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Expression analysis of cell wall assembly and remodelling-related genes in *Arabidopsis* roots subjected to boron stress and brassinosteroid at different developmental stages

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ABSTRACT

Plant cell walls are affected by many biotic and abiotic stress conditions. The aim of this study is to determine the effects of 24-Epibrassinolide (EBL) on some cell wall-related genes in root tissue of five- and ten-week-old *Arabidopsis thaliana* plants exposed to boron (B) deficiency (0 µM) or toxicity (3000 µM) at the transcriptional level. Expressions of the genes that encode cellulose synthase (*CESA1, CESA4, CESA6* and *CESA8*), cellulose synthase-like (*CSLB5*), expansin (*EXPA5, EXPA8* and *EXPA14*) and cell wall protein (*SEB1*) decreased under conditions of B deficiency and toxicity. EBL treatments, in general, led the expressions of these genes to reduce significantly. Expressions of xyloglucan endotransglucosylase/hydrolase genes (*XTH21* and *XTH23*) changed only under conditions of B toxicity. Boron stress and/or EBL treatments caused different responses in expression of pectin methylesterase (*PME2* and *PME41*) genes. As a result of B stress, the expression levels of investigated genes changed more in roots of five-week-old plants than in roots of ten-week-old plants. Results of the present study include new findings that support the ability of BRs to increase molecular aspects of tolerance to stress in plants.

Keywords: 24-Epibrassinolide, *Arabidopsis thaliana*, boron, cellulose synthase, gene expression, expansin, pectin methylesterase, xyloglucan endotransglucosylase/hydrolase

Introduction

Plant cell walls are one of the main barriers to abiotic and biotic stresses (Han *et al.* 2012). It can be suggested that the genes which can synthesize or hydrolize plant cell wall components may promote stress tolerance by means of changes in composition of the cell wall since they show different expressions under different stress conditions (Houston *et al.* 2016). Boron (B), which is essential for plants, is taken up from soil in the form of boric acid (BA) (Brown *et al.* 2002). Depending on the B concentration in the soil, BA is taken through different mechanisms such as (i) passive process, (ii) channel-mediated facilitated diffusion and (iii) under B limit conditions via BOR transporters (Miwa & Fujiwara 2010). Primary function of B in higher plants is to form borate esters with rhamnogalacturonan II (RGII) (Kobayashi *et al.* 1996). Construction of this structure is critical for cell wall composition and function, and the complex controls cell wall porosity and strength (Camacho-Cristobal *et al.* 2011). Boron deficiency (Camacho-Cristobal *et al.* 2011) and B toxicity (Kasajima & Fujiwara 2007) cause expressions of the genes involved in numerous physiological processes to change.

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Brassinosteroids (BRs), a group of steroidal hormones in plants, regulate many different developmental and physiological processes (Divi *et al.* 2016). In addition, it has been revealed that exogenous BR application and genetic manipulation of the genes controlling endogenous BR level increase crop tolerance under various abiotic stress conditions and promote biomass productivity (Sharma *et al.* 2013; Ahammed *et al.* 2015). In plants, leucine-rich repeat receptor-like kinase (LRR-RLK) BRASSINOSTEROID INSENSITIVE1 (BRI) perceives BR signal at the cell surface (Li & Chory 1997). Binding between BR and BRI1 results in BRI1 autophosphorylation and association and transphosphorylation with coreceptor BRI1-ASSOCIATED KINASE1 (BAK1). Inactivation of the negative regulator BRASSINOSTEROID INSENSITIVE1 kinase and finally, dephosphorylation and stabilization of transcription factors BRASSINAZOLE-RESISTANT1 (BZR1) and BRI1-EMS-SUPPRESSOR1 (BES1) occur (Li & Jin 2007). In order to affect the expression of BR-regulated genes, activated BZR1 and BES1 bind the promoters of BR-regulated genes directly (Wang *et al.* 2014). According to recent evidence, it is suggested that there is an association between BR signaling pathways and cell wall remodeling (Rao & Dixon 2017).

Transcriptional changes under differential abiotic stress conditions were discussed in detail (Gehan *et al.* 2015) however; there are very few studies that investigated these changes in the context of cell wall-related genes (Houston *et al.* 2016). The aim of this study is to determine expression levels of some *cellulose synthase, expansin, xyloglucan endotransglycosylase/hydrolase, pectin methylesterase* and cell wall protein coding genes under B deficiency and toxicity conditions and also possible effects of 24-Epibrassinolide on these genes under B stress in *Arabidopsis* roots at different developmental stages using real-time PCR.

Materials and methods

Plant material and treatments

Arabidopsis thaliana (L.) Heynh Columbia (Col 0) ecotype was cultured in hydroponic system (Surgun & Bürün 2015) in liquid medium containing 1.75 mM sodium phosphate buffer (pH 5.8), 1.5 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 3 mM KNO_3, 6.7 µM Na_2EDTA, 8.6 µM $FeSO_4$, 10.3 µM $MnSO_4$, 150 µM H_3BO_3, 1.0 µM $ZnSO_4$, 24.0 nM (NH4)6Mo_7O_{24}, 130 nM $CoCl_2$ and 1 µM $CuSO_4$ (Fujiwara *et al.* 1992) during five-weeks and ten-weeks. The culture medium changed with fresh medium two times in a week. Afterwards, five-week-old and ten-week-old *Arabidopsis* seedlings were transferred to the identical medium without boric acid (BA) (0 µM) or including high concentration of BA (3000 µM) and/or 1 µM 24-Epibrassinolide (EBL) hormone and incubated for 24 hours. The full-strength medium described earlier was used as the control medium. After 24 hours roots of plants were harvested from each treatment and frozen in liquid nitrogen for RNA extraction. All experiments were carried out in a growth chamber (Aralab, Portugal) under 16- h light (150 µM m-2 s-1) and 8-h dark photoperiod at 22±2 °C.

RNA extraction

Total RNA from root of *Arabidopsis* seedlings was isolated using Plant RNA Miniprep Kit (Zymo Research, USA) according to manufacturer's manual. Total RNA concentration and purity were assesed at 260 nm and 260 nm / OD 280 nm respectively, using Multiskan FC Mikroplate Photometer (Thermo, Germany). The integrity of isolated RNA samples was assessed by electrophoresis on 1.2 % agarose gels.

cDNA synthesis and real-time PCR assay

Genes of interest [*cellulose synthase* and *cellulose synthase-like* (*CESA1, CESA4, CESA6, CESA8* and *CSLB5*), *expansin* (*EXPA5*, *EXPA8* and *EXPA14*), *xyloglucan endotransglycosylase/hydrolase* (*XTH16, XTH21* and *XTH23*), *pectin methylesterase* (*PME2* and *PME41*) and cell wall protein (*SEB1*) coding genes] were determined based on previous literatures (Yokoyama & Nishitani 2001; Müssig *et al.* 2002; Hamann *et al.* 2004; Camacho-Cristobal *et al.* 2008). Primers for some genes were designed using Primer3. Primers spanning one intron were designed to minimize effect of DNA contamination in RNA samples. The following gene-specific primers were used: *CESA1* (At4g32410): 5´-ACTGGTTCCAATGGCGAAGAAC-3´ and 5´-AACCGAGGTCAACCACAAAG-3´; *CESA4* (At5g44030): 5´-CATTCGTCAAAGATCGCAGA-3´ and 5´-CCAACCTTCTTCAGGCTTCT-3´; *CESA6* (At5g64740): 5´-CGTGGACCTCTCTACCGCTCA-3´ and 5´-AGAAGAGCGCCATGAAGAGG-3´; *CESA8* (At4g18780): 5´-CTTATGGAGAATGGCGGTGT-3´ and 5´- AACCCGTCAAAATGTCTTCG-3´; *CSLB5* (At4g15290): 5´-TTATTGCCTCTTGCCTGCTT-3´ and 5´- CAGTGCATCCCCACAAGTGTG-3´; *XTH16* (At3g23730): 5´-TGAGCTTAATGCTTATGGGAGAA-3´ and 5´- CTCTGGTGGGAATCCTTGAG-3´; *XTH21* (At2g18800): 5´-GGGTGTGGCTTATCCAAAGA-3´ and 5´-GGTCCCTGTGACCAGTTTGT-3´; *XTH23* (At4g25810): 5´-CAAGAACAGATGAGATGGGTACAGAAT-3´ and 5´-CGCAGCTAAGCACTCGCGT-3´; *EXPA5* (At3g29030): 5´-CCGGTATCATTCCCGTTATG-3´ and 5´- AATTTTGCCCCCAATTTCTC-3´; *EXPA8* (At2g40610): 5´-CAACCATCACCGTCACAGCTA-3´ and 5´-TGAAGAGGAGGATTGCACCAA-3´; *EXPA14* (At5g56320): 5´-TTCACGATCAACGGTCATTC-3´ and 5´- GCCAACGTGTATTGGTTCCT-3´; *PME2* (At1g53830): 5´-ATGTTCTTGGGAGATGGCCG-3´ and 5´-TCGACGGTTCCGGTTATGTG-3´, *PME41* (At4g02330): 5´-TGGACCACTTTCAACTCCG-3´ and 5´-GGTTCAACAACCTCGTCTATG-3´ and *SEB1* (*COBL7*) (At4g16120): 5´-GGTACCGTTTTCGCTGGTTA-3´ and

5´-TTTGGGACATTTCCATCCAT-3´. To eliminate DNA contamination, total RNA samples treated with DNase I (Thermo, Germany) according to the instructions manual and one microgram of DNase I-treated RNA from each sample was reverse-transcribed to cDNA using RevertAid Reverse Transcriptase (Thermo, Germany) and oligo $(dT)_{18}$ primer. cDNA was diluted 1:20 with RNase-free water prior use in RT-PCR reaction.

RT-PCR reaction contained 5 µl 2 X SYBR Green qPCR Master Mix (Thermo, Germany), 0.375 µl forward and reverse primer (10 pmol), 3 µl cDNA and 1.25 µl nuclease free water in each reaction for *CESA1, CESA4, CESA6, CSLB5, EXPA8, EXPA14, XTH16, XTH23, PME41, SEB1* and *Actin2* genes. Five µl 2 X SYBR Green qPCR Master Mix, 0.375 µl forward and reverse primer (10 pmol), 3 µl cDNA (diluted 1:20), 0.75 µl MgCl2 (25 mM) and 0.50 µl nuclease free water was contained in PCR reaction for the other genes (*CESA8, EXPA5, PME2 and XTH21*). The real-time PCRs were perfomed using CFX Connect real-time PCR Detection System (Bio-Rad, Germany) as follows: 3 min at 94 oC (initial denaturating step), 30 (*CESA1, CESA4, CESA6, CESA8, CSLB5, EXPA5, EXPA8* and *EXPA14*) – 35 cycles of (*XTH16, XTH21, XTH23, PME2, PME41, SEB1* and *Actin2*) 30 sec at 94 °C (denaturation), 30 sec at 50 °C (annealing) for *Actin2, CESA8, EXPA5, XTH21* and *PME2*; 53.8 °C for *CESA4, CESA6, CSLB5* and *EXPA8*; 57.8 °C for *CESA1, EXPA14, XTH16* and *XTH23*; 58.7 °C for *PME41* and *SEB1* and 45 sec at 70 °C (extension). *Actin2* (At3g18780) (forward primer: TGCCAATCTACGAGGGTTTC; reverse primer: TTCTCGATGGAAGAGCTGGT) was considered as a reference gene to normalize gene expression. Bio RAD CFX Manager 3.1 software (Bio-Rad, Germany) relative quantification analysis was used to determine the relative expression level of genes (control as 100). Three biological replicates for each sample were used for RT-PCR assay and three technical replicates for each biological replicate were analysed.

Statistical analysis

Normal distribution of each data set was analyzed using Shapiro-Wilk's and Bartlett's tests. Data are the mean ± standard error (SE). Statistical significance was tested by one-way analysis of variance (ANOVA) and differences between group means were evaluated using Duncan multiple range test. In all analyses, the significance level was set at 0.01.

Results

Cellulose synthase genes

While expression of *CESA1* gene in root tissues of five-week-old seedlings in BA-free medium (0 µM BA) did not change, EBL added to 0 µM BA medium caused the expression to reduce by 1.88 comparing to the control. Boron toxicity (3000 µM) and EBL hormone co-applied with 3000 µM BA reduced the expression of *CESA1* gene in a statistically significant way (Fig. 1A). Boron stress (deficiency or toxicity) and/or EBL applications that were applied for 24 hours led transcript level of *CESA4* gene to decrease at similar rates (at the rates of 2.06-2.46) (Fig. 1A). As a result of 3000 µM BA application, expression of *CESA8* gene was decreased by 1.90 when compared to control (Fig. 1A). EBL application in roots of five-week-old seedlings reduced expression of *CSLB5* gene by 6.15, while 0 µM BA + EBL application reduced it by 7.66 (Fig. 1A). 3000 µM BA reduced the expression level of this gene by 2.22, and 3000 µM BA + EBL application resulted in a dramatic decrease (22 -fold) (Fig. 1A).

Boron deficiency and toxicity in root tissues of ten-week-old plants did not change the expression of *CESA1* gene, while 3000 µM BA + EBL application resulted in a 2-fold reduction in expression (Fig. 1B). In ten-week-old

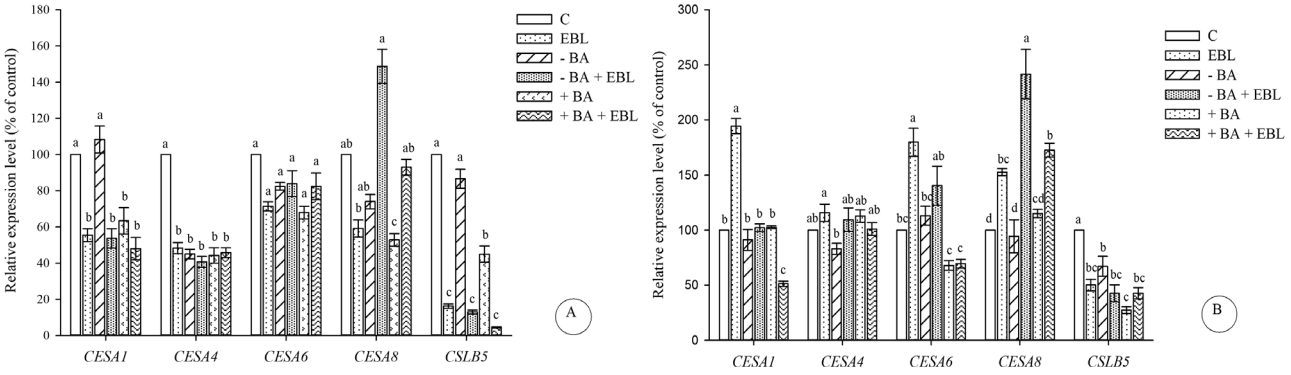

Figure 1. Relative expression levels of *cellulose synthase (CESA)* and *cellulose synthase-like (CSL)* genes in five- and ten-week-old *Arabidopsis thaliana* roots. Effects of 24-Epibrassinolide (EBL) (C: Control, EBL: 1µM, -BA: 0 µM boric acid, +BA: 3000 µM boric acid) on mRNA level of genes under boron stress. **A.** Expression levels of *CESA* and *CSLB5* genes in five-week-old plants and **B.** Expression levels of *CESA* and *CSLB5* genes in ten-week-old plants. Relative expression levels were determined with reference to controls (taken as 100 %). Each value in the graph shows the mean with the standard error (SE). The means denoted by the letters on the bars represent significance differences at $P < 0.01$ according to Duncan's multiple range tests.

plants, expression of *CESA6* gene gave similar results to expression profile determined in five-week-old plants and the applications carried out did not change the level of expression of this gene in a statistically significant way (except alone EBL application in ten-week-old plants) (Fig. 1B). 0 and 3000 μM BA applications did not alter the *CESA8* gene expression comparing to the control, whereas EBL addition to B stress mediums induced an increase in the expression levels (Fig. 1B). Boron stress led to a reduction in mRNA level of *CSLB5* gene (Fig. 1B). 0 μM BA + EBL application reduced the expression of *CSLB5* gene by 2.34 and combined application of 3000 μM BA and EBL decreased the expression by 2.37 (Fig. 1B).

Expansin genes

EXPA5 and *EXPA8* genes showed similar expression profiles in roots of five-week-old plants. Boron deficiency led to decrease in mRNA levels of both genes. However, 0 μM BA + EBL and 3000 μM + EBL applications caused the expression of these genes to be reduced further (Fig. 2A). While the expression level of *EXPA14* gene was reduced by 4.34 as a result of application of EBL alone, a reduction of about 6-fold was determined after application of 3000 μM BA and EBL together (Fig. 2A).

Expression of *EXPA5* gene in ten-week-old plants gave different results than the expression profile determined in five-week-old plants. Alone EBL application performed for 24 hours increased the expression of *EXPA*5 gene by 12-fold (Fig. 2B). 0 μM BA application did not change the expression level of *EXPA5* gene but an increase in the expression at the rate of 2.93 was observed after 0 μM BA + EBL application (Fig. 2B). As a result of 3000 μM BA + EBL application, the expression of *EXPA8* gene showed a 2-fold increase comparing to the control (Fig. 2B). While the transcript level of *EXPA14* gene did not change in BA-free medium, it was determined that the expression was reduced when co-applied with EBL. 3000 μM BA and EBL application performed with 3000 μM BA resulted in similar reduction in the expression of *EXPA14* gene (by 3.98 and 4.41, respectively) as compared with control (Fig. 2B).

Xyloglucan endotransglycosylase/hydrolase genes

EBL treatment alone reduced the expression of *XTH16* gene by 3.05 in roots of five-week-old plants. 3000 μM BA application decreased the expression of *XTH16* gene by 3.13, and 3000 μM BA + EBL application reduced expression by 6.54 (Fig. 3A). Expression level of *XTH21* gene changed as a result of high concentration BA (3000 μM) treatment. EBL application combined with 3000 μM BA resulted in further reduction in the expression of this gene compared to 3000 μM BA application alone (Fig. 3A). When 1 μM EBL was applied alone, mRNA level of *XTH23* gene increased by 19-fold. Addition of EBL hormone to BA-free or high concentration BA containing medium led to dramatic increases in the expression of this gene (Fig. 3C).

Boron stress and/or EBL treatments in roots of ten-week-old plants showed statistically significant reductions in the expression of *XTH16* gene comparing to the control. 0 μM BA and 0 μM BA + EBL applications resulted in similar decreases (by 2.28 and 2.65, respectively) in the expression of this gene. On the other hand, 3000 μM BA application led to further reduction in the expression level of *XTH16* gene compared to other applications (Fig. 3B). 3000 μM BA application caused the expression of *XTH21* gene to decrease by 3.15 comparing to the control (Fig. 3B). 1 μM EBL application increased the expression level of *XTH23* gene by 23.01 when compared to control (Fig. 3D).

Cell wall protein and pectin methylesterase genes

In five-week-old plants, the expression level of *SEB1* (*COBL7*) gene was found to be reduced as a result of

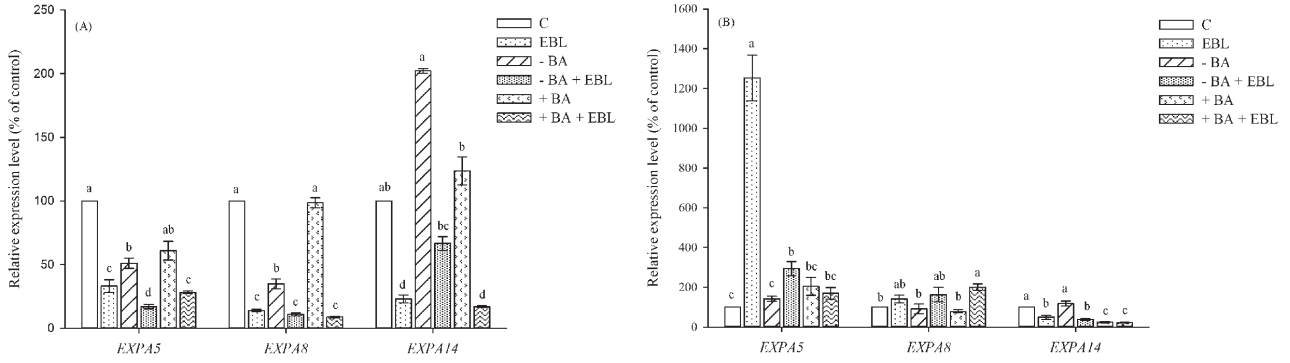

Figure 2. Relative expression levels of *expansin (EXP)* genes in five- and ten-week-old *Arabidopsis thaliana* roots. Effects of 24-Epibrassinolide (EBL) (C: Control, EBL: 1μM, -BA: 0 μM boric acid, +BA: 3000 μM boric acid) on mRNA level of genes under boron stress. **A.** Expression levels of *EXP* genes in five-week-old plants and **B.** Expression levels of *EXP* genes in ten-week-old plants. Relative expression levels were determined with reference to controls (taken as 100 %). Each value in the graph shows the mean with the standard error (SE). The means denoted by the letters on the bars represent significance differences at $P < 0.01$ according to Duncan's multiple range tests.

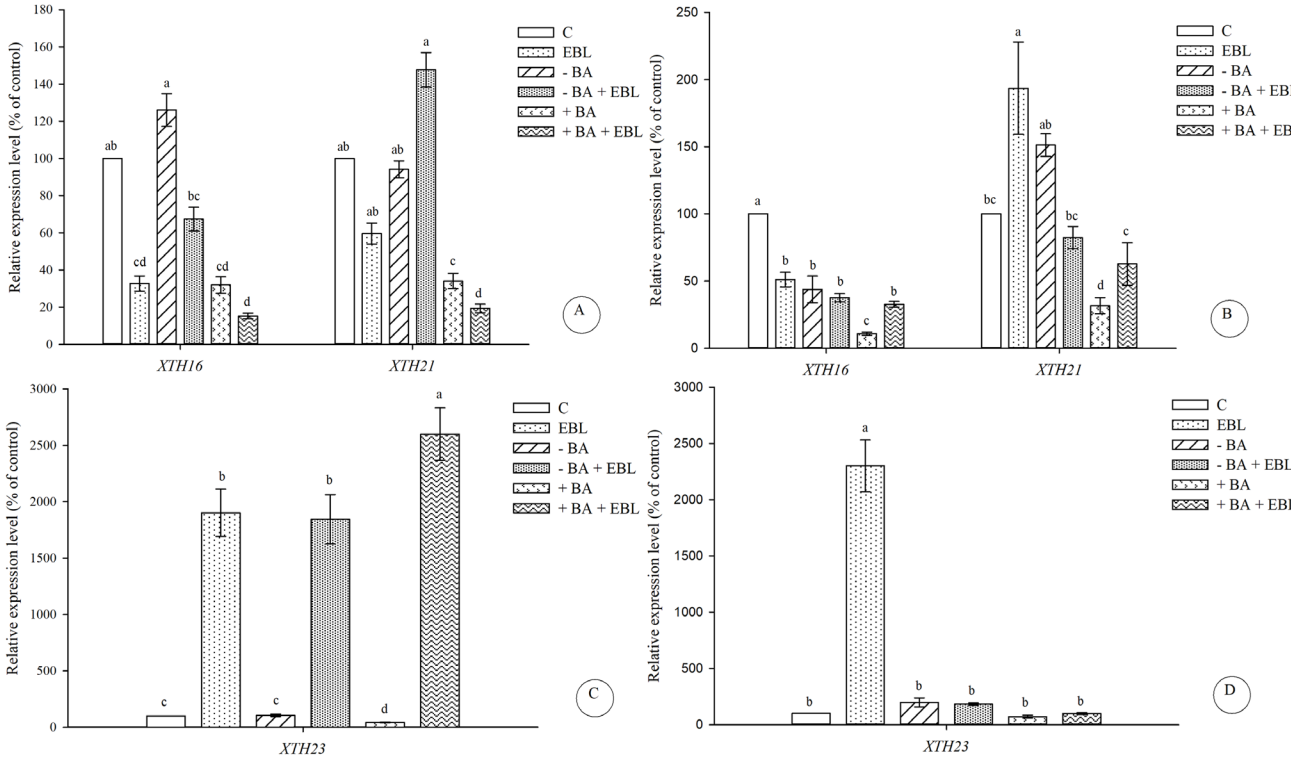

Figure 3. Relative expression levels of *xyloglucan endotransglucosylase/hydrolase* (*XTH*) genes in five- and ten-week-old *Arabidopsis thaliana* roots. Effects of 24-Epibrassinolide (EBL) (C: Control, EBL: 1μM, -BA: 0 μM boric acid, +BA: 3000 μM boric acid) on mRNA level of genes under boron stress. **A.** Expression levels of *XTH16* and *XTH21* genes in five-week-old plants, **B.** Expression levels of *XTH16* and *XTH21* genes in ten-week-old plants, **C.** Expression level of *XTH23* gene in five-week-old plants, and **D.** Expression level of *XTH23* gene in ten-week-old plants. Relative expression levels were determined with reference to controls (taken as 100%). Each value in the graph shows the mean with the standard error (SE). The means denoted by the letters on the bars represent significance differences at $P < 0.01$ according to Duncan's multiple range tests.

EBL application alone by 7.94 comparing to the control, whereas co-application of EBL with 0 or 3000 μM BA led to reductions at the rates of 2.12 and 6.43, respectively (Fig. 4A). In ten-week-old plants, EBL application increased the expression of *SEB1* gene by 1.84, which is statistically significant (Fig. 4B).

Expression profile of *PME2* gene showed similarity (except 3000 μM BA application in ten-week-old plants) at both different developmental stages (in five- and ten-week-old plants). When EBL hormone was applied alone or co-applied with 0 or 3000 μM BA, it led to a reduction in the expression level of *PME2* gene (Fig. 4C-D). BA-free or high concentration BA applications did not alter the expression of *PME41* gene in five-week-old plants but it was determined that B deficiency increased the expression level in ten-week-old plants. On the other hand, EBL applications performed with B stress at both developmental stages led to an increase in the expression level (Fig. 4C-D).

Discussion

Plant cell walls are consisted of carbonhydrate polymers, structural proteins and lignin in variable amounts. Cell walls have critical importance for cell shape and provide mechanical strength by resisting turgor pressure (Tenhaken 2015). In addition, it constitutes one of the first lines of defense against stress (Kesten *et al.* 2017). Abiotic stress conditions (drought, osmotic stress and salinity) reduce cell turgor and can lead to physical damage such as loss of fragments from the wall, separation of binding areas of wall components, reduced bonding between the cell wall and plasma membrane (Hamann 2015). Perception of abiotic stresses in the cell wall is carried out by different receptor-like kinases, which are members of integral plasma membrane protein family. Receptor-like kinases are reported to perceive changes in the environment outside the cell and send these signals into the cell either through second messengers such as reactive oxygen species or by phosphorylating transcription factors or other unidentified signaling proteins (Lindner *et al.* 2012). Since cell wall changes are not easy to analyze, the genes as part of cell wall metabolism were primarily focused on in the investigations about cell wall under abiotic stress conditions (Tenhaken 2015). In previous studies, it has been reported that the effects of abiotic stresses changes depending on plant developmental stage (Gall *et al.* 2015). On the other hand, it was determined that BR-induced stress tolerance depends

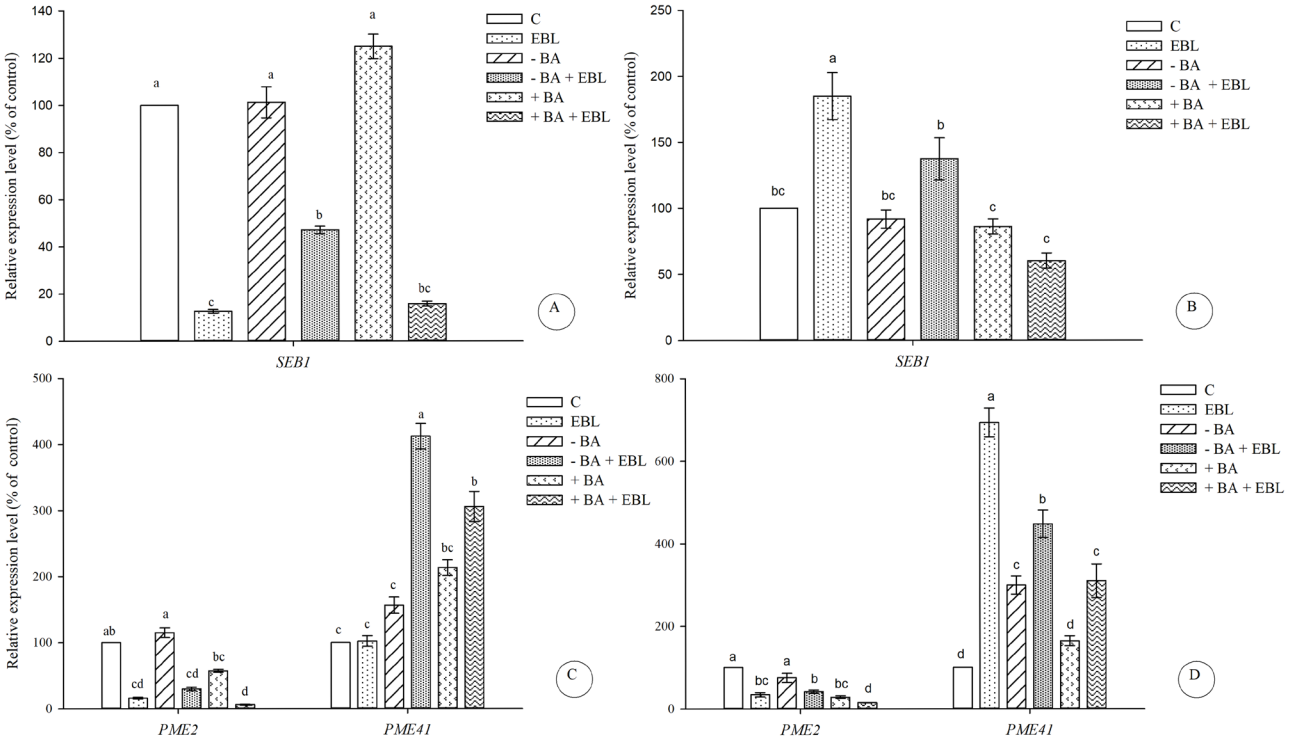

Figure 4. Relative expression levels of cell wall protein coding-gene (*SEB1*) and *pectin methylesterase* (*PME*) genes in five- and ten-week-old *Arabidopsis thaliana* roots. Effects of 24-Epibrassinolide (EBL) (C: Control, EBL: 1µM, -BA: 0 µM boric acid, +BA: 3000 µM boric acid) on mRNA level of genes under boron stress. **A.** Expression level of *SEB1* gene in five-week-old plants, **B.** Expression level of *SEB1* gene in ten-week-old plants, **C.** Expression levels of *PME* genes in five-week-old plants and **D.** Expression levels of *PME* genes in ten-week-old plants. Relative expression levels were determined with reference to controls (taken as 100 %). Each value in the graph shows the mean with the standard error (SE). The means denoted by the letters on the bars represent significance differences at $P < 0.01$ according to Duncan's multiple range tests.

on the developmental stage at which BR applied to the plant (Ahammed *et al.* 2015). In this study, which was carried out by taking these into consideration, it was aimed to determine the effect of EBL hormone on expression levels of some genes in *Arabidopsis* roots within the scope of cell wall assembly and remodelling under B deficiency and toxicity conditions at two different developmental stages.

Cell wall polysaccharides are divided into three classes as cellulose, hemicellulose and pectin based on its chemical structure. In primary cell wall in dicotyledons, weight and main load-bearing structure make up cellulose primary structure. Cellulose is synthesized by cellulose synthase (CesA) enzymes in the plasma membrane (Wang *et al.* 2016). In this study, expressions of *CESA* genes showed differences depending on the plant developmental stage at which the plant was exposed to B stress. This may be because the composition of cell wall may change during different developmental stages (Popper *et al.* 2011). While expression of *CESA* genes was not affected by B stress in roots of ten-week-old plants, B toxicity suppressed the expressions of *CESA1* and *CESA8* genes in root tissues of five-week-old plants and the expression of *CESA4* gene was decreased under both B toxicity and deficiency conditions. Abiotic stresses affect cytoskeleton dynamics negatively and change amount of cell wall components. Cellulose is an important component of cell wall alterations that are necessary as a response to changing abiotic stresses (Wang *et al.* 2016). Piro *et al.* (2003) have shown that cell wall polysaccharides (cellulose, hemicelluloses and pectins) are reduced in apical and subapical root segments of wheat under drought stress conditions. In a study by Aquea *et al.* (2012), it was determined that B toxicity regulates cell cycle and the expressions of key cell cycle genes are reduced. It was reported that changes in the cell cycle were a general mechanism in adaptation to stress. Recent developments demonstrate that cellulose biosynthesis in the primary cell wall is regulated by phytohormone pathways. Stress-induced degradations in the cell wall alter cellulose synthesis and microtubule regulation and trigger phytohormone-based stress response pathways (Kesten *et al.* 2017). In their study on *Arabidopsis* hypocotyls, Sanchez-Rodriguez *et al.* (2017) stated that BIN2 is activated in the absence of BR and it reduces activity of cellulose synthase complex (CSC) by phosphorylating *CESA1*. They reported that BIN2 was inactive, CSC was active in the presence of BR in the medium (10 nM) and thus cellulose synthesis increased. In our study, it was determined that expressions of investigated *CESA* genes (except *CESA8* gene) were generally reduced

by EBL applications with B stress in *Arabidopsis* roots. In our previous study, effects of high concentration B and/or EBL applications on expression levels of B transporters and some stress-related genes in leaf and root tissues of *Arabidopsis thaliana* were investigated and, in this study, it was determined that effect of EBL on expressions of investigated genes changed depending on concentration and tissues (Surgun *et al.* 2016). *Cellulose synthase-like* (*CSL*) genes, which show sequence similarity with *CESA* genes (Cutler & Somerville 1997), are suggested to encode glycan synthases that polymerize the backbone of non-cellulosic cell wall polysaccharides (Somerville *et al.* 2004). *CSLB5* gene showed similar expression profiles in root tissues in five- and ten-week-old plants and significant decreases (except 0 μM BA application in five-week-old plants) in the expression level of *CSLB5* were determined under B stress. In a different study, it was determined that the expression level of *CSLB5* gene in root tissues of *Arabidopsis* plants was reduced significantly as a result of exposure to B deficiency for 6 and 24 hours and it was revealed that B might have a role in the synthesis of cell wall components (Camacho-Cristobal *et al.* 2008). In this study, EBL applications combined with B deficiency or toxicity reduced the expression level of *CSLB5* gene significantly at both developmental stages as compared with control. Plant roots stop or inhibit elongation under conditions of B toxicity and deficiency, which depends on cell expansion rather than cell division (Wang *et al.* 2010; Liu *et al.* 2014). In line with this evidence, it is suggested that cell wall properties change and thus biosynthesis of cellulose, an important cell wall component, is reduced under stress conditions based on the findings of the present study. On the other hand, exogenous EBL application may be supportive to the mechanism of tolerance to stress in roots by suppression of expressions of some *CESA* and *CSL* genes.

Expansins play an important role in root growth and development (Guo *et al.* 2011). It has been revealed that expressions of two expansin genes (*AtEXP7* ve *AtEXP18*) in *Arabidopsis thaliana* are closely related to root hair initiation (Cho & Cosgrove 2002). Similarly, it has been determined that *HvEXPB1* expansin gene is root-specific and related to root hair formation in barley (Kwasniewski & Szarejko 2006). These results support the roles of expansins in the cell wall synthesis during cell division. In addition, expansins are cell wall loosening proteins that induce extension of plant cell walls under stress conditions (Peape *et al.* 2004). In present study, *EXPA5* and *EXPA8* genes were determined to be suppressed in B-free medium in roots of five-week-old plants. Yu *et al.* (2014) determined global transcription profiles using microarray method in *B. napus* seedlings exposed to heat stress and found that the expression of *EXPA5* gene was reduced 10-fold comparing to the control. In a study carried out by Camacho-Cristobal *et al.* (2008), it was reported that expressions of *EXPA14* and *EXPB1* genes were decreased under B deficiency. It has been suggested that mechanical signal can be quickly transferred to the plasma membrane via arabinogalactan-proteins by reduction in tensile strength of cell wall under B deficiency conditions (Camacho-Cristobal *et al.* 2011). According to these results, it can be considered that the suppression of expansin genes may be due to inhibition of cell division and elongation in roots under B stress (Wang *et al.* 2010; Li *et al.* 2015). In this study, while the effects of EBL applications co-applied with B stress on expansin genes change depending on the developmental stage at which the application is performed led to a reduction in expression of expansin genes generally in five-week-old plants. This effect of EBL may be explained by BR-induced ethylene production. Many studies have shown that BRs promote ethylene biosynthesis in different plants (Arteca & Arteca 2001; Müssig *et al.* 2003; Joo *et al.* 2006). Paepe *et al.* (2004), by means of microarray analyses, have revealed that some of α-expansin genes (*EXP5* and *EXP11*) are regulated by ethylene. Similar results were obtained from different studies and it has been determined by transgenic approaches that ethylene regulates the expression of *EXPA5* negatively in *A. thaliana* (Son *et al.* 2011).

Structural changes of cell wall components are regulated by enzymatic modifications and wall-modifying enzymes have important roles on cell wall plasticity (Burstin 2000). In this study, it was determined that expressions of *XTH* genes of which expression levels were investigated were only suppressed under B toxicity at both developmental stages (except *XTH23* gene in ten-week-old plants). This is important and some *XTH* genes may be used as specific markers for B toxicity after detailed studies. Genetic responses of specific organs of barley ears to drought stress were investigated by Abebe *et al.* (2010) in detail. Continuation of growth is actualized by constant modification of cellulose, hemicellulose and pectin via enzymes in the primary cell wall. Xyloglucan endotransglycosylases (XETs) break β-(1→4) bonds of xyloglucans and allows easy elongation of the cell wall (Ober & Sharp 2007). However, it has been reported that drought stress suppresses cell expansion by reducing turgor pressure and it has been revealed that expressions of some *XET* and cell wall biosynthesis genes are reduced under drought stress (Abebe *et al.* 2010). The fact that EBL applications generally reduce the expressions of *XTH* genes (except *XTH23* gene) under boron toxicity conditions significantly can be interpreted as BRs are effective in re-regulation of network of microfibrils cross-linked with xyloglucans, and on cell wall plasticity under stress conditions. On the other hand, some of the genes of which expression levels were investigated within the scope of the study were determined increase in expressions after EBL applications alone and EBL applications combined with stress conditions. Ahammed *et al.* (2015) reported that BRs-induced stress responses may be changed depending on the plant species, developmental stages, plant species and environmental conditions as well. Also, increases in expression level of different cell-wall

related genes as a result of EBL treatments may be providing sufficient cell wall component to maintain the architecture of existing cells under stress conditions (Xie *et al.* 2011).

Pectin methylesterases (PMEs) catalyze specific demethylestirification of pectic polysaccharides in the plant cell wall (Pelloux *et al.* 2007) and have roles in different physiological processes such as tuber productivity, root development, stem growth, and fruit softening (Wolf *et al.* 2003). Enzymatic activity of PMEs leads to loosening or stiffening in the cell wall depending on convenience of apoplastic pH and divalent cations (Wolf *et al.* 2003). In present study, *PME* genes of which expression levels were investigated gave different responses to B stress. mRNA level of *PME2* gene was reduced under B toxicity conditions, whereas EBL applications along with B stress led to further reduction in *PME2* gene expression. Expression of *PME41* gene increased as a result of B deficiency conditions in ten-week-old plants and also EBL applications with boron stress increased the expression levels in both developmental stages. In a study conducted on *Cucumis sativus*, PME activity and expression of *CsPME* genes were determined to increase under high B concentration in the roots (Wang *et al.* 2010). Yang *et al.* (2008) reported increased PME activity in rice roots exposed to Al stress. On the other hand, application of heat stress led the expression of *PME35* gene to decrease by about 10-fold in *B. napus* seedlings (Yu *et al.* 2014). As is seen in above mentioned studies, different responses in different *PME* genes were determined in different plants depending on the type of stress and tissue. Wolf *et al.* (2003) have reported that expressions of *PME* genes are regulated strongly specifically to the tissue. This shows the complexity of cell wall response to abiotic stresses. In order to control pectin-dependent cell wall integrity, BR signaling in *Arabidopsis* is concomitant with the modification of methyl-esterified homogalacturonans (HGs) (Wolf *et al.* 2012). Qu *et al.* (2011) have determined that the regulation of *PME* genes in *Arabidopsis* is dependent on BR under chilling stress conditions and this is probably actualized by regulation of *ATPME41* expression.

In addition to CESA proteins, there are a number of other proteins involved in microfibril formation; however, none of them has been linked to cellulose synthase complex (Liepmann *et al.* 2010). COBRA (COB) proteins were discovered as a consequence of scanning of mutants with root cell expansion abnormalities (Benfey *et al.* 1993). *COB* encodes a small protein found on extracellular surface of the plasma membrane however the role of this protein in cell-wall deposition or polymerization is uncertain (Roudier *et al.* 2002). In *Arabidopsis* genom, there are eleven members of COBRA-LIKE (COBL) family (Brown *et al.* 2005). In present study, expression level of *SEB1* (*COBL7*) gene differed depending on the developmental stage of stress application. On the other hand, B stress in five-week-old plants did not change the expression of *SEB1* gene, whereas EBL appeared to affect the expression of *SEB1* gene at transcriptional level. This is the first finding suggested in this context while more detailed investigations on *SEB1* gene are necessary.

Conclusion

Genetic and transgenic approaches suggest that cell wall modification has a significant effect on stress tolerance. It was determined that expressions of genes related to cell wall in *Arabidopsis* roots were generally reduced under boron stress. BRs have been shown to affect expressions of investigated genes at transcriptional level. However, extensive future researches are needed to understand how BRs regulate the genes related to the cell wall and crosstalk between other plant hormones in this regulation.

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