common feature of the region. Some part of Central Anatolia region is under threat due to B toxicity and considered as the major constraints for crop production. Leaching of B from soil profile or application of some organic compounds to immobilize is an expensive way of decreasing B level of soil and plants can also be used to overcome the problem that show different tolerance range [Metwally et al. 2012]. Safflower could be used to some extend in reducing B level of soil in Central Anatolia.

The factors affecting B content in plant include B concentration, moisture, soil type (pH, organic matter content, texture, and alkalinity/calcareousness) and plant species [Kabata-Pendias 2001]. Under these circumstances, screening of suitable safflower varieties to B provides an alternate to grow B tolerant plants without significant reduction in yield. Furthermore, it is possible to check the B toxicity on uptake of P, Ca, K, Na and B by safflower cultivars cultivated under Central Anatolian (hot Mediterranean/dry-summer subtropical climatic; Köppen-Geiger classification: Csa) conditions. To our best knowledge, there is no report to date which provides informations about B requirement by safflower. The study was designed with aim to screen the safflower cultivars to B toxicity at early growth stage in order to find the best cultivar for B rich soils of Central Anatolia region.

MATERIALS AND METHODS

Experimental design and growth conditions

Turkish safflower cultivars 'Balci, 'Yenice' and 'Remzi Bey' were grown under controlled temperature (24 ±1°C), humidity (45%) and 16 h light photoperiod in greenhouse conditions. The soil used in the experiment was clay in texture (sand/clay; 23 : 45, by dry weight) with 12.0 g kg^{-1} organic matter (Walkley-Black), 1.1 g kg^{-1} total nitrogen (Kjeldahl), 11 mg kg^{-1} sodium bicarbonate ($NaHCO_3$)-available P, 71.5 g kg^{-1} calcium carbonate ($CaCO_3$), 0.18 mg kg^{-1} B and 250 mg kg^{-1} amonium acetate (NH_4OAc)-extractable K (rich in potassium). The pH of the experimental soil was 7.9 (1 : 2.5 soil/water) with a saturation extract of 0.19 dS m^{-1} electrical conductivity (EC). The water used in the experiment was analysed according to Maxfield and Mindak [1985] and found B free with 47.5 mS cm^{-1} electrical conductivity (EC) value (WTW 3.15 conductivity meter). The soil characteristics were determined according to methods detailed in Page et al. [1982].

Pots made from polyvinyl chloride (17.0 cm deep × 16 cm top diameter × 11 cm bottom diameter and approximately 2.3 L in volume) lined with polyethylene bags were filled with 2 kg air-dried experimental soil. After application of respective doses of boron as H_3BO_3 dissolved in water, basal doses of nitrogen (N) and phosphorus (P) were also applied at the rate of 100 and 50 mg kg^{-1} dissolved in water, respectively, were left to dry. Thereafter, the soil in each pot was mixed uniformly to get uniform distribution of B and other nutrients prior to sowing. Each pot filled with soil was planted with four seeds. Each treatment was replicated five times with one plant per replicate that was maintained after thinning. Plants were grown for

8 weeks after emergence and water content of the soil was maintained at 70% of field capacity by irrigating with B free water during the experiment.

Sampling and measurements

After 8 weeks of growth in the greenhouse, chlorophyll content index (CCI) was estimated with a hand-held chlorophyll meter (CCM-200 plus, Opti-Sciences Inc., Tyngsboro, MA, USA). Five matured leaves from each plant were selected and measured with CCM-200 plus [Al-Shatti et al. 2014]. Five repetitions were made for sampling to avoid chloroplasts movement and measurements were made early in the morning at 8.00–9.00 Turkish Standard Time. Plant height of the shoots were measured at the end of the experiment just before harvesting the plants.

Harvested shoot samples were washed twice with deionized water and shoots were weighed by using sensitive electronic balance to obtain fresh weight of the shoots. Thereafter, shoots were immediately placed individually in paper bags and left to dry in an oven at 70°C for two days followed by measuring the dry weight of the shoots. Dried samples were ground into fine powder and kept in the dark at room temperature until analysis.

Boron (B), phosphorus (P), potassium (K), calcium (Ca), and sodium (Na) ions analyses. The ground samples were weighed as 500 mg and these were incinerated with the dry-ash method in a muffle furnace at 500°C for 6 h. The ash was dissolved in 0.1 M hydrochloric acid (HCl) and the nutrients (B, P, K, Ca and Na) were analysed using ICP-OES (Perkin Elmer Optima 2100 DV; Waltham, MA) as described by Çikili et al. [2015].

Analysis of data

The experimental layout consisted of completely randomized factorial design with three safflower cultivars (Balci, Yenice and Remzi Bey), and seven B levels (0, 4, 8, 16, 32, 64 and 128 mg kg^{-1}). This experimental plan generated 21 treatments. Each treatment was replicated 5 times and consisted of 5 plants (replications). Data of the experiment were subjected to analysis of variance (ANOVA) using MSTAT-C package program (Michigan State University) and the significance of differences among treatments was tested using the Duncan's Multiple Range Test at the 0.01 probability level.

RESULTS

Results on CCI clearly deflected the clear impact ($P < 0.05$) of B levels on all cultivars that was gradually decreased with the increased B level for cv. Remzi Bey and cv. Yenice compared to control treatments. Contrarily, increased CCI contents were recorded up to 8 mg B kg^{-1} treatment in cv. Balci followed by sharp decline. The lowest chlorophyll content values for all cultivars were obtained at 128 mg B kg^{-1} treatment. Comparing cultivars, cv. Balci was more responsive to B compared to other cultivars and showed maximum CCI value on all B concentrations (fig. 1).

All safflower plants showed survival when exposed to all B concentrations used in this study. Although, plant showed survival at highest B treatment (128 mg kg^{-1}), it resulted in significant reduction in plant growth for all cultivars. Among cultivars, cv. Balci responded better at higher concentration of 128 mg B kg^{-1} and yielded plant height of 10.3 cm compared to cv. Remzi Bey and cv. Yenice which were 8.7 cm and 3.51 cm, respectively. Contrarily, maximum longer shoots were obtained at 4 mg kg^{-1} B from all cultivars. Comparing cultivars, cv. Yenice produced shorter shoots on all mediums supplemented with different B concentrations. In general, shoot length ranged 10.3–31.6 cm for cv. Balci, 8.7–25.5 cm for cv. Remzi Bey and 3.5–20.6 cm for cv. Yenice (tab. 1).

The addition of B exerted positive effects on shoot fresh weight and dry weight when applied at the rate of 4 mg B kg^{-1}. Maximum fresh weight was recorded 7.77, 6.90 and 5.73 g $plant^{-1}$ (tab. 1) for cv. Balci, cv. Remzi Bey and cv. Yenice, respectively. Whereas, maximum dry weight for Balci, Remzi Bey and Yenice was scored 0.80, 0.77 and 0.59 g $plant^{-1}$, respectively (tab. 1). It was also interesting to note that both fresh and dry weight at 4 mg B kg^{-1} was relatively more than control. Results also revealed that further increase of B concentration (8 mg kg^{-1}

Table 1. Growth parameters of safflower cultivars affected by different boron levels

Cultivar	Added B (mg kg^{-1})	Plant height (cm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
Balci	0	27.0 b*	7.27 a*	0.77 a*
	4	31.6 a	7.77 a	0.80 a
	8	24.4 bc	4.81 b	0.43 cd
	16	21.5 cd	2.74 cd	0.27 ef
	32	16.9 ef	2.63 cd	0.25 ef
	64	16.6 ef	1.21 ef	0.12 fgh
	128	10.3 gh	0.68 f	0.07 gh
Remzi Bey	0	20.1 de	5.70 b	0.51 bc
	4	25.5 b	6.90 a	0.77 a
	8	19.8 de	3.42 c	0.25 ef
	16	18.5 de	2.55 cd	0.22 efg
	32	17.7 def	2.06 de	0.19 e–h
	64	17.5 def	1.07 ef	0.12 fgh
	128	8.7 h	0.31 f	0.04 h
Yenice	0	17.8 def	4.76 b	0.52bc
	4	20.6 cde	5.73 b	0.59 b
	8	18.8 de	4.94 b	0.46 bc
	16	16.2 ef	4.60 b	0.45 bc
	32	13.7 fg	2.98 cd	0.29 de
	64	11.6 gh	2.18 de	0.24 ef
	128	3.5 i	0.31 f	0.04 h

Different letters above columns indicate significance levels of means according to Duncan's Multiple Range Test.
* – $P < 0.01$

and above) was detrimental and resulted in reduced fresh and dry weight which showed decreased pattern with increased B concentration irrespective of cultivar type. Negative impact of B on fresh and dry weight was at peak when used at 128 mg kg^{-1}.

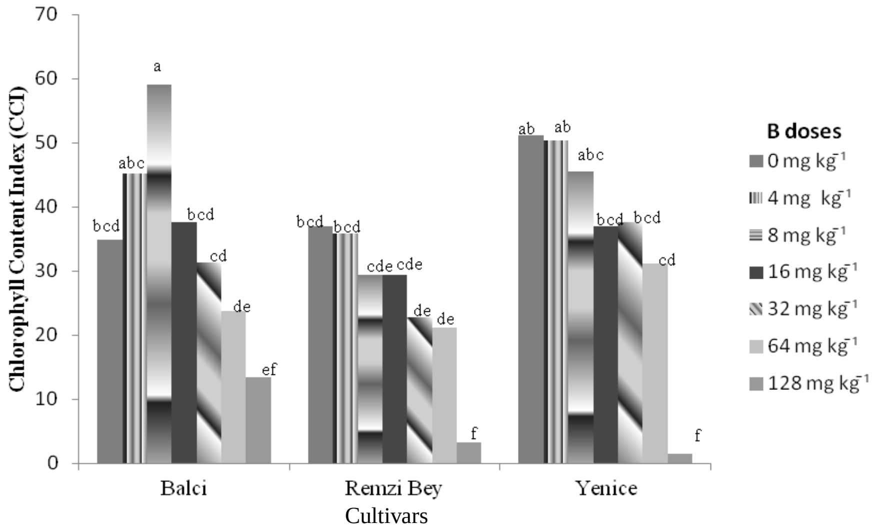
Mineral constituents of the plants. Results on B content of plants showed positive corelation with B doses which increased with increased B concentration with maximum content at 128 mg B kg^{-1} treatment for all cultivars. Among cultivars, maximum B content (1835.2 mg kg^{-1}) was obtained from cv. Remzibey followed by cv. Yenice (1247.0 mg kg^{-1}) and cv. Balci (861.7 mg kg^{-1}) at 128 mg kg^{-1} of B. Results on Na content for cv. Balci and cv. Yenice showed the same trend of increased content with increase of B concentration and maximum content was scored 3905.8 mg kg^{-1} for Balci and 1324.0 mg kg^{-1} for Yenice at 128 mg kg–1, respectively. Contrarily, maximum Na content by Remzi Bey cv. was recorded 2117.0 mg kg^{-1} at 64 mg B kg^{-1}.

K content of the cultivars decreased with the increasing levels of added B that was recorded less than control for all cultivars. The minimum K content was obtained from cv. Balci at 128 mg B kg^{-1} treatment and scored as 27.8 g kg^{-1}. The Ca content of the cultivars declined with increase of B concentration with minimum content was recorded by the application of B up to 128 mg kg^{-1}. Ca content ranged 11.3–8.1,

Table 2. Content of different nutrients of safflower cultivars under different boron levels

Cultivar	Added B (mg kg^{-1})	B (mg kg^{-1})	Na (mg kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	P (g kg^{-1})
Balci	0	55.5 g*	566 h*	67.7 abc*	11.3 abc*	3.2 b–e*
	4	65.0 g	645 gh	60.2 a–e	9.8 efg	3.1 cde
	8	88.9 g	780 fgh	65.2 a–d	9.4 fgh	3.1 b–e
	16	116.7 g	870 fgh	68.8 ab	8.1 hij	3.4 bcd
	32	229.6 f	1230 c–g	62.8 a–d	8.2 hij	3.5 bcd
	64	796.4 c	930 e–h	60.1 a–e	8.4 hij	2.6 de
	128	861.7 c	3906 a	27.8 g	7.3 j	1.2 f
Remzi Bey	0	55.2 g	1025 d–h	72.1 a	10.4 b–f	3.2 b–e
	4	76.9 g	1750 bc	59.6 a–e	11.0 a–e	3.4 b–e
	8	86.5 g	1151 c–h	71.0 a	10.2 c–f	3.2 b–e
	16	121.4 g	1285 c–g	68.0 abc	9.9 ef	3.4 b–e
	32	258.4 ef	1620 bcd	67.7 abc	10.0 def	3.6 bcd
	64	549.6 d	2117 b	45.4 f	8.5 g–j	3.9 a–d
	128	1835.2 a	1557 b–e	46.0 ef	7.7 ij	2.9 de
Yenice	0	53.9 g	753 fgh	69.2 ab	12.2 a	4.8 a
	4	70.6 g	780 fgh	53.9 c–f	10.8 b–e	4.9 a
	8	86.5 g	1180 c–h	55.8 b–f	11.5 ab	4.4 ab
	16	133.5 g	888 fgh	58.6 a–f	10.2 c–f	4.3 abc
	32	326.8 e	880 fgh	55.3 b–f	11.3 a–d	3.7 a–d
	64	473.4 d	725 fgh	52.6 def	9.4 fgh	3.3 b–e
	128	1247.0 b	1324 c–f	57.8 a–f	8.7 ghi	2.1 ef

Different letters above columns indicate significance levels of means according to Duncan's Multiple Range Test. * – $P < 0.01$



Different letters above columns indicate significance levels of means according to Duncan's Multiple Range Test ($P < 0.05$)

Fig. 1. Chlorophyll content index (CCI) of safflower cultivars under different boron levels

10.4–7.7 g kg^{-1} dry weight and 12.2–8.7 g kg^{-1} dry weight, respectively, for cv. Balci, Remzi Bey and Yenice. Results on P content of the shoots showed variable effects of B with respect to cv. type. The highest P content was noted for cv. Yenice at 4 mg B kg^{-1} treatment with an average value of 4.9 g kg^{-1} (tab. 2). Comparing cultivars, P content of Yenice cv. was relatively high up to 32 mg B kg^{-1} concentration.

DISCUSSION

Diversities among the cultivars for all the studied characters were observed under different B treatments. The chlorophyll content index showed differences among cultivars that changed and decreased with the increasing levels of B. Decrease in CCI at high level of B especially at 128 mg kg^{-1} might be due to accumulation of high B concentration in the old mature leaves that also led to necrosis on leaf edges. This might be due to introduction of B into the transpiration stream that accumulates at the end of the stream [Tanaka and Fujiwara 2008] that led to the excessive production of reactive oxygen species (ROS) causing damage to cells in leaves and subsequently to cell death. Similarly, negative effect of B on chlorophyll synthesis was also observed in potato by Ayvaz et al. [2016] and in barley by El feky et al. [2012].

Low chlorophyll results in low photosynthesis and that affects plant growth negatively. Results showed that decreased CCI at 128 mg B kg^{-1} treatment led to the minimum plant height for all cultivars. Toxic B levels can also cause physiological and metabolic problems related to genotoxicity thus limiting crop productivity. But genotoxic impact of B is unclear. Ayvaz et al. [2016] highlighted the genotoxic evidence with inhibited multiplication of cells and elongation of shoots. Increasing B levels up to 8 mg kg^{-1} or beyond were even toxic for cv. Balci, Remzi Bey and Yenice was even toxic for cells that subsequently had negative impact on plant height. Similarly, reduction in plant height was noted at high concentration of B in barley [El Feky et al. 2012] and cotton [Ahmed et al. 2008].

As plant height was affected by increased B concentration, shoot fresh and dry weight was also decreased with increased B concentration above 4 mg B kg^{-1}. Inhibited growth by B toxicity can lead to multiple biotic and abiotic stresses that is the main reason of decrease in shoot fresh and dry weight. Similar reduction in shoot fresh weight with the increasing level of B was also observed in tomato and pepper [Eraslan et al. 2007]. Reduction caused in shoot fresh weight by toxic B levels could be due to inhibition in cell growth and multiplication. A similar response on dry weight to excessive B has been reported in wheat [Metwally et al. 2012] and cotton [Ahmed et al. 2008]. However, application of 4 mg B kg^{-1} had positive effects on both fresh and dry weight compared to control treatment for all cultivars.

In our study, higher levels of B resulted in higher B accumulation in plants but the variation was genotype specific in agreement with Atalay et al. [2003]. B content in shoots of cv. Balci was recorded lower than other genotypes at 128 mg kg^{-1} B application to soil. It was reported earlier that B content in the shoots of tolerant genotypes is lower than non-tolerant genotypes [Nable et al. 1997, Reid and Fitzpatrick 2009]. It was further noted that accumulation of low B in some plants is due to the ability of plant to limit B uptake by roots and carry it to shoots [Reid and Fitzpatrick 2009]. This study reports higher accumulation of B with the higher B treatments which are in line with the studies of Karabal et al. [2003], Ruiz et al. [2003], Cervilla et al. [2007] and Korzeniowska [2008] on other plants.

High levels of B concentration in soil led to significant increase in content of Na in shoots. However, Na content in safflower cultivars differed with increasing level of B. The gradual increase in Na content showed contradiction to the findings of Tariq and Mott [2006] who reported decreased Na content in radish plant with increasing B levels. Contrarily, enhanced Na concentration with increasing level of B is reported for peanut [Çıkılı et al. 2015].

The concentration of K in shoots decreased significantly at 128 mg B kg^{-1}. Results also reveal that the reduction of K in all cultivars differed with the level of B. The system regarding K-B synergism is based on enhanced K uptake with the increasing B levels that caused Ca concentration depression because of the antagonistic effect [Lopez-Lefebre et al. 2002, Tariq and Mott 2006, Ahmed et al. 2008, Tariq et al. 2010]. Our results are contradictory to above mentioned reports about the role of toxic B in K uptake and accumulation. The possible reason might be the difference of plant type, concentration, substrate or culture conditions used in this study.

Ca content of cultivars decreased significantly with the increasing levels of B treatment. The explanation of decreased Ca seems to be connected with the increase in Na content of cultivars due to the high B and Na. Consequently, it has negative impact on Ca uptake and it reduces the quantity of Ca ions in the root medium which is available for uptake by the plant [Flowers 2004]; that results in formation of ROS with negative impact on growth parameters. Similar decrease in Ca concentration with increasing B levels was also determined in *Vicia faba* [Muhling et al. 1998] and cotton [Ahmed et al. 2008]. B and Ca have similarites such as low mobility, high extra-cytoplasmic concentration and growth alterations during deficiency [Bonilla et al. 2004]. It is well known that B deficiency causes abnormal changes in the Ca metabolism of cell wall. Also, plants that were supplied with inadequate quantity of B accumulated higher Ca concentration in tissues [Ahmed et al. 2011].

The decrease of P concentent because of high B supply was observed in all cultivars. Decrease of P because of excess B was also reported in cotton [Ahmed et al. 2008]. On the contrary, increase in P concentration due to high B supply was reported in tobacco by Lopez-Lefebre et al. [2002].

CONCLUSION

This study presents the screening of safflower cvs to B stress in order to find the best cv. for Central Anatolian region of Turkey. It is concluded that all cvs grow well on medium supplemented with 4 mg kg^{-1} of B. On the basis of this conclusion, all these cvs are suitable for the areas containing B around 4 mg kg^{-1} for cultivation. Furthermore, cv. Balci has the less potential to accumulate B in shoots and it has a better potential to tolerate B compared to other cultivars and could be grown under B contaminated soils up to 32 mg kg^{-1}. These results suggested that balci safflower cv. has potential to use for phyto remediation of B toxic soils that have similarities to soil we used in the experiment (alkaline and including high available K). However, there is need to do more extensive work on B uptake and its impact on accumulation of B and other minerals in the safflower.

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