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RESPONSE OF WINTER CANOLA VARIETIES TO BORON STRESS DURING GERMINATION AND SEEDLING GROWTH STAGE

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Abstract: The objective of the study was to determine the effects of boron concentrations on germination and seedling growth of winter canola varieties under laboratory conditions. Seeds of four winter canola varieties (KWS Cyrill CL, Miranda, PT264, and NK Caravel) were germinated between papers with different boron levels (0, 20, 40, 60, 80, and 100 mg B L^{-1}) consisting of sodium borate ($Na_2B_8O_{13}.4H_2O$) at 20°C for 7 days. The germination percentage, mean germination time, germination index, seedling growth parameters, and dry matter were measured. The results showed that germination percentage, mean germination time, and germination index were negatively affected by increasing B concentrations. When B levels increased, root and shoot lengths and weights were also inhibited, while the responses of canola varieties differed. B levels had a significant effect on shoot length, which decreased from 5.15 cm to 1.82 cm and root length from 4.99 cm to 2.59 cm. Under boron stress, KWS Cyrill CL germinated higher and developed longer roots and shoots. Differences in both germination and seedling growth among canola cultivars were observed at 80 mg B L^{-1} and higher. It was concluded that there was a genotypic variation among canola varieties concerning boron toxicity and that KWS Cyrill CL was more tolerant to high boron concentrations than the other varieties.

Keywords: *Brassica napus* L., Germination, Seedling growth, Boron, Toxicity

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1. Introduction

Vegetable oils play a crucial role in maintaining a healthy and balanced human diet. The production of edible oils is required to meet the demands of both private households and industries, and at the same time creates promising employment opportunities (Safdar et al., 2023). Canola (*Brassica napus* L.) is a globally important oilseed crop used for human consumption, a source of protein-rich animal feed, and a renewable resource for biodiesel production (Zhao et al., 2020). It contains 40-46% high-quality oil, and its meal has a protein content of 38-40% (Samreen et al., 2022). In 2022, the sowing area of canola in Türkiye was 41.100 ha, and seed production was 150.000 tons (TÜİK, 2023).

Boron (B), an important micronutrient for crop plants, stimulates plant growth and yield. It plays a key role in many physiological processes such as protein formation, cell division, cell wall construction, cell membrane integrity, and root growth (Marschner, 1995; Gupta, 2016). However, high levels of boron (B) inhibit germination and growth of crops, along with other abiotic stresses like extreme temperatures, drought, salinity, flooding, and heavy metal (Mozafar, 1993; Rerkasem et al., 1997; Landi et al., 2019). B requirements vary for different crops, and the optimal concentrations for one species may be toxic to another (Blevins and Lukaszewski, 1998). The concentration of boron in arable soil ranges from 1 to 467 mg kg^{-1}, with a general variation between 0.5 and 5 mg kg^{-1} (Gupta, 2016). In our country, 46.2% of soils suffer from B deficiency, while 19.4% and 3.3% of the total area have excessive and toxic B concentrations (Kıllıoğlu, 2022). However, if a soluble B fertilizer is applied near the seeds, it may cause toxicity in crops, depending on cultivar susceptibility (Metwally et al., 2018). In canola, there are large variations among cultivars in tolerance to B toxicity compared to barley and wheat (Hughes-Games, 1991). Öztürk et al. (2010) found that canola seed yield decreased by 31% at a toxic level of 15 kg B ha^{-1}. In contrast, it supports plant growth and oil content of canola. Ma et al. (2015) found that foliar application of boron increased canola seed yield by 10%, although no improvement was observed with soil B application. Manaf et al. (2019) determined that oil content was increased by application of 2 kg B ha^{-1}, while Eggert and von Wirén (2016) demonstrated that canola seed harvested from B-supplied mother plants produced better seedling growth and development, without improving germination performance. In this study, the response of four winter canola varieties to different concentrations of boron (B) was evaluated during germination and seedling growth stages.

2. Materials and Methods

A laboratory experiment was conducted at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University in 2023. The seeds of winter canola varieties KWS Cyrill CL, Miranda, PT264, and NK Caravel from different seed companies and sodium borate (20.9% $Na_2B_8O_{13}.4H_2O$) were used as materials.

The seeds were germinated at six boron levels of 0, 20, 40, 60, 80, and 100 mg B L^{-1} prepared from Etidot-67 ($Na_2B_8O_{13}.4H_2O$). Distilled water was used as a control. Four replicated fifty seeds (4×50) from each canola variety were spread between three filter paper sheets with a dimension of 20 × 20 cm and each paper was watered with 7 mL for respective boron solutions. They were put into sealed plastic bags after the filter papers were rolled. The packages were incubated at 20°C for 7 days under dark conditions. The appearance of two millimeters of radicle hook was evaluated as the germination criterion. Germination percentage (GP), mean germination time (MGT), and germination index (GI) were also calculated using equations 1 and 2 (ISTA, 2018), and 3 (Salehzade et al, 2009):

$$GP\ (\%)=\frac{Germinated\ seeds\ at\ final\ day}{Total\ seeds}\times 100 \quad (1)$$

$$MGT\ (day)=\frac{\sum Dn}{\sum n} \quad (2)$$

where n represents the seed number germinated on day D, and D refers to the number of days since the initiation of the germination test.

$$GI=\frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ first\ count}+\cdots+\frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ final\ count} \quad (3)$$

On the final day, ten seedlings were randomly selected from each B level to measure the root length, shoot length, the fresh and dry weight of the seedlings. The seedlings were exposed to drying in an air oven at a temperature of 80°C for a period of 24 h.

2.1. Statistical Analysis

The study was designed as a two-factor factorial in a completely randomized design with four replicates. Data were analyzed using the MSTAT-C software, and differences were compared using the Least Significant Differences (LSD) test at a 5% level.

3. Results

There were significant differences among winter canola varieties and boron levels for germination percentage, mean germination time, and germination index (Table 1). KWS Cyrill CL had the highest germination percentage and germination index, while it also had the shortest mean time for germination. Increasing B levels caused a reduction in germination percentage and the lowest germination percentage (88.0%) and index (20.1) were obtained at 100 mg B L^{-1}. Mean germination time was delayed under B stress and reached the maximum at 80 mg B L^{-1}. Increased boron levels significantly inhibited seedling growth parameters of winter canola varieties and the interaction of variety × boron level was significant (Table 1). Seedling growth decreased as boron levels increased: however, an increase was observed in shoot length, and seedling fresh weight at a boron level of 60 mg L^{-1}. No significant effect of boron levels on the seedling dry weight was determined. Among the winter canola varieties, KWS Cyrill CL had a longer shoot and root length and a higher fresh and dry weight of seedlings than the other varieties, while Miranda had a longer root and a higher dry weight. Root growth declined with higher boron levels than 60 mg L^{-1}. Seedling fresh weight also increased up to 60 mg L^{-1} and then decreased.

Table 1. Analysis of variance and main effects of canola variety and boron level on the investigated characteristics.

Factors	GP (%)	MGT (day)	GI	SL (cm)	RL (cm)	SFW (mg plant^{-1})	SDW (mg plant^{-1})	DM (%)
Varieties (A)								
NK Caravel	89.4^{b}	2.59^{a}	19.4^{c}	3.50^{b}	4.03^{b}	451^{b}	36.1^{c}	8.1^{c}†
Miranda	90.1^{b}	2.51^{ab}	22.1^{b}	3.64^{b}	4.89^{a}	388^{c}	37.9^{b}	10.4^{a}
KWS Cyrill CL	95.7^{a}	2.24^{c}	25.2^{a}	4.29^{a}	4.74^{a}	483^{a}	46.0^{a}	9.7^{b}
PT264	95.1^{a}	2.44^{b}	22.3^{b}	3.08^{c}	3.65^{c}	366^{d}	35.7^{c}	9.9^{ab}
Boron doses (B)								
Control	94.6^{a}	2.16^{c}	25.8^{a}	5.15^{a}	4.99^{a}	428^{c}	39.4	9.4^{c}
20 mg B L^{-1}	94.0^{a}	2.49^{b}	23.0^{b}	4.07^{c}	4.79^{a}	459^{b}	38.8	8.7^{d}
40 mg B L^{-1}	94.6^{a}	2.48^{b}	22.4^{b}	3.72^{d}	5.07^{a}	418^{c}	38.3	9.2^{cd}
60 mg B L^{-1}	92.6^{ab}	2.40^{b}	22.1^{b}	4.53^{b}	4.85^{a}	509^{a}	39.3	7.7^{e}
80 mg B L^{-1}	91.6^{b}	2.63^{a}	20.3^{c}	2.47^{e}	3.68^{b}	383^{d}	40.2	10.6^{b}
100 mg B L^{-1}	88.0^{c}	2.51^{ab}	20.1^{c}	1.82^{f}	2.59^{c}	334^{e}	37.5	11.5^{a}
Analysis of variance								
A	**	**	**	**	**	**	**	**
B	**	**	**	**	**	**	*ns*	**
A×B	**	**	**	**	**	**	**	**

**= significant at 1%, *ns*= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05. (GP: germination percentage, MGT= mean germination time, GI= germination index, SL= shoot length, RL= root length, SFW= seed fresh weight, SDW= seed dry weight, DM= dry matter).

The interaction of variety × boron level on germination and seedling growth parameters is shown in Figure 1. Boron levels significantly affected the germination and seedling growth parameters of winter canola varieties. Germination percentage decreased when B levels increased, but KWS Cyrill CL was the least affected variety (Figure 1A). Surprisingly, Miranda had a germination percentage of 90% in the control, which increased to 96.5% at 40 mg B L^{-1}. Depending on the germination percentage, the germination index was changed. At all B levels, KWS Cyrill CL had the highest germination index, while PT264 had the lowest germination index (Figure 1C). Mean germination time was delayed with increasing B, and the longest mean germination time was observed at 100 mg B L^{-1} (Figure 1B). At a boron level of 60 mg L^{-1}, shoot length was induced, while higher levels inhibited it considerably (Figure 1D). KWS Cyrill CL had the highest shoot length under all boron levels. At the highest boron level, the shoot length was reduced in Miranda by 27%, NK Caravel by 30%, KWS Cyrill CL by 36%, and PT264 by 55%. The root length was depressed by increasing boron levels except for 60 mg L^{-1} at which it was promoted (Figure 1E), except for PT264. Miranda and KWS Cyrill CL had the longest length of root at 60 mg B L^{-1}. The seedling fresh weight fluctuated by changes in shoot and root depletion. Although KWS Cyrill CL had the highest fresh weight at all levels of boron (except for 80 mg L^{-1} boron level), generally seedling fresh weight induced at 60 mg B L^{-1}, but higher levels resulted in a significant decline (Figure 1F). Moreover, a heavier seedling fresh weight was obtained from PT 264 under boron stresses compared to the control. Similarly, KWS Cyrill CL had the highest seedling dry weight at all levels of boron (Figure 1G). All varieties had the lowest dry matter at 60 mg B L^{-1}, while Miranda produced the highest dry matter under all boron levels (Figure 1H).

4. Discussion

This study focused on the toxic effects of boron concentrations on germination and early seedling growth parameters of winter canola varieties. Our results showed that increasing boron levels led to a decrease in germination percentage and germination index. This result confirmed the findings of Turhan and Kuşçu (2021), who determined significant differences among B levels for germination percentage, index, energy, and mean germination time in watermelon, pepper, and eggplant, although they used B doses up to 16 mg L^{-1}. Also, Archana and Pandey (2016) reported that the maximum germination was recorded in a non-boron solution, while a significant reduction was observed especially at 330 mM. Contrarily, Kaya et al. (2023) found no significant reduction in germination percentage of sunflower, soybean, and opium poppy up to 90 mg B L^{-1}. This means that plant species showed sensitivity to B during germination phases and canola was more sensitive than the mentioned species. On the other hand, retarded mean germination time and decreased germination index were recorded under B stresses in this study. Kaya et al. (2023) reported similar results in sunflower and opium poppy.

Seedling growth, including shoot and root length, as well as seedling fresh and dry weight of canola varieties, proved to be more sensitive to boron levels compared to germination because the shoot length of the canola varieties declined significantly. No significant changes were identified in control and at 60 mg B L^{-1}. Our results agree with the findings reported by Metwally et al. (2018), who observed a linear decrease in shoot growth of canola with increasing B levels and found higher levels than 25 mg B kg $soil^{-1}$ to be toxic. In addition, Kaya (2023) reported a promoter effect of 20 mg B L^{-1} on seedling growth of melon cultivars, but higher levels were also found to be toxic. Similarly, Kaya et al. (2023) found a reduction in shoot length of sunflower at 60 and 90 mg B L^{-1}, while a precise B level leading to a decline in root and shoot length of soybean could not be detected, which can be explained by the toxic level of boron depending on the plant species. Our results showed that there were significant differences among canola varieties in terms of B concentrations and KWS Cyrill CL was the least affected variety by high B concentrations. This result confirms the finding of Öztürk et al. (2010) who found that two cultivars were not affected by a toxic B level.

5. Conclusion

The results indicated that boron levels adversely influenced the germination characteristics of winter canola varieties and they declined at 80 mg B L^{-1}. Seedling growth was more sensitive to B stress than the germination traits of canola, and shoot length was more adversely inhibited by boron levels than root length. In addition, there was genotypic variation among canola varieties in terms of tolerance to boron toxicity, with KWS Cyrill CL germinating and growing better than the others. It was concluded that the susceptibility of canola varieties to boron toxicity varies and that tolerant varieties should be preferred on boron-affected soils.

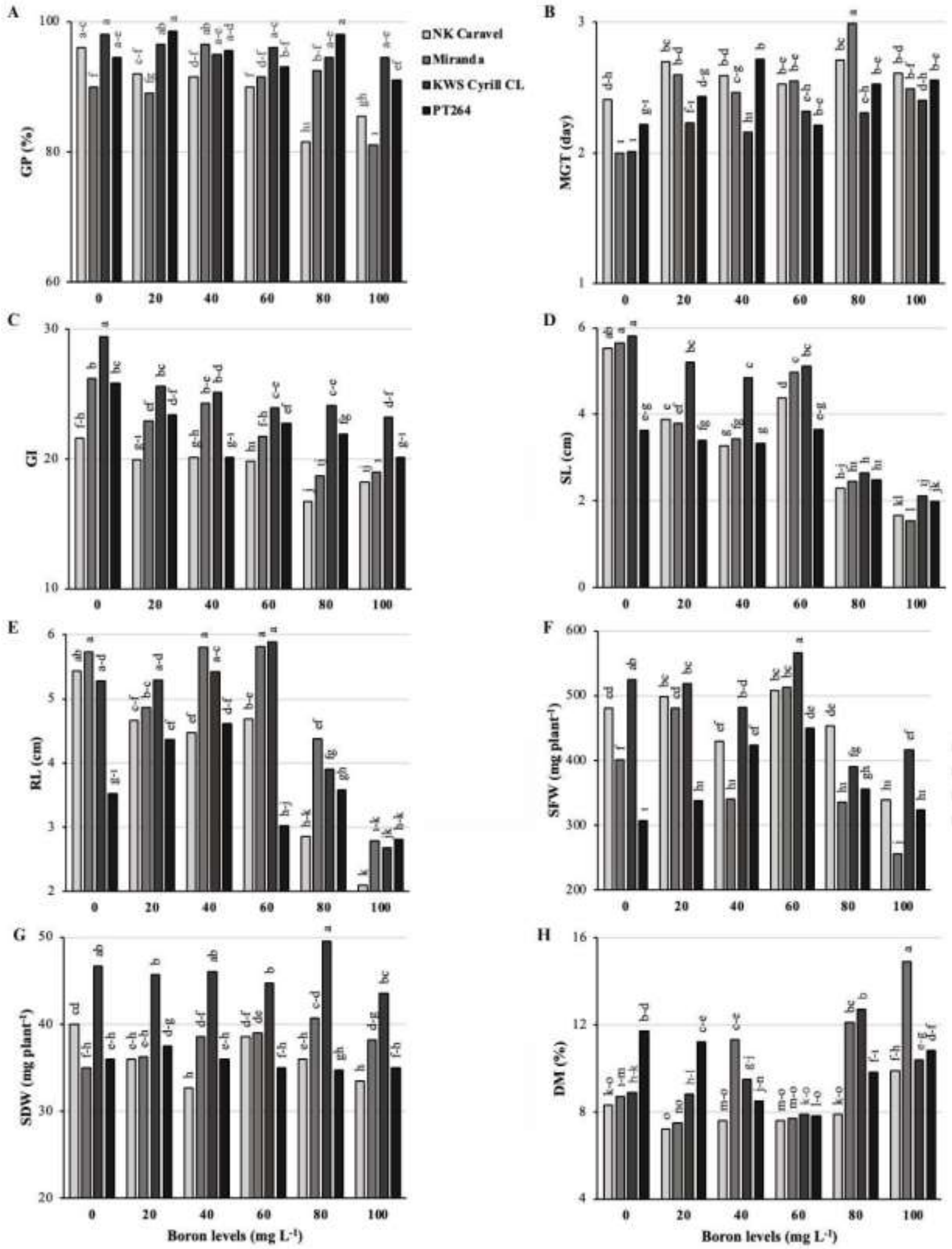

Figure 1. Effects of different boron concentrations on germination percentage (A), mean germination time (B), germination index (C), shoot length (D), root length (E), seedling fresh weight (F), seedling dry weight (G) and dry matter (H) of winter canola varieties. GP= germination percentage, MGT= mean germination time, GI= germination index, SL= shoot length, RL= root length, SFW= seed fresh weight, SDW= seed dry weight, DM= dry matter. Letter(s) on each bar shows significance levels at 5%.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	E.Y.	P.H.	M.D.K.	E.G.K.
C	40	0	60	0
D	40	30	0	30
S	0	0	50	50
DCP	40	40	0	20
DAI	50	0	50	0
L	40	30	20	10
W	50	10	40	0
CR	50	0	50	0
SR	20	20	20	40
PM	20	20	60	0
FA	0	0	80	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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