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ORIGINAL ARTICLE

Bottom-cold stress was less harmful than cold-air stress on tomato seedling production treated with boric acid

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Abstract

Boron is beneficial element can help plants face chilling stress. This research was arranged using factorial experiment based on randomized complete block design on *Lycopersicon esculentum* var. Infinity in Isfahan University of Technology due to having a new idea about bottom-cold and cold-air stress tomato seedling with boron application. There were two treatments having different boron (B1) concentrations (B1) 50.5 mM, and (B2) 75.82 mM. Three temperature treatment used as following: (1) the cold treatment used with vegetation chambers with low temperature (10 °C) (cold air), (2) the low nutrient solution (10 °C) temperature but the aerial part exposed to optimum temperature (bottom-cold) and (3) the last part, was the control plant (opt) keep in optimum root and shoot temperature (22 °C). Some physiological and biochemical characteristics were measured. The results were shown that boron uptake decreased in cold or stress as well as the water status of the plant which is suffering from cold-air stress greater than bottom-cold. Boron application, especially in higher concentration, improved some deleterious effect of cold stress, especially in the bottom-cold. The reason may refer to keeping photosynthesis traits in better level with B application. Cold-air stress, increased stress indices such as antioxidant and proline as well as glucose level and saturated/unsaturated fatty acid greater than bottom-cold stress. It was concluded that tomato was more resistant to bottom-cold stress than cold-air stress. Boron application increased the boron of leaves more effectively in the bottom-cold consequently increase plant tolerance to a chilling condition at the bottom-cold too.

Keywords Chilling stress · Optimum temperature · Photosynthesis · Sugar

Abbreviations

B	Boron
Ψleaf	Leaf water potential
EL	Electrolyte leakage
RWC	Relative water content
CF	Chlorophyll fluorescence
Chi	Chlorophyll index
DW	Dry weight
LST	Shoot zone
LRT	Root zone
Pn	Photosynthesis
gs	Stomatal conductance
Ci	CO_2 intracellular of stomata
FA	Fatty acid
MDA	Malondialdehyde
RCBD	Randomized complete block design

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Introduction

Healthy seedling production is a prerequisite for increasing yield. Seedlings could be grown in a different substrate, which is important for producing vigorous seedlings in nurseries (Sterrett 2001). In Iran, tomatoes are grown over about 139 thousand hectares with an average yield of about 34.4 tons ha^{-1} (Anonymous 2008) and an average of the yield is below than average of yield being achieved in some of the developed countries of the world. The tomato yield in leading producing countries such as the Netherland is nearly 231 tons per hectare in 2007.

For the best seedling growth, adjusting the root zone temperatures could improve germination percentage and uniformity of seedling growth. It is highly recommended that using bottom-heat for plug production of the seedlings. Because many seedlings are produced in the winter

and early spring, once seeds germinate, the temperature is low in many nurseries. Although using bottom-heating for seedling production is usually costly, it improves plant quality and greatly improves rooting, which thereby decreases germination to transplant duration (Vocanson and Jeuffroy 2008). The numerous plant species exhibit a positive linear relationship between temperature and seedling growth after emergence (Chen et al. 2019). The increase in growth of *M. oleifera* seedlings enhanced with the increase in temperature especially between 10 and 30 °C. On the other hand, beyond this temperature due to changes in metabolic pathways growth decreases in *Chrysanthemum morifolium* changes of growth physiological and anatomical changes in cold air (chilling) were more investigated. Many researchers have revealed that the delay of seedling growth by low temperature is related to inadequate nutrient uptake, probably due to root damage (Paredes and Quiles 2017). Photosynthesis is one of the first and main physiological processes affected by low temperature (Mohabbati et al. 2013). Changes in protein (Annikki et al. 2002), proline contents (Kaur et al. 2011), soluble sugars and MDA (Phornvillay et al. 2019) that are commonly observed due to enhancement of chilling tolerance in plants. Moreover, some anatomical and morphological changes, such as enhanced leaf epidermis, and reduction cell wall thickness of leaf at low temperatures in different species during growth (Maria et al. 2001).

To a lesser extent, the effect of cold temperature was investigated in the root zone. One of the main difficulties in commercial tomato seedling production is the limiting of plant height which varies with temperature. Irrigation with 5–15 °C water increased shoot and root dry weight and stem length result in better seedling for transplanting of tomato (Biddington and Dearman 1985).

On the other hand, there were few reports revealed that irrigation with cold water could not affect on seedling growth. They showed that when seedlings irrigation with 5 °C water, the temperature in the substrate did not lower than 18–20 °C. These temperatures did not inhibit growth and reduced dry weight and leaf chlorophyll content (Biddington and Dearman 1985).

Recent research results have also considerably improved our knowledge of B uptake and transport and its effect on stress condition (Frommer and von Wiren 2002). Boron roles in cell wall formation, cellular membrane functions, and antioxidant defense systems were proved (Frommer and von Wiren 2002). On the other hand, it was reported that the low temperature in the root zone affects B transport partitioning in the shoot (Matzner and Comstock 2001). B deficiency caused significant reduction in the net photosynthetic rate, transpiration and gs in turmeric (*Curcuma longa* L.) (Dixit et al. 2002), soybean (*Glycine max* L.) (Liu 2000), sunflower (*Helianthus annuus* L.) and citrus (*Citrus sinensis*) (Papadakis et al. 2004). Boron helped in maintaining the integrity of plasma membranes (Cakmak et al. 1995) and prevention of oxidation of phenolic compounds and the production ROS (Zhao and Oosterhuis 2003), the formation of leafy and flower buds, vascular tissue regeneration, root growth, nucleic acid metabolism, carbohydrates, lipids, glucose and protein, cell membrane permeability, auxin hormonal mechanism, phenolic compounds, changes in the pattern of proteins (Demiray et al. 2011) and affect sugar movement and metabolism (Marschner 1995). Also, B increases the resistance of plants to stresses, including chilling stress (Papadakis et al. 2004).

Tomato (*Lycopersicon esculentum* Mill) considered one of the cold-sensitive plant (Hannan et al. 2007). Cold stress reduces pure Pn and stomatal conductance in tomato (Starck et al. 2000). There are two methods of seedling production in Iran: first, plug cultivation in the soilless substrate; the second, cultivation in the soil in an open area or greenhouse without a heating system. In both methods, roots received cold stress in the first method with decreasing nutrient solution's temperature which kept outside the greenhouses and in the second method, by decreasing soil temperature in early season production especially at night.

Therefore, tomato seedling exposed to both air and soil chilling stress in the early growth stage. Some seedling producers use bottom-heat to promote growth and prevent chilling stress of the substrate in the early morning. But the others believe that dropping soil temperature would not happen as the variance of soil temperature is very limited. In the pre-test, we monitor the soil and air temperature. The results showed the decreasing soil temperature to average 10 °C during seedling production. Therefore, the aim of this research was to rebuild real condition in seedling production and investigate the comparison effect of root zone cold (bottom-cold) with natural cold air on seedling production and the possible effect of boron on the improvement of negative effects of cold stress exposed to root or whole plant on tomato seedling growth.

Materials and methods

The experiment was arranged according to factorial experiment based on randomized complete block design (RCBD) with 6 treatments and 13 replications in each treatment on *Lycopersicon esculentum* var. Infinity in the research greenhouse of Isfahan University of Technology, Isfahan, Iran. Tomato seeds were grown in peat and perlite (1:1 V/V) and after 4 weeks' growth transferred to the hydroponic container filled with 1 L Johnson nutrient solution (Jones 2005) including (mM) $MgSO_4$:2, KH_2PO_4:1, H_3BO_3:50, $MnCl_2$: 10, $CaCl_2$: 1, $MnSO_4$:10, $CuSO_4$:1.5, $ZnSO_4$: 0.8, Na_2MoO_4: 0.4, $Co(NO_3)_2$: 0.1, KNO_3: 10, $FeCl_3$:0.1, EDTA: 0.3 with different H_3BO_3 concentration; control was equal to boron

in Johnson nutrient solution (B1) 50.5 mM, and the other B concentration were (B2) 75.82 mM. Plants keep in the nutrient solution with boron treatment equipped with 5 min airing in every 15 min was provided for 10 days and then temperature treatment started. One-third of plant was transferred to vegetation chambers with low temperature (10 °C) (cold air) that all plant parts exposed to low temperature, and two-third of plant exposed to just low nutrient solution temperature (10 °C) but the aerial part exposed to optimum temperature (bottom-cold) and the last plants, was the control plants (opt) keep in the optimum root and shoot temperature (22 °C). For adjusting temperature treatments, using incubator with (EYELA LTI-1000 SD) 14-h photoperiod by photosynthetic photon-flux density of 270 µmol m^{-2} s^{-1} with low (10 °C) and optimum (22 °C) temperature and a humidity of 70%. The root zone temperature treated in low (10 °C) in the water bath for 24 h.

The effect of stress and B on some photosynthesis and growth traits

Growth trait assay: When temperature treatments were finished, the shoots of seedling were separated from the roots and after weighted, they were oven dried at 70 °C to measure the dry weight of shoots and roots.

Photosynthesis traits assay: Gas exchange parameters including photosynthesis rate, transpiration, stomata conductivity and intercellular CO_2 of stomata were measured from three replications per treatment by a portable photosynthesis meter (Li-Cor Li-3000, USA) in a sunny day. Photosynthetically active radiation (PAR) intensity was 1000 µmol m^{-2} s^{-1} and CO_2 concentration was 350 µmol mol, the same leaves of each plant were used for chlorophyll measurement using chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA). Mesophyll conductance (mmol CO_2 m^{-2} s^{-1}) was measured according following formula:

Photosynthetic rate/sub-stomatal CO_2 concentration (Ahmadi and Siosemardeh 2005).

The effect of stress and B on stress indices (electrolyte leakage, antioxidant, proline, RWC)

Relative water content: Relative water content (RWC) was calculated using method of Filella et al. (1998): the 7 mm leaf discs were weighed (FW) then hydrated until saturation for 48 h at 5 °C in darkness (TW). The leaf discs were dried in an oven at 105 °C for 24 h (DW). The following formula were used for presenting RWC (Filella et al. 1998):

$$\mathrm{RWC}\% = (\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW}) \times 100.$$

Leaf water potential (Ψleaf): Ψleaf measured following method, Midday (11:30–12:30 h) Ψleaf was measured using a Scholander pressure chamber. Two to four mature and fully exposed leaves in each treatment were detached from the shoot and placed in the pressure chamber (Laboratory Plant Water Status Console, Santa Barbara, USA) (Turner 1988).

Chlorophyll fluorescence (Fv/Fm): Maximum photochemical quenching Fv/Fm was measured by chlorophyll fluorescence (model OS-30, Minolta Corp). Chlorophyll fluorescence measured by portable fluorescence monitoring system (RS232, Handy PEA, UK). A clip was placed on the leaf for 30 min for dark adaptation. The initial (F0), maximum (Fm) and the maximum quantum efficiency of the photosystem-II (Fv/Fm) was reported according to Yu et al. (2002).

Antioxidant activity: The antioxidant activity of tomato leaves was determined by Koleva et al. (2002). Three mg of leaves dissolved methanol then blended with 0.6 mL of DPPH solution. After 30 min, the absorbance of the supernatant was recorded at 515 nm by the spectrophotometer (UV 160A- Shimadzu Corp., Kyoto, Japan). The methanol was used for a blank.

Phenolic content: The Folin–Ciocalteu method used for measuring the total phenolic content. The absorbance was measured at 725 nm with a spectrophotometer (UV 160A-Shimadzu Corp., Kyoto, Japan). The results were expressed in gallic acid equivalents (mg/100 g fresh weight) using a gallic acid standard curve (McAdam et al. 2016).

Proline: Proline concentrations were determined by a fast, simple and accurate method based on the reaction of proline with acid ninhydrin (Bates et al. 1973).The leaf with sulfosalicylic acid 3% was used to homogenize the leaves at 4 °C. Then, the solution incubated and centrifuged at 5000 rpm for 20 min. The supernatant mixed with 2.5% ninhydrin, 60% phosphoric acid (v/v) and 1 ml of glacial acetic acid (100%). The absorbance waves at 518 nm were measured by spectrophotometer (UV 160A- Shimadzu Corp., Kyoto, Japan).

Electrolyte leakage (EL): EL was measured using an electrical conductivity meter using the method described by Lutts et al. (1995). Leaf pieces shake with distilled water at 100 rpm for 24 h at room temperature. The initial conductance measured using a conductivity meter. The tubes were then autoclaved at 115 °C for 10 min and final readings were EL (%) = initial measurements/final measurements × 100.

The effect of stress and B on biochemical and B concentration (protein, sugar, FA)

Protein: Total soluble protein content was measured according to Bradford (1976) using bovine serum albumin as a protein standard. Na-Phosphate buffer (pH = 7.2) for leaf homogenization and Coomassie Brilliant Blue G-250 as dye and albumin as a standard were used. The absorbance was measured using spectrophotometer (V UV 160A- Shimadzu Corp., Kyoto, Japan) at 595 nm.

Saturated/unsaturated fatty acid (FA)%: The composition of the FA and the ratio of saturated/unsaturated% were measured by the Gas Chromatograph (Agilent 6890N) equipped with a flame ionization detector (FID) and HP-88 capillary column (100 m × 250 µm) (Motamedi et al. 2019). FA methyl esters produced with methylation with sodium methoxide (0.5 N). The carrier gas, was nitrogen with a flow rate of 1.1 ml min^{-1}. The oven temperature was 5 °C min^{-1} from 150 °C/1 min to 190 °C/2 min, and then to 240 °C/ 8 min. The injector and detector temperatures were 150 and 250 °C, respectively.

Glucose and fructose measurement: Leaf sample was homogenized with ethanol (80%) by stirring and sonication (10 min) centrifuged at 4 °C in 3000 rpm for 10 min. The supernatant solution was injected into the 20 µl loop with 5 ml syringe filters containing 0.2 ml membrane filters. A high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) equipped with a refractive index detector. The HPLC column was SCR-101 N (30 cm × 9.7 mm i.d) fitted with a guard column SCR (N) (5 cm × 4 mm i.d). The mobile phase was deionized water at a flow rate of 0.7 ml min^{-1} and 60 °C (Adams et al. 2008).

The effect of stress and B on anatomical changes

Anatomical traits: To investigate the anatomical structure of the leaves (length of spongy parenchyma, length of palisade parenchyma and thickness of upper and under epidermis), the samples were fixed in 70% alcohol, cutting by blade manually and after removing the color with bleach, coloring with methylene blue and carmen-zagi, and after permanent fixation on the lam, were examined under an optical microscope at 40 × magnification (Leica Galen III). Anatomical traits were observed by an optical microscope at 40X magnification (Leica Galen III) and measured through the Edn-2 software (Polic et al. 2009).

Statistical analysis: Data were analyzed with SAS (Ver.9.1) after checking data normality and means separated with the least significant difference by LSD test at the 5% level. The glucose and fructose were measured in the best treatment and results were analyzed with *T* test with SAS (Ver.9.1).

Result

The main effect of stress and B on biochemical, anatomical and physiological parameters of tomato

The two-way ANOVA table showed that the main effect of temperature status, B conc. and the interactive effect was significant in all of parameters except the effect of B conc. on antioxidant and the effect of temperature on dry weight which was not significant (Table 1). The interactive effect of temperature status and B conc. on length of palisade and spongy parenchyma and thickness of upper and lower epidermis was significant (Table 1). The main effect of cold stress showed that chlorophyll content decreased in cold-air/bottom. Chlorophyll fluorescence, RWC, Ψleaf increased at bottom-cold and RWC and Ψleaf decreased at Opt. condition. CF decrease and EL increased with bottom-cold and it was the same in other treatments (Table 2).

Proline and antioxidant activity and total phenol was highest in cold air and then in bottom-cold. Protein and starch increased in bottom-cold; and cold air decreased protein. Pn was highest in Opt. condition, and gs and Ci were increased in bottom-cold. B Conc. decreased in leaves when plant exposed to bottom-cold (Table 3).

Boron enhanced shoot DW, root DW, ChI, RWC, CF, and Ψ leaf; boron decreased EL (Table 4).

With increasing B conc. proline and phenol content decreased, and antioxidant did not change significantly. Protein, Pn, gs, Ci, starch, and B conc. increased when exogenous-B applied (Table 5).

The interactive effect of stress and B on biochemical and physiological parameters of tomato

Glucose increased in cold air and fructose enhanced in Opt. The less glucose and fructose was seen in bottom-cold (Table 6). Root and shoot dry weight increased in B1 in all temperature status. The highest dry weight of root was seen in Opt × B1, and shoot dry weight was maximum at bottom-cold × B1 (Fig. 1).

CF increased with boron application in all temperature status. Antioxidant increased in cold air and bottom-cold when B was used; it was lowest in opt. temperature in both B0 and B1. The highest antioxidant and phenol was at cold air without boron application. The phenol and EL were decreased with B1 in all treatment. Proline was highest in cold air and decreased in all other treatments (Fig. 2a–e). RWC increased with B and the highest was at cold air and bottom-cold with B. The reverse result from RWC was seen

Table 1 Analysis of variance effect of cold air/bottom-cold and boron on some parameters of tomato

Source	df	DW of Shoot	DW of Root	ChI	CF	RWC	EL	Proline	Ψ leaf	Antioxidant activity	Phenol content	Protein	Pn	gs	Ci
Treatment	1	3.69^{ns}	2.56^{ns}	1890.47**	1.60*	1388.33**	1871.34*	12.61**	1569.09**		113.94^{ns}	8279.8**	26589**	24.00**	23876*
B conc	1	12.04**	9.30**	3537.41**	6.39**	1500**	6816*	8.44**	7.21^{ns}		2274.27**	92622.2**	124880**	100.90**	387239**
T×B	2	0.76**	0.47**	80.41**	9.28**	126.77**	1266.36**	2.48**	1145.21**		22.53**	76.1**	3466**	5.05**	9303**
Error	22	0.05	0.05	33.34	1.28	21.37	11.71	0.09	5.84		9.52	802.8	1276	1	3625
CV		18.71	23.55	9.24	13.62	6.13	12.07	-13.07	7.5		19.53	22.27	24.78	20.34	15.12

Source	df	Starch	Saturated/unsaturated	B conc	Proline	Length of spongy parenchyma	Length of palisade parenchyma	Thickness of upper epidermis	Thickness of lower epidermis
Treatment	1	3212.08*	23874^{ns}	2943.8*	51865.8**	179.36^{ns}	928.42^{ns}	92.21^{ns}	57.48^{ns}
B conc	1	9210.73**	34297^{ns}	22632.1**	49335.3**	286.74^{ns}	934.71^{ns}	135.74^{ns}	126.61^{ns}
T×B	2	898.68**	654**	2581.3**	28431.8**	62.39**	347.62**	26.97**	28.95**
Error	22	90.86	14.56	210.5	523.5	9.51	116.29	7.51	5.80
CV		18.67	7.54	55.25	24.9	8.44	10.79	10.75	10.39

ns no significant, *significant at 5% and **significant at 1%

for Ψleaf i.e., Ψleaf was greater when B was applied and did not significantly change in bottom-cold; it was more negative in opt. and cold air without B (Fig. 3a–b). All photosynthesis traits (Pn, gs, Ci, ChI) was increased with B in all temperature status. It was greater in Pn and gs than ChI. The highest Pn was at cold-bottom and opt. at B1. The gs increased in bottom-cold with B1; Ci in cold air and bottom-cold with B1; and ChI in Opt with B1 (Fig. 4a–d). Starch, protein, and B concentration of leaves was increased when exogenous-B was applied and all of them was highest in bottom-cold × B1. Saturated/unsaturated FA decreased in bottom-cold and increased in the cold air dramatically. Boron has no effect on saturated/unsaturated FA (Fig. 5a–d).

The interactive effect of stress and B on anatomical changes

Length of spongy parenchyma, length of palisade parenchyma, the thickness of the upper epidermis, and thickness of lower epidermis increased with boron application. The highest spongy parenchyma, length of palisade parenchyma was seen in opt × B1; they are decreased with cold air and bottom-cold, although the boron increased them. The thickness of the upper epidermis and thickness of the lower epidermis was highest in bottom-cold × B1 and was lowest at Opt. (Fig. 6a–d).

Discussion

The effect of stress and B on photosynthesis and growth

Photosynthesis is the major physiological indices change with low temperature (Mohabbati et al. 2013). Both root and shoot chilling can cause a reduction in leaf gs, in bean (*Phaseolus vulgaris*), maize and tomato (Matzner and Comstock 2001; Aroca et al. 2003; Bloom et al. 2004).

The main result of temperature treatment revealed that photosynthesis traits were more affected by cold air compared with bottom-cold. The bottom-cold increased transpiration more than cold air in tomato var. Infinity. The reason was to explain with Bloom et al. (2004) that stomata keep open in a chilling-sensitive tomatoes when root temperature reach to 5 °C, but they were not seen in a chilling-tolerant tomatoes (Bloom et al. 2004). Chilling reduced the CO_2 assimilation and inactivation of the photosystem-II (P680) reaction center (Arato et al. 2004), B deficiency deteriorate these oxidative effects at chilling condition. Because the Boron deficiency changes photosynthetic rate, transpiration and gs in turmeric (*Curcuma longa* L.); (Dixit et al. 2002) sunflower (*Helianthus annuus* L.) and citrus (*Citrus sinensis*) (Papadakis et al. 2004). Photosynthesis traits (Pn,

Table 2 The main effect of cold air and bottom-cold on some parameters of tomato

Treatments	ChI (SPAD value)	CF (Fv/Fm)	RWC (%)	EL (%)	Ψleaf (MPa)
Cold air	51.5^{c}	0.7^{b}	77.5^{b}	33.36^{a}	− 2.17^{b}
Bottom-cold	65.88^{b}	0.7^{b}	82.59^{a}	34.5^{a}	− 1.7^{a}
Opt	70.02^{a}	0.8^{a}	66.30^{c}	17.20^{b}	− 3.25^{c}

Within a column means followed by the same letter are not significantly different at $P<5\%$ according to LSD test

Table 3 The main effect of cold air and bottom-cold on some parameters of tomato

Treatments	Proline (μmolg^{-1}FW)	Antioxidant activity (% DPPH)	Phenol content (mg GAE g^{-1} W)	Protein (mg g^{-1} FW)	Pn (μmco$_2$ m^{-2} s^{-1})	gs (mmolH$_2$O m^{-2} s^{-1})	Ci (μmol mol^{-1})	Starch (mg/g dry weight)	B conc. (mg/kg)
Cold air	149.54^{a}	41.99^{a}	18.50^{a}	107.51^{c}	11.01^{c}	3.75^{c}	394.5^{b}	44.49^{b}	22.5^{ab}
Bottom-cold	53.07^{c}	24.70^{c}	14.88^{b}	148.16^{a}	13.97^{b}	5.92^{a}	434.4^{a}	65.66^{a}	16.45^{b}
Opt	73.01^{b}	30.01^{b}	14^{b}	126.03^{b}	18.24^{a}	5.1^{b}	365.6^{b}	43.01^{b}	39.82^{a}

Within a column means followed by the same letter are not significantly different at $P<5\%$ according to LSD test

Table 4 The main effect of boron on some parameters of tomato

B conc. (ppm)	Shoot DW (g)	Root DW (g)	ChI (SPAD value)	CF (Fv/Fm)	RWC (%)	EL (%)	Ψleaf (MPa)
B0	0.85^{b}	0.63^{b}	54.79^{b}	0.7^{b}	70.46^{b}	39.01^{a}	− 2.75^{b}
B1	1.75^{a}	1.42^{a}	70.14^{a}	0.8^{a}	80.46^{a}	17.69^{b}	− 2^{a}

Within a column means followed by the same letter are not significantly different at $P<5\%$ according to LSD test

Table 5 The main effect of boron on some parameters of tomato

B conc. (ppm)	Proline (μmolg^{-1}FW)	Antioxidant activity (% DPPH)	Phenol content (mg GAE g^{-1} FW)	Protein (mg g^{-1} FW)	Pn (μmco$_2$m^{-2} s^{-1})	Gs (mmolH$_2$O m^{-2} s^{-1})	Ci (μmolmol^{-1})	Starch (mg/g dry weight)	B conc. (mg/kg)
B0	120.55^{a}	32.58^{a}	21.95^{a}	87.94^{b}	9.89^{b}	3.62^{b}	317.83^{b}	38.66^{b}	6.83^{b}
B1	63.2^{b}	31.89^{a}	9.64^{b}	166.52^{a}	18.93^{a}	6.22^{a}	478.5^{a}	63.44^{a}	45.68^{a}

Within a column means followed by the same letter are not significantly different at $P<5\%$ according to LSD test

Table 6 The effect of cold air/bottom-cold and boron on sugar content of tomato at 0.5 ppm (B1)

	Opt	Cold air	Bottom-cold
Glucose	0.24^{b}	7.87^{a}	0.017^{c}
Fructose	0.32^{b}	5.02^{a}	0.05^{c}

Within a row means followed by the same letter are not significantly different at $P<5\%$ according to LSD test

gs, Ci) and chlorophyll content were improved in each cold air, bottom-cold and optimum treatments when B was used. The improving of Pn and gs was greater in bottom-cold than other treatments when B was used. The reason could explain

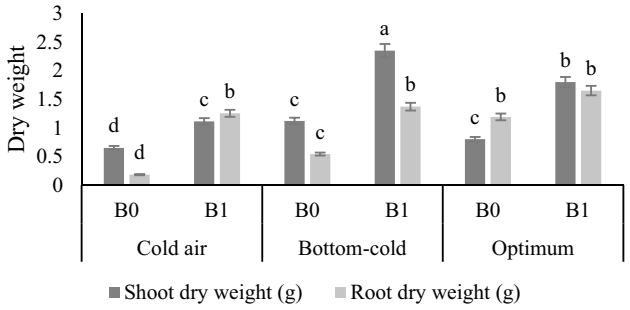

Fig. 1 The effect of cold air/bottom-cold and boron on shoot dry weight and root dry weight

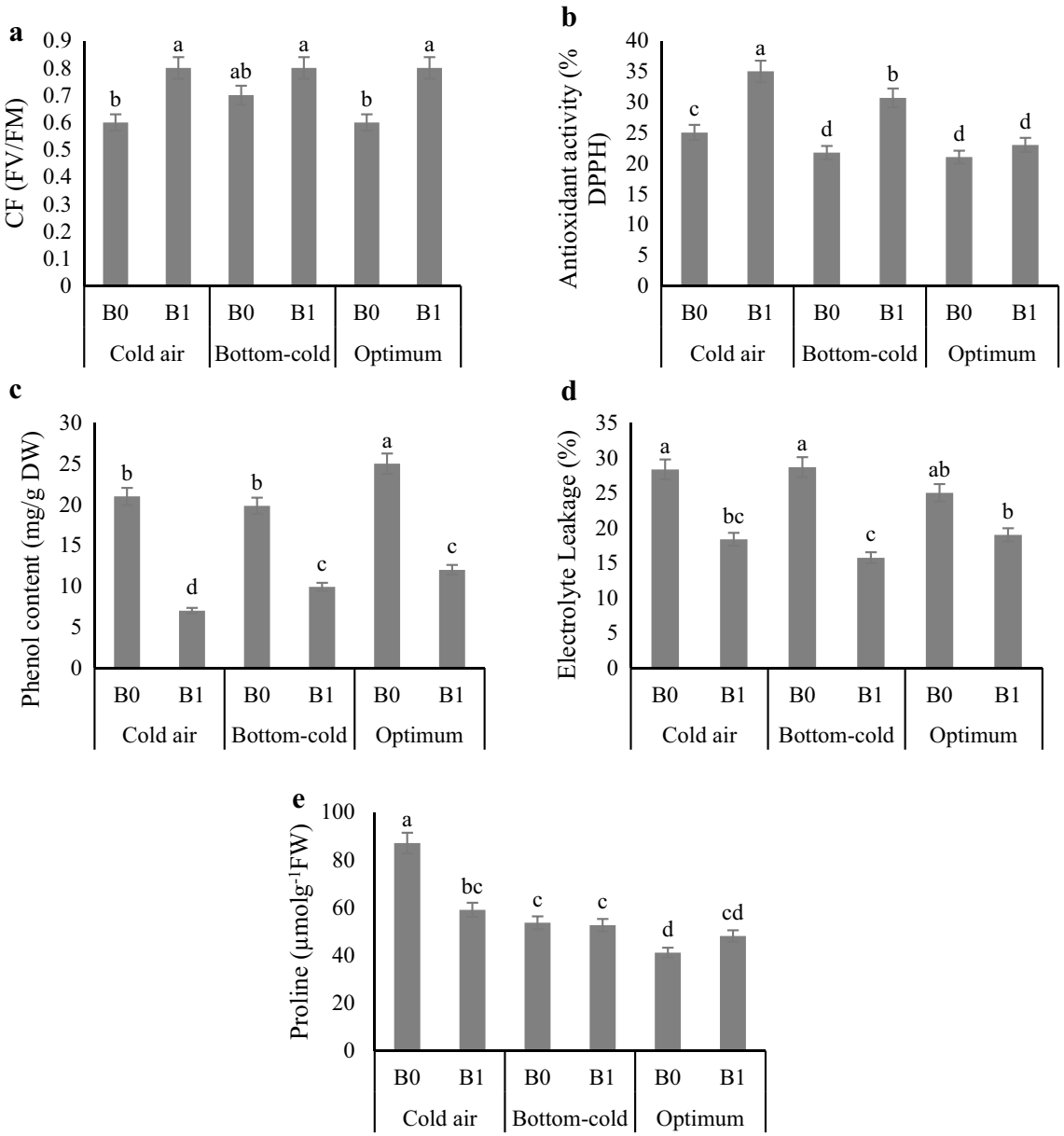

Fig. 2 The effect of cold air/bottom-cold and boron on CF (**a**), antioxidant activity (**b**), phenol content (**c**), electrolyte leakage (**d**) and proline (**e**)

that by maintaining membrane integrity and photosynthate translocation (Liu 2000). It seems that applying B could not improve stomata opening and so the transpiration (Koç et al. 2010; Aghaee et al. 2011). Chlorophyll is the main pigments of Pn in the chloroplast, (Zhang et al. 2009). Therefore, increasing the chlorophyll content positively correlated with photosynthetic rate; therefore, influence photosynthetic efficiency (Thomas et al. 2005) and could be increase biomass production (Wang et al. 2001). The same trend in our result showed that with increasing chlorophyll using B treatments the Pn improved result in increase of the growth. It was observed that fresh and dry weight of tomato increased when B was added in the nutrient solution too. B deficiency result in reducing the dry weights of root, stem, and leaf, as well as reducing the CO_2 assimilation and stomatal conductance of soybean; decrease of leaf area, fresh and dry mass (Dixit et al. 2002). Various varieties of soybean increased main stem length, DW of roots with B in nutrient solution (Liu et al. 2005). Second, the balanced consumption of boron, improves the transfer of photosynthetic materials and increase the amount of dry matter. Moreover, direct effect of Boron increased cell growth and differentiation in meristems, lastly, fresh and dry weights of tomato increased at 100 and 200 ppm due to the effect of B on nucleic acid synthesis, cell division, the uptake of calcium and transport of carbohydrates in plant (Jeanine et al. 2003).

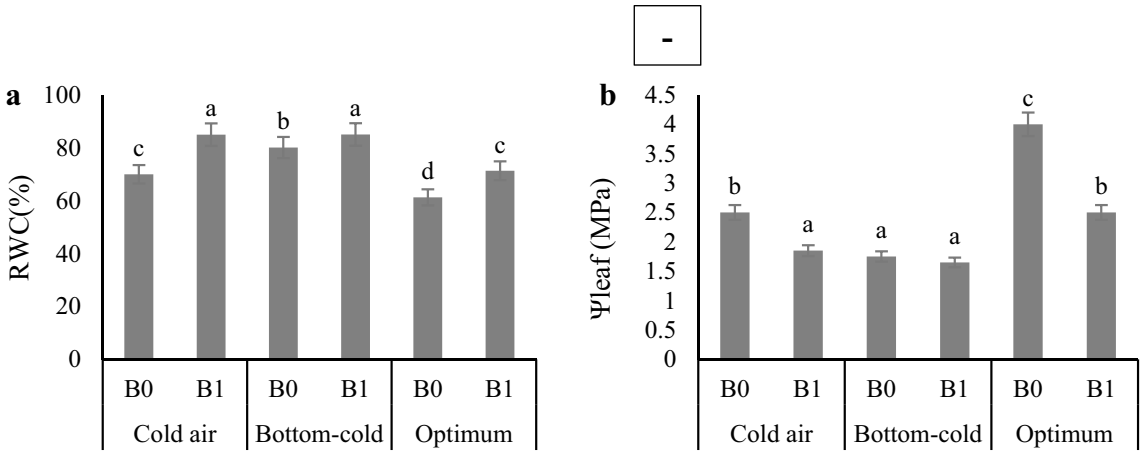

Fig. 3 The effect cold air/bottom-cold and boron on RWC (**a**) and Ψleaf (**b**). Ψleaf is minus and the significant letter is based on minus numerical

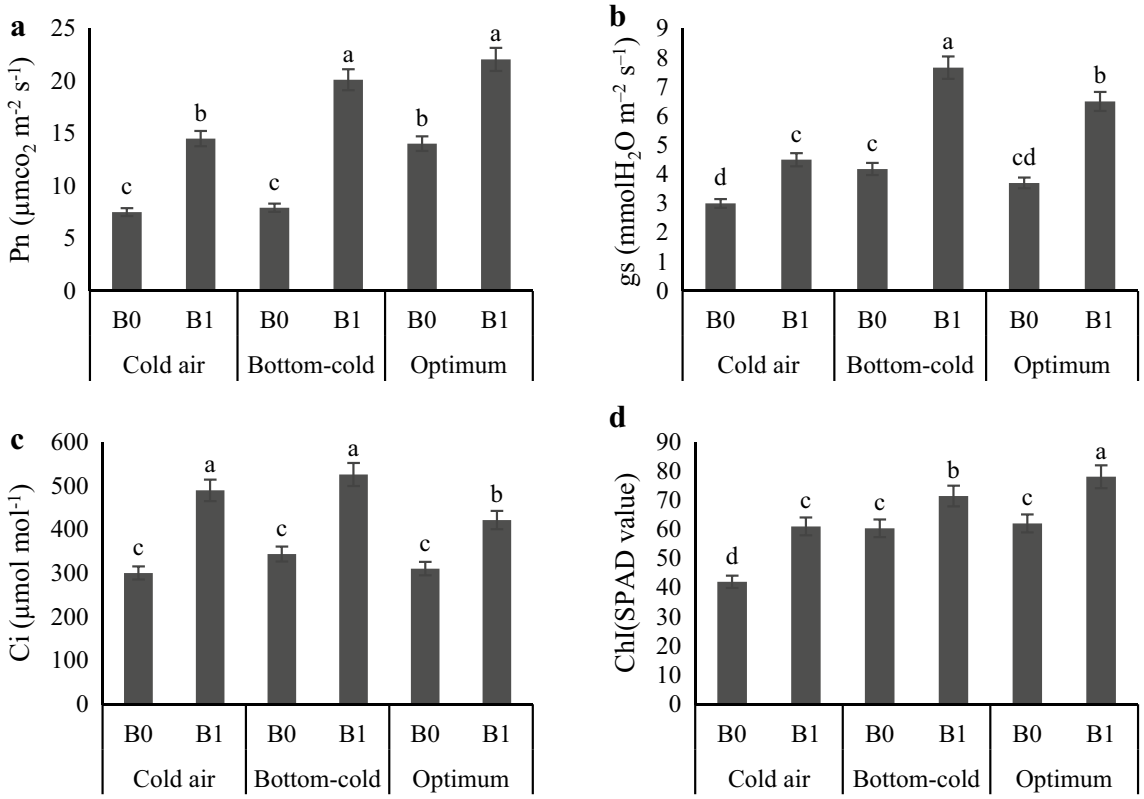

Fig. 4 The effect cold air/bottom-cold and boron on Pn (**a**), Gs (**b**), Ci (**c**) and ChI (**d**)

The effect of stress and B on stress indices (electrolyte leakage, antioxidant, proline, RWC)

Boron deficiency in the root decreases the level of antioxidants. On the other hand, chilling-induced oxidative damage in root result in the decrease of plasma membrane integrity in which is sensitive responses of the plasma membrane to B deficiency which is showed the importance of B the structural and functional plasma membrane (Cakmak and Romheld 1997). When root and shoot exposed to cold stress, the gs decreased in some species, such as bean (*Phaseolus vulgaris*), maize and tomato (Matzner and Comstock 2001; Aroca et al. 2003; Bloom et al. 2004). Therefore, leaf transpiration is minimal. Cold stress decreases root hydraulic

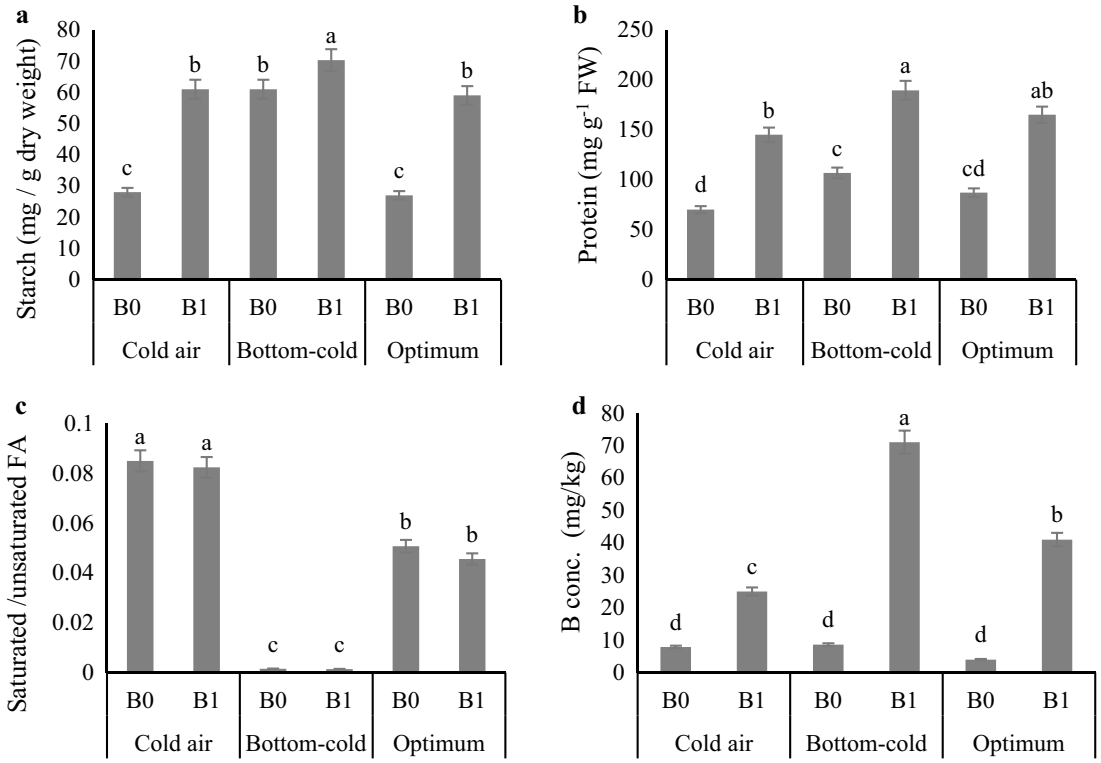

Fig. 5 The effect of cold air/bottom-cold and boron on starch (**a**), protein (**b**), saturated/unsaturated FA (**c**) and B conc. (**d**)

conductance especially in chilling-sensitive species, result in decreasing water absorption and movement and impairs stomatal function which leads to excessive water loss and leaf wilting (Bloom et al. 2004) the same was happening in deficiency of boron and cold stress. Hajiboland and Farhanghi (2010) reported that B deficiency reduce the K + uptake into the guard cells result in the reduction of membrane integrity and, thus, stimulate passive leakage of K + from guard cells to open stomata. Chilling-tolerant maize showed lower transpiration and higher water potentials comparing chilling-sensitive ones (Han et al. 2008). Stomata closed in tolerant cultivar ('LA 1778') *Lycopersicon esculentum* when root temperature declined to 5 °C (Bloom et al. 2004). The same results showed that when chilling-tolerant maize genotype keep in 5 °C for 30 h their root hydraulic conductance increase comparing with the control after an initial decline in root hydraulic conductance (Aroca et al. 2001).

Boron can increase the antioxidant activities and decreased the leaves phenolic compound. They alleviated ROS damage to improve the photosynthetic rate and reduces cell damage (Waraich et al. 2011).

Cold air was a greater effect on antioxidant activity, even when boron was applied. Antioxidant activity increased in WL and Lt stress (Waraich et al. 2011). The reason was to explain by Lukaszewski and Blevins (1996). They revealed that oxidative damage in leaves is much more than damage to roots, because in addition that the leaves exposed to chilling, have to face reactive oxygen species created from Pn.

Water deficits, salinity, and chilling stress cause accumulation of proline in higher plants. At low temperatures, proline accumulates in plants such as potato hybrids which accumulated proline in their leaves of when exposed to cold acclimation (Van-Swaaij et al. 1985; Gilmour et al. 2000).

The effect of stress and B on biochemical and B concentration (protein, sugar, FA)

It was observed that the starch, protein and B concentration was highest in bottom-cold with B. On the other hand, starch accumulation increased in plant in both cold/bottom-cold stress in tomato; the highest glucose was at the cold-air, it seems that under cold-air stress, the starch change to glucose to increase acclimation of plant to cold stress and boron facilitate sugar movement to leaves for improving stress (Waraich et al. 2011; Marschner 1995). It was suggested that the hydrolysis of starch and sugars conversion result in sugars increase (Ingram et al. 1997) leading osmoregulation role of sugar as osmotic to protect some macromolecules and stabilized membrane structures by interact with polar head groups of phospholipids and prevent membrane fusion (Phillips et al. 2002). It has been proved that soluble sugars have a key role in cold acclimation, means that increase in

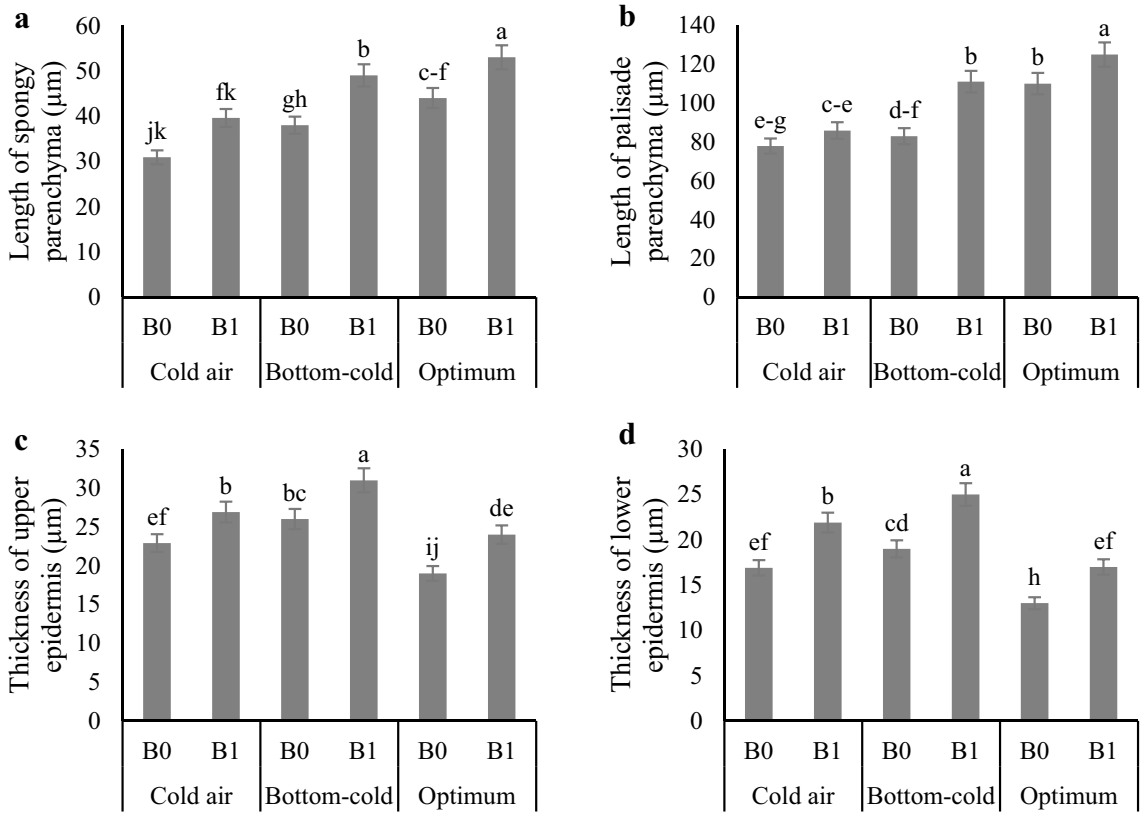

Fig. 6 The effect cold air/bottom-cold and boron on length of spongy parenchyma (**a**), length of palisade parenchyma (**b**), thickness of upper epidermis (**c**) and thickness of lower epidermis (**d**)

freezing tolerance related to collection of soluble sugars, particularly sucrose (Rutten and Santarius 1992). Chilling causing accumulation of starch, sucrose, and hexoses, of muskmelon leaves (Paul 1991). It was observed that the bottom-cold and optimum condition which have more photosynthesis was poor in sugar content especially fructose content. It was explained previously that sugars accumulated which could adverse impact on Pn at cold acclimation (Lu and Huang 2003). On the other hand, soluble sugars are regulated photosynthetic activity. The photosynthetic decreased by accumulating sugars to prevent sugar export out of the leaf (Strand et al. 2003).

As it is reported previously, the sucrose, glucose, and fructose content are further susceptible to than raffinose and stachyose to cold stress (Bellaloui et al. 2012). Foliar B application increased sucrose, glucose, and fructose levels showing that B involve in carbohydrate metabolism. It seems that boron in the phloem can complex sucrose and impacting sugar metabolism (Marschner 1995). Demiray et al. (2011) showed that the protein profile of tomato seedlings changes at all the applied boron concentrations.

Cold acclimation proteins such as heat shock proteins may play a physiological role in protecting cell organism from deleterious effect of cold stress (Teigen et al. 2015). Chang et al. (2001) showed that proteins were about 60% higher than the control seedlings in cold-acclimated of mung bean seedlings. In the current research, protein content raised in bottom-cold stress. On the other hand, protein content in boron treated plant was greater in bottom-cold stress to cold-air stress. It was proven that protein, sugar and proline contribute to an enhancement of chilling resistance (Annikki et al. 2002). In cold-sensitive plants such as tomato, B uptake and transport from root to shoot limited, and B partitioning into leaves lessened in low temperature (Ye et al. 2000). In agreement with this result, we observed that cold stress decreased B concentration of leaves and bottom-cold decreased B concentration of leaves more than cold air and optimum condition, although when exogenous-B was applied the B concentration of leave was greater in bottom-cold compare with cold air. On the other hand, chilling change the shoot hydraulic conductance and the transpiration intensity, so the total B transportation into the shoot change. Transpiration in the leaves changes the B uptake by root and loading into the xylem (Huang et al. 2001). Reduction of transpiration reduced B-sink strength in leaves results in the decrease in B uptake and, partitioning to leaves (Huang et al. 2001) like it was reported in the sunflower under low root zone temperature (12 °C) (Ye et al. 2000).

The effect of stress and B on anatomical changes

The low temperature increased leaf epidermis and cell wall thickness in different species (Maria et al. 2001). Cold stress changes the epidermal cell wall structure result in modifications in properties of the outer epidermal cells. Its role is to control the expanding force of turgid, and concentrated cells placed beneath the epidermis (Marzanna et al. 1999). We observed the same results as the Maria et al. (2001) declare which show that the increase in thickness of upper and lower epidermis cell in the bottom-cold was greater which may result in more tolerate to stress.

Conclusion

It was concluded that tomato was more resistant to bottom-cold stress than cold-air stress. Boron application increased the boron of leaves more effectively in the bottom-cold consequently increase plant tolerance to a chilling condition at the bottom-cold too. The reason may refer to increase the protein content. Due to more sensitive of leaf to root the antioxidant level of the shoot was higher than root even when exposed to boron. On the other hand, boron transporting was more destructive in the cold air rather than bottom-cold and suppress water content and leave water potential of leaves greater in cold-air stress than bottom-cold. Therefore, it is suggested using boron in cold soil could be as effective as bottom-heat with lower expense but it is not as efficient in cold air temperature.

Author contribution statement FD: performed experiments and doing the measurements and helped shape the writing. MH: designed and planned the experiments, analysed data and took the lead in writing the manuscript and co-wrote the paper. All authors discussed the results and contributed to the final manuscript.

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