TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2020) 44: 153-166
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doi:10.3906/bot-1910-26

Acquisition of boron tolerance by salt pretreatment in two sunflower cultivars

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Received: 20.10.2019 • **Accepted/Published Online:** 21.02.2020 • **Final Version:** 17.03.2020

Abstract: This study was carried out to investigate the ameliorative effects of salt pretreatment against B toxicity in two sunflower cultivars (*Helianthus annuus* L. 'Sanbro' and 'Tarsan-1018') differing in salt tolerance. Seedlings were grown in perlite with modified half-strength Hoagland's solution for 15 days and then they were divided into two groups: salt-pretreated (75 mM NaCl for 5 days) and B-treated (control, 2, 4, and 8 mM B for 10 days). In both cultivars, the biomass of root and shoot decreased depending on B accumulation, especially at 8 mM. The translocation factor values indicated that B uptaken by roots of the genotypes were translocated to the leaves. High B accumulation adversely affected the water balance and membrane integrity of the leaves. Additionally, toxic B levels caused changes in the some JIP tests and slow fluorescence parameters (ABS/RC, TR_0/RC, ET_0/RC, RE_0/RC, DI_0/RC, Area, φ_{E0}, φ_{R0} and j_{D0}, ΦPSII, ETR) of both cultivars and these changes led to a significant decrease in photosynthetic performance (PI_{ABS} and PI_{TOTAL}). Salt pretreatment ameliorated the damaging effects of toxic B on membrane integrity, water content, and the photosynthetic process; decreased B accumulation; and improved the membrane stability. Both cultivars acquired tolerance against B toxicity with salt pretreatment and survived in increasing boron toxicity. We conclude that sunflower can be used for phytoremediation purposes for boron-contaminated soils. Additionally, this study is the first report to reveal that moderate salt stress pretreatment alleviates B toxicity and provides tolerance to B. This alleviation might be achieved by NaCl to decrease the boron uptake from the roots.

Key words: Boron toxicity, JIP test, photochemical activity, salt pretreatment, slow and fast chlorophyll *a* fluorescence, sunflower (*Helianthus annuus* L.)

1. Introduction

Boron (B) is an essential micronutrient for growth at low concentrations and the optimum concentration range of plant-available B is very narrow (Brown et al., 2002; Miwa and Fujiwara, 2010; Tomić et al., 2015; Landi et al., 2019). For this reason, it can be toxic for plants when its concentration in the rhizosphere exceeds a given threshold value (Bañón et al., 2012; Landi et al., 2012). B is involved in important physiological and biochemical functions including nucleic acid, carbohydrate, phenol, and protein metabolism; cell wall synthesis and stability; membrane integrity and function; and photosynthesis, leaf expansion, root elongation, and flower and fruit development. These functions are negatively affected by toxic concentrations (Ayvaz et al., 2016; Uluisik et al., 2018).

B exists primarily as boric acid (H_3BO_3) in soil and can be easily leached under heavy rainfall conditions (Shorrocks, 1997; Öz et al., 2014). On the other hand, under low rainfall conditions, B is not sufficiently leached from the rhizosphere and may consequently accumulate to toxic levels (Reid, 2010). High concentrations of B may accumulate both naturally in the soil, derived from marine evaporites and marine argillaceous sediments, and also from various anthropogenic sources such as fertilizers, irrigation water, mining, fly ash, and industrial chemicals (Nable et al., 1997). Although in many parts of the world natural B levels in the soil are insufficient for potential crop production, B toxicity is a crucial problem that significantly limits crop yield in agricultural areas of Australia, North Africa, and West Asia, which all have alkaline and saline soils (Ben-Gal and Shani, 2002; Camacho-Cristóbal et al., 2008). In addition to that, B toxicity in arid and semiarid soils is a major problem for plant development (Nable et al., 1997; Ayvaz et al., 2016), like salinity, which is a major abiotic stress for plant growth and crop productivity in both irrigated and nonirrigated lands. High salt concentrations cause ion imbalance (increase in Na^+, Cl^-, B, etc.) and hyperosmotic stress in plants (Gondim et al., 2012; Negrao et al., 2017; Munns et al., 2020). B is removed more slowly than Na^+, Cl^-, and SO_4^{2-} ions during leaching; therefore, it accumulates in higher concentrations in association with saline soil (Nable et al., 1997). Recent reports revealed that

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there is an interaction between salinity and B for some plants (Alpaslan and Gunes, 2001; Yermiyahu et al., 2008; Masood et al., 2012; Rodríguez-Hernández et al., 2013; Farooq et al., 2015; Liu et al., 2018; Zhen et al. 2019). Most of these studies showed that B and salinity when simultaneously administered have an antagonistic effect on plants. Besides that, there are no reports available on the effects of salt pretreatment in relation to B toxicity.

Sunflower (*Helianthus annuus* L.) is one of the most important and largest sources of agricultural oil-seed crops in the world due to its high content of unsaturated fatty acids (Gholinezhad et al., 2015; Umar et al., 2019). While the world sunflower production shows a fluctuating pattern since 2010, reports show that approximately 47 million tons of sunflower seeds were produced in 2016 (http://www.fao.org). Although sunflower is grown globally and performs well in most temperate climates (Seiler et al., 2017), the development of this plant is largely affected by excess B and/or salt (Liu and Shi, 2010; Tassi et al., 2017). The purpose of this study was to understand whether or not salt pretreatment had an ameliorative effect on B toxicity in two sunflower varieties with different salt tolerance levels by means of growth and photosynthetic performance.

2. Materials and methods

2.1. Plant materials, growth, and treatment conditions

Two sunflower cultivars [*Helianthus annuus* L.; the salt-sensitive Sanbro and the salt-tolerant Tarsan-1018, (Mutlu, 2003)] were used in this study. The seeds of the two cultivars were provided by the Trakya Agricultural Research Institute of Turkey. Seeds were surface-sterilized [5% (v/v) sodium hypochlorite (NaOCl) solution for 3 min] and imbibed in distilled water for 2 h. After incubation, 5 seeds were sown in plastic pots (14 cm in diameter and 13 cm in height) filled with perlite. The pots were watered every other day with half-strength Hoagland's solution. The plants were grown in a controlled growth chamber, with a temperature regime of 25 ± 1 °C, a 16-h photoperiod, 40 ± 5% humidity, and 200 μmol m^{-2} s^{-1} light intensity.

The salt pretreatment concentration was determined in preliminary studies as 75 mM. Fifteen-day-old seedlings were exposed to four treatments as follows: The first group of plants (C) was kept for control purposes. The second group of plants (SP) was subjected to salt pretreatment (75 mM NaCl for 5 days). The third group of plants (B) was subjected to only different concentrations of boron (2, 4, and 8 mM H_3BO_3) for 10 days with half-strength Hoagland's solution. The last group of plants (SP-B) was subjected to boron treatments followed by SP. At the end of the experiments, plants were harvested for further analyses.

2.2. Growth parameters

Shoot (stem + leaf) and root lengths of the sunflower seedlings were measured (cm $plant^{-1}$) and then these parts of three plants from each group were taken randomly to determine the fresh weights (g $plant^{-1}$). They were kept at 80 °C in an oven for 48 h to measure their dry weights (g $plant^{-1}$).

2.3. Water content of the leaves

Leaf discs [(R = 1 cm in the middle of the leaf) from each treatment and 4 replications] were used to determine the water status of the leaves. To determine the relative water content (RWC) of leaves according to Farrant (2000), the following formula was used: [(FW – DW)/(HW – DW) × 100], where FW, HW, and DW are the leaf disc fresh, hydrated, and dry weights, respectively. The hydrated weight of discs was measured after 24 h of immersion in distilled water at room temperature. Dry weight of discs was recorded gravimetrically after 48 h at 80 °C in an oven. The leaf water potential (Yw) was measured by a WP4 Dewpoint Potential Meter (WP4-T/Operator's Manual Version 2.2, Decagon Devices, Inc.).

2.4. Boron content

After harvesting, the seedlings were washed three times in deionized water and then the leaf, stem, and root tissues were collected separately and dried at 80 °C for 48 h. The dried tissues were ground to a powder and dried powder samples were burned in a muffle furnace at 550 °C for 5 h. Then the samples were digested with 1 mL of concentrated HNO_3 for 15 min. The extracts were transferred to volumetric flasks and 25 mL of distilled deionized water was added. After 30 min, the samples were filtered using Whatman filter paper and stored in plastic vials until analyzed. The B content (mg kg^{-1} DM) in the tissues was quantified using inductively-coupled plasma-atomic emission spectroscopy analysis (ICP-AES, IRIS Intrepid, Thermo Elemental, USA), after 0.2 g of dried samples were ashed at 550 °C for 5 h and dissolved in 0.1 N HNO_3. Subsequently, B translocation factor (TF) and total B accumulation (TA) of the root and shoot (stem + leaves) were calculated (ppm per $plant^{-1}$) were determined from the obtained data according to Al Chami et al. (2015) with minor modification. TF, which is described as the ratio of B concentration in shoots to that in roots, was used to estimate the translocation of B from roots to aboveground parts of plants.

2.5. Membrane stability

Membrane damage in the leaf tissues [5 leaf disks (R = 1 cm) of each treatment and 4 replications] of cultivars was measured as leakage of UV-absorbing substances according to the method of Redmann et al. (1986). Leaf discs were cut and put into tubes containing distilled water, which were shaken for 24 h. The discs were then transferred to liquid nitrogen and the absorbance values

of the solutions at 24 h before and after incubation in liquid nitrogen for 20 min were measured using a UV-Vis spectrophotometer at 280 nm. The relative leakage ratio (RLR) was calculated using the following equation: RLR = A280/A′280, where A280 and A′280 are the first and the last absorbance values at 280 nm, respectively.

2.6. Photosynthetic pigment analyses

The middle leaf part was used to determine the photosynthetic pigments [chlorophylls (a + b), chlorophyll a/b ratio, and carotenoids (x+c)]. Leaf disks [1 leaf disk (R = 1 cm) of each treatment and 6 replications] were extracted in 100% acetone and centrifuged at 3500 rpm for 5 min. Absorbance of the sample extracts was recorded at 470, 644.8, and 661.6 nm using a Shimadzu Mini-1240 UV-Vis spectrophotometer. The contents of pigments were calculated using adjusted extinction coefficients (Lichtenthaler, 1987).

2.7. Chlorophyll (Chl) a fluorescence measurements

2.7.1. Slow Chl a fluorescence measurements

Chl a fluorescence measurements were performed with a portable, modulated fluorescence monitoring system (FMS-2, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) on the fully expanded leaves of the sunflower genotypes, which were dark-adapted using leaf clips for at least 30 min. The initial (minimum) Chl fluorescence (F_O) and the maximum fluorescence (F_M) were recorded using a measuring beam (0.2 μmol photons m^{-2} s^{-1}) and a saturating actinic light pulse (PPFD of 7500 μmol photons m^{-2} s^{-1} for 1 s), respectively. Light-induced changes in Chl fluorescence following actinic illumination (300 μmol photons m^{-2} s^{-1}) were determined prior to the measurement of F_O' (min. Chl fluorescence in light saturated state) and F_M' (max. fluorescence in light-saturated state). The actual photochemical efficiency of the PSII photochemistry in the light-adapted state [ΦPSII = $(F_M' - F_S)/F_M'$] was calculated from F_M' and F_S values according to Genty et al. (1989). After the actinic light was switched off, the leaves were illuminated by far-red light (7 μmol photons m^{-2} s^{-1}) to oxidize the photosynthetic electron transport chain and to determine the accurate min. F_O'. The electron transport rate at the given PAR (ETR) was determined from the following equation: ΦPSII × PAR × 0.84 × 0.5 (Genty et al., 1989).

2.7.2. Fast (polyphasic) Chl a fluorescence measurements

The polyphasic OJIP fluorescence transient was measured with a Handy PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) fluorimeter. All measurements were conducted on dark-adapted (for 30 min) leaves. The measurement consisted of a single strong 1-s light pulse (peak 650 nm, 3000 μmol m^{-2} s^{-1}, an excitation intensity sufficient to ensure the closure of all PSII reaction centers) provided by an array of three light-emitting diodes. Photoinduced polyphasic Chl a fluorescence transients (OJIP), flux ratios or quantum yields (φ_{E0}, quantum yield for the electron transport from Q_A^- to PQ; φ_{D0}, quantum yield of energy dissipation; RE_0/ABS, the quantum yield of electron transport from Q_A^- to the PSI end electron acceptors), specific energy fluxes (ABS/RC, apparent antenna size of an active PSII; TR_0/RC, maximum trapped exciton flux per active PSII; ET_0/RC, the flux of electrons transferred from Q_A^- to PQ per active PSII; RE_0/RC, the electron transport from Q_A^- to end electron acceptors at the PSI acceptor side; DI_0/RC, the flux of energy dissipated in processes other than trapping per active PSII), and photosynthetic performance indexes [PI_{TOTAL}, total performance index, indicator of the overall functional activity of photosystems and intersystem electron transport chain; PI_{ABS}, performance index, an indicator of PSII functional activity; 10RC/ABS, the density of the active photosystems; $\varphi_{P0}/(1 - \varphi_{P0})$, the efficiency of the primary photochemistry or trapping; $\psi_0/(1 - \psi_0)$, the capacity of electron transport to proceed beyond Q_A^-; $d_{R0}/(1 - \delta_{R0})$, the efficiency of intersystem electron transport to PSI end electron acceptors], and some other parameters (Area, the area above the induction curve of chlorophyll fluorescence; ΔV_{IP}, the amplitude of relative variable fluorescence of I–P) were calculated and specific characteristics of the light phase of photosynthesis were analyzed according to the JIP test as described in detail by Tsimilli-Michael et al. (2000), Strasser et al. (2004, 2010), and Goltsev et al. (2016).

2.8. Statistical data analysis

The experiments were performed in a completely randomized design with 3 replicates using SPSS 20.0 (IBM Corp., Armonk, NY, USA) to determine the differences between the cultivars and the treatments. Statistical variance analysis was performed using ANOVA and the results were compared with the least significant difference (LSD) and Tukey's test at the 5% level.

3. Results

3.1. Growth parameters

Shoot and root lengths of the two cultivars significantly decreased with increasing B concentrations (approx. 10%–43% and 4%–40%, respectively), except for the root length of Tarsan-1018 at 2 mM B (Table 1). It was observed that the shoot length of Tarsan-1018 and the root lengths in both cultivars were significantly higher in all SP-B treatments compared to their B treatments (about 6%–13% and 9%–34%, respectively). Also, fresh and dry weights of the shoots and roots declined by 12%–43% and 10%–43%, respectively, with increasing B concentrations in both cultivars. Fresh and dry weights of shoots and roots were higher in Tarsan-1018 than in Sanbro. Increased B concentration caused growth inhibition in sunflower

Table 1. The growth parameters of the sunflower cultivars exposed to salt pretreatment (SP) and/or B treatments. Different letters mean a significant difference between the treatments ($P < 0.05$, Tukey's test, ANOVA).

Cultivars	Treatment	Length of shoot (cm plant^{-1})	Length of root (cm plant^{-1})	Fresh weight of shoot (g plant^{-1})	Fresh weight of root (g plant^{-1})	Dry weight of shoot (g plant^{-1})	Dry weight of root (g plant^{-1})
Sanbro	Control	16.2* ± 0.3^{a}	15.7** ± 0.2^{a}	6.1** ± 0.1^{a}	3.0** ± 0.1^{a}	0.64** ± 0.01^{a}	0.14** ± 0.00^{a}
	SP	15.4 ± 0.2^{b}	15.6 ± 0.3^{a}	5.6 ± 0.1^{ab}	2.8 ± 0.1^{a}	0.58 ± 0.01^{b}	0.13 ± 0.00^{b}
	2 mM B	14.5 ± 0.2^{c}	13.1 ± 0.4^{b}	5.3 ± 0.3^{b}	2.6 ± 0.0^{b}	0.52 ± 0.01^{c}	0.10 ± 0.00^{c}
	SP-2 mM B	14.3 ± 0.5^{c}	15.1 ± 0.2^{a}	5.5 ± 0.1^{b}	2.7 ± 0.0^{b}	0.55 ± 0.01^{d}	0.11 ± 0.00^{d}
	4 mM B	12.4 ± 0.2^{d}	11.2 ± 0.1^{c}	4.4 ± 0.1^{c}	2.0 ± 0.0^{c}	0.42 ± 0.00^{ef}	0.09 ± 0.00^{e}
	SP-4 mM B	12.5 ± 0.3^{d}	13.4 ± 0.2^{b}	4.5 ± 0.1^{c}	2.2 ± 0.0^{c}	0.44 ± 0.00^{e}	0.10 ± 0.00^{c}
	8 mM B	10.5 ± 0.2^{e}	8.7 ± 0.2^{d}	4.1 ± 0.1^{c}	1.7 ± 0.0^{d}	0.40 ± 0.01^{f}	0.06 ± 0.00^{f}
	SP-8 mM B	10.2 ± 0.1^{e}	9.5 ± 0.3^{d}	4.1 ± 0.1^{c}	1.8 ± 0.0^{d}	0.41 ± 0.01^{f}	0.08 ± 0.00^{g}
Tarsan-1018	Control	21.0 ± 0.2^{a}	15.2 ± 0.2^{a}	7.1 ± 0.1^{a}	3.1 ± 0.1^{a}	0.78 ± 0.00^{a}	0.15 ± 0.00^{a}
	SP	14.9 ± 0.3^{b}	15.2 ± 0.3^{a}	6.8 ± 0.2^{b}	3.1 ± 0.1^{a}	0.62 ± 0.01^{be}	0.15 ± 0.00^{a}
	2 mM B	14.8 ± 0.3^{b}	14.6 ± 0.4^{a}	5.3 ± 0.0^{c}	2.8 ± 0.0^{bd}	0.57 ± 0.01^{c}	0.14 ± 0.00^{b}
	SP-2 mM B	15.7 ± 0.3^{c}	15.2 ± 0.3^{a}	6.7 ± 0.1^{b}	3.1 ± 0.0^{a}	0.64 ± 0.01^{b}	0.15 ± 0.00^{a}
	4 mM B	11.8 ± 0.2^{d}	13.1 ± 0.3^{b}	4.8 ± 0.2^{d}	2.6 ± 0.0^{cdf}	0.44 ± 0.01^{d}	0.11 ± 0.00^{c}
	SP-4 mM B	13.9 ± 0.2^{e}	14.5 ± 0.1^{a}	5.9 ± 0.0^{e}	2.7 ± 0.0^{df}	0.61 ± 0.01^{e}	0.12 ± 0.01^{d}
	8 mM B	10.5 ± 0.2^{f}	11.2 ± 0.2^{c}	4.0 ± 0.1^{f}	2.2 ± 0.0^{e}	0.40 ± 0.00^{f}	0.10 ± 0.00^{e}
	SP-8 mM B	11.9 ± 0.2^{d}	13.4 ± 0.3^{b}	5.3 ± 0.0^{c}	2.7 ± 0.0^{f}	0.55 ± 0.00^{c}	0.12 ± 0.00^{d}
LSD 5%		0.74	0.94	0.58	0.16	0.03	0.01

*: Each value represents the mean of 9 replicates (n = 9) and its standard error (±SE).
**: Each value represents the mean of 3 replicates (n = 3) and its standard error (±SE).

cultivars as determined in the dry weights of shoot and root (about 19%–49% and 7%–56%, respectively) (Table 1). The reduction in the biomass of both cultivars was observed in all B treatments compared to controls. B-induced shoot biomass reduction was significantly ameliorated by SP applications in Tarsan-1018, especially at highly toxic B concentrations, compared to Sanbro. This amelioration effect of the SP was found to be significant for the root biomass of both cultivars.

3.2. Water content of leaves

While the leaf water potential of both cultivars gradually decreased for all B applications, SP treatments maintained the leaf water potential of cultivars at control levels up to SP-8 mM B treatment (Figure 1A). RWC was diminished significantly due to the increased toxic B concentrations (more than 9%), but it was significantly higher in the SP-B treatments (Figure 1B). Leaf water potential and the RWC of both cultivars were significantly decreased in high B-treated groups while they were maintained at control levels in salt-pretreated (SP) groups (Figures 1A and 1B).

3.3. Boron contents

B contents of shoots and roots were evaluated by the determination of total B accumulation (TA) and translocation factor (TF) in all treatment groups (Table 2). TA in shoots and roots of both cultivars increased with increasing B concentration in nutrient solution. The shoot accumulation (leaves > stems) was higher than the root accumulation at all B treatments. While Sanbro significantly accumulated higher B in the shoot (e.g., approx. 18-fold at 8 mM B) than Tarsan-1018 (approx. 13-fold) compared with those of the controls, the root B content was much higher in Tarsan-1018 (e.g., about 33-fold at the highest B level) than in Sanbro (e.g., about 21-fold). B accumulation in shoots and roots of both cultivars was remarkably low in salt-pretreated groups (more than avg. 35% at highly toxic B levels). Both cultivars translocated large amounts of B, which were uptaken by roots and transferred particularly to leaves. TF of the cultivars was decreased in 4 mM B-treated groups, while it was increased with 8 mM B. With SP-B treatment, the TF of both genotypes was increased with increasing B concentrations. These results indicated that Sanbro has greater B transport capability (roots < shoots) than Tarsan-1018 in both applications (SP-B and B) (Table 2).

3.4. Membrane stability

Relative electrolyte leakage in both cultivars was increased with increasing B concentrations, whereas SP-B treatments

Table 2. Total B accumulation (TA) and the translocation factor (TF) of the sunflower cultivars exposed to toxic B with or without salt pretreatment.

Cultivars	Treatments	TA of leaves (mg kg^{-1} DW)	TA of stem (mg kg^{-1} DW)	TA of shoot (mg kg^{-1} DW)	TA of root (mg kg^{-1} DW)	TF
Sanbro	Control	100.9* ± 5.5	9.8 ± 0.5	110.7 ± 5.1	17.3 ± 0.9	6.5 ± 0.7
	Salt (NaCl)	95.9 ± 9.68	2.3 ± 0.1	98.1 ± 9.8	18.9 ± 0.2	5.2 ± 0.5
	2 mM B	1351.9 ± 38.3	245.2 ± 6.4	1597.1 ± 33.3	470.1 ± 4.9	3.4 ± 0.1
	SP-2 mM B	564.8 ± 21.1	94.8 ± 5.2	659.6 ± 22.9	134.3 ± 5.1	4.9 ± 0.1
	4 mM B	1456.9 ± 11.9	441.4 ± 9.0	1898.2 ± 9.0	667.0 ± 14.1	2.9 ± 0.1
	SP-4 mM B	912.7 ± 37.2	224.6 ± 14.3	1137.3 ± 47.9	170.7 ± 6.1	6.7 ± 0.1
	8 mM B	2551.9 ± 82.9	582.3 ± 19.8	3134.2 ± 69.2	853.4 ± 12.4	3.7 ± 0.1
	SP-8 mM B	1655.7 ± 60.9	473.7 ± 14.5	2129.4 ± 68.9	327.9 ± 16.0	6.5 ± 0.3
Tarsan-1018	Control	94.9 ± 6.2	7.2 ± 0.4	102.1 ± 6.0	22.1 ± 1.3	4.7 ± 0.5
	Salt (NaCl)	92.5 ± 5.7	4.4 ± 0.2	96.9 ± 5.6	24.9 ± 0.6	3.9 ± 0.3
	2 mM B	1146.0 ± 16.3	212.1 ± 2.3	1358.1 ± 17.4	551.0 ± 7.9	2.5 ± 0.1
	SP-2 mM B	378.6 ± 1.1	61.1 ± 5.1	439.7 ± 5.7	183.9 ± 3.1	2.4 ± 0.1
	4 mM B	1150.9 ± 52.6	275.1 ± 7.7	1426.0 ± 47.4	887.1 ± 6.8	1.6 ± 0.1
	SP-4 mM B	589.0 ± 14.5	177.6 ± 12.2	766.6 ± 16.2	250.6 ± 0.5	3.1 ± 0.1
	8 mM B	2151.1 ± 30.8	401.9 ± 15.2	2553.1 ± 40.9	1127.1 ± 34.7	2.3 ± 0.1
	SP-8 mM B	1230.1 ± 42.0	381.1 ± 8.1	1611.2 ± 49.9	513.6 ± 15.4	3.1 ± 0.1
LSD 5%		139.1	37.8	46.6	141.1	1.4

* Each value represents the mean of 3 replicates (n = 3) and its standard error (±SE).

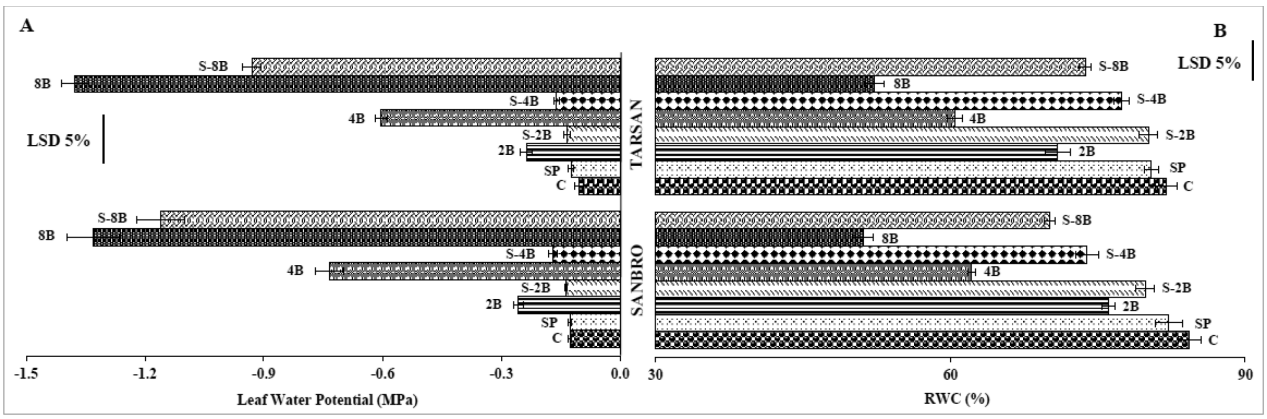

Figure 1. Effect of B treatments with or without salt pretreatment on the leaf water potential (A) and relative water contents (RWC) (B) in sunflower cultivars. Each data point is the average of three replicates for leaf water potential and four replicates for RWC, and the error bars represent the standard error (±SE). C, Control; SP, salt pretreatment; 2B, 2 mM H_3BO_3; S-2B, 75 mM NaCl + 2 mM H_3BO_3; 4B, 4 mM H_3BO_3; S-4B, 75 mM NaCl + 4 mM H_3BO_3; 8B, 8 mM H_3BO_3; S-8B, 75 mM NaCl + 8 mM H_3BO_3.

significantly decreased the levels of leakage (Figure 2). In the SP-B treatment, electrolyte leakage in leaves was lower than 30% compared to B applications and this value was at the control level only for SP-2 mM B for both cultivars. Therefore, SP treatment ameliorated B-induced cellular membrane damage of sunflower cultivars leaves. However, both cultivars showed similar electrolyte leakage responses in all treatments (B and SP-B) (Figure 2).

3.5. Photosynthetic pigment contents

Total chlorophyll contents (a + b) of the sunflower cultivars were significantly reduced in B-treated groups and the rate of decline was higher in Sanbro (approx. 50%) than

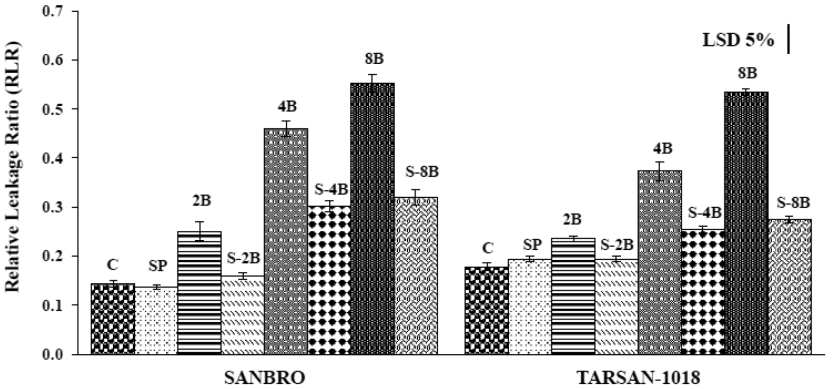

Figure 2. Electrolyte leakage in the leaves of sunflower cultivars exposed to salt pretreatment and/or B treatments. Each data point is the average of four replicates and the error bars represent the standard error (±SE). See Figure 1 for explanation of legends.

Tarsan-1018 (approx. 19%) at highly toxic B levels (Figure 3A). The chlorophyll contents of both genotypes were significantly decreased by B toxicity, thereby leading to chlorosis or necrosis in leaves. Salt pretreatment alleviated the adverse effect of B toxicity on the total chlorophyll content of both cultivars.

The decrease due to B toxicity in the chlorophyll a/b ratio, which is an indicator of the antenna size of PS I and PS II, was not generally significant in both cultivars. However, in the SP-B treatment, this ratio was higher only at SP-4 mM B in Sanbro and at SP-8 mM B in Tarsan-1018 among B applications (Figure 3B). Additionally, carotenoid contents were not changed significantly in both cultivars for all treatments (Figure 3C).

3.6. Chl a fluorescence

Photosynthetic apparatus could be substantially limited by stressful conditions and this limitation reflects on the status of plant physiology. To evaluate the effects of boron treatments (B and SP-B) on photosynthesis of the two sunflower cultivars, Chl a fluorescence measurements were performed. The values of the slow fluorescence parameters (ΦPSII and ETR) of both sunflower genotypes displayed significant variations under B and SP-B treatments compared to the controls (Figures 4A and 4B). Only 2 and 8 mM B treatments significantly decreased the actual photochemical efficiency of the PSII (ΦPSII) of Sanbro. On the other hand, almost all the treatments significantly increased the ΦPSII values in Tarsan-1018, except 8 mM B (Figure 4A). No significant changes were observed in the ETR of Sanbro, except those of 2 and 8 mM B treatments, which decreased the ETR. Almost all treatments led to a significant increase in the ETR of Tarsan-1018, except 8 mM B (Figure 4B).

The Kautsky curve is known as the fluorescence induction curve, which defines the characteristic changes in the fluorescence intensity when a dark-acclimated leaf is illuminated (Goltsev et al., 2016) and comprises fast (O-J-I-P curve) and slow phases. The shape of the polyphasic fluorescence rise reflects the changes in the redox state of photosystems, which in turn affects the quantum yield of fluorescence. This result indicates the changes in the OJIP curve's shape; B and SP treatments compared with the controls are clearly shown in Figures 5A and 5B. The OJIP curve for salt-treated plants exhibited a similar trend with the control. B treatments decreased the I-P amplitude, whereas salt pretreatment increased the amplitude in both sunflower cultivars. These results indicate that salt pretreatment alleviates the toxic effects of boron. Also, toxic B concentrations (4 and 8 mM B) more negatively influenced the IP phase of the curve as compared to other treatments.

As shown in Figures 6A–6D, salt pretreatment increased all of the photosynthetic capacity/efficiency values in both sunflower genotypes. The specific energy flux per reaction center (RC) for absorption (ABS/RC), trapped energy flux (leading to Q_A^- reduction) per RC (TR_0/RC), energy dissipation per RC (DI_0/RC), and quantum yield of energy dissipation (φ_{D0}) were significantly increased at 4 and 8 mM B and these parameters were lower in SP-B treatments compared to B applications in both cultivars (Figures 6A and 6B). The electron transport flux further than Q_A^- per RC (ET_0/RC) of both cultivars did not change significantly in all B treatments. However, quantum yield for the electron transport from Q_A^- to PQ (φ_{E0}), quantum yield for the reduction of PSI end electron acceptors per photon absorbed (RE_0/ABS), electron transport from Q_A^- to end electron acceptors at the PSI acceptor side (RE_0/RC), ΔV_{IP} as a measure for the leaf PSI content, and the size of the plastoquinone pool (Area) significantly decreased, especially at the more toxic 8 mM B level (Figures 6A and 6B).

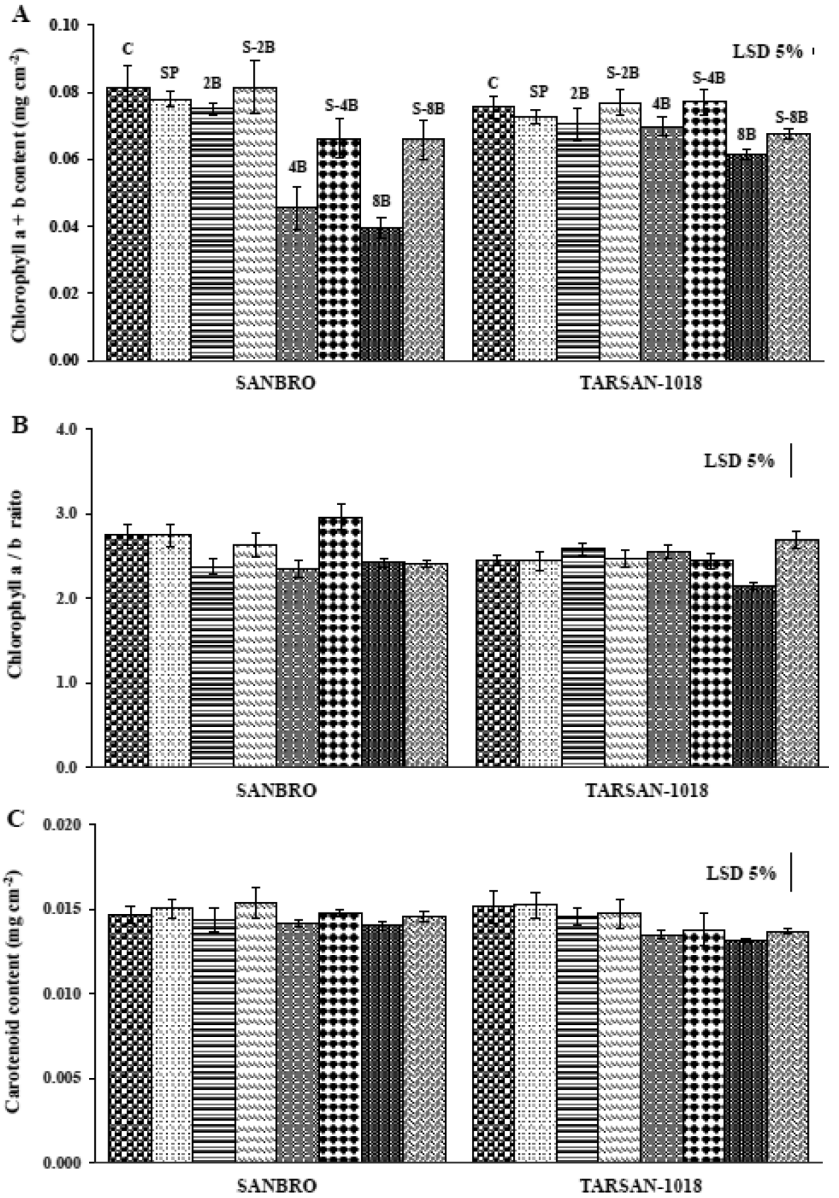

Figure 3. Changes in the pigment contents [chlorophyll a + b (A) and carotenoid (C) contents] and chlorophyll a/b ratio (B) of sunflower cultivars exposed to salt pretreatment and/or B treatments. Each data point is the average of six replicates and the error bars represent the standard error (±SE). See Figure 1 for explanation of legends.

Two photosynthetic performance indexes [PI_{ABS} and PI_{TOTAL} (the former is an indicator of PSII functional activity and the latter is the indicator of the overall photosynthetic activity from PSII to PSI)] decreased with increasing B concentrations in both cultivars (Figures 6C and 6D). The density of the active photosystems (10RC/ABS), the efficiency of the primary photochemistry or trapping [$\varphi_{P0}/(1 - \varphi_{P0})$], electron transport activity [$\psi_0/(1 - \psi_0)$], and the efficiency of intersystem electron transport to PSI end electron acceptors [$\delta_{R0}/(1 - d_{R0})$], which are the performance indexes' partial components, were significantly reduced, especially at the highest B treatment in both cultivars. According to these results, salt pretreatment alleviated the B toxicity in both sunflower cultivars (Figures 6C and 6D).

4. Discussion

Plants generally encounter various environmental stressors simultaneously in nature and the effect of one stress on plants can be modified by other cooccurring

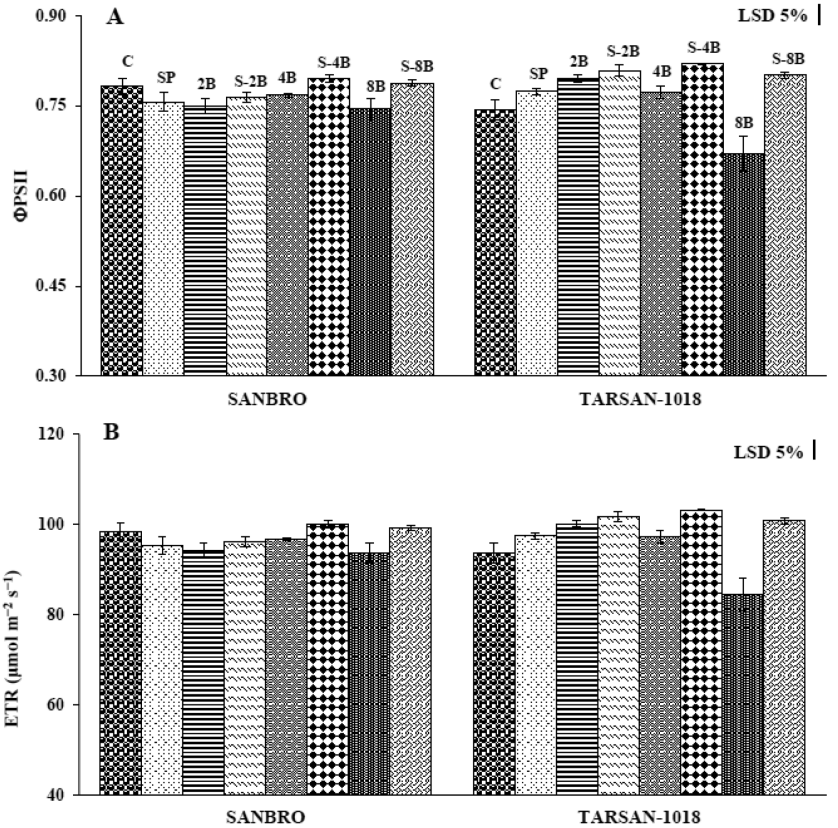

Figure 4. Effect of B treatments with or without salt pretreatment on the FPSII: the actual photochemical efficiency of PSII (A) and ETR; electron transport rate of sunflower cultivars (B). See Figure 1 for explanation of legends.

stresses (Mittler, 2006). It was proposed that exposure to one stress by pretreatment approach might result in better performance and increase survival rates of plants under other environmental stresses. Thus, the growth and photosynthetic responses of the two sunflower cultivars were studied in order to gain better insight into the mechanisms that define tolerance for B toxicity and determine the level of ameliorated effect of salt pretreatment on the adverse effects of B toxicity. B accumulation was found higher in shoots (leaves + stems) than in the roots, but the accumulation trend in different plant parts was similar in both cultivars with all B applications; i.e. all B applications caused accumulation (Table 2). B is taken from the soil in the form of boric acid by roots and is forwarded to the xylem for transport to the shoots (Safari et al., 2017; Uluisik et al., 2018). In this case, the results could explain that B is transported through the xylem by transpiration streams. The influx of B to the roots without energy consumption could explain the increment of B content in sunflower organs due to B applications. When the cultivars are compared, Sanbro had higher B content in the shoots, whereas Tarsan-1018 had higher B content in the roots. TF values supported that both cultivars transported B from the roots to the shoots and this translocation was higher in Sanbro as compared to Tarsan-1018. This observation indicated that Tarsan-1018 could reduce B intake to roots, restrict the transport of B to the leaves, and avoid toxic effects of B in aboveground organs. These results were consistent with earlier works conducted by Alparslan and Gunes (2001) and Öz et al. (2014). In addition, salt pretreatment (SP) decreased the B uptake and/or might enhance the B efflux and ameliorate the toxic effect of B in both cultivars (Table 2). It was suggested that B tolerance might be related to the ability to restrict B accumulation in both roots and shoots (Bonilla and González-Fontes, 2011). Salt pretreatment provided B tolerance in cultivars by limiting the B accumulation in tissues and organs in present study. There are also conflicting reports related to the interaction between the effects of salt stress and the toxic boron levels on plant metabolisms. It was found that salt stress decreased the toxic effect of boron on the growth of various plants (Ben-Gal and Shani, 2002; Edelstein et al., 2005). In the present study, we hypothesized that salt pretreatment at moderate levels could mitigate the toxic effects of B and provide tolerance against this stress in plants. The results

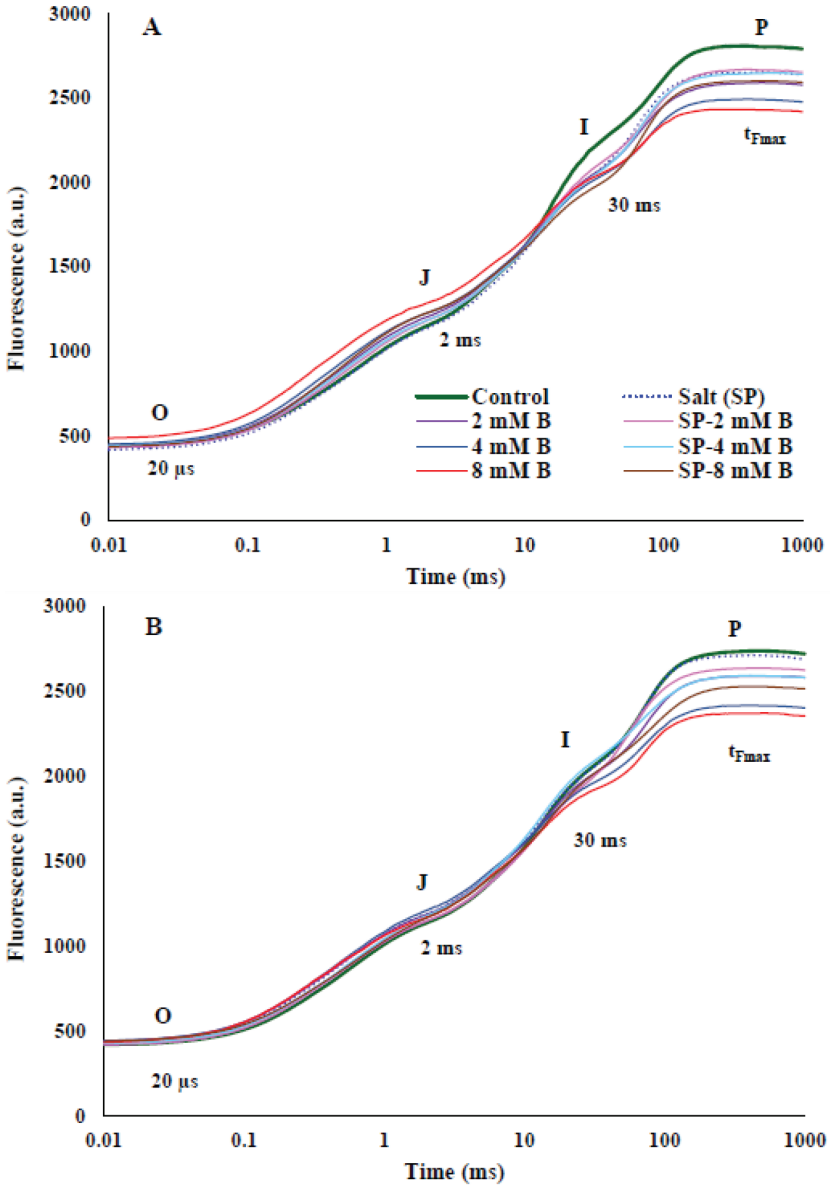

Figure 5. The polyphasic Chl a fluorescence rise OJIP of Sanbro (A) and Tarsan-1018 (B) recorded under B treatments with or without SP.

obtained indicate that the salt pretreatment mitigates the toxic effects of B.

Adesodun et al. (2010) studied the uptake and translocation of metals (Pb and Zn) in the shoots and roots of sunflower species grown in soil contaminated with metals. As a result of these studies, it was found that the TF for these metals was greater than 1. This is an indication of these metals having high mobility in these plants. Compatible with these results, TF values of both cultivars under B applications are greater than 1 in this study. These results suggest that B was easily transported in the plants, the phytoextraction capacity of sunflower could be high, and it may be able to clean toxic B from soils with higher B content. On the other hand, it was found that an increase of electrolyte leakage in the leaves was induced by B accumulation and the membrane leakage was higher in Sanbro compared to Tarsan-1018 (Figure 2). In addition to this, salt pretreatment reduced B accumulation and enhanced the membrane stability in both cultivars (Table 2; Figure 3). Bastias et al. (2004) reported that B is uptaken from plant roots by passive or active processes via BOR transporters and aquaporin. Bastias et al. (2004) also suggested that the functionality of aquaporins and/or channel-mediated water permeation decreased in NaCl-

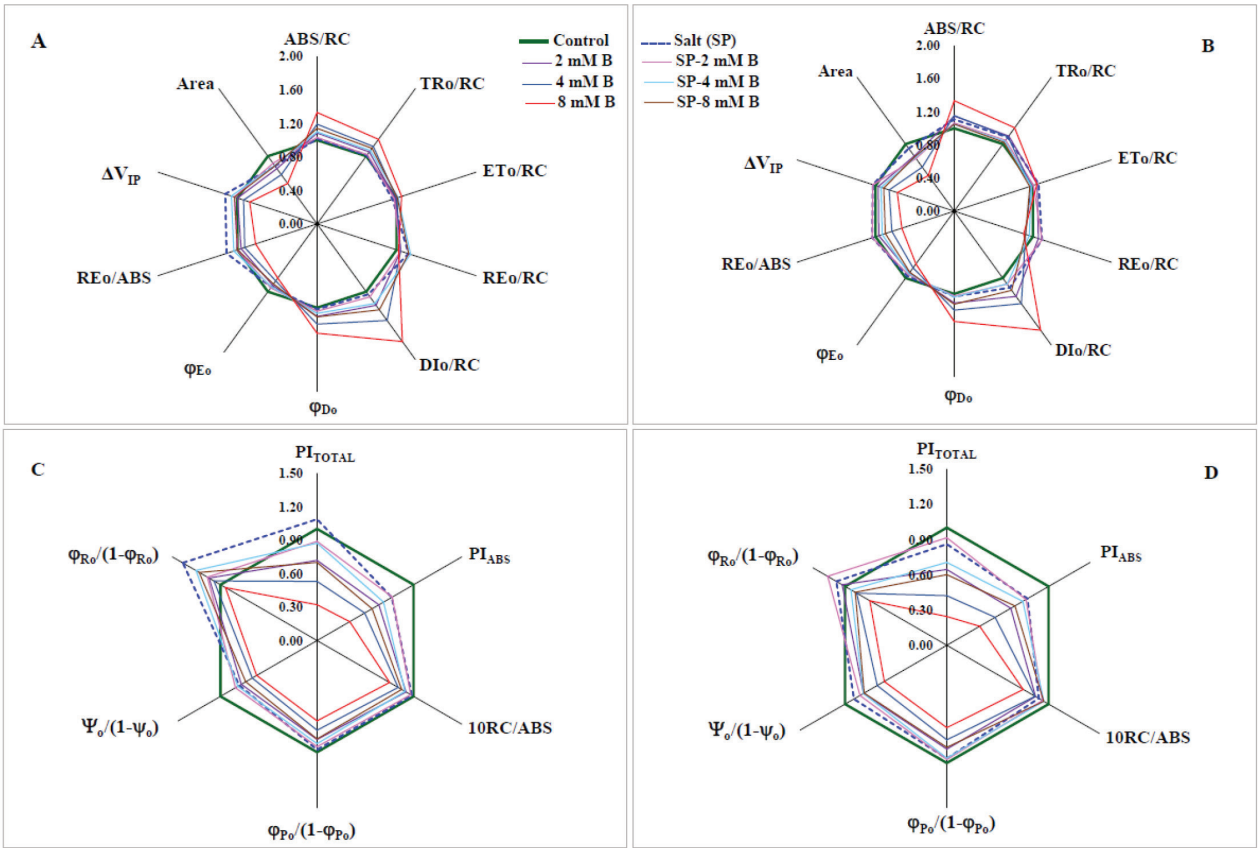

Figure 6. Effect of B treatments with or without salt pretreatment on the specific energy fluxes per reaction center and quantum yields of Sanbro (A) and Tarsan-1018 (B), and the performance indexes (PI_{TOTAL} and PI_{ABS}) and their partial components (Sanbro, C and Tarsan-1018, D). Descriptions of the selected JIP test parameters are explained in Section 2.

treated plants. In the present study, salt pretreatment might have decreased the functionalities of membrane transporters; therefore, this alteration could provide an explanation of the reasons why B contents were reduced in salt-pretreated plants compared to B application in the plant tissues. Hence, in salt pretreatment, the TF values increased in both genotypes compared with the B applications. In this study, an increase in the salt ions in the roots with salt pretreatment might have reduced B transport from soil to the plants, despite increased B accumulation in the shoot. Also, the salt pretreatment enhanced the membrane stability and provided an obvious reduction of electrolyte leakage in the leaves under B toxicity. Furthermore, this pretreatment mitigated the effect of toxicity better in Tarsan-1018 (about 47% at the highest B level) than Sanbro (about 41%) (Figure 2).

Shoot and root growth were negatively affected by B stress in both cultivars (Table 1). Similar results were reported in tomato, cucumber (Alpaslan and Gunes, 2001), wheat (Öz et al., 2014; Kayıhan et al., 2017), and pomegranate (Sarafi et al., 2017) under toxic B conditions. The length and the fresh and dry weights of the shoots in Tarsan-1018 (salt-tolerant) were more reduced with increasing B concentrations than in Sanbro. However, some researchers indicated that genotypic variations were used effectively as an indicator of tolerance in the elongation of roots (Jefferies et al., 1999; Choi et al., 2007). In this study, the shoot and root growth of Tarsan-1018, which was exposed to salt pretreatment, provided some improvement of B toxicity compared to that of Sanbro. Plants have to maintain water balance under environmental stress conditions. In the present study, B application in toxic levels reduced the leaf water content of both genotypes. The SP showed more beneficial effects on leaf water content in Tarsan-1018 than Sanbro (Figure 1).

Photosynthesis is one of the most fundamental processes of plant metabolisms, which are highly susceptible to and severely affected by toxic B levels (Han et al., 2009). Chl fluorescence measurements represent a reliable approach for examining the photochemical efficiency of leaves and provides detailed information about the structure and the functionality of the PSII reaction centers (Kalaji et al., 2016). While toxic boron levels reduced the photosynthetic efficiency in both

cultivars, as was expected, the salt pretreatment improved the efficiency and the boron tolerance of the cultivars (Figures 4 and 5). Toxic B levels affected slow fluorescence parameters. On the other hand, salt pretreatment ameliorated this toxic B effect (Figures 4A–4F). The genotypes also exhibited the typical polyphasic rise of the OJIP transient under control and B toxicity conditions with or without SP. A typical OJIP curve presents three main phases: the O-J phase indicates reduction for the primary electron acceptor PSII as QA and gives information about the antenna size. The J-I phase is related to reduction for the plastoquinone pool. The last one, the I-P phase states the reduction rate of the PSI acceptor side with the fully reduced plastoquinone pool or is related to the PSI RC (reaction center) content (Ceppi et al., 2012; Goltsev et al., 2016; Ripoll et al., 2016; Kalaji et al., 2018). The shape of the OJIP curve gives information about the photosynthetic activity of the photosystems. In this study, the I-P phase of the curve of cultivars was negatively influenced, especially under 4 and 8 mM B treatment. The salt pretreatment mitigated the adverse effect of B (Figures 5A and 5B). The reduction in IP amplitude due to toxic B levels may be related to the re-reduction of plastocyanin and $P700^{+}$ and/or a decreased electron flux towards cyclic electron flow (CEF) and/or lowered electron transfer efficiency towards PSI end electron acceptors and/or decrease in PSI content (Figures 6A and 6B). Han et al. (2009) demonstrated that IP amplitude was decreased by boron stress treatments in *Citrus* plants.

The OJIP transients were further analyzed with the JIP test. The specific energy fluxes (ABS/RC, φ_{D0}, DI_0/RC, and TR_0/RC, excluding ET_0/RC and RE_0/RC) were significantly increased in high B-treated groups of both cultivars (Figures 6A and 6B). Strasser et al. (1999) proposed that significant increment in ABS/RC could indicate a reduction in antenna size, which might result from the inactivation of PSII. Moreover, the decrease in chlorophyll contents (a + b) of the sunflower cultivars due to B toxicity is consistent with the increment in ABS/RC (Figure 3A) and the decrease in 10RC/ABS (Figures 6C and 6D). This decrease in pigment contents may be related to reductions in photosynthesis due to damage of the thylakoid membranes, or a typical indication of B stress as a result of photooxidation, chlorophyll degradation, or deterioration of membrane integrity (Figures 2, 3A, 6C, and 6D). However, the chlorophyll a/b ratio, which is an indicator of the antenna size of PS I and PS II, did not significantly change in both cultivars under all treatments (Figure 3B). The unchanged chlorophyll a/b ratio indicates that the chlorophyll pigments of the core antenna and the outer antenna were diminished by approximately the same level. In contrast to our results, Kayıhan et al. (2017) proposed that B increased the ratio of Chl a/b in wheat. Franić et al. (2017) expressed that the decrease in the concentration of reaction centers per chlorophyll (10RC/ABS) reflects susceptibility to photoinhibition and inactivation of reaction centers to form heat sinks in order to dissipate the excess of absorbed light. Mohapatra et al. (2010) suggested that reduced ET_0/TR_0 as in this study (Figures 6C and 6D) points toward the reduction of electron transport in functional PS II in both cultivars. These results demonstrated that the B treatments inhibited photosynthetic activity. Also, an increase in energy dissipation (φ_{D0} and DI_0/RC) shows that in high B-treated groups the dissipation of excess energy as heat and energy transfer to systems rather than to electron transport/photochemistry was induced (Strasser et al., 2010). Meanwhile, the increase in TR_0/RC indicates that the B treatments induced the increment in the rate of an excitation trapped by the reaction center (Bussotti et al., 2007). However, carotenoids that act as light-harvesting pigments and can provide the protection of the photosynthetic pigments and the membrane integrity did not significantly change in the present study (Figure 3C). The performance (vitality) indexes (PIs – PI_{ABS} and PI_{TOTAL}), which exhibit the plant performance and are the most used and sensitive JIP parameters, were decreased in sunflower cultivars and this decline indicates that the photosynthetic activities of the cultivars were significantly influenced with B applications in this study (Figures 6C and 6D). On the other hand, the PIs of both cultivars showed similar behavior against B toxicity and the damaging effect of B was reversed by salt pretreatment (Figures 6C and 6D). Estaji et al. (2019) suggested that the reduction of vitality indexes (PI_{ABS} and PI_{TOTAL}) under stress conditions reflects the reduction in overall photosynthetic performance in association with the decrease of electron transport capacity. In addition to the above comments, Lepeduš et al. (2009) reported that the changes in PI_{ABS} were related to DI_0/RC and TR_0/RC. Also, the B application caused a decrease in Area, which is the number of available electron acceptors in PSII and ETR, and the alteration of ET_0/RC, which showed that the B treatments affected the electron transport further than Q_A^-. In addition to this, ΦPSII, the actual photochemical efficiency of PSII photochemistry in the light-adapted state, was affected by especially toxic B. These effects were found to be more apparent in Tarsan-1018 than Sanbro (Figures 4A, 4B, 6A, and 6B). However, a slight increase and/or stability in PI_{TOTAL}, ΔV_{IP}, and RE_0/RC may indicate a slight increase and/or protected capacity of the PSI electron acceptor side. Consequently, it was revealed that B toxicity caused disruption in photosystem functionality (decrease in performance indexes) and salt pretreatment alleviated the effects of toxicity. Cultivars try to overcome excitation energy pressure by triggering the dissipation of excess energy to reduce the damage to the photosynthetic

apparatus. Salt pretreatment enhanced photosynthetic efficiency in both genotypes that were exposed to toxic boron levels. Additionally, the differences in PIs and other parameters showed that Sanbro had slightly better performance in the transfer of electrons than Tarsan-1018 in salt-pretreated groups.

In conclusion, toxic B levels adversely affected growth (length, fresh and dry weights of shoots and roots), water content, B contents of shoots and roots, membrane stability, photosynthetic pigment content, and photosynthesis of these two sunflower cultivars. This study demonstrated that salt pretreatment improved both cultivars' capacity to cope with B toxicity via enhancement of photosynthetic efficiency and growth, B sequestration, etc. Another important result obtained from this study is that the salt tolerance character of the tolerant cultivar did not provide an advantage with SP application against B toxicity in Tarsan-1018. Accordingly, the salt-tolerant cultivar (Tarsan-1018) exhibited similar responses to the salt-sensitive one at high B concentrations in salt-pretreated groups. In light of our findings, both of these sunflower cultivars can be used for remediation purposes of soils with excess B content. The present study seems to be the first report to provide information on the effects of salt pretreatment mitigation of B toxicity and enhancement of B tolerance in plants.

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