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Original Scientific Paper

Boron toxicity tolerance in barley may be related to intrinsically higher levels of reactive oxygen species in the shoots

Şükrü Serter Çatav[1]*, Eda Çetin[2], Emre Vural[2] and Betül Bürün[1]

1 Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Turkey
2 Department of Molecular Biology and Genetics, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Turkey
* Correspondence: sertercatav@mu.edu.tr

ABSTRACT:
There is considerable intra-and interspecific variation in boron (B) toxicity tolerance in crop plants. In this study, we aimed to investigate the mechanisms involved in tolerance to excess B in barley (*Hordeum vulgare*) in the early stages of plant development. To do this, B-sensitive (Bülbül-89) and B-tolerant (Tarm-92) barley cultivars were grown hydroponically under control and B stress conditions (10 mM H_3BO_3) for 4 or 7 days. The hydrogen peroxide (H_2O_2), malondialdehyde (MDA), total phenolic, total flavonoid, anthocyanin, proline, and total sugar contents, as well as DPPH radical scavenging capacity, were then determined for both cultivars. Our results showed that B treatment led to significant increases in the B concentration of the barley cultivars for both exposure times. However, there were no drastic differences in the B concentration of the roots and shoots between the sensitive and tolerant cultivars. While the dry root weight of Bülbül-89 was reduced after 7 days of B stress ($p < 0.05$), such a decrease was not observed in Tarm-92. The H_2O_2, MDA, proline, total sugar, and anthocyanin contents of both cultivars increased considerably in response to excess B during at least one treatment period ($p < 0.05$). The H_2O_2 content of Tarm-92 under control and B stress conditions was significantly greater than that of Bülbül-89, but there was no difference in the MDA content and radical scavenging capacity between the two cultivars. Finally, a 35% increase was found in the total flavonoid content of the Tarm-92 seedlings exposed to B stress for 4 days. In conclusion, the findings of this study suggest that tolerance to B toxicity in barley seedlings may be related to their capacity to tolerate higher levels of reactive oxygen species.

Keywords:
boron toxicity, tolerance mechanisms, barley, reactive oxygen species, antioxidant capacity, compatible solutes

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INTRODUCTION

Although there has been much recent debate about the essentiality of boron (B) to plants (McGrath 2020), B is thought to play a key role in maintaining of cell walls and integrity of membranes by forming complexes with glycosylinositol phosphorylceramides and rhamnogalacturonan II (Voxeur & Fry 2014). In addition, B has also been shown to be involved in a number of metabolic and physiological processes in plants, such as phenol metabolism, sugar transport, indole acetic acid metabolism, root elongation, nitrogen fixation, and pollen tube growth (González-Fontes *et al.* 2008; Landi *et al.* 2019). On the other hand, excess B can hinder plant growth and reduce crop yield, especially in arid and semiarid regions (Brdar-Jokanović 2020). The negative impacts of B toxicity on plants include root growth inhibition, decreased nitrate uptake, necrosis in the leaves, lower photosynthetic capacity, oxidative injury, genotoxicity, and alterations to antioxidant status (Cervilla *et al.* 2009; Çatav *et al.* 2018, 2022; Papadakis *et al.* 2018).

There is substantial genotypic variation in the tolerance of barley, rice, and wheat to B toxicity (Nable 1988; Torun *et al*. 2006; de Abreu Neto *et al*. 2017). It has been demonstrated that several mechanisms may participate in improving tolerance to excess B in these cereals. The primary mechanism is thought to be associated with either reduced uptake and/or enhanced efflux of B from the roots (Reid 2013; Princi *et al*. 2016). This is mainly attributed to differences in the expression patterns of genes encoding borate exporters and boric acid channels among susceptible and tolerant cultivars (Schnurbusch *et al*. 2010a). However, some studies have found no significant relationship between tissue B concentration and B toxicity symptoms (Torun *et al*. 2003; Ochiai *et al*. 2008). The redistribution of B from the symplast to the apoplast in the leaves by efflux transporters was reported to increase tolerance to B toxicity in barley and wheat (Reid & Fitzpatrick 2009). This mechanism was proposed as a plausible explanation for why cereal genotypes with similar leaf B concentrations could display distinct toxicity profiles. In addition, Ochiai *et al*. (2011) showed that the suppression of the *BET1* (*Boron Excess Tolerant1*) gene, which encodes a NAC-like transcription factor, improves tolerance to excess B in rice. Furthermore, transcriptomic analysis of B-sensitive and B-tolerant rice genotypes revealed that the genes related to transcriptional regulation, redox homeostasis, and biochemical binding played an important role in B toxicity tolerance (de Abreu Neto *et al*. 2017).

Antioxidants and compatible solutes (e.g. glycine betaine, proline, putrescine, and sucrose) are considered to be major components of the plant defence system against abiotic stresses (Kumar *et al*. 2018; Hasanuzzaman *et al*. 2020). Previous studies have indicated that B toxicity could cause alterations in oxidative metabolism, antioxidant status, proline content, and soluble sugar levels (Landi *et al*. 2019; Çatav *et al*. 2022). However, there is limited information available concerning the role of antioxidants and compatible solutes in the B toxicity tolerance capacity of cereals (Karabal *et al*. 2003). In this study, therefore, we aimed to ascertain whether there is a marked difference between sensitive and tolerant barley cultivars in terms of oxidative and antioxidative status and compatible solute content. We hypothesised that any differences in the constitutive levels of these biochemical parameters might play a role in tolerance to excess B in barley seedlings.

MATERIALS AND METHODS

Studied cultivars, growing conditions, and experimental design. In this study, seeds of the studied barley cultivars (*Hordeum vulgare* L. cv. Bülbül-89 and cv. Tarm-92) were purchased from the Republic of Turkey's General Directorate of Agricultural Enterprises (TIGEM). Seeds sterilised with sodium hypochlorite were germinated in plastic containers containing filter papers saturated with distilled water at 20°C in darkness for 7 days. Uniform seedlings were placed in hydro-pots filled with expanded clay pebbles. The hydro-pots were then transferred into hydroponic systems containing 2500 mL of quarter-strength Hoagland's nutrient solution (pH: 5.8). The seedlings were grown at 22 ± 1°C under a 16-h photoperiod with light intensity of 115 µmol/s/m^2 for 3 days. After the acclimation period, a 2 × 2 × 2 factorial design was employed to understand the B toxicity-related responses in the barley cultivars. Treatment (0 and 10 mM H_3BO_3; Öz *et al*. 2009), exposure period (4 and 7 days; Karabal *et al*. 2003), and cultivar (B-sensitive: Bülbül-89 and B-tolerant: Tarm-92; Torun *et al*. 2003; FCCRI 2023) were selected as the three independent variables. Seedlings from both barley cultivars were subjected to treatments for 4 or 7 days under the growth conditions explained above. Three replicates (pots) of 8 seedlings were used per treatment condition, and the experiment was repeated 3 times independently. Half of the seedlings were immediately packed following harvest and stored at -30°C prior to biochemical analyses, while the other half were used for the measurement of the growth parameters (length and dry weight) and B concentration.

Determination of B concentration. The dried roots and shoots (approx. 100 mg) of the barley cultivars were subjected to acid digestion in a microwave system. Subsequently, the azomethine-H method was used for the quantification of B (µg g^{-1} DW) present in the samples (Banuelos *et al*. 1992; Çatav *et al*. 2018).

Physiological and biochemical parameters. The concentrations of photosynthetic pigments in the barley leaves were measured by the method outlined in Sumanta *et al*. (2014).

The barley shoots were homogenised in 0.1% trichloroacetic acid solution at a ratio of 1/10 (w/v) for the assessment of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents. The homogenates were centrifuged at 10,000 rpm for 30 min at 4°C, and the resultant supernatants were used in both assays. The MDA and H_2O_2 levels were estimated using the thiobarbituric acid and oxidation of potassium iodide methods, respectively, as previously described (Du & Bramlage 1992; Velikova *et al*. 2000).

The shoot samples were homogenised in methanol at a ratio of 1/10 (w/v) for the measurement of the total phenolic and flavonoid contents as well as the free radical scavenging activity. The homogenates were then centrifuged at 13,000 rpm for 15 min at 4°C, and the resultant supernatants were used in these assays. The total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu and aluminum chloride methods, respectively, and expressed as µg caffeic acid equiva-

Table 1. The results of three-way analyses of variance regarding the single and combined effects of the independent factors (C: cultivar, T: treatment, and E: exposure period) on growth and physiological characteristics.

		Source of variation						
Parameters		**C**	**T**	**E**	**C x T**	**C x E**	**T x E**	**C x T x E**
B concentration (Root)	%	0.61	74.75	3.34	0.87	7.93	10.78	0.07
	p	*	****	****	*	****	****	ns
B concentration (Shoot)	%	0.26	92.39	0.09	0.05	0.01	1.66	4.93
	p	*	****	ns	ns	ns	****	****
Root length	%	9.98	19.84	3.09	0.00	0.34	2.72	0.00
	p	*	**	ns	ns	ns	ns	ns
Shoot length	%	16.97	2.52	35.71	1.13	3.54	4.24	1.79
	p	****	ns	****	ns	*	*	ns
Seedling length	%	17.93	9.66	23.56	0.46	2.38	4.62	0.90
	p	***	**	****	ns	ns	*	ns
Root weight	%	54.68	3.45	1.24	1.27	0.43	7.67	0.00
	p	****	*	ns	ns	ns	**	ns
Shoot weight	%	43.35	1.36	17.88	0.06	0.06	4.69	1.79
	p	****	ns	****	ns	ns	*	ns
Seedling weight	%	50.83	1.94	13.85	0.00	0.12	5.87	1.18
	p	****	ns	****	ns	ns	**	ns
Chlorophyll a	%	4.87	63.35	1.79	6.96	1.39	3.58	6.69
	p	*	****	ns	**	ns	*	**
Chlorophyll b	%	2.28	35.30	0.71	0.67	1.76	4.04	0.11
	p	ns	**	ns	ns	ns	ns	ns
Total chlorophyll	%	4.62	61.78	1.66	5.56	1.58	4.00	4.89
	p	*	****	ns	*	ns	ns	*
Carotenoids	%	3.29	7.62	1.98	5.16	0.00	0.00	31.29
	p	ns	ns	ns	ns	ns	ns	**
H_2O_2	%	50.74	14.53	14.76	7.85	7.68	0.61	0.22
	p	****	****	****	****	****	ns	ns
MDA	%	0.10	35.96	23.22	0.56	0.87	22.78	9.04
	p	ns	****	****	ns	ns	****	***
Phenolics	%	8.49	31.61	12.12	0.02	6.27	1.70	7.68
	p	ns	**	*	ns	ns	ns	ns
Flavonoids	%	33.21	36.33	0.36	2.39	4.98	6.61	0.30
	p	****	****	ns	ns	*	*	ns
Anthocyanin	%	7.82	71.96	1.66	0.80	0.00	0.53	0.40
	p	*	****	ns	ns	ns	ns	ns
DPPH	%	4.62	16.23	6.65	7.58	12.47	3.81	23.47
	p	ns	**	ns	*	*	ns	**
Proline	%	0.02	67.73	4.95	0.62	5.42	0.03	15.55
	p	ns	****	**	ns	**	ns	****
Total sugar	%	0.79	59.19	2.69	0.46	7.59	11.36	7.99
	p	ns	****	ns	ns	**	***	**

"%" indicates the percent of the total variation. *P* values are represented by asterisks and ns (ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$).

Table 2. The growth parameters of the barley cultivars grown under control and excess B conditions for 4 or 7 days. The data are presented as mean ± SEM (n = 6 replicates of 4 seedlings per treatment group). For each growth parameter, values with different superscript letters are significantly different from one other ($p < 0.05$).

		Control		B toxicity	
Parameter	**Cultivar**	**4-day**	**7-day**	**4-day**	**7-day**
Root length (mm)	Bülbül-89	146 ± 10^{ab}	160 ± 9^{a}	133 ± 12^{ab}	131 ± 5^{ab}
	Tarm-92	129 ± 6^{ab}	147 ± 7^{ab}	116 ± 7^{b}	119 ± 8^{b}
Shoot length (mm)	Bülbül-89	226 ± 3^{bcd}	278 ± 16^{a}	246 ± 9^{ab}	251 ± 5^{ab}
	Tarm-92	201 ± 8^{cd}	261 ± 12^{ab}	188 ± 3^{d}	237 ± 8^{abc}
Seedling length (mm)	Bülbül-89	373 ± 11^{abc}	438 ± 22^{a}	379 ± 12^{abc}	382 ± 9^{abc}
	Tarm-92	330 ± 14^{cd}	408 ± 19^{ab}	303 ± 10^{d}	357 ± 16^{bcd}
Root dry weight (mg)	Bülbül-89	7.9 ± 0.5^{ab}	8.7 ± 0.3^{a}	7.8 ± 0.3^{ab}	7.2 ± 0.3^{bc}
	Tarm-92	5.5 ± 0.1^{d}	6.7 ± 0.4^{bcd}	6.1 ± 0.2^{cd}	5.8 ± 0.4^{d}
Shoot dry weight (mg)	Bülbül-89	22.3 ± 0.9^{bc}	28.9 ± 1.9^{a}	24.6 ± 0.7^{ab}	25.0 ± 1.1^{ab}
	Tarm-92	17.7 ± 0.9^{cd}	22.4 ± 1.3^{bc}	17.2 ± 0.5^{d}	20.4 ± 0.8^{bcd}
Seedling dry weight (mg)	Bülbül-89	30.2 ± 1.0^{bc}	37.6 ± 1.9^{a}	32.4 ± 0.9^{ab}	32.2 ± 1.3^{ab}
	Tarm-92	23.3 ± 1.0^{d}	29.1 ± 1.6^{bc}	23.3 ± 0.6^{d}	26.2 ± 1.1^{cd}

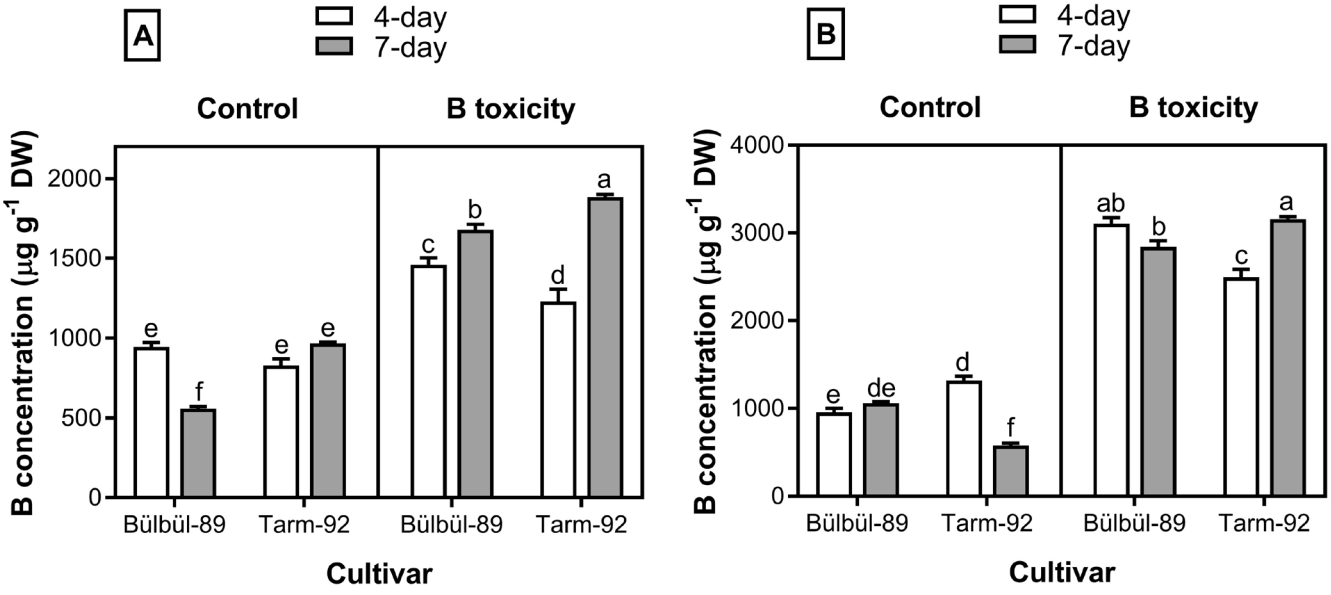

Fig. 1. The B concentration of the root (A) and shoot (B) tissues in the barley cultivars grown under control and B stress conditions for 4 or 7 days. Each bar represents mean ± SEM (n = 3). Different letters denote significant differences ($p < 0.05$) among the mean values according to Tukey's test.

lent (CAE) and quercetin equivalent (QE) per g of fresh weight (FW) (Pourmorad *et al*. 2006; Küçükakyüz & Çatav 2021). The free radical scavenging activity was determined by the DPPH method described in Çatav *et al*. (2021) with minor modifications. In brief, 1600 µL of 0.2 mM DPPH solution was mixed with 400 µL of supernatant, and the absorbance was read at 517 nm.

The anthocyanin level in the barley shoots was estimated according to the procedure outlined in Kayihan (2021) and expressed as absorbance units per g of FW.

The proline content of the barley shoots was evaluated at 508 nm using the colorimetric acid-ninhydrin reagent (Shabnam *et al*. 2016).

The total sugar content of the barley shoots was measured by the phenol-sulfuric acid reaction procedure (Dubois *et al*. 1956). Briefly, the samples were homogenised in 80% hot ethanol at a ratio of 1/10 (w/v). After centrifugation, the diluted supernatant (200 µL) was mixed with 200 µL of 5% phenol solution and 1000 µL of 98% H_2SO_4. The absorbance of the mixture was

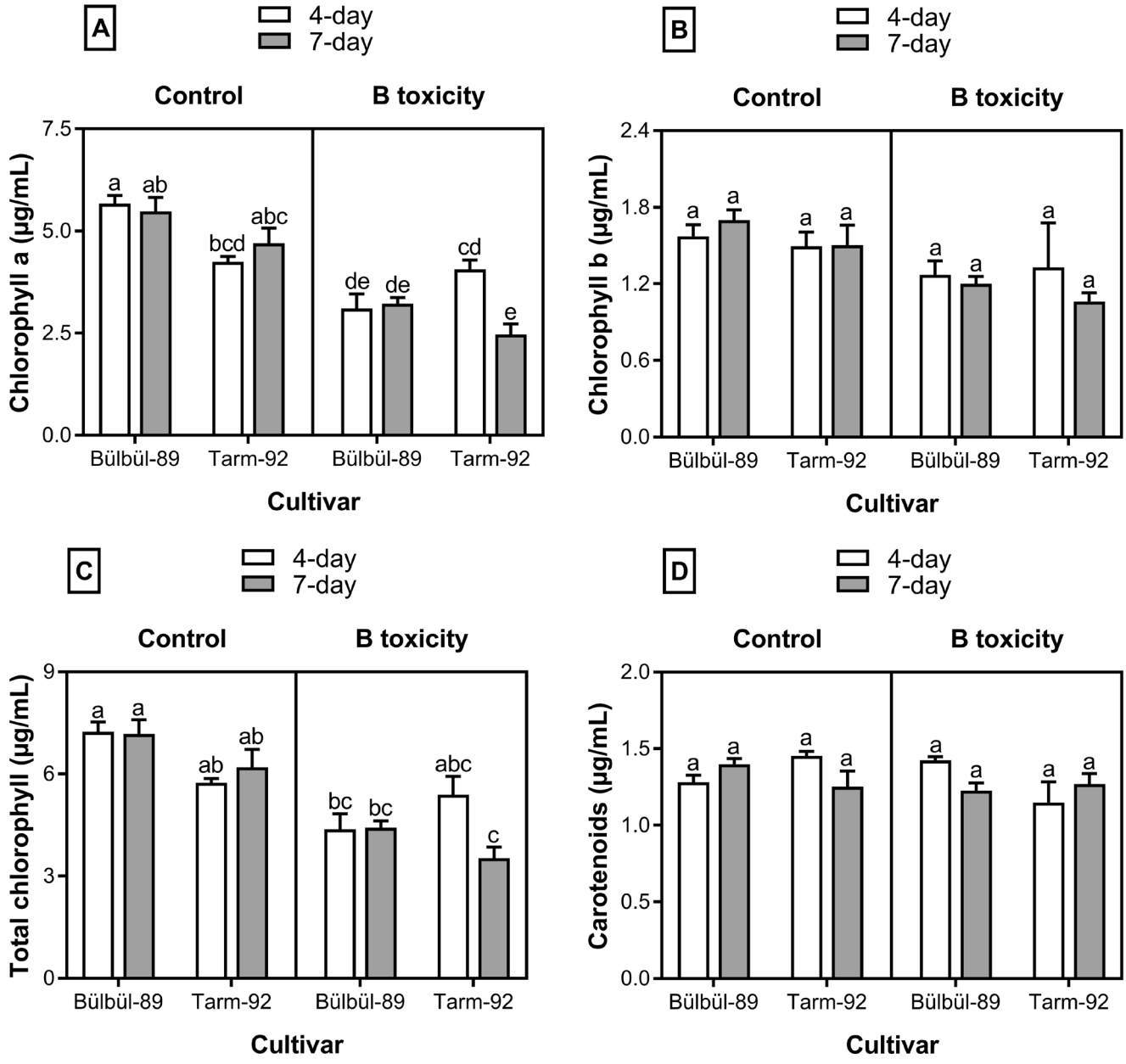

Fig. 2. The chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoid (D) concentrations of the barley cultivars grown under control and B stress conditions for 4 or 7 days. Each bar represents mean ± SEM (n = 3). Different letters denote significant differences ($p < 0.05$) among the mean values according to Tukey's test.

recorded at 490 nm following 30 min of incubation at room temperature.

Data analysis. Three-way ANOVA analyses were performed to evaluate the separate and interactive effects of the treatment, exposure period, and cultivar on the dependent measures. The assumptions for parametric tests were checked prior to each analysis, and Tukey's HSD test was used for pairwise comparisons (α = 0.05).

RESULTS

B concentration. Under non-stress conditions, the B concentration ranged from 557 to 967 µg g^{-1} DW for the roots and from 579 to 1319 µg g^{-1} DW for the shoots in the studied cultivars (Fig. 1). The treatment period was found to have a certain impact on the level of B in the roots and shoots of the control plants. For instance, the B concentration of the roots in Bülbül-89 and the shoots

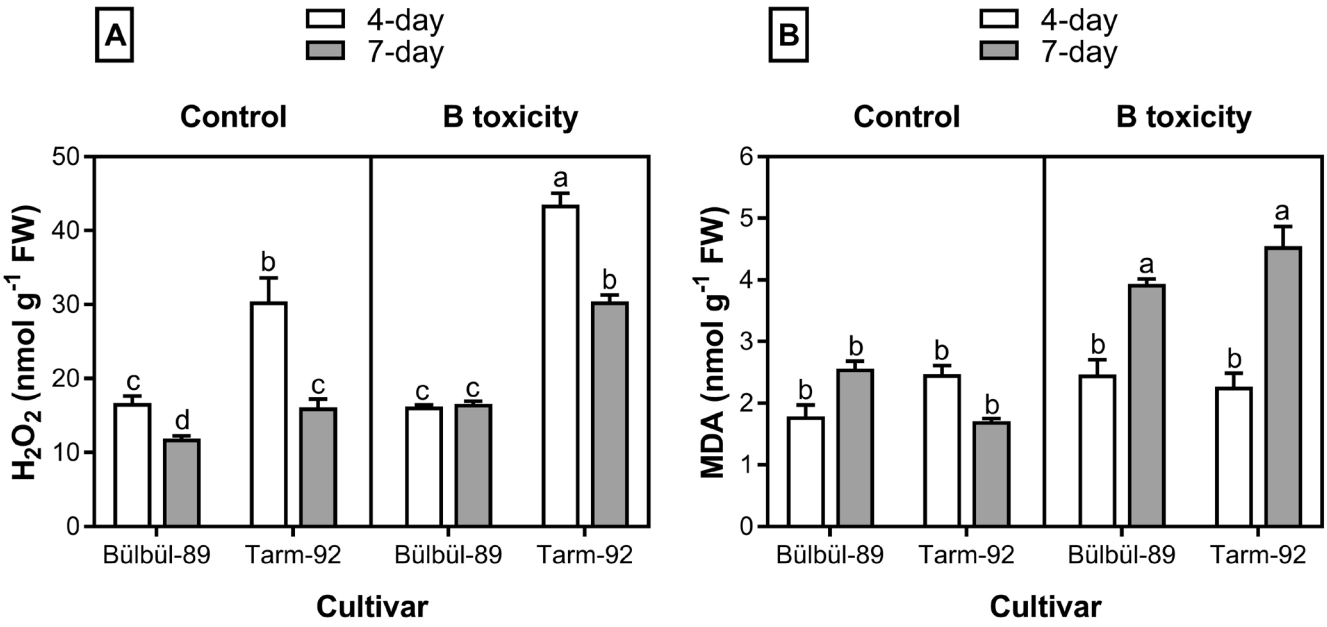

Fig. 3. The H_2O_2 (A) and MDA (B) levels of the barley cultivars grown under control and B stress conditions for 4 or 7 days. Each bar represents mean ± SEM (n = 3). Different letters denote significant differences ($p < 0.05$) among the mean values according to Tukey's test.

in Tarm-92 markedly decreased with an increase in treatment time (Fig. 1A & B). B stress resulted in drastic increases in the concentration of B in both the roots (1.48- to 3.01-fold) and shoots (1.89- to 5.45-fold) of the barley seedlings. The highest level of B in the roots was observed after 7 days of treatment with 10 mM H_3BO_3 for both the cultivars (Fig. 1A). The interaction of the treatment, exposure period, and cultivar had a significant effect on the B concentration in the shoot tissue (Table 1; Fig. 1B). Overall, we did not find any dramatic differences in terms of B tissue levels between Bülbül-89 and Tarm-92.

Growth parameters. The growth measurements of the barley cultivars exposed and unexposed to B stress for 4 or 7 days are presented in Table 2. When compared with the control groups, B stress did not induce significant changes in the root, shoot, and total seedling lengths of the barley cultivars for the same exposure time ($p > 0.05$). In addition, no significant differences were observed with regard to the length parameters between Bülbül-89 and Tarm-92 under optimal growth conditions. However, Bülbül-89 had longer shoots than Tarm-92 after 4 days of treatment with 10 mM H_3BO_3.

The presented results show that the cultivar explains most of the variation in the dry weight measurements (Table 1). In general, the root, shoot, and total seedling dry weights of Bülbül-89 were higher than those of Tarm-92 under both non-stress and stress conditions (Table 2). On the other hand, the root dry weight of Bülbül-89 was notably reduced after 7 days of exposure to 10 mM H_3BO_3 compared to the control group ($p < 0.05$). Finally, B stress did not affect the shoot dry weight of the barley cultivars for both exposure times.

Photosynthetic pigments. The photosynthetic pigment concentrations of the barley cultivars treated with or without boric acid (10 mM) for 4 or 7 days are shown in Fig. 2. While B toxicity caused significant decreases (38–45%) in the concentrations of chlorophyll a and total chlorophyll in the leaves of Bülbül-89 for both treatment periods, this decrease was only observed after 7 days of exposure in Tarm-92 (Fig. 2A & C). On the other hand, no changes in the concentrations of chlorophyll b and carotenoids were observed after 4 or 7 days of B treatment (Fig. 2B & D). On the whole, there were few or no differences in the level of photosynthetic pigments between the sensitive and tolerant barley cultivars (Table 1).

H_2O_2 and MDA contents. The cultivar was found to account for most of the variation in the H_2O_2 content (Table 1; Fig. 3A). The concentration of H_2O_2 in the shoots of Tarm-92 under control and B stress conditions was remarkably higher than that of Bülbül-89 for both exposure times. In contrast, the cultivar had no significant effect on the MDA level (Table 1). The results showed that 7 days of B treatment gave rise to prominent increases in the H_2O_2 (1.39- to 1.89-fold) and MDA (1.53- to 2.66-fold) levels of both Bülbül-89 and Tarm-92 (Fig. 3A &

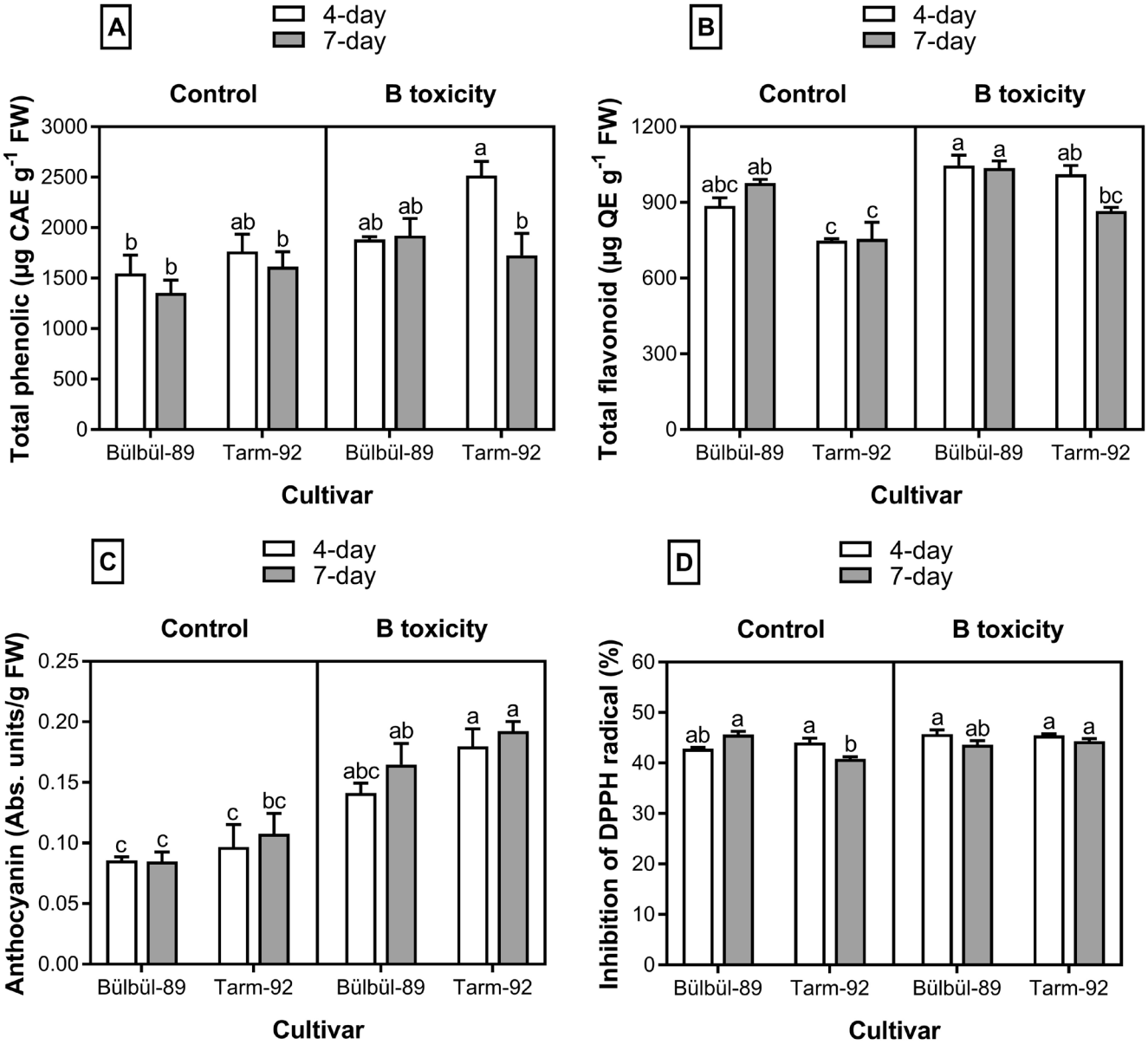

Fig. 4. The total phenolic (A), total flavonoid (B), and anthocyanin (C) contents, and the DPPH radical scavenging activity (D) of the barley cultivars grown under control and B stress conditions for 4 or 7 days. Each bar represents mean ± SEM (n = 3). Different letters denote significant differences ($p < 0.05$) among the mean values according to Tukey's test.

B). Furthermore, a notable increase was detected in the H_2O_2 content of Tarm-92 subjected to 10 mM H_3BO_3 for 4 days ($p < 0.05$).

Phenolic compound content and free radical scavenging activity. The phenolic, flavonoid, and anthocyanin contents, as well as the DPPH radical scavenging activity of the barley cultivars exposed and unexposed to B stress for 4 or 7 days are shown in Fig. 4. The findings of the present study indicate that B toxicity results in slight non-significant increases in the total phenolic content of Bülbül-89 and Tarm-92 for both the treatment periods (Fig. 4A). The cultivar exerted a marked influence on the total flavonoid content (Table 1). Bülbül-89 exhibited a higher flavonoid concentration than Tarm-92 after 7 days of growth under both control and B stress conditions. Moreover, 4 days of B treatment caused a considerable increment (35%) in the flavonoid content of Tarm-92 relative to the control (Fig. 4B). B stress also produced dramatic increases in the level of anthocyanin of the barley cultivars in the range of 64–95.2% (Fig. 4C). Finally, our results revealed that the interaction of the

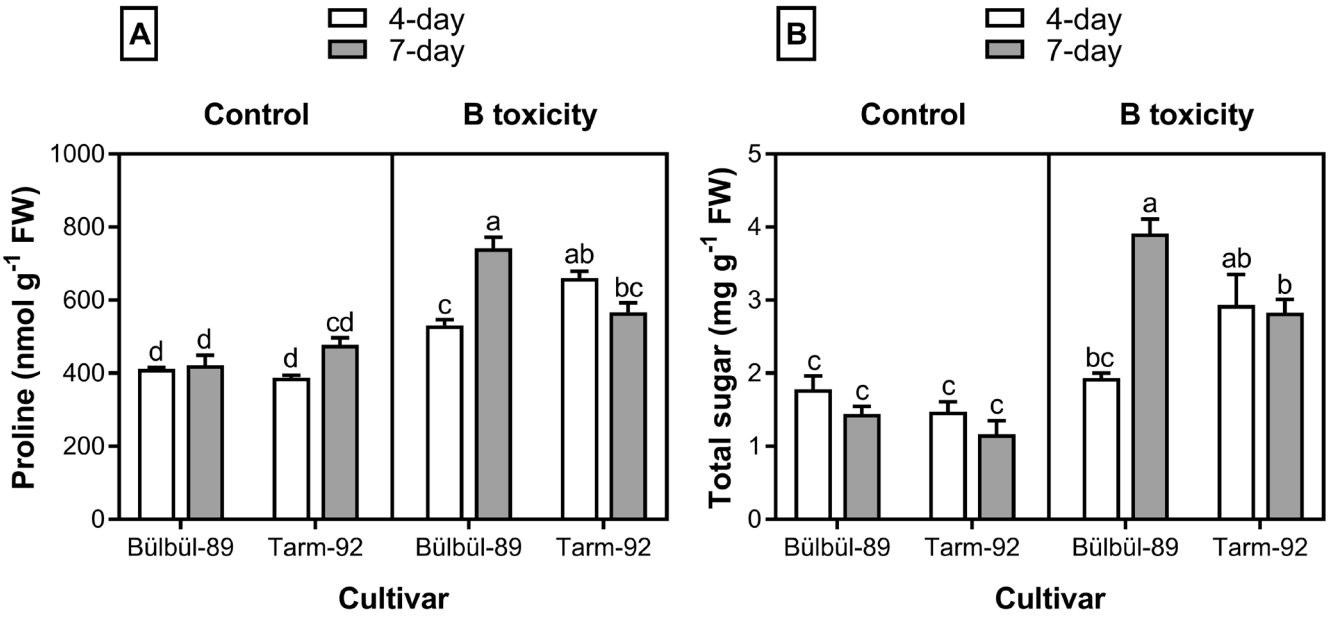

Fig. 5. The proline (A) and total sugar (B) contents of the barley cultivars grown under control and B stress conditions for 4 or 7 days. Each bar represents mean ± SEM (n = 3). Different letters denote significant differences ($p < 0.05$) among the mean values according to Tukey's test.

treatment, exposure time, and cultivar had the highest impact on DPPH activity (Table 1). In this regard, a slight but significant rise was observed in the DPPH activity of Tarm-92 after 7 days of exposure to excess B (Fig. 4D).

Compatible solute content. The proline and total sugar levels of the barley cultivars treated with or without boric acid (10 mM) for 4 or 7 days are illustrated in Fig. 5. There were no differences ($p > 0.05$) in the proline and total sugar contents between the cultivars under control conditions. The levels of both parameters increased considerably in response to B toxicity for at least one treatment period. In particular, 2.44- and 2.72-fold increases were seen in the total sugar content of Tarm-92 and Bülbül-89, respectively, after 7 days of B treatment. Lastly, most of the interactions among the independent variables had a significant effect on the proline and total sugar levels (Table 1).

DISCUSSION

B toxicity is an important constraint to crop growth and yield in saline-alkaline soils of arid and semi-arid areas (Landi *et al*. 2019; Pandey *et al*. 2019). However, it has been shown that there is great variability in the tolerance of cereal cultivars to B toxicity (Nable 1988; Torun *et al*. 2003, 2006; de Abreu Neto *et al*. 2017). For instance, Nable (1988) found a 78% and 86% decline in the root and shoot dry weights of B-sensitive barley cultivar Schooner grown under 5 mM H_3BO_3 for 35 days, respectively. On the other hand, only a 6% decrease was observed in the plant dry weight of the B-tolerant barley cultivar Sahara 3763. Torun *et al*. (2003) also reported significant differences in terms of grain yield among Turkish barley cultivars exposed to excess B. In their study, Bülbül-89 and Tarm-92 were classified as B-susceptible and B-tolerant barley cultivars, respectively. Consistent with their findings, we demonstrated here that exposure to 10 mM H_3BO_3 for 7 days caused a prominent decrease in the root dry weight of B-sensitive cultivar Bülbül-89.

Maintaining a lower concentration of B in the root and shoot tissues is considered a primary mechanism for tolerance to B toxicity in cereal species (Reid 2013). It has been suggested that the genes *HvBot1* and *HvNIP2;1* encode a borate exporter and a boric acid channel, respectively, thus playing a crucial role in reducing B concentration in barley roots (Schnurbusch *et al*. 2010a). In this context, Sutton *et al*. (2007) revealed that the transcript level of *HvBot1* in the B-tolerant cultivar Sahara was considerably higher than that of the B-susceptible cultivar Clipper under excess B conditions. In addition, when compared to Clipper, a lower expression of the *HvNIP2;1* gene was observed in the Sahara roots (Schnurbusch *et al*. 2010b). On the other hand, some studies failed to find a significant association between the B concentration in tissues and the tolerance of cereals to B toxicity (Torun *et al*. 2006; Ochiai *et al*. 2011). In accordance with the latter reports, we did not detect

any dramatic differences between the sensitive and tolerant barley cultivars with regard to tissue B concentrations. A possible explanation for this discrepancy is that several mechanisms may participate in the tolerance of cereals to excess B. For example, the redistribution of B in the leaves is recognised as another important mechanism contributing to the mitigation of B toxicity in barley and wheat cultivars (Reid & Fitzpatrick 2009).

Reactive oxygen species (ROS) are involved in the regulation of various cellular processes (e.g. signal transduction, differentiation, and cell death) in plants at basal levels (Mittler 2017). However, abiotic stress conditions can increase the generation of ROS by different mechanisms, including the over-reduction of the electron transport chain in chloroplasts and mitochondria and thiol depletion (Ercal *et al.* 2001; You & Chan 2015). Cytotoxic levels of ROS have been shown to be detrimental to biomolecules and cells (Hasanuzzaman *et al.* 2020). In this study, a remarkable rise was found in the H_2O_2 and MDA contents of the barley cultivars exposed to excess B for 7 days. Our results are in agreement with previous findings that B toxicity causes oxidative stress in plants, such as maize, pepper, tomato, and wheat (Cervilla *et al.* 2007; Çatav *et al.* 2018, 2022; Kaya *et al.* 2018).

In recent years, growing interest has focused on understanding whether there is a relationship between steady-state or basal levels of ROS and abiotic stress tolerance in plant species. In this regard, Puckette *et al.* (2007) found a positive correlation between ozone tolerance and constitutive levels of ROS in *Medicago truncatula* accessions. Similarly, it was shown that ozone-tolerant common bean and wheat varieties had intrinsically higher levels of ROS compared to ozone-sensitive ones (Caregnato *et al.* 2013; Yadav *et al.* 2019). In addition, Kaur *et al.* (2016) and Saini *et al.* (2018) reported that in rice, salt-tolerant cultivars exhibited higher H_2O_2 concentrations and NADPH oxidase activity than salt-susceptible cultivars. Our results, indicating that the H_2O_2 content of B-tolerant Tarm-92 was markedly greater than that of B-sensitive Bülbül-89 under optimal growth conditions, are compatible with these studies. Taken together, it seems that high endogenous levels of ROS may have a priming effect on abiotic stress-induced responses in plants.

Enhanced antioxidant capacity plays a vital role in the alleviation of ROS-induced oxidative injury in plants (Soares *et al.* 2019). It has been demonstrated that there may be differences in antioxidant capacity among sensitive and tolerant genotypes subjected to abiotic stress conditions, such as salinity, drought, and aluminium toxicity (Gossett *et al.* 1994; Giannakoula *et al.* 2010; Avramova *et al.* 2017). For instance, Gossett *et al.* (1994) revealed that catalase activity and α-tocopherol content in salt-sensitive cotton varieties were significantly lower than those in salt-tolerant varieties. In addition, Avramova *et al.* (2017) suggested that drought tolerance in maize hybrids was related to higher antioxidant activity. However, little is known about the possible relationship between B toxicity tolerance and antioxidant capacity in cereal cultivars. In a study exploring the antioxidant response of barley to excess B, Karabal *et al.* (2003) found small differences in the activities of glutathione reductase and superoxide dismutase among susceptible and tolerant cultivars. On the other hand, the ascorbate peroxidase and catalase activities of the studied cultivars displayed a similar pattern of response to high B levels. They concluded from their data that B toxicity tolerance might not be associated with differences in antioxidant enzyme profiles. In the current study, we measured DPPH radical scavenging activity and total phenolic and flavonoid contents in order to determine the antioxidant status of the barley cultivars. Our results show that there are no apparent differences in the radical scavenging activity and total phenolic content between B-sensitive Bülbül-89 and B-tolerant Tarm-92. Nevertheless, Bülbül-89 exhibited a significantly higher flavonoid content than Tarm-92, while a notable increase was observed in the flavonoid content of Tarm-92 after 10 mM H_3BO_3 treatment.

Anthocyanins are a major class of flavonoids with diverse functions in plants, including pollination, the protection of the photosynthetic apparatus, and scavenging free radicals (Liu *et al.* 2018; Berland *et al.* 2019). Landi *et al.* (2014) reported that anthocyanins displayed a protective effect against photo-oxidative stress caused by B toxicity in basil. Anthocyanins are also known to form complexes with metalloids (e.g., B and Ge), and it has been proposed that the sequestration of anthocyanin-B complexes to the vacuoles may improve tolerance to B toxicity (Landi *et al.* 2015; Estévez *et al.* 2021). In accordance with this assumption, Kayihan (2021) demonstrated that excess B induced the expression of the genes (*C4H*, *4CL3*, *TT13*, and *TT19*) responsible for anthocyanin biosynthesis and transport and increased the leaf anthocyanin level in *Arabidopsis thaliana*. In this study, we also found a dramatic rise in the anthocyanin levels of the barley cultivars exposed to B stress. This increase, however, was slightly more pronounced in Tarm-92. These findings collectively suggest that more attention should be paid to anthocyanin-mediated responses in plants grown under B toxicity.

The accumulation of proline and sugars in plant tissues is an important defence mechanism against environmental stresses, such as drought, salinity, and B toxicity (Kumar *et al.* 2018; Çatav *et al.* 2022). Proline has been shown to play a key role in several processes, including osmoregulation, ROS scavenging, metal chelation, and the stabilisation of proteins (Kaur & Asthir 2015; Meena *et al.* 2019). In addition, sugars are also known to contribute to the maintenance of osmotic potential in plants (Sami *et al.* 2016). In this study, the re-

sults indicate that exposure to excess B for 4- or 7-days results in a considerable increase in the proline and sugar contents of the barley cultivars. On the other hand, no significant differences were detected in the level of these compatible solutes between Bülbül-89 and Tarm-92.

CONCLUSION

This research showed that prolonged exposure to 10 mM H_3BO_3 induced root growth inhibition in B-sensitive Bülbül-89. The results also revealed that there were no remarkable differences in tissue B concentrations, compatible solute content, DPPH radical scavenging activity, and total phenol content between B-susceptible and B-tolerant barley cultivars. However, Tarm-92 exhibited a significantly higher level of H_2O_2 than Bülbül-89. In addition, a prominent escalation was found in the total flavonoid content of the Tarm-92 seedlings subjected to B toxicity for 4 days. Despite only analysing several parameters in relation to the oxidative and antioxidative status, our data suggest that differences in the constitutive levels of H_2O_2 may account for genotypic variation in the tolerance of barley cultivars to B toxicity. Further studies with a larger number of sensitive and tolerant genotypes are required to determine a causal relationship between steady-state ROS levels and B toxicity tolerance in cereal species.

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REZIME

Botanica SERBICA

Tolerancija toksičnosti bora kod ječma može biti povezana sa postojanjem suštinski viših nivoa reaktivnih vrsta kiseonika u izdancima

Şükrü Serter Çatav, Eda Çetin, Emre Vural i Betül Bürün

Postoje značajne intra-i interspecifične varijacije u toleranciji toksičnosti bora (B) u biljnim kulturama. U ovoj studiji, imali smo za cilj da istražimo mehanizme uključene u toleranciju viška B kod ječma (*Hordeum vulgare*) u ranim fazama razvoja biljaka. Da bi se to postiglo, sorte ječma osetljive na B (Bulbul-89) i B-tolerantne (Tarm-92) su uzgajane hidroponski pod kontrolom i uslovima stresa borom (10 mM H_3BO_3) na 4 ili 7 dana. Vodonik peroksid (H_2O_2), malondialdehid (MDA), ukupni fenoli, ukupni flavonoidi, antocijani, prolin i ukupni šećeri, kao i kapacitet uklanjanja radikala DPPH su određeni kod oba kultivara. Naši rezultati su pokazali da je tretman B doveo do značajnog povećanja koncentracije B kod kultivara ječma u oba vremena izlaganja. Međutim, nije bilo drastičnih razlika u koncentraciji B u korenu i nadzemnom delu između osetljivih i tolerantnih sorti. Dok je masa suvog korena Bulbul-89 smanjena nakon 7 dana B stresa ($p < 0,05$), takvo smanjenje nije primećeno kod Tarm-92. Sadržaj H_2O_2, MDA, prolina, ukupnog šećera i antocijana oba kultivara je značajno povećan kao odgovor na višak B tokom najmanje jednog perioda tretmana ($p < 0,05$). Sadržaj H_2O_2 kod Tarm-92 u kontrolnim i uslovima stresa borom bio je značajno veći nego u Bulbul-89, ali nije bilo razlike u sadržaju MDA i kapacitetu uklanjanja radikala između dva kultivara. Konačno, utvrđeno je povećanje ukupnog sadržaja flavonoida za 35% u sadnicama Tarm-92 koje su bile izložene B stresu tokom 4 dana. Zaključno, nalazi ovog rada sugerišu da tolerancija na B toksičnost kod sadnica ječma može biti povezana sa sposobnošću tolerisanja viših nivoa reaktivnih vrsta kiseonika.

Ključne reči: toksičnost bora, mehanizmi tolerancije, ječam, reaktivne kiseonične vrste, antioksidativna aktivnost, kompatibilni rastvori