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Evaluation of photosynthetic performance of wheat cultivars exposed to boron toxicity by the JIP fluorescence test

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Abstract

The changes in growth and photosynthetic performance of two wheat (*Triticum aestivum* L.) cultivars (Bolal-2973 and Atay-85) differing in their sensitivity to boron (B) toxicity were investigated under toxic B conditions. Eight-day old seedlings were exposed to highly toxic B concentrations (5, 7.5, and 10 mM H_3BO_3) for 5 and 9 days. Fast chlorophyll *a* fluorescence kinetics was determined and analysed using JIP test. Growth parameters, tissue B contents, and membrane damage were measured at two stress durations. The photochemical performance of PSII was hindered more in the sensitive cultivar (Atay-85) than that of the tolerant one (Bolal-2973) under B toxicity. The increase in the B concentration and stress duration caused membrane leakage in both cultivars. However, higher membrane damage was observed in Atay-85 compared to Bolal-2973. Additionally, significant reduction of growth parameters was observed in both cultivars at toxic B concentrations. The accumulation of B was higher in shoots than in roots of both cultivars. Nevertheless, Atay-85 translocated more B from roots to leaves compared to Bolal-2973. The advantages of certain JIP test parameters were demonstrated for evaluation of PSII activity in plants exposed to B stress. Evaluation of photosynthetic performance by JIP test as well as assessment of growth and tissue B content might be used to determine the effects of B toxicity in wheat. The results indicated lesser sensitivity to B toxicity in Bolal-2973 compared to Atay-85.

Additional key words: chlorophyll fluorescence; fluorescence transient; performance index; photosynthesis.

Introduction

In soil solution, B exists mainly as a boric acid (H_3BO_3), which can be leached easily under high rainfall conditions, leading to deficiency in plants (Shorrocks 1997). On the contrary, under low rainfall conditions, insufficient leaching of H_3BO_3 may lead to accumulation of B which is toxic to plants (Nable *et al.* 1997). Boron toxicity is a worldwide problem that reduces significantly crop yields in agriculture. It is characterized by alkaline and saline soils together with very scarce leaching. Sources of high B include over-fertilization and/or irrigation with water containing high concentrations of B (Nable *et al.* 1997). Boric acid is permeable through the plasma membrane, allowing passive diffusion. Additionally, membrane-located channel proteins facilitate passive transport (Miwa and Fujiwara 2010). Both boric acid and borate anion can readily react with a wide variety of biological molecules having two hydroxyl groups in *cis*-configuration (Reid *et al.* 2004). To date, a component of cell wall, rhamno-galacturonan II (RG-II), which exists as a dimer cross-linked by borate esters at apiose residues, remains the only known complex containing B in higher plants (Kobayashi *et al.* 1996, O'Neill *et al.* 2004).

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Abbreviations: ABS – absorption; CS – cross section; ET – electron transfer/transport; F_0 – initial fluorescence intensity; F_M – maximal fluorescence intensity; ICP-ASE – inductively coupled plasma-atomic emission spectroscopy; OJIP – fluorescence transient with O-J-I-P phases; PI – performance index; Q_A – primary quinone acceptor of photosystem II; Q_B – secondary quinone acceptor of photosystem II; RC – reaction centre; TR – trapping flux; φ_{E0} or ET_0/ABS – quantum yield of electron transport; φ_{P0} or TR_0/ABS – maximum quantum yield of primary photochemistry.
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Boron accumulation in many plants follows a pattern from leaf base to tip, the latter having a high B content. This leads to typical toxicity symptoms on leaves, which appear as marginal or tip chlorosis and necrosis (Gupta 1983). Toxic B content has different effects on plant physiological and biochemical processes including disruption of growth, cell wall development, and cellular division as well as reduction in chlorophyll (Chl) contents, photosynthetic rates, and lignin contents (Nable *et al.* 1997, Reid *et al.* 2004). Accordingly, a reduced growth of shoots and roots is typical for plants exposed to high B concentrations. Growth inhibition is proposed to be caused by binding of B to ribose, either as free sugar or within RNA, NADH, or NADPH (Nable *et al.* 1997, Han *et al.* 2009). Although growth is rapidly inhibited by internal B concentrations in the range of 1–5 mM, this inhibition is not attributable to effects of B on either energy supplies or inhibition of protein synthesis (Reid *et al.* 2004). Additionally, there is no evidence to support the hypothesis that toxicity in leaves is due to osmotic stress induced by the accumulation of B (Reid *et al.* 2004, Reid 2007). The toxicity to mature tissues is rather due to the accumulated retardation of many cellular processes, enhanced by photooxidative stress (Reid *et al.* 2004).

Chl fluorescence, which is a noninvasive and quantitative tool, enables determination of changes in the photosynthetic processes (Oxborough 2004). It is particularly suitable for investigation of the effects of B toxicity that induces chlorosis or necrosis in leaves. It has been suggested that the kinetics of the Chl *a* fluorescence is sensitive to stress and can reflect the physiological status and response of plants under various stresses (Bussotti *et al.* 2007, Oukarroum *et al.* 2007, Eullaffroy *et al.* 2009). An analysis of the fast OJIP fluorescence kinetics has been developed previously. The analysis, called JIP test, quantifies the *in vivo* energy fluxes passing through the reaction centres (RCs) and photosystems and evaluates plant photosynthetic performance (Strasser and Strasser 1995, Strasser *et al.* 2000). The JIP test links different steps and phases of the transient with the redox states of PSII. Concomitantly, it correlates the phases with the efficiencies of electron transfer (ET) in the intersystem chain between PSII and PSI and to the end electron acceptors at the PSI acceptor side (Strasser *et al.* 2004). All photosynthetic reactions should be reflected in the shape of the OJIP fluorescence kinetics. Although the analysis of polyphasic Chl *a* fluorescence rise with JIP test is a reliable technique to screen for changes in photosynthetic performance, it is practical to use also complementary techniques, such as measurements of transmission signal at 820 nm. These measurements allow verification of interpretations derived from fluorescence measurements and provide further information on changes related to electron flow through PSI (Schansker *et al.* 2005, Lazár 2006, 2009; Ceppi *et al.* 2012).

Cases of B deficiency or toxicity in agricultural land were reported in different parts of the world including USA, China, Russia, Southern Australia, as well as Central Anatolia of Turkey. Central Anatolia, which encompasses 3.5 Mha agricultural land, is specifically important since it is the cereal storehouse of Turkey. Wheat (*T. aestivum* L.), the main cereal grown in this region, is sensitive to high concentrations of B (Schnurbusch *et al.* 2010). The effects of excessive B have been investigated in several plant species (Karabal *et al.* 2003, Ardic *et al.* 2009, Guidi *et al.* 2011). Additionally, Chl fluorescence has been used for evaluation of damage induced by B in various plants (Papadakis *et al.* 2004, Landi *et al.* 2013a,b). However, there is limited data available on photosynthetic response of wheat under B toxicity. Therefore, in the present work, the changes in growth and photosynthetic performance of two wheat cultivars differing in their sensitivity to B toxicity were determined under toxic B conditions. Particular parameters of Chl fluorescence were used to determine the sensitivity of wheat cultivars to B toxicity and to describe differences in photosynthetic performance. Additionally, the relationship between B contents in seedlings and sensitivity to B toxicity were evaluated. We also demonstrated the advantages of certain JIP test parameters for evaluation of PSII activity of plants exposed to B stress.

Materials and methods

Plant materials, growth and treatment conditions: In this study, two local wheat (*T. aestivum* L.) cultivars of different sensitivity to B, *i.e.*, Bolal-2973 (tolerant) and Atay-85 (sensitive) (Mahboobi *et al.* 2001), were used. Seeds, obtained from the Agriculture and Livestock, Ministry of Food (Republic of Turkey), were surface-sterilized with 2% sodium hypochlorite solution for 20 min with gentle shaking, and subsequently rinsed with sterile water three times. Surface sterilized seeds were transferred to plastic trays containing a nutrient solution (pH 5.8) including 1.25 mM KNO_3, 1.15 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4, 2.3 μM $MnCl_2$, 0.2 μM $ZnSO_4$, 0.1 μM $CuSO_4$, 0.1 μM Na_2MoO_4, 10 μM Fe-EDTA, and 10 μM H_3BO_3 (Hoagland and Arnon 1950). The solution was replaced with a fresh one every 3 d. The seedlings were grown at 22 ± 2°C with 16-h light (400 μmol m^{-2} s^{-1} PAR) and 8-h dark photoperiod with 40 ± 5% relative humidity in a controlled growth chamber.

After 8 d of growth, the seedlings were transferred to sterile, hydroponic cultures for B treatment, which was applied immediately after transfer as a nutrient solution containing three different H_3BO_3 concentrations (5 mM, 7.5 mM, and 10 mM) for another 5 and 9 d under the same physical conditions. The concentrations of H_3BO_3 were selected to achieve B accumulation in plant tissues at early seedling stage under laboratory conditions. Control groups

were transferred to nutrient solutions without extra H_3BO_3. The second and third leaves of 13- and 17-d-old plants were used in the experimental analyses.

Growth parameters: The root and shoot lengths [mm per plant] of wheat seedlings were recorded. Six plants for each group were sampled randomly to determine total fresh mass (FM) [mg(FM) plant^{-1}] at the end of stress treatments and subsequently kept at 80°C for 48 h to measure the dry mass (DM) [mg(DM) per plant].

Boron contents: After 13 and 17 d of growth (5 and 9 d of B treatment), the seedlings were rinsed three times in deionized water, and then the shoot and root tissues were collected separately and dried at 80°C for 48 h. The dried tissues were ground to powder with liquid nitrogen. The B content [mg kg^{-1}(DM)] in tissues was quantified using inductively coupled plasma-atomic emission spectroscopy (*ICP-AES, IRIS Intrepid, Thermo Elemental*, USA) analysis after 0.2 g of dried samples were ashed at 500°C for 5 h and dissolved in 0.1 N HNO_3.

Membrane stability: Membrane damage in leaf tissues (1 cm segments of leaves) of the cultivars were measured indirectly as leakage of UV-absorbing substances according to the method of Redmann *et al.* (1986). For measurement of the leakage, samples were gently shaken in 10 ml distilled water for 24 h at room temperature on an orbital shaker and subsequently the absorption (C_1) was determined at 280 nm (*UV mini 1240* spectrophotometer, *Shimadzu*, Japan). Then the samples were frozen with liquid nitrogen for complete membrane disintegration, and the second absorption (C_2) was recorded after incubation of freezing-killed samples in 10 ml of distilled water with gentle shaking for 24 h at room temperature. The total injury to the membranes as a relative leakage ratio was calculated by dividing C_1 by C_2.

Chlorophyll (Chl) *a* fluorescence measurements: The polyphasic, OJIP fluorescence transient measurements were performed with a *Handy PEA* (*Hansatech Instruments Ltd.*, Norfolk, UK) fluorimeter on selected leaves of the cultivars at room temperature. Following a 30-min dark adaptation, samples were illuminated with continuous light (650 nm peak wavelength; 3,000 μmol(photon) m^{-2} s^{-1} maximum light intensity for 1 s) provided by three LEDs, and the Chl *a* fluorescence signals were recorded according to Strasser and Strasser (1995). The OJIP transient was analysed by the JIP test based on the energy fluxes theory for biomembranes in a photosynthetic sample, which leads to the equations and calculations for the efficiencies for the whole energy cascade from absorption to the reduction of the end electron acceptors at the PSI acceptor side and the performance indexes (Strasser *et al.* 2004). The fluorescence parameters (Table 1) were calculated with *Biolyzer* software package and also according to the JIP test (Strasser *et al.* 2004).

Statistical analysis: The experiments, arranged in a completely randomized design, were repeated three times. Differences among treatments and cultivars were tested using *MINITAB* statistical programme. Statistical variance analysis (*ANOVA)* of the data was performed and data were compared using *Tukey*'s test at the 5% level.

Results and discussion

The effects of B toxicity on some parameters of growth and physiology were investigated in two wheat cultivars at two stress durations (5 and 9 d). Toxicity of B induced marginal or tip chlorosis and necrosis in leaves. Although the difference between two cultivars was not visually distinguishable, symptoms of toxicity were observed in the first-emerging leaves at the end of stress period (Fig. 1). A primary phenotypic effect of B toxicity is reduced root growth compared to that of plants grown at optimal concentrations of B (Reid *et al.* 2004, Choi *et al.* 2007). Toxicity treatments in our study led to gradual decrease in the root length of both cultivars compared with the controls (Table 2). The inhibition of root growth was found for the extremely toxic (10 mM B) concentration at both stress durations. Reduction of growth in roots as a consequence of B toxicity has previously been observed in wheat (Kalayci *et al.* 1998), barley (Karabal *et al.* 2003), and tomato (Cervilla *et al.* 2009). Genotypic variation in root elongation has been effectively used as an indicator of B tolerance (Jefferies *et al.* 1999, Choi *et al.* 2007). Similarly, in our study, the sensitive wheat cultivar, Atay-85, showed the most reduced root length at 10 mM B, where 10 and 15% reductions were observed after 5 and 9 d, respectively. Besides the root growth, shoot elongation in both cultivars was reduced by B toxicity (Table 2). The reduction in the shoot growth was dependent on B concentrations and treatment durations. Under the extreme condition (10 mM B for 9 d), the inhibition of the shoot growth reached the highest value (12%) in Atay-85. Sotiropoulos *et al.* (2002) suggested that the restriction of growth at high concentrations of B results from the reduced photosynthetic rate and water use efficiency. The effects of B on FM and DM of root and shoot tissues reflected the effects of B on root and shoot growth (Table 2). The shoot FM and DM of both cultivars gradually decreased with increasing concentrations and stress durations (Table 2). Treatment with 10 mM B resulted in the pronounced reduction of both FM and DM in Atay-85. The strong influence of B toxicity on growth parameters of wheat is in agreement with previous studies (Chantachume *et al.* 1995, Campbell *et al.* 1998).

Under all toxic treatments and exposure durations, B contents in root and shoot tissues of both cultivars progressively increased in a concentration-dependent manner (Fig. 2). The increase of B content in roots was

Table 1. Summary of the formula and terms of JIP test parameters and their description using data extracted from the chlorophyll *a* fluorescence transient (Strasser *et al.* 2000, 2004). * when expressed per CS_M, F_0 is replaced by F_M (Strasser *et al.* 2000)

Parameter	Formula/Explanation
Extracted and technical fluorescence parameters	
F_0	Initial fluorescence intensity, when all RCs of PSII are open
F_{300}	Fluorescence intensity at 300 μs
F_J	Fluorescence intensity at the J-step (at 2 ms)
F_I	Fluorescence intensity at the I-step (at 30 ms)
F_M	Maximal fluorescence intensity, when all RCs of PSII are closed
t_{FM}	Time to reach F_M, in ms
V_J	$(F_J - F_0)/(F_M - F_0)$, relative variable fluorescence at the J-step (2 ms)
V_I	$(F_I - F_0)/(F_M - F_0)$, relative variable fluorescence at the I-step (30 ms)
V_K	$(F_{300} - F_0)/(F_M - F_0)$, relative variable fluorescence at the K-step (300 μs)
M_0 or $(dV/dt)_0$	$4\ (F_{300} - F_0)/(F_M - F_0)$, approximated initial slope (in ms^{-1}) of the fluorescence transient V = f(t)
Area	Total complementary area between fluorescence induction curve and F_M
S_M	$Area/(F_M - F_0)$, normalized total complementary area above the OJIP (reflecting multiple-turnover Q_A reduction events) or total electron carriers per RC
Quantum efficiencies or flux ratios	
φ_{P0} or TR_0/ABS	$1 - F_0/F_M$ or F_V/F_M, maximum quantum yield of primary photochemistry at t = 0
φ_{E0} or ET_0/ABS	$(1 - F_0/F_M) \times \psi_0$, quantum yield for electron transport at t = 0
ψ_0 or ET_0/TR_0	$1 - V_J$, probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
δ_{R0} or RE_0/ET_0	$(1 - V_J)/(1 - V_I)$, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end final electron acceptors
φ_{R0} or RE_0/ABS	$\varphi_{P0} \times \psi_0 \times \delta_{R0}$, the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors
Specific fluxes or specific activities	
ABS/RC	$M_0 \times (1/V_J) \times (1/\varphi_{P0})$, absorption flux per RC at t = 0 or a measure for an average antenna size
TR_0/RC	$M_0 \times (1/V_J)$, trapped energy flux per RC at t = 0
ET_0/RC	$M_0 \times (1/V_J) \times \psi_0$, electron transport flux per RC at t = 0
Phenomenological fluxes or phenomenological activities	
ABS/CS_0	F_0 or other useful expression, absorption flux per CS at t = 0*
TR_0/CS_0	$\varphi_{P0} \times (ABS/CS_0)$, trapped energy flux per CS at t = 0
ET_0/CS_0	$\varphi_{P0} \times \psi_0 \times (ABS/CS_0)$, electron transport flux per CS at t = 0
RC/CS_0	$\varphi_{P0} \times (V_J/M_0) \times F_0$, amount of active PSII RCs per CS at $t = t_{FM}$
PI_{abs}, PI_{total}	$(RC/ABS) \times [\varphi_{P0}/(1 - \varphi_{P0})] \times [\psi_0/(1 - \psi_0)] \times [\delta_{R0}/(1 - \delta_{R0})]$, total PI, measuring the performance up to the PSI end electron acceptors

more pronounced in Bolal-2973 compared to Atay-85 (Fig. 2*A*). On the other hand, under all treatments, Atay-85 displayed higher B contents in the shoots compared to Bolal-2973 (Fig. 2*B*). B-tolerant cultivars of wheat have been identified; their tolerance appears to be associated with a reduced accumulation of B in shoots (Nable 1988, Paull *et al.* 1988). Additionally, membrane-located transporters, with a function in B translocation, have been determined in *Arabidopsis*, rice, and barley (Miwa and Fujiwara 2010). Involvement of these transporters as well as aquaporins in tolerance mechanisms has been proposed (Takano *et al.* 2005, Sutton *et al.* 2007). In barley, the physiological basis for tolerance of toxicity and reduced B accumulation in tolerant varieties were proposed to be associated with the active efflux of B from root cells by regulated expression of *HvBot1* and *HvNIP2;1* (Hayes and Reid 2004, Sutton *et al.* 2007, Schnurbusch *et al.* 2010). Although a gene (*TaBor2*) coding for a B transporter has been cloned from cDNA prepared from roots of wheat grown under B toxic conditions, less is known about the molecular basis of tolerance in wheat (Reid 2007, Schnurbusch *et al.* 2010). Besides, *TaBor2* in wheat might have a function in translocation of B under toxic conditions. In our study, relatively high shoot B content in sensitive Atay-85 and the high root B content in tolerant Bolal-2973 suggest that the tolerant cultivar Bolal-2973 was able to restrict the transport of B to shoots to avoid toxic effects of B in above ground tissues. This hypothesis should be verified in succeeding molecular studies on B transporters and aquaporins from wheat *via* investigation of transcriptional or translational regulation of these proteins under B toxicity.

Toxicity of B led to increased membrane permeability as indicated by electrolyte leakage in Atay-85 under all treatments (Fig. 3). On the other hand, the increase in electrolyte leakage of Bolal-2973 leaves was significant

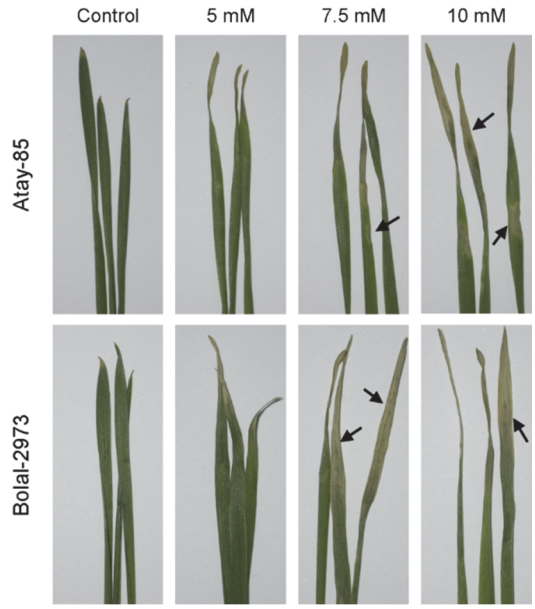

Fig. 1. The visual symptoms of B toxicity observed in leaves of wheat cultivars exposed to B toxicity after 5 d of 5, 7.5, and 10 mM H_3BO_3 treatment. First-emerging leaves of randomly selected seedlings are displayed. *Arrows* indicate zones of chlorosis or necrosis.

only under the extreme toxicity treatment (10 mM B for 9 d). Excessive B-mediated membrane damage was also previously reported in tomato, cucumber (Alpaslan and Gunes 2001), barley (Karabal *et al.* 2003), and grapevine (Gunes *et al.* 2006). Our results suggest that a protective mechanism in tolerant wheat cultivar reduced membrane permeability and maintained membrane stability under B toxicity. This should be investigated in further physiological experiments.

Various abiotic stresses directly or indirectly affect photosynthetic activity of plants and alter Chl *a* fluorescence kinetics. The analysis of changes in Chl *a* fluorescence kinetics supplies valuable information on structure and activity of PSII (Oukarroum *et al.* 2007). The measurements of Chl *a* fluorescence kinetics in our study indicated alterations in the efficiency of the photosynthetic apparatus of B-treated cultivars. The fluctuations of JIP test parameters are displayed in Fig. 4. The value of each parameter was normalized according to that of control. Initial fluorescence (F_0) represents the fluorescence emission by the excited antenna Chl *a* molecules before the migration of excitation to the reaction centres (RCs) (Krause and Weis 1991). F_0 was measured at 20 μs when all RCs are open. F_0 values of Atay-85 increased with increasing B concentrations 5 and 9 d after onset of treatments (Fig. 4*A*,*B*). The increase in F_0 value was significant in Bolal-2973 after 7.5 and 10 mM B treatments at both durations (Fig. 4*C*,*D*). F_0 is considered to be a measure for the initial distribution of energy and the efficiency of excitation capture in PSII. Value of F_0 increases when the number of functional Chls not connected to the RCs of PSII increases. An increase in F_0 can be interpreted as a physical separation of LHCII from the PSII core complexes and an irreversible damage to PSII (Čajánek *et al.* 1998). A higher increase in F_0 was observed in Atay-85 compared to Bolal-2973 under all toxic B concentrations at both stress durations (Fig. 4). This result indicated that the highly toxic B treatments exceeded the photoprotective capacity and thus resulted in a higher degree of damage in Atay-85 compared to Bolal-2973.

Table 2. The growth parameters of wheat cultivars exposed to B toxicity after 5 and 9 d of boric acid (H_3BO_3) treatment. Each value represents the mean ± SD of six independent replicates. Values that are indicated with *different letters* in a column are significantly (P<0.05) different from each other according to the *Tukey*'s test in conjunction with *ANOVA*.

Cultivars	Duration [d]	Treatment [mM(H_3BO_3)]	Root length [mm per plant]	Shoot length [mm per plant]	Total fresh mass [mg per plant]	Total dry mass [mg per plant]
Atay-85	5	0 (control)	183 ± 2^{a}	255 ± 8^{a}	278 ± 5^{a}	28.0 ± 0.5^{a}
		5	173 ± 5^{b}	253 ± 2^{a}	269 ± 2^{b}	27.2 ± 0.4^{a}
		7.5	169 ± 3^{b}	237 ± 5^{b}	259 ± 5^{c}	25.9 ± 0.5^{b}
		10	165 ± 3^{b}	232 ± 4^{b}	254 ± 2^{c}	24.8 ± 0.3^{c}
	9	0	199 ± 5^{a}	313 ± 8^{a}	310 ± 2^{a}	29.1 ± 0.7^{a}
		5	182 ± 4^{b}	297 ± 6^{b}	270 ± 5^{b}	28.4 ± 0.2^{a}
		7.5	174 ± 3^{bc}	293 ± 8^{b}	252 ± 5^{c}	26.4 ± 0.6^{b}
		10	169 ± 3^{c}	275 ± 9^{c}	234 ± 4^{d}	24.5 ± 0.3^{c}
Bolal-2973	5	0	207 ± 9^{a}	322 ± 12^{a}	316 ± 6^{a}	30.2 ± 0.2^{a}
		5	195 ± 4^{b}	309 ± 6^{b}	306 ± 2^{b}	29.8 ± 0.5^{ab}
		7.5	194 ± 4^{b}	308 ± 7^{b}	301 ± 2^{bc}	29.0 ± 0.6^{b}
		10	191 ± 5^{b}	307 ± 6^{b}	294 ± 3^{c}	27.8 ± 0.4^{c}
	9	0	216 ± 4^{a}	402 ± 3^{a}	343 ± 4^{a}	31.3 ± 0.3^{a}
		5	207 ± 5^{b}	395 ± 2^{b}	325 ± 3^{b}	30.1 ± 0.1^{b}
		7.5	199 ± 2^{bc}	393 ± 2^{b}	312 ± 4^{c}	29.7 ± 0.2^{b}
		10	196 ± 2^{c}	391 ± 1^{b}	298 ± 2^{d}	28.1 ± 0.5^{c}

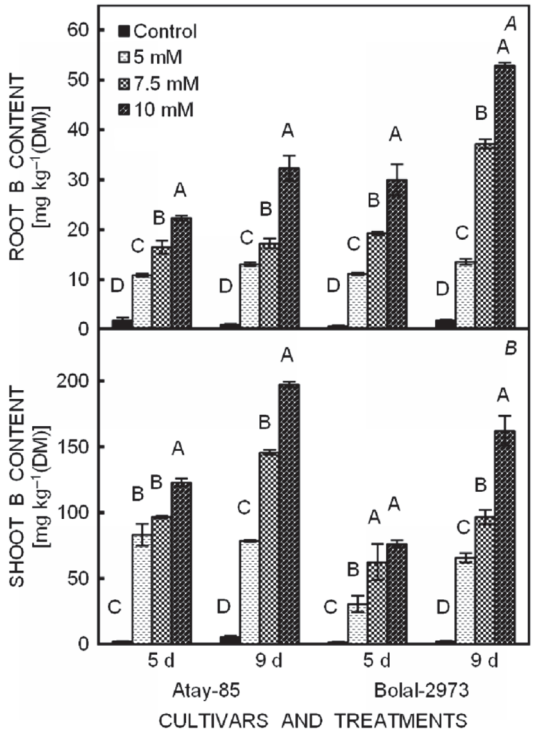

Fig. 2. The B content of root (*A*) and shoot (*B*) tissues of wheat cultivars exposed to B toxicity after 5 and 9 d of 5, 7.5, and 10 mM H_3BO_3 treatment. Columns and vertical bars represent mean values and SD, respectively (n = 6). Columns that are indicated with *different letters* are significantly (P<0.05) different from each other according to the *Tukey*'s test in conjunction with an *ANOVA*.

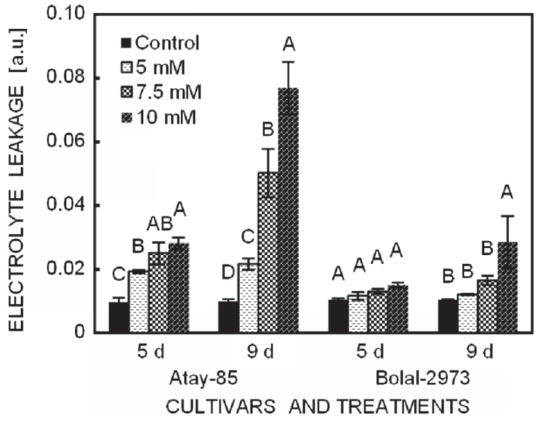

Fig. 3. The electrolyte leakage in leaves of wheat cultivars exposed to B toxicity after 5 and 9 d of 5, 7.5, and 10 mM H_3BO_3 treatment. Columns and vertical bars represent mean values and SD, respectively (n = 6). Columns that are indicated with *different letters* are significantly (P<0.05) different from each other according to the *Tukey*'s test in conjunction with an *ANOVA* (a.u. – arbitrary units).

The ratio of $TR_0/ABS = \varphi_{P0} = 1 - F_0/F_M = F_V/F_M$ is the most widely used PSII efficiency indicator (Thach *et al.* 2007) and describes the maximum quantum yield of the primary photochemical reaction (Maxwell and Johnson 2000). The TR_0/ABS value of Atay-85 decreased more drastically than that of Bolal-2973 (Fig. 4). Application of 10 mM H_3BO_3 caused 10.5% reduction in TR_0/ABS in Atay-85, and 5.86% reduction in Bolal-2973 after 9 d of B treatment. The severity of the decrease in TR_0/ABS suggests that the donor side is relatively more affected than the acceptor side. In the present study, reduction of TR_0/ABS ratio was attributed to an important increase in F_0 recorded in both cultivars subjected to B treatments. However, this was neither at a level that was damaging nor indicative of cessation of PSII activity in both cultivars. The ratio of $ET_0/ABS = (1 - F_0/F_M) \times (1 - V_J) = \varphi_{E0} = \varphi_{P0} \times \psi_0$ represents the quantum yield of electron transport beyond Q_A of PSII. Quantum yield of electron transport of PSII in Bolal-2973 decreased significantly only after 5 mM B treatment at both stress durations. Similar response was determined in Atay-85 only after 5 d of treatment. On the other hand, ET_0/ABS of Atay-85 decreased significantly with increasing B concentration after 9 d.

Quantum yield for reduction of end acceptors at PSI electron acceptor side (RE_0/ABS) of Atay-85 decreased with increasing B concentrations after 5 d of stress. This response was also significant after 9 d of treatment. The RE_0/ABS value of Bolal-2973 increased significantly only under highly toxic B concentrations (7.5 and 10 mM B) after 5 d of treatment compared with control. There was no significant change in the RE_0/ABS at 9 d in Bolal-2973 when compared with control. B stress also reduced photosynthetic electron transport by decreasing the electron flux per RC between the PSI and PSII. It caused a remarkable decrease in RE_0/ET_0, the reduction of end acceptors at PSI acceptor side, only in the 9 d-treated plants of Atay-85. On the other hand, RE_0/ET_0 in Bolal-2973 was not affected with B toxicity at both stress durations. It was concluded that B stress resulted in a decrease in the efficiency of electron transfer from Q_A^- to Q_B, indicating an inhibition of electron transport after Q_A^-. Effective antenna size of an active RC (ABS/RC) can be expressed as the total number of photons absorbed by the Chl molecules divided by the total number of active RCs, and therefore a measure of the average antenna size is obtained (Strasser *et al.* 2004). However, the photons are absorbed by Chl molecules associated with both active and inactive RCs. The ABS/RC of Atay-85 increased with increasing B concentrations after 5 d of treatment compared with control, and this response was more pronounced after 9 d. On the other hand, there were no significant variations in the ABS/RC after 5 d in Bolal-2973 when compared with the control. The ABS/RC value of Bolal-2973 increased significantly only after 9 d. The significant increase in ABS/RC of Atay-85 might indicate a decrease in the antenna size and could result from PSII inactivation and excitation energy transfer from inactive PSII to active PSII units. This response was determined in Bolal-2973 only after 9 d of stress period.

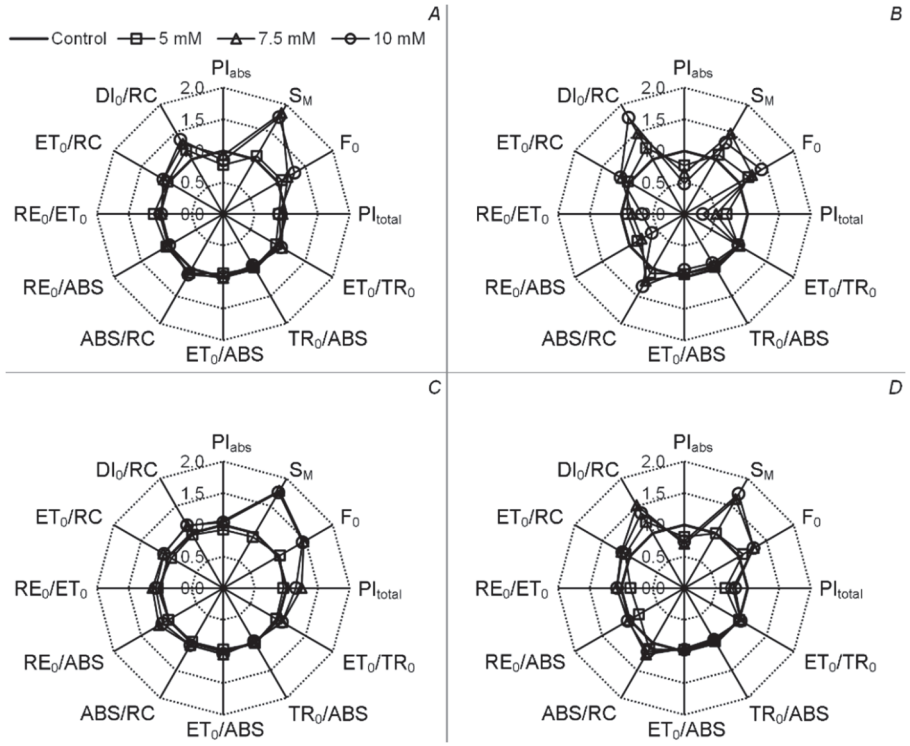

Fig. 4. The radar-plot presentation of selected JIP test parameters quantifying the photosynthetic efficiencies of dark-adapted leaves of Atay-85 (*A*,*B*) and Bolal-2973 (*C*,*D*) exposed to B toxicity after 5 d (*A*,*C*) and 9 d (*B*,*D*) of 5, 7.5, and 10 mM H_3BO_3 treatment. Mean values of parameters were plotted relative to their respective control values ($n = 6$).

Dissipation refers to the loss of absorbed energy by heat and fluorescence emission and energy transfer to systems other than electron transport. It is represented by the equation, $DI_0/RC = (ABS/RC) - (TR_0/RC)$, where ABS/RC is the effective antenna size of an active RC and TR_0/RC is the maximal trapping rate of PSII (Strasser *et al.* 2000). The dissipation per RC (DI_0/RC) describes the ratio of the total dissipation of untrapped excitation energy from all RCs to the number of active RCs (Strasser *et al.* 2000, Eullaffroy *et al.* 2009). In our study, an activation of dissipation process (increase of DI_0/RC) was observed in both cultivars at 9 d of stress compared to 5 d under high toxic B concentrations. The DI_0/RC value of both cultivars increased with increasing B concentrations compared with control at both treatment durations. But this increase was more pronounced in Atay-85 compared to Bolal-2973. Additionally, alteration of DI_0/RC value in Bolal-2973 under highly toxic B treatments was found to be insignificant at the end of the treatment period. An increase in the effective dissipation of an active RC (DI_0/RC) reflects the loss of connectivity that trapped energy does not go to RC and is dissipated. When the excitation energy in the antenna of the RCs is in excess of required for trapping, excess energy is probably dissipated as heat. In Atay-85, the reductions in F_V/F_M and RE_0/ET_0 were correlated with an increase in DI_0/RC suggesting that B treatments induced the dissipation of damaging excess energy.

Strauss *et al.* (2006) reported that the performance index (PI) can be a suitable parameter for evaluating a large number of genotypes for stress tolerance. In the current study, the effect of B toxicity on PSII activity was quantified by the values for PI_{abs} and PI_{total}. The PI is the overall expression of the three functional steps (energy, absorption energy trapping, and energy conversion into the electron transport). The PI_{total}, measuring the performance up to the PSI end electron acceptors, is the combination of all the parameters mentioned above (Strauss *et al.* 2006). Therefore, the changes in all the OJIP parameters are reflected in the expression of PI_{total}. The effect of B treatment for 5 d on PI_{abs} and PI_{total} values was not found to be significant among both cultivars. On the other hand, B stress promoted a significant reduction in PI_{abs} and PI_{total} of Atay-85 under all treatments compared with the control plants, while it had no remarkable effect on PI_{abs} and PI_{total} of Bolal-2973 among B treatments (Fig. 4). Analysis and evaluation of polyphasic Chl fluorescence parameters provided distinction for tolerance to B toxicity where the tolerant wheat cultivar Bolal-2973 exhibited a better photosynthetic performance compared to the sensitive cultivar Atay-85.

Total electron carriers per RC (S_M) of both cultivars exhibited similar behaviour in comparison with controls under B treatments. Toxic B concentrations (7.5 and 10 mM B) increased S_M values in both cultivars at 5 and 9 d of stress duration compared to corresponding controls. Toxic effects of 5 mM B did not significantly alter the S_M values in both cultivars.

Overall, it was found that B toxicity caused retardation of growth in wheat cultivars, specifically in the sensitive cultivar, Atay-85, employed in this study. Additionally, total B contents of roots and shoots indicated a possible restriction of B transport to shoots in the tolerant cultivar, Bolal-2973, to avoid toxic effects of B in above ground tissues. Moreover, remarkable and more pronounced

fluctuations in the values of fluorescence parameters were determined in Atay-85 compared to Bolal-2973 under all B treatments. Photosynthetic performance of Atay-85 determined by JIP test was affected negatively under B toxicity. Bolal-2973 cultivar can be successfully grown in B-rich areas. In conclusion, evaluation of photosynthetic performance by JIP test as well as assessment of growth and tissue B content might be used to determine the effects of B toxicity in wheat.

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