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STUDIES ON DIFFERENTIAL RESPONSE OF SPRING CANOLA CULTIVARS TO BORON TOXICITY

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STUDIES ON DIFFERENTIAL RESPONSE OF SPRING CANOLA CULTIVARS TO BORON TOXICITY

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□ *Although many states recommend boron (B) fertilizer for many field crops, information about B toxicity of canola is lacking. This experiment was carried out at Central Anatolia, Turkey from 2002 to 2003, to determine genotypic range in B efficiency of eight spring canola cultivars, to identify the B-inefficient cultivars and to identify specific responses. The cultivars were grown under B moderate deficiency (extractable B 0.56 mg kg^{-1}) and toxic B applied (15 kg B ha^{-1}) conditions. According to the results, seed yield varied significantly among the cultivars and B application decreased the seed yield by 31% on average. Also, toxic B application reduced protein and oil contents similar to seed yield, and increased leaf B concentration in all varieties. This study has shown that leaf B concentration has increased considerably when B is applied to Pactol and Star cultivars, but seed yield of +B and −B has not shown significantly a change. It is possible to say that Star and Pactol—which have not been affected by the toxic B application—are genotypes that are tolerant to B toxicity and may be cultivated at B toxic lands.*

Keywords: boron toxicity, interaction, oil ratio, protein ratio, seed yield, canola

INTRODUCTION

Boron (B) is an essential element for plants and its deficiency affects plant growth and yield in many parts of the world. Soils vary greatly in B content. Some contain insufficient B to support normal plant growth. Other soils contain excessive amounts and cause B toxicity in some plants. Soil concentration range between B deficiency and toxicity is narrow; deficiency occurs at <0.5 mg kg $^{-1}$ hot water soluble B (Rashid et al., 1997a, 1997b; Rashid et al., 2002) while toxicity could occur at >5.0 mg kg $^{-1}$ (Yau et al., 1997).

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Boron toxicity has long been recognized as a common mineral nutritional problem, particularly in arid and semiarid regions where B levels are frequently high in the soil or irrigation waters around the world, causing significant decreases in growth and yield as reported for many countries (Nable et al., 1997). For example, soils in Iraq, Syria, India, South Australia, and Turkey have above average B levels with some sites having toxic levels (Sillanpaa, 1982; Rashid and Ryan, 2004). Also, B toxicity has been reported as an important constraint to crop production in Turkey, particularly in Central Anatolia (Sillanpaa, 1982; Kalaycı et al., 1998). The soils in Central Anatolia are typical of those in arid and semi-arid regions. They have low organic matter, high free-lime content, high pH, and usually a fine texture. Gezgin et al. (2002) surveyed B contents of 898 soil samples representing Central Southern Anatolian soils. According to the survey, the concentration of extractable B with 0.01 M mannitol in soil samples ranged from 0.01 to 63.9 mg kg $^{-1}$ soil with a mean value of 2.48 mg kg $^{-1}$. Nearly 10% of the soils sampled in Central Anatolia contained more than 5 mg extractable B per kg soil (Gezgin et al., 2002) which is a widely accepted critical concentration for occurrence of B toxicity in crop plants (Nable et al., 1997), but this result varies widely with species. Despite its considerable agronomic importance, our understanding of B toxicity is rather fragmented and limited (Ross et al., 1997).

Canola quality rapeseed (<2% erucic acid in the oil, and <30 μmole glucosinolate g^{-1} of meal) is one of the main oil crops world-wide but is a new crop for Turkey. There is very limited information regarding optimum micronutrient fertility levels.

Canola requires B in concentrations greater than 0.5 mg kg^{-1} of hot water-extracted B (HWB) through all growth stages—vegetative and flowering—in the soil than most other crops to complete its growth and development (Liu, 1995). Existence of larger variations in tolerance of canola to B toxicity than barley and wheat has been reported by several researchers (Hughes-Games, 1991; Hocking et al., 2003). Tolerance was reported to be related to the origin of a cultivar (Moody et al., 1988; Yang et al., 1993; Xu and Wang, 1998; Du et al., 2002). Some cultivars can grow normally in soil with 0.25 mg kg^{-1} HWB, whereas majority of cultivars will suffer seed yield loss, or even die at this level (Wang and Lan, 1995). Because of variation among varieties, new plant genotypes could be developed with higher genetic ability to tolerate B toxicity in soils. Several screening studies have been conducted to determine the extent of genotypic variation in tolerance to B toxicity in different crop species such as wheat (Paull et al., 1988; Nable and Moody, 1992; Yau et al., 1995; Jamjod, 1996; Kalaycı et al., 1998; Torun et al., 2006), barley (Kluge and Podlesak, 1985; Nable, 1988; Mahalakshmi et al., 1995; Rehman et al., 2006), pea (Bagheri et al., 1992; Avcı and Akar, 2005), tomato (Güneş et al., 2000), groundnut (Lauter et al., 1989), and turnip (Kaur et al., 2004). These studies showed existence of a large genotypic variation in susceptibility to B toxicity.

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Data are not available concerning the sensitivity of canola cultivars grown in Turkey to B toxicity. Our objective was to evaluate the risks of B toxicity and determinate B-inefficient cultivars of canola. The present study was carried out to investigate the differential response of different spring canola cultivars (eight cultivars of each) to B toxicity in field under irrigated conditions in a typical Central Anatolian soil low in extractable B and with relatively high lime content.

MATERIALS AND METHODS

The field experiments were carried out in soil containing 0.56 mg of B kg^{-1} (Table 1) extracted using 0.01 M Mannitol + 0.01 M calcium chloride ($CaCl_2$) solution (Cartwright et al., 1983) before reading in inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Varian-Vista Model, Palo Alto, CA) during the 2002 and 2003 growing seasons at the Research Institute of Rural Affairs, Konya, Turkey. Organic matter was determined by the ModifiedWalkley-Black procedure and $CaCO_3$ was determined by Sheibler's Calsimeter method (Black, 1965). Phosphorus was measured by Olsen method according to the Black (1965). Ca, Mg, K and Na were determined by ICP-AES (Varian Vista Model) (Soltanpour and Workman, 1981) following extraction by 1 N CH3 COONH4 (pH, 7) according to the Black (1965). Plant available concentrations of Fe, Zn, Mn, and Cu in soils were determined according to the method of Lindsay and Norvell (1978) by extraction with DTPA. Other soil characteristics are given in Table 1. Extractable B levels of the experimental soils were low according to the critical

TABLE 1 Selected physical and chemical properties of topsoil samples (0–30 cm depth) collected from the experimental area (mean of soil samples collected before sowing each year)

Property	Mean	Property	Mean
pH	7.6	Mg (cmol (+) kg^{-1})[1]	5.3
$CaCO_3$ (%)[1]	20.7	K (cmol (+) kg^{-1})[1]	0.6
E.C (dS m^{-1})	0.94	Na (cmol (+) kg^{-1})[1]	0.13
Organic matter (%)[1]	1.4	P (mg kg^{-1})[1]	8.5
Sand (%)	26.7	B (mg kg^{-1})	0.56
Silt (%)	68.1	Mn (mg kg^{-1})[1]	2.3
Clay (%)	5.2	Zn (mg kg^{-1})[1]	0.3
Ca (cmol (+) kg^{-1})[1]	20.2	Fe (mg kg^{-1})[1]	0.4

[1]Organic matter was determined by the Modified Walkley-Black procedure and $CaCO_3$ was determined by Sheibler's Calsimeter method (Black, 1965). Phosphorus was measured by Olsen method according to Black (1965). Ca, Mg, K and Na were determined by ICP-AES (Varian Vista Model) (Soltanpour and Workman, 1981) following extraction by 1 N CH_3 $COONH_4$ (pH, 7) according to Black (1965). Plant available concentrations of Fe, Zn, Mn, and Cu in soils were determined according to the method of Lindsay and Norvell (1978) by extraction with DTPA.

levels indicated by Reisenauer et al. (1973) and Keren and Bingham (1985) for many crops.

Eight spring canola cultivars ('Marinka', 'Briol', 'Pactol', 'Helios', 'Star', 'Prota', 'Spok' and 'Semu 209/82') were studied. Plants were grown with (+B = 15 kg B ha $^{-1}$) and without (−B) B applications.

The experiments were performed in a split plot design in randomized complete blocks with three replications. Boron treatments were administered to main plots where the sub-plots contained plant cultivars. Before sowing in both years, B at a rate of 15 kg ha^{-1} was broadcasted onto the soil surface using borax ($Na_2B_4O_7$ 10 H_2O), followed by incorporation to a 0–20 cm depth of soil prior to sowing.

Plots were sown in five rows (30 cm apart and 1–2 cm deep) with 2.5 m long (1.5 × 2.5 = 3.75 m^2) on 13 April in the first year, and on 17 April in the second year. Lines were drawn and seeds were sown by hand. Plants within rows were spaced 15 cm apart by thinning at 2 to 4 leaf stage.

Plots were basically fertilized with 120 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5. Entire quantities of phosphorous fertilizers and 60 kg ha^{-1} of the nitrogenous fertilizers were applied in bands as ammonium phosphate (18% N, 46% P_2O_5), by a driller during the sowing. Fifty percent of the remaining quantities of nitrogen were dispersed onto the soil surface in the form of ammonium sulfate (21% N) before flowering.

Routine management practices were followed. Plots were irrigated with sprinklers 3 times: a) after sowing, b) during flowering, and c) during pod filling. Crops were harvested at maturity for seed yield by hand on 23 July in the first and 30 July in the second year, and yield was adjusted to 9% moisture level (Yusuf and Bullock, 1993). Harvested area (HA) of a plot was 1.35 m^2 of the internal part after removing the two outer rows.

Normally receiving about 112 mm of total precipitation annually based on a 30 year average from 1974 to 2003, the area received 33 mm higher and 28 mm lower precipitation than the long- term average for 2002 and 2003, respectively. Temperatures during the study period were similar to the 30 year average for the area. The mean growing season temperatures from April to August were 18.2°C and 19.0°C for 2002 and 2003, respectively.

The following measurements were obtained annually:

1. Seed yield was measured at maturity in plants in each HA harvested, seeds were separated and the data were expressed as kg ha^{-1}.
2. B concentration of leaf was during the heading stage, 20 youngest leaves from main shoots of each plot were composited, washed with deionized water and oven dried at 70°C for 48 h for dry weights. Samples were finely ground and 0.5 g of plant material was digested with concentrated nitric acid (HNO_3) in a microwave system. The B in extracts was analyzed using an ICP-AES (Varian-Vista Model; Varian Inc., Palo Alto, CA, USA) device (Nyomora et al., 1997).

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3. Oil content was measured through Soxhlet apparatus oil extraction using petroleum ether (40–60°C) (AOAC, 1970).
4. Protein content was estimated using Kjeldahl method, N × 6.25, and protein concentration was determined as percentage (Diepenbrock and Geisler, 1979; Bilsborrow et al., 1993).

Data were analyzed as a split plot design using a computerized statistical software package (MSTATC; East Lansing, MI). All statistically significant main effects interactions were considered. Differences among treatments were tested by analysis of variance and were compared using Least Significant Difference (LSD) Tests at the 0.01 or 0.05 level of significance.

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RESULTS AND DISCUSSION

Seed Yield

The effect of year on seed yield of all cultivars was not significant. On the other hand, cultivar, B × cultivar interactions were significant for seed yield. Cultivars showed significantly varying responses to toxic B treatment. All cultivars, except for ‘Pactol’ and ‘Star’, showed significant yield decreases when treated with +B. Following the B application, the most important yield decrease is determinated with that of ‘Spok’, ‘Marinka’, ‘Briol’, ‘Prota’ and ‘Semu 209/82’ cultivars (52, 46, 45, 45, and 41%, respectively) over the control when applied with +B. However, seed yield of ‘Pactol’ and ‘Star’ has not changed significance at −B and +B doses (Table 2). This result makes these two varieties significant in regard to tolerance to B toxicity.

Significant genotype variation has been observed in regard to reaction to borax application in studies conducted on canola, but with respect to on B deficiency (Sakal et al., 1991; Xue et al., 1998; Yang et al., 1993; Stangoulis et al., 2000). Very few studies have been conducted on canola regarding applied B as borax toxicity. Wang et al. (1999) reported that application of borax, at 3.3 kg B ha^{-1}, significantly reduced canola yield in only one out of 11 experiments. In the single experiment where B toxicity depressed growth, the effect was relatively small, equivalent to only 5% of maximum seed yield. Moreover, application rates of up to 6.6 kg B ha^{-1} did not cause any depression in oilseed rape yield in a single experiment. In addition, there was no indication that a total of 9.9 kg B ha^{-1} depressed seed yield of canola. The risk of B toxicity from the soil application of borax at 4–8 times the minimum rates required to correct deficiency was not as high as previously assumed. Therefore, while soil application of B fertilizer at 1.10–1.65 kg ha^{-1} can be recommended to correct B deficiency of oilseed rape, even at rates substantially higher, oilseed crops are unlikely to exhibit B toxicity symptoms or decrease yield (Wang et al., 1999). In our study, application of a dose of 15 kg ha^{-1} B was far above this amount and caused significant decreases in yield (Table 2). As can be seen from Table 2, ‘Star’ and ‘Pactol’ can be

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TABLE 2 Seed yield (kg ha^{-1}) and Leaf B concentration (mg kg^{-1}) of eight canola cultivars when grown in two consecutive years with two levels of B supply (kg B ha^{-1}). Values are means of two years

Cultivar	Seed Yield (kg ha^{-1}) +B	Seed Yield (kg ha^{-1}) −B	Significance of differences between +B & −B	B Concentration in Leaf (mg kg^{-1}) +B	B Concentration in Leaf (mg kg^{-1}) −B	Significance of differences between +B & −B
Marinka	820	1525	**	54	36	**
Briol	682	1248	**	30	26	NS
Pactol	2095	2183	NS	59	22	**
Helios	1298	1601	**	58	36	**
Star	1538	1598	NS	58	34	**
Prota	1349	2469	**	86	36	**
Spok	738	1559	**	56	34	**
Semu 209/82	904	1525	**	52	31	**
Mean	1178	1713		57	32	
LSD	$Lsd_{\%1}$ C = 288; $Lsd_{\%1}$ B × C = 408			$Lsd_{\%1}$ C = 11.80; $Lsd_{\%1}$ B × C = 16.69; $Lsd_{\%1}$ Y × B × C = 23.60		

+B = Boron application (0.56 mg kg^{-1}B content soil + 15 kg ha^{-1} B application), −B = Control (0.56 mg kg^{-1} B content soil).

LSD = Least significant difference for comparisons between individual means; C; B × C; indicates cultivar (C) main effect, interaction of B application (B) with cultivars.

**Significant at $P < 0.01$, NS not significant.

grown successfully under high natural or fertilizer B conditions because of their tolerance to B oversupply. From this point of view, both genotypes can be considered B-inefficient having high agronomic values and may serve as significant parental materials for development of B-inefficient genotypes (Kaur et al., 2004).

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Effects of Applied B on Leaf Boron Concentrations of Canola Cultivars

Boron application significantly ($P < 0.01$) increased leaf B concentrations in all canola cultivars. Compared to the control, higher leaf B concentrations were obtained from all cultivars when treated with +B. 'Prota' contained the highest leaf B concentration (86 mg kg^{-1}) in +B while the lowest leaf B concentration (22 mg kg^{-1}) was found in 'Pactol' in control (Table 2). However, 'Pactol' had the highest rate of increase in leaf B accumulation (167%) as a result of B application.

Year × B × cultivar interaction was also significant ($P < 0.01$) for leaf boron concentration (data not shown). In general, leaf B concentration was lower in the first year than the second year. While −B and +B application did not change the leaf B content of any cultivar in the first year (2002), +B application increased significantly leaf B concentration of all varieties in the year 2003. It is thought that this difference is caused by climate; for annual precipitation (83 mm) and relative humidity (41%) of 2002 has been considerably lower than that of 2003 (144 mm, 50%, respectively). Considering year × B × cultivar interaction, Prota contained the highest leaf B (139 mg kg^{-1}) in the second year with +B.

Seed yields of canola varieties exhibited a polynomial curve in comparison to leaf B concentrations (Figure 1). Considering the mean of years and

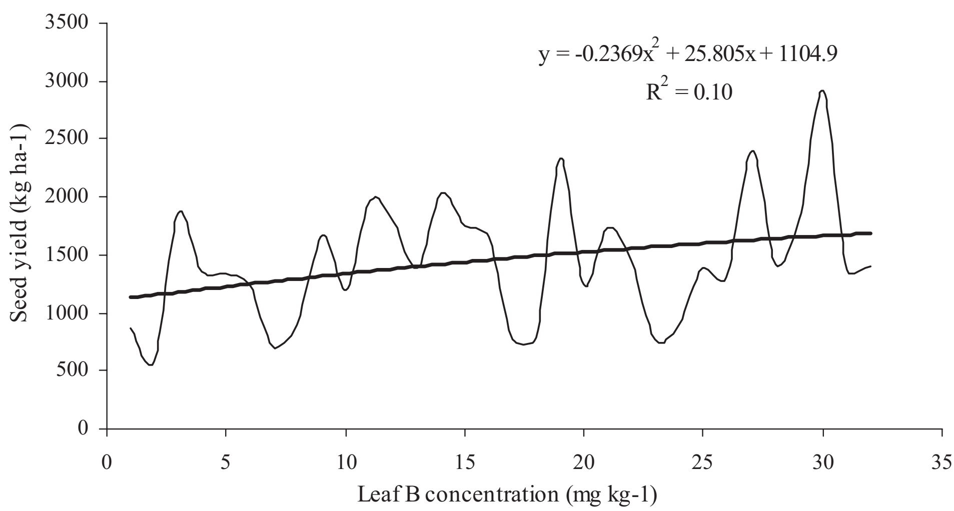

FIGURE 1 Effects of leaf B concentrations on the seed yield of canola cultivars.

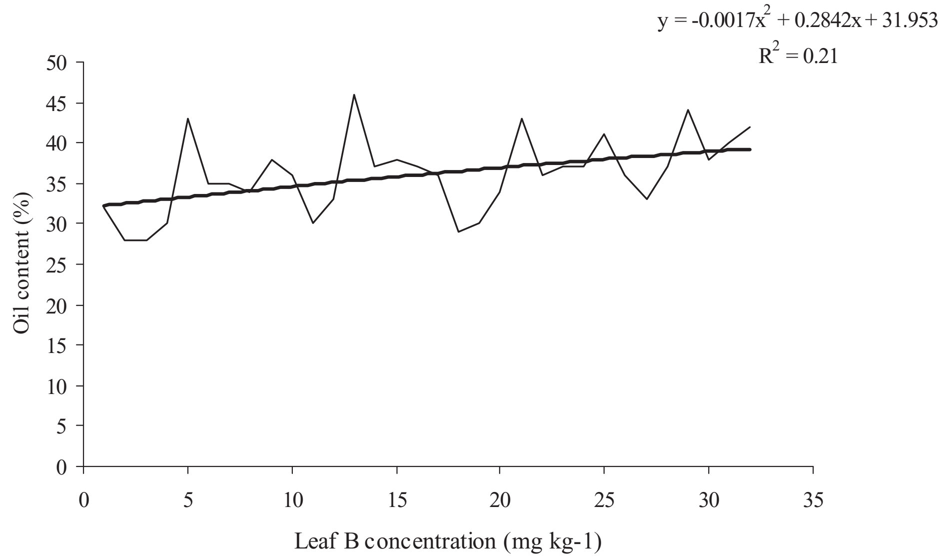

FIGURE 2 Effects of leaf B concentrations on the oil content of canola cultivars.

all cultivars, the regression equation for canola leaf B concentrations was: $y = -0.2369x^2 + 25.805x + 1104.9$, where × was leaf B concentration (mg kg^{-1}) and y was the seed yield (kg ha^{-1}). For oil content, $y = -0.0017x^2 + 0.$ $2842x + 31.953$ can indicate the leaf B concentration as the mean of years and cultivars (Figure 2). For protein content, $y = 0.0044x^2 - 0.\ 2331x + 24.495$ can indicate the leaf B concentrations as mean of years and cultivars (Figure 3). In cultivars, leaf B concentration was related more to oil content ($R^2 = 0.21$, Figure 2) than the seed yield and protein content with a weaker same relation ($R^2 = 0.10$, Figures 1 and 3).

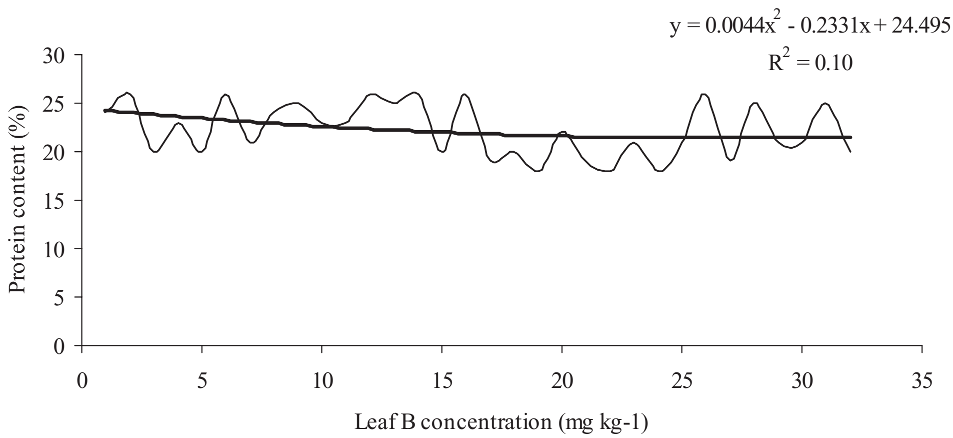

FIGURE 3 Effects of leaf B concentrations on the protein content of canola cultivars.

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TABLE 3 Relationships between leaf B concentration (x) (mg kg^{-1}) and seed yield (y) (kg ha^{-1}) of 8 canola varieties grown in two consecutive years with two levels of B supply (kg B ha^{-1})

Cultivars	Mean (Years)	Cultivars	Mean (Years)
Marinka	$y = 16{,}429x^2 - 77{,}171x + 1193{,}6$ $R^2 = 0.0443$	Star	$y = -43{,}679x^2 + 358{,}41x + 976{,}2$ $R^2 = 0.6685$
Briol	$y = -19{,}196x^2 + 200{,}57x + 554{,}3$ $R^2 = 0.1786$	Prota	$y = -40{,}018x^2 + 438{,}18x + 982{,}3$ $R^2 = 0.2169$
Pactol	$y = -49{,}857x^2 + 407{,}51x + 1469{,}2$ $R^2 = 0.7179$	Spok	$y = 4{,}7857x^2 + 18{,}586x + 1011{,}2$ $R^2 = 0.0444$
Helios	$y = 18{,}839x^2 - 132{,}96x + 1629{,}3$ $R^2 = 0.0637$	Semu	$y = 6{,}6429x^2 - 7{,}4429x + 1139{,}8$ $R^2 = 0.0465$

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Varying with the genotypes, leaf B concentrations were increased up to certain points in +B that in turn had negative contributions to seed yield of cultivars. However, while leaf B concentration has increased considerably when B is applied to 'Pactol' and 'Star' cultivars, seed yield of +B and –B has not shown a significant change. The regression equation for cultivars leaf B concentrations given in Table 3. As shown, leaf concentrations were related more to grain yield ($R^2 = 0.72$ and 0.67, respectively) in 'Pactol' and 'Star' cultivars than in the others. This result shows that these two varieties are tolerant to B toxicity and may be cultivated at boron toxic lands (Tables 2 and 3).

Boron concentrations in leaves increase with by age and in the same leaf, B accumulates at terminal sites of transpiration stream, such as leaf tips, causing non-homogenous B distribution particularly in mature and older leaves. In a mature or old leaf B concentrations at the base and tip portions varied 100 fold (Oertli, 1994). Oertli (1994) observed that toxic B concentrations in old leaves and deficient B concentrations in growing young leaves occurred concurrently in the same plant which was transferred from nutrient solutions containing very high B concentrations to B deficient solution. Stangoulis et al. (2000) determined that B concentration of young canola cultivar leaves ranged from 13–18 mg kg^{-1} in 0.1–0.2 mg B kg^{-1} growing condition. Reuter et al. (1997) and Wei et al. (1998) reported that these levels of B in young leaves indicate marginal B deficiency in canola cultivars. Other researchers reported that for the canola cultivar, the critical range of 10–14 mg kg^{-1} B dry matter concentration is recommended for B deficiency diagnosis at vegetative growth stages up to stem elongation. B deficiency symptoms were not observed in control (−B) plants that contained more than 22 mg kg^{-1} B, which was reported as a critical level for canola leaf (Reuter et al., 1997 and Wei et al., 1998).

In our study, B concentration was found to be 22 mg kg^{-1} at the lowest and 36 mg kg^{-1} at the highest in −B (control) in canola varieties. The reasons for the different responses may be related to soil physical and chemical property differences. No B deficiency symptoms were observed in control plants (−B). Therefore, increases in leaf B concentrations achieved through additional B applications had a negative rather than positive effect on varieties in terms of yield and quality. The values stated above by researches as B deficiency in canola leaves lend support to a lack of deficiency symptoms in −B in our study.

Oil Content

The seed oil content of canola cultivars was influenced significantly by B treatment. Boron application significantly ($P < 0.01$) decreased oil content in all cultivars. In addition, significant B application × cultivar interactions were registered in oil content (Table 4).

The quality of canola is mainly controlled by its genetics (Yang et al., 1993). In the present experiments, oil contents varied widely among cultivars, and relatively slightly with high B treatments. High B fertilizer decreased significantly the oil content of all cultivars. The lowest decrease in oil content was observed in 'Prota' by 5% while the highest decrease was in 'Briol' variety by 21% as a result of a high B treatment. The highest oil rate was found in the 'Star' variety (44.23%) with a decrease of 5%. These results indicate that toxic level B fertilizer could reduce the canola quality and maintain the stability of genetic characteristics of canola.

TABLE 4 Oil content (%) and protein content (%) of eight canola cultivars when grown in two consecutive years with two levels of B supply (kg B ha^{-1}). Values are means of two years

Cultivar	Oil content (%)		Significance of differences between +B & −B	Protein content (%)		Significance of differences between +B & −B
	+B	−B		+B	−B	
Marinka	34.25	39.80	**	22.14	23.58	NS
Briol	29.03	36.86	**	23.15	24.81	NS
Pactol	29.82	32.33	**	19.25	21.72	NS
Helios	32.83	35.46	**	23.09	26.37	NS
Star	43.01	45.44	**	20.16	23.75	NS
Prota	36.24	38.19	**	22.51	24.19	NS
Spok	36.27	39.58	**	21.64	22.95	NS
Semu 209/82	35.66	39.82	**	21.75	23.41	NS
Mean	34.64	38.44		21.71	23.85	
LSD	$Lsd_{\%1}$ C = 1.58; $Lsd_{\%1}$ B × C = 2.23			$Lsd_{\%1}$ C = 2.39		

+B = Boron application (0.56 mg kg^{-1}B content soil + 15 kg ha^{-1} B application), −B = Control (0.56 mg kg^{-1} B content soil).

LSD = Least significant difference for comparisons between individual means; C; B × C; indicates cultivar (C) main effect, interaction of B application (B) with cultivars.

**Significant at $P < 0.01$, NS not significant.

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Protein Content

The protein content of canola cultivars was influenced significantly by B treatment. Boron application significantly ($P < 0.01$) decreased protein content in cultivars (Table 4). The highest decrease in protein content as a result of B treatment was found in the 'Star' variety of 15% while the lowest decrease was in the 'Spok' variety by 6%. The highest value in the study in terms of protein content was determined in the Helios variety by 24.73% and the reaction of this variety to high B treatment emerged in the form of a 12% decrease in protein content. The protein contents of the varieties used in the study varied between 20.48 and 24.73%. Ilisulu (1970) stated that the commonest substance in canola seeds after oil was protein and that it generally constituted one-fifth of the seed. Weiss (1983) reported that protein content in canola seeds was 25% on average. As some researches (Schuster, 1970; Atakişi, 1977) stated, although protein content may be affected by environmental conditions, they vary to a great extent depending on the genetic properties of variety.

CONCLUSION

Studies on canola regarding B are limited in the whole world and almost all of them are about the effects of B deficiency. Studies concerning the effects of B toxicity in canola are next to nothing and most of these were conducted on monocotyl plants, especially cereals.

The present study revealed that there is a wide variation in canola in terms of B toxicity as in B deficiency. The results of this study could be beneficial to canola breeders, particularly, those who are interested in improving their germplasms showing B efficiency and toxicity. Currently high B fertilization did not appear to be the most common practise in cultivated soils containing very low levels of available B. However, cultivation of high B tolerant genotypes in soils with moderately high levels of soil B can offer advantages.

In this research showed that Pactol and Star canola cultivars can be successfully grown under high B conditions without important seed yield losses. From this point of view, both cultivars can be considered B-inefficient. In addition, both cultivars may serve as suitable parental materials for the development of B-inefficient genotypes for B toxicity. Other cultivars showing sensitivity to B toxicity (e.g., 'Spok', 'Marinka', 'Briol', 'Prota', and 'Semu 209/82′) can be grown under normal B conditions for adequate crop yield.

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