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Original Scientific Paper

The effect of 24-Epibrassinolide on gene expression related to cell walls under boron deficiency and toxicity in the leaves of *Arabidopsis thaliana*

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ABSTRACT:
The changing composition of plant cell walls allows for the continuation of the existing structure under normal conditions and also the protection of physical integrity under altering environmental conditions. In this study, the possible effects of the 24-Epibrassinolide (EBL) hormone under boron (B) deficiency and toxicity conditions on the expression of cell wall-related genes [*cellulose synthase* (*CESA*), *expansin* (*EXP*), *xyloglucan endotransglucosylase/hydrolase* (*XTH*) and *pectin methylesterase* (*PME*)] were investigated in the rosette leaves of *Arabidopsis thaliana*. For this purpose, 0 or 3000 μM of boric acid (BA) and/or 1 μM of EBL were applied to the plants which were grown in a hydroponic medium for five and ten weeks. While B-toxicity elevated the mRNA levels of the *CESA4* and *CESA8* genes in the leaves of the five-week-old plants, B-stress (B-deficiency and -toxicity) caused an increase in the expression of the *CESA4, CESA6*, and *CESA8* genes in the ten-week-old plants. The transcript levels of the *EXPA5* gene increased under B-stress in the ten-week-old plants whereas the expression of the *EXPA8* gene decreased when compared to the control at two developmental stages. Co-treatment of EBL and B-stress strongly elevated the transcript level of the *EXPA5* gene in the ten-week-old plants and the *EXPA8* gene at both developmental stages. The *EXPA14* and *XTH23* genes exhibited distinct expression profiles under B-deficiency and -toxicity in both the five- and ten-week-old plants. The transcript level of the *XTH21* gene was upregulated in the leaves of the plants exposed to B-stress. The mRNA level of the *PME2* and *PME41* genes was generally upregulated in response to B-stress in both the five- and ten-week-old plants. 24-Epibrassinolide alone and in combination with B-stress led to a remarkable increase in the expression of the *XTH* and *PME* genes compared to the control. These results demonstrate that cell wall genes generally show a similar pattern of expression at both developmental stages and the EBL hormone induces changes in the expression levels of cell wall-related genes under B-stress.

Keywords:
Brassinosteroid, cellulose synthase, expansin, gene expression, pectin methylesterase, xyloglucan endotransglucosylase/hydrolase

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INTRODUCTION

Boron (B) is one of the essential micronutrients for plant growth. Plants regulate boric acid (BA) homeostasis by uptake and efflux transporters (Takano *et al*. 2008). The main function of B in plants is the maintenance of the structure and the functioning of the cell walls (García-Sánchez *et al*. 2020). Boron deficiency and toxicity cause changes in the subcellular structure and compositions of cell walls (Wu *et al*. 2018a; Riaz *et al*. 2021).

The cell wall is a structure with a composite material containing cellulose microfibrils and non-cellulosic

neutral polysaccharides in a pectin matrix, with cross-linked structural proteins and lignin content specific to tissue and organs. Cell walls can be quickly remodelled when stimulated by developmental, abiotic, or biotic stimuli (Houston *et al*. 2016; Xu *et al.* 2020). One of the two mechanisms occurring in many cases in the cell wall which allows for plant adaptation and/or resistance to abiotic stresses is an increased degree of Rhamnogalacturonan-I (RG-I) branching and sustainability of cell wall plasticity depending on the increased level of expansin (EXP) and the xyloglucan endotransglucosylase/hydrolase (XTH) level. The other is improved cell wall stiffening by the strengthening of the secondary wall through lignin and hemicellulose accumulation (Gall *et al*. 2015). However, molecular mechanisms of cell wall remodelling under stress conditions have not been sufficiently highlighted yet (Rui & Dinneny 2020).

Reactive oxygen species (ROS), cell wall remodelling enzymes, plant hormones, and different wall sensors play coordinately during abiotic stress, resulting in modifications to the cell wall thus enabling plants to survive adverse conditions (Novakovic *et al.* 2018). Brassinosteroids (BRs) play an important role in the regulation of plant growth by promoting cell expansion and differentiation (Höfte 2015). Furthermore, several studies have highlighted that tolerance in an array of plant species against several abiotic stresses such as high temperature, drought, heavy metals, cold, and salinity was induced by BRs (Divi & Krishna 2009; Ahammed *et al.* 2020). The membrane steroid receptor Brassinosteroid Insensitive 1 (BRI1) binds directly to the BR ligand, activating a signal cascade in the cytoplasm which leads to the transcription of BR-responsive genes that regulate cellular growth and tolerance to various stresses (Planas-Riverola *et al.* 2019). Recent investigations have shown the relationship between BR signaling pathways and cell wall remodelling under stress conditions. However, detailed mechanisms of BR-regulated stress tolerance at the cell wall level still remain largely unknown (Tenhanken 2015).

It is estimated that about 10% of the plant genome is dedicated to the assembly and remodelling of cell walls throughout growth (McCann & Carpita 2008). In addition, the fact that the cell wall of *Arabidopsis thaliana* (L.) Heynh resembles the cell walls in many crop plants and trees has made *A. thaliana* a model plant frequently chosen to investigate cell walls (Liepman *et al.* 2010). The effects of numerous forms of abiotic stress vary according to stress intensity and duration, plant species, and developmental stage (Gall *et al.* 2015). When the cell is faced with stress factors, specific transcriptional responses affect the generation of specific cell wall proteins, leading to essential changes in the cell wall construction (Klis *et al.* 2006). Several cell wall-related genes have been shown to contribute to altering cell-wall composition under stress conditions in order to maintain cell-wall integrity (Wu *et al.* 2018b). The present study focuses on how B-stress (deficiency and toxicity) and/or 24-Epibrassinolide treatments affect the expression levels of some cell wall-related genes in the leaves of *A. thaliana* at different developmental stages.

MATERIALS AND METHODS

Plant growth and treatments. Plants of *A. thaliana* ecotype Columbia (*Col 0*) were grown hydroponically for five and ten weeks in MGRL liquid medium containing 1.75 mM sodium phosphate buffer (pH 5.8), 1.5 mM $MgSO_4$, 2.0 mM $Ca(NO_3)_2$, 3.0 mM KNO_3, 67 µM Na_2EDTA, 8.6 µM FeSO4, 10.3 µM $MnSO_4$, 150 µM H_3BO_3, 1.0 µM $ZnSO_4$, 24 nM $(NH_4)_6Mo_7O_{24}$, 130 nM $CoCl_2$ and 1.0 µM $CuS0_4$ (Fujiwara et al. 1992). The five- and ten-week-old plants were treated with MGRL medium including 0, 150 or 3000 µM boric acid (BA) (Kasajima & Fujiwara 2007) referred to as no-B, control-B, or high-B conditions, respectively, combined with or without 1 µM 24-Epibrassinolide (EBL), an active brassinosteroid, for 24 h. The preparation of the stock solution of the EBL hormone was carried out in accordance with Ali et al. (2008). The harvested rosette leaves were frozen in liquid nitrogen and stored at -80°C until molecular analysis. Three hydroponic tanks were used per treatment containing 12 plants each. The plants were grown at 22±2°C for a 16-h light (150 µM m^{-2} s^{-1}) and 8-h dark photoperiod in a growth chamber.

RNA extraction and cDNA synthesis. The total RNA was extracted from the rosette leaves of the treated and control plants by utilizing a ZR Plant RNA Miniprep Kit (Zymo Research, USA) in compliance with the protocol. The concentration and purity of the extracted RNA samples were evaluated by means of a Multiskan FC Mikroplate Photometer (Thermo, Germany) and checked by agarose gel electrophoresis. The residual DNA was removed by enzymatic digestion with DNaseI (Thermo, Germany). Reverse transcription of one microgram total RNA into complementary DNA (cDNA) was performed using oligo $(dT)_{18}$ primers and RevertAid Reverse Transcriptase (Thermo, Germany) as per the instructions.

Quantitative Real-Time PCR assay. Quantitative Real-Time PCR (qRT-PCR) was performed by means of the CFX Connect Real-Time PCR System (Bio-Rad, Germany). Primers of *cellulose synthase* (*CESA1, CESA4, CESA6,* and *CESA8*), *expansin* (*EXPA5, EXPA8,* and *EXPA14*), *xyloglucan endotransglucosylase/hydrolase* (*XTH21* and *XTH23*) and *pectin methylesterase* (*PME2* and *PME41*) genes were designed using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). In order to detect genomic DNA contamination, the primers were chosen to span exon-intron boundaries. The sequences and annealing temperature of the primers are given in

Table 1. The real-time PCR reaction was done as noted in the previous study (İşkil & Surgun-Acar 2018). The conditions for the RT-PCR were as follows: 94°C for 3 min, 30 (*CESA* and *EXP* genes) – 35 cycles (*XTH, PME* and *Actin2* genes), 94°C for 30 sec, 50 - 58.7°C for 30 sec and 70°C for 45 sec. *Actin2* was used as a reference gene and run with each PCR analysis. The RT-PCR assay was performed using three biological replicates for each treatment and two technical replicates for each biological replicate. The calculations of the relative expression level of the genes were done by Bio RAD CFX Manager 3.1 software as $2^{-\Delta\Delta Cq}$ formula (Livak & Schmittgen 2001) and normalizations were carried out by the mean of $2^{-\Delta\Delta Cq}$ values for the reference gene in each sample (Remans *et al*. 2012).

Statistical analysis. The normality and homoscedasticity of the data were tested by Shapiro-Wilk's and Bartlett's tests, respectively. The data are presented as means ± standard error (SE). The differences between the mean gene expression values in the groups were examined using Duncan's multiple range tests ($P < 0.01$).

RESULTS

In this study, the expression levels of certain cell wall-related genes were determined in the rosette leaves of five- and ten-week-old plants subjected to B-stress combined with or without the EBL hormone using qRT-PCR.

Boron toxicity (3000 µM BA) enhanced the expression of the *CESA4* and *CESA8* genes by 1.84 and 2.34-fold, respectively, in the leaves of the five-week-old plants. Co-treatment of EBL and B-toxicity resulted in the increased expression of the *CESA4* and *CESA8* genes in comparison to B-toxicity and the control samples, respectively (Fig. 1A). The transcript levels of the *CESA4, CESA6*, and *CESA8* genes increased by 4.44, 1.91 and 3.19-fold in the plants subjected to B-deficiency and 3.28, 1.81, and 2.45-fold as a result of B-toxicity, respectively, in the ten-week-old plants (Fig. 1B). The expression of the *CESA6* gene was upregulated in the leaves of the plants exposed to 3000 µM BA + EBL treatment as compared with the control (Fig. 1B).

EBL application alone caused a 2.74-fold increase in the expression of the *EXPA8* gene as compared with the

Table 1. The sequences and optimal annealing temperature of the primers utilized in this study.

Sequences ID	GenBank accession no	Gene name	Sequence (5'- 3')	Annealing temperature (°C)	Gene description
At4g32410	NM_119393.3	*CESA1*	F-ACTGGTTCCAATGGCGAAGAAC R-AACCGAGGTCAACCACAAAG	57.8°C	Cellulose synthase 1
At5g44030	NM_001344528.1	*CESA4*	F-CATTCGTCAAAGATCGCAGA R-CCAACCTTCTTCAGGCTTCT	53.8°C	Cellulose synthase A4
At5g64740	NM_125870.3	*CESA6*	F-CGTGGACCTCTCTACCGCTCA R-AGAAGAGCGCCATGAAGAGG	53.8°C	Cellulose synthase 6
At4g18780	NM_117994.4	*CESA8*	F-CTTATGGAGAATGGCGGTGT R-AACCCGTCAAAATGTCTTCG	50.0°C	IRX1 cellulose synthase family protein
At2g18800	NM_127436.2	*XTH21*	F-GGGTGTGGCTTATCCAAAGA R-GGTCCCTGTGACCAGTTTGT	50.0°C	Xyloglucan endotransglucosylase/ hydrolase 21
At4g25810	NM_118713.5	*XTH23*	F-CAAGAACAGATGAGATGGGTACAGAAT R-CGCAGCTAAGCACTCGCGT	57.8°C	Xyloglucan endotransglycosylase 6
At3g29030	NM_113824.3	*EXPA5*	F-CCGGTATCATTCCCGTTATG R-AATTTTGCCCCCAATTTCTC	50.0°C	Expansin A5
At2g40610	NM_129623.3	*EXPA8*	F-CAACCATCACCGTCACAGCTA R-TGAAGAGGAGGATTGCACCAA	53.8°C	Expansin A8
At5g56320	NM_001345194.1	*EXPA14*	F-TTCACGATCAACGGTCATTC R-GCCAACGTGTATTGGTTCCT	57.8°C	Expansin A14
At1g53830	NM_104260.5	*PME2*	F-ATGTTCTTGGGAGATGGCCG R-TCGACGGTTCCGGTTATGTG	50.0°C	Pectin methylesterase 2
At4g02330	NM_116466.4	*PME41*	F-TGGACCACTTTCAACTCCG R-GGTTCAACAACCTCGTCTATG	58.7°C	Plant invertase/pectin methylesterase inhibitor superfamily
At3g18780	NM_001338359.1	*Actin2*	F-TGCCAATCTACGAGGGTTTC R-TTCTCGATGGAAGAGCTGGT	50.0°C	Actin2

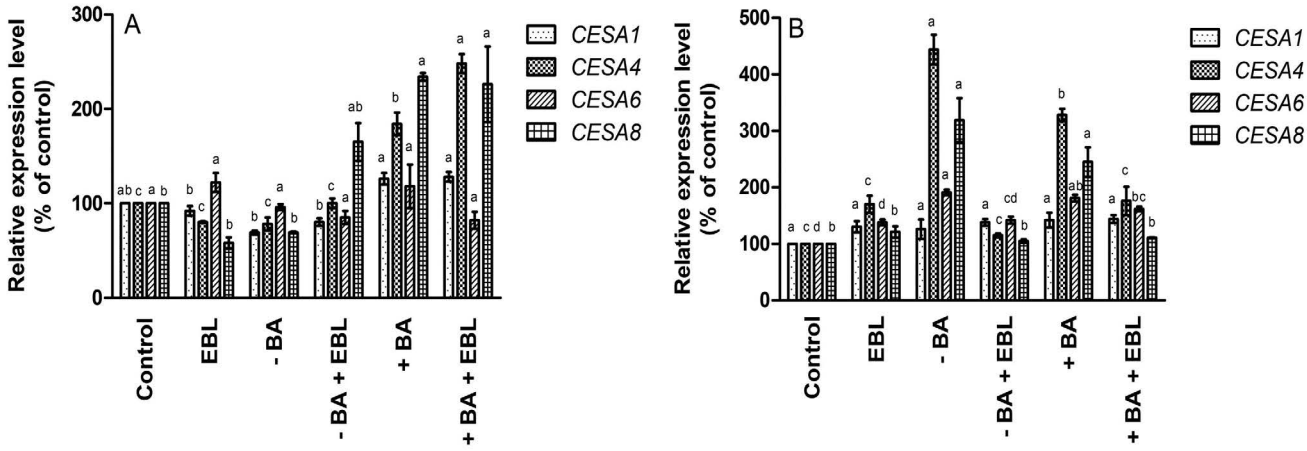

Fig. 1. The relative expression levels of *cellulose synthase* (*CESA*) genes in the leaves of five- and ten-week-old seedlings subjected to BA deficiency (- BA) or BA toxicity (+ BA) and/or 24-Epibrassinolide (EBL) treatments for 24 h. A) Expression levels of *CESA* genes in the five-week-old plants. B) Expression levels of *CESA* genes in the ten-week-old plants. The relative expression levels were calculated with reference to the controls (taken as 100%). Each value in the graph shows the mean with standard error (SE). The different letters above the bars show the statistical differences according to Duncan's multiple range tests ($P < 0.01$) among the treatments for each gene.

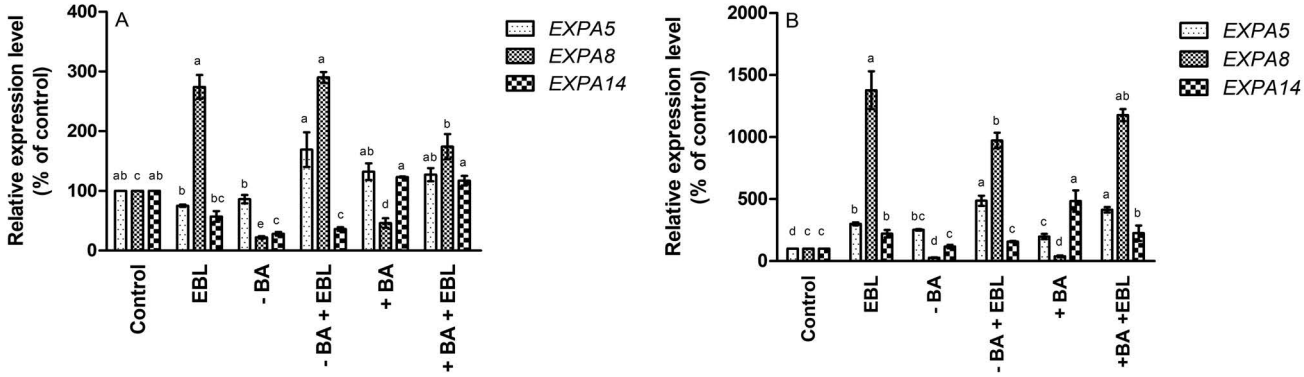

Fig. 2. The relative expression levels of *expansin* (*EXP*) genes in the leaves of the five- and ten-week-old seedlings subjected to BA deficiency (- BA) or BA toxicity (+ BA) and/or 24-Epibrassinolide (EBL) treatments for 24 h. A) Expression levels of *EXP* genes in the five-week-old plants. B) Expression levels of *EXP* genes in the ten-week-old plants. The relative expression levels were calculated with reference to the controls (taken as 100%). Each value in the graph shows the mean with standard error (SE). The different letters above the bars show the statistical differences according to Duncan's multiple range tests ($P < 0.01$) among the treatments for each gene.

control in the five-week-old plants. Treatment with B-deficiency and B-toxicity decreased the expression of the *EXPA8* gene at rates of 4.54 and 2.17 in comparison to the control, respectively; while EBL caused an increase in expression levels when compared with both the control and stress treatments alone (Fig. 2A). Boron deficiency and co-treatment of B-deficiency and EBL reduced the transcript level of the *EXPA14* gene when compared to the control samples in the leaves of the five-week-old plants (Fig. 2A). The expression of the *EXPA* genes was enhanced as a result of EBL treatments alone in the ten-week-old plants (Fig. 2B). Boron stress upregulated the expression level of the *EXPA5* gene compared with the control, however, combined treatment with EBL and B-stress caused an increase in expression levels at higher rates (Fig. 2B). Boron stress decreased the expression levels of *EXPA8* compared with the control, whereas the EBL hormone caused a remarkable rise in the expression levels of *EXPA8* where it was applied under stress (Fig. 2B). Boron toxicity served to enhance the mRNA level of the *EXPA14* gene by 4.84-fold in the leaves of the ten-week-old plants. Co-treatment of EBL and B-toxicity was observed to promote the mRNA level of the *EXPA14* gene compared with the control (Fig. 2B).

The expression of the *XTH21* gene was elevated by 2.70-fold with EBL, by 1.83-fold with B-deficiency and by 4.33-fold with the co-treatment of EBL and B-deficiency compared to the control in the five-week-old

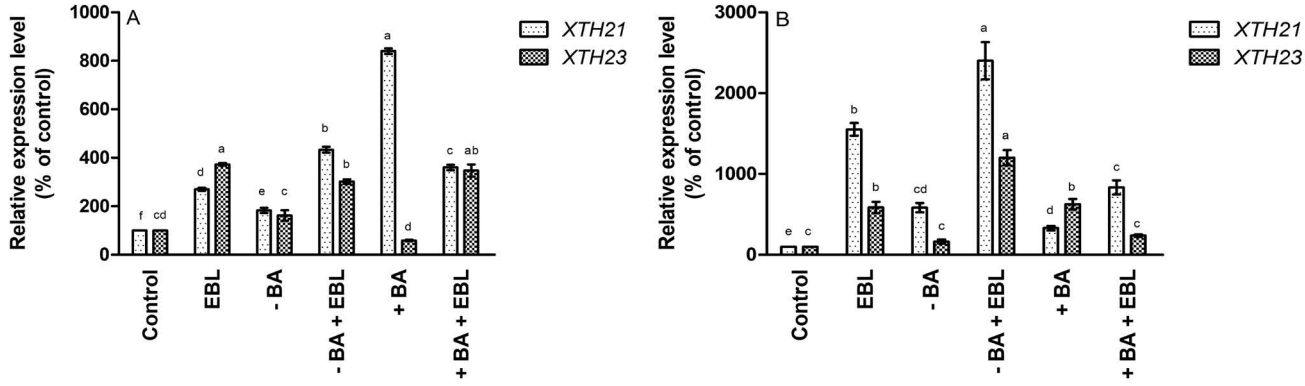

Fig. 3. The relative expression levels of *xyloglucan endotransglucosylase/hydrolase* (*XTH*) genes in the leaves of five- and ten-week-old seedlings subjected to BA deficiency (- BA) or BA toxicity (+ BA) and/or 24-Epibrassinolide (EBL) treatments for 24 h. A) Expression levels of *XTH* genes in the five-week-old plants. B) Expression levels of *XTH* genes in the ten-week-old plants. The relative expression levels were calculated with reference to the controls (taken as 100%). Each value in the graph shows the mean with standard error (SE). The different letters above the bars show the statistical differences according to Duncan's multiple range tests ($P < 0.01$) among the treatments for each gene.

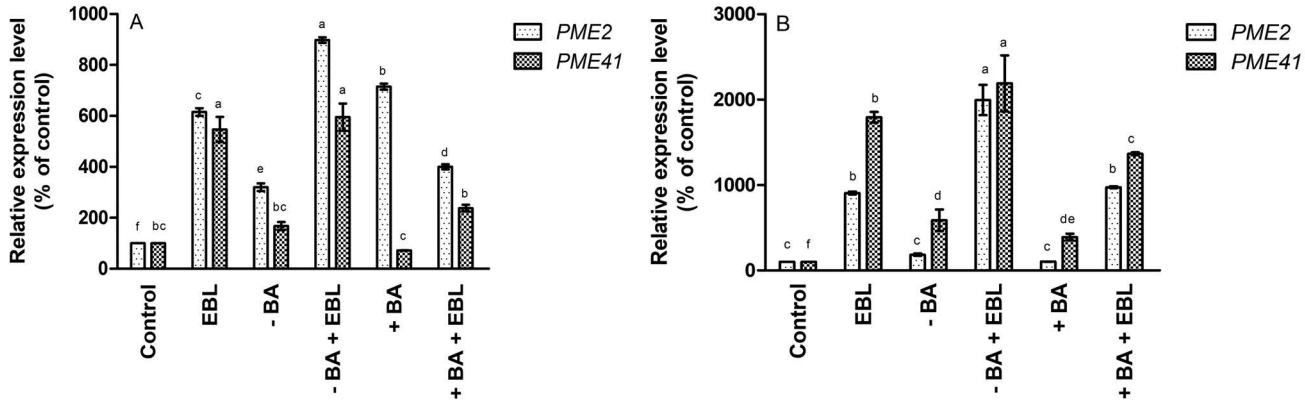

Fig. 4. The relative expression levels of *pectin methylesterase* (*PME*) genes in the leaves of the five- and ten-week-old seedlings subjected to BA deficiency (- BA) or BA toxicity (+ BA) and/or 24-Epibrassinolide (EBL) treatments for 24 h. A) Expression levels of *PME* genes in the five-week-old plants. B) expression levels of *PME* genes in the ten-week-old plants. The relative expression levels were calculated with reference to the controls (taken as 100%). Each value in the graph shows the mean with standard error (SE). The different letters above the bars show the statistical differences according to Duncan's multiple range tests ($P < 0.01$) among the treatments for each gene.

plants. While B-toxicity increased the mRNA level of the *XTH21* gene by 8.40-fold, the combined treatment of B-toxicity and EBL increased it by 3.60-fold (Fig. 3A). The mRNA level of the *XTH23* gene remained stable as a result of B-deficiency and toxicity applications in comparison to the control, whereas the mRNA level increased at rates of 3.02 and 3.48, respectively when applied with EBL in the leaves of the five-week-old plants (Fig. 3A). EBL treatment alone elevated the expression level of the *XTH21* and *XTH23* genes in the leaves of the ten-week-old plants. The transcript level of the *XTH21* gene increased in response to B-stress treatment compared with the control plants. The combined treatment of EBL with B-stress caused a sharp rise in the expression of the *XTH21* gene in the ten-week-old plants (Fig. 3B). Boron toxicity increased the expression level of the *XTH23* gene by 6.27-fold, whereas 0 μM BA + EBL treatment led to an increase in the expression level of the gene transcripts of 12-fold (Fig. 3B).

The expression levels of the *PME2* and *PME41* genes rose by 6.15 and 5.47-fold, respectively, as a result of EBL application in the five-week-old plants. Treatment with B-deficiency and B-toxicity dramatically increased the transcript level of the *PME2* gene at rates of 3.20 and 7.15, respectively and treatment of EBL with B-stress promoted the expression of the *PME2* gene when compared with the control in the leaves of the five-week-old plants (Fig. 4A). While B-stress did not change the ex-

pression of the *PME41* gene, the combined treatment of EBL and B-stress upregulated the expression levels of *PME41* compared with the control (Fig. 4A). 24-Epibrassinolide treatment alone enhanced the mRNA level of the *PME2* gene by 9.07-fold, and when combined with 0 and 3000 μM BA lead to an increase in the expression rates of 19.96 and 9.74, respectively in the leaves of the ten-week-old plants (Fig. 4B). The *PME41* gene also exhibited an increased expression level as a result of the applications. Boron deficiency and toxicity increased the transcript level of the *PME41* gene at rates of 5.89 and 3.91, respectively. EBL treatment alone resulted in a 17.93-fold rise in the mRNA level of *PME41*, while the treatment of EBL with 0 and 3000 μM BA increased the expression level by 21.90 and 13.66-fold, respectively, in the leaves of the ten-week-old plants (Fig. 4B).

DISCUSSION

Advances in plant genomics over the last years have merged with forward and reverse genetic analyses and molecular details of cellulose biosynthesis have been detailed. The synthesis of cellulose microfibrils in plants is carried out by the cellulose synthase complex (CSC) localized in the cell membrane in relation to the sucrose synthase. Each CSC consists of 6 subunits, each of which includes 6 CESA proteins (Lerouxel *et al.* 2006). The *Arabidopsis thaliana* genome was determined to include 10 different *CESA* genes (Somerville *et al.* 2006). In the present study, the mRNA levels of the *CESA4, CESA6* and *CESA8* genes in the rosette leaves of the plants generally increased under B-stress in both developmental stages. Increased cellulose synthesis may result in continued cell growth under stress conditions by sustaining cell wall integrity and turgor pressure (Gall *et al.* 2015). Advanced spectroscopic analyses (Fourier transform infrared and X-ray photoelectron spectroscopy) conducted on orange plants revealed increased relative cellulose and hemicellulose contents in the cell walls under B-deficient conditions (Liu *et al.* 2014). In another study, the importance of the *CESA6* and CSI_1 genes were determined for maintaining cellulose synthesis under salt stress conditions in *A. thaliana* as a result of forward genetic analysis (Zhang *et al.* 2016). On the other hand, in a later study, the expression of the *CESA* genes was not affected by B-stress in ten-week-old plants, while B-toxicity suppressed the expressions of the *CESA1* and *CESA8* genes in the roots of five-week-old *A. thaliana* (İşkil & Surgun-Acar 2018). Combined treatment of EBL and B-toxicity led to an increase in the mRNA level of the *CESA4* gene compared with B-toxicity treatment alone in the plant leaves. It was revealed by Xie *et al.* (2011) that BR signaling triggers the transcription factor BRI1-EMS-Suppressor 1 (BES1) and BES1 stimulates the promoters of most *CESA* genes to enhance their expression and cellulose biosynthesis in the aerial parts of *Arabidopsis* seedlings.

Expansins, a gene superfamily, consist of three subfamilies which are α-expansin (*EXPA*), β-expansin (*EXPB*) and expansin-like (*EXLA* and *EXLB*) genes (Han *et al.* 2012). Expansins break the hydrogen link between cellulose microfibrils and matrix glycans through cell growth and play a role in cell wall loosening without causing hydrolytic degradation (Park *et al.* 2010). Expansins regulate fruit softening, organ development, germination, and stress responses in plants depending on their ability to promote cell wall loosening (Wieczorek *et al.* 2006). In this study, while the expressions of the *EXPA5* and *EXPA14* genes increased under B-stress and B-toxicity respectively, in the ten-week-old plants, the mRNA level of the *EXPA8* gene decreased in the plants under B-deficiency and toxicity conditions at two developmental stages. The expression level of the *EXPA14* gene reduced in the leaves of the five-week-old-plants exposed to B-deficiency. 24-Epibrassinolide treatments with B-stress generally led to increased mRNA levels of the investigated *EXP* genes. İşkil & Surgun-Acar (2018) reported that the effects of EBL on *EXP* genes under B-stress change depending on the developmental stage in the roots of *Arabidopsis* plants. Park *et al.* (2010) showed that *EXPA5* is a growth-regulating gene in *A. thaliana* and the transcription of this gene is regulated by the Brassinazole Resistant 1 (BZR1) transcription factor in the BR signal transduction pathways. It was revealed that Homolog of BEE2 interacting with IBH1 (HBI1), Paclobutrazol Resistant1 (PRE1), and ILI1 Binding bHLH Protein1 (IBH1) modulates cell elongation in response to light, BRs, and gibberellic acid (GA). In this interaction, BZR1 can positively regulate HBI1 which stimulates cell expansion by binding to the promoter of the *EXP* genes (*EXP1* and *EXP8*), and consequently inducing their expression (Bai *et al.* 2012). It was concluded from the findings that exogenous EBL application to the plants exposed to B-deficiency and -toxicity may stimulate cell wall loosening. However, the findings on the *EXP* genes need to be studied further with a focus on EBL and B-stress.

Abiotic stress conditions lead to stiffening by the cross-linking of ROS and peroxidases with phenolic compounds and glycoproteins of the cell walls. In this case, by acting together, xyloglucan modifying enzymes and expansin allow stressed organs to expand as a result of cell wall loosening (Tenhaken 2015). The expression of the *XTH21* gene was upregulated in the rosette leaves of the plants under B-stress conditions in both developmental stages. It was also found that B-toxicity increased the transcript level of the *XTH23* gene in the ten-week-old plants. It was demonstrated that several abiotic stress conditions (cold, osmotic stress, salt, and drought) caused an increased expression of one or several *XTH* gene family members (Chan *et al.* 2011). The cross-linking of each gene in the *XTH* family which catalyse either the molecular grafting or disassembly of xyloglucan occurs within the cellulose/xyloglucan framework (Yokoyama &

Nishitani 2001). A notable increase was observed in the transcripts of *OsXETs*, a xyloglucan-modifying enzyme, in rice plants exposed to different forms of abiotic stress and it served as a general stress marker gene (Dong *et al.* 2011). The overexpression of *CaXTH3* in *Solanum lycopersicum* L. revealed that enhanced salt tolerance involved cell-wall elasticity for mitigating the effects of stress (Choi *et al.* 2011). 24-Epibrassinolide alone and combined with B-stress remarkably upregulated the expression of the *XTH21* and *XTH23* genes compared with the control and stress treatments. Yokoyama & Nishitani (2001) found that the mRNA level of the *XTH23* gene is regulated by plant growth regulators and the maximum increase in the transcript level was observed as a result of the 1 μM brassinolide (BL) treatment when compared with other plant hormones. In another study, the *XTH19* and *XTH23* genes were induced by salt stress via the key BR signalling pathway transcription factor BES1, thus contributing to lateral root adaptation to salt (Xu *et al.* 2020). The regulation of *XTH* expression by BRs may induce modification of the interaction between xyoglucan and cellulose microfibrils to change cell wall stiffness (Rao & Dixon 2017).

Pectic polysaccharides include RG-I, Rhamnogalacturonan-II (RG-II), xylogalacturonan (XGA) and homogalacturonan (HG) polymers as backbone units containing various galacturonic acid molecules (Rao & Dixon 2017). The looseness and stiffness of the pectic matrix are regulated by the degree of methylesterification in HGs, which is controlled by the balance of activity between pectin methylesterase enzymes (Wolf *et al.* 2012). Pectin methylesterases play a role in different developmental processes in the cell wall and responses to biotic and abiotic stresses (Qu *et al.* 2011). In the present study, an enhanced expression level was observed in the *PME2* and *PME41* genes under B-stress conditions in both the five- and ten-week-old plants. The expression of some genes in the five- and ten-week-old plants responded differently to B-deficiency and -toxicity at the transcriptional level. This phenomenon may have resulted from the changeability of cell wall composition at various developmental stages (Popper *et al.* 2011). Pectin methylesterase activity and *PME* transcript levels were observed to increase in different plant species in response to chilling stress, which resulted in enhanced cold tolerance (Solecka *et al.* 2008; Qu *et al.* 2011). 24-Epibrassinolide applications caused the expressions of *PME2* and *PME41* to increase markedly in the present study. Similar findings were reported by İşkil & Surgun-Acar (2018) who observed a significant upregulation in the expression of the *PME41* gene with EBL supplementation under B-stress. Sun *et al.* (2010) investigated the direct transcriptional targets of BR signalling and found an increase in the expression level of *PME* genes. In order to decrease the stiffness of the pectic matrix and induce cell wall loosening under both normal and stress conditions in *Arabidopsis*, BR-receptor kinase BRI1-Associated Kinase (BAK1) is capable of repressing the activity of PME inhibitors by directly interacting with cell membrane receptor-like protein RLP44 (Wolf *et al.* 2014). In this case, *PME* genes can be considered as candidate genes in stress tolerance directly or indirectly provided by BRs.

CONCLUSION

The present study showed that B-stress affected the expression levels of cell wall-related genes at the transcriptional level and the majority of these genes exhibited a similar response to B-deficiency and B-toxicity. 24-Epibrassinolide mostly upregulated the expression level of genes related to cell wall assembly and remodelling under B-stress in the rosette leaves of the five- and ten-week-old plants. In addition to the progress made over the last decade regarding the effects of abiotic stresses on the metabolism of cell walls, physiological, biochemical, proteomic, and genetic approaches are expected to yield worthwhile results in order to allow a deeper understanding of the complex structure of cell walls and their response to abiotic stresses with plant hormone treatments.

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REZIME

Botanica SERBICA

Efekat 24-Epibrazinolida na ekspresiju gena povezanih sa ćelijskim zidom u uslovima nedostatka i toksičnih nivoa bora u listovima *Arabidopsis thaliana*

Rabia İşkil i Yonca Surgun-Acar

Promenljiv sastav ćelijskog zida biljaka omogućava produžetak postojeće strukture u normalnim uslovima, a takođe i zaštitu fizičkog integriteta u promenljivim uslovima sredine. U ovoj studiji je istraživan potencijalni efekat 24-Epibrasinolidnog (EBL) hormona u uslovima nedostatka bora (B) i uslova toksičnosti na ekspresiju gena odgovornih za ćelijski zid [*celulozna sintaza* (*CESA*), *ekspanzin* (*EXP*), *ksiloglukan endotransglukozilaza/hidrolaza* (*XTH*) i *pektin metil esteraze* (*PME*)], u listovima rozete *Arabidopsis thaliana*. U ovu svrhu je 0 ili 3000 μM borna kiselina (BA) i/ili 1 μM EBL primenjena na biljke koje su rasle u hidroponskom medijumu tokom pet i deset nedelja. Dok je toksičnost borom izazvala porast mRNA nivoe *CESA4* i *CESA8* gena u listovima biljaka starih pet nedelja, stress izazvan borom (njegov nedostatak i toksičnost) doveo je do povećanja ekspresije CESA4, CESA6, i CESA8 gena u biljkama starim deset nedelja. Nivo transkripcije *EXPA5* gena izazvan stresom B kod biljaka starih deset nedelja je porastao, dok se ekspresija *EXPA8* gena smanjila u poređenju sa kontrolom oba razvojna stadijuma. Zajednički tretman EBL i stresa B znatno je povećao nivo transkripcije *EXPA5* gena u biljkama starim 10 nedelja, kao i *EXPA8* gena kod oba razvojna stadijuma. *EXPA14* i *XTH23* geni pokazali su različite profile ekspresije pri nedostatku B, kao i pri njegovim toksičnim nivoima u biljkama starim pet i deset nedelja. Nivo transkripcije *XTH21* gena je pojačano regulisan u listovima biljaka izloženim stresu izazvanim B. Nivo mRNA kod gena *PME2* i *PME41* se generalno povećava kao odgovor na stress izazvan B u biljkama starim pet i deset nedelja, respektivno. 24- Epibrazinolid samostalno i u kombinaciji sa stresom izazvanim B doveo je do značajnog povećanja ekspresije *XTH* i *PME* gena u poređenju sa kontrolom. Ovi rezultati pokazuju da ćelijski zid generalno pokazuje slični obrazac ekspresije u oba razvojna stadijuma i da EBL hormon izaziva promene u nivoima ekspresije gena povezanih sa ćelijskim zidom pod B-stresom.

Ključne reči: Brasinosteroid, celulozna sintaza, ekspanzin, ekspresija gena, pektin metil esteraza, ksiloglukan endotransglukozilaza/hidrolaza