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Analysis of Effects of 24-Epibrassinolide and Boron Treatments on the Expression of *CycD3;1, TCH4* and *KOR* Genes in *Arabidopsis thaliana* (L.) Heynh Seedlings using Semi-Quantitative RT-PCR

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Abstract
Brassinosteroids (BRs) play pivotal roles in plant cell division and cell elongation. Although boron (B) is an essential element for plants, excess B is toxic. In this study, we investigated the effects of 24-Epibrassinolide (EBL) on expression of cell cycle (*CycD3;1*) and cell wall-related (*TCH4* and *KOR*) genes in leaf and root tissues of *Arabidopsis thaliana* (L.) Heynh seedlings under high concentrations of boric acid (BA) using semi-quantitative reverse transcriptase – polymerase chain reaction (RT-PCR). *A. thaliana* seedlings were grown in a hydroponic culture for four weeks, and then transferred to liquid media containing BA (50 and 100 mg L^{-1}) and/or EBL (0.01 and 1 µM) and incubated for 60 hours. In root tissues, BA treatments decreased the expression of *CycD3;1* gene, whereas 0.01 µM EBL treatments increased the expression level of this gene when compared with plants exposed to BA alone. 1 µM EBL alone or in combination with BA treatments significantly led to down regulation of *CycD3;1* gene. On the other hand, application of 1 µM EBL resulted in significant increase of mRNA levels of *KOR* and *TCH4* genes in root tissues. In leaf tissues, no significant differences were observed in the expression levels of *CycD3;1* and *KOR* genes as a result of all treatments. BA treatments increased the expression levels of *TCH4* gene significantly when compared to control in leaf tissues. To conclude, the results showed that EBL in general positively regulate the expression of cell cycle and cell-wall related genes under high B stress and stress-free conditions.
Keywords: *Arabidopsis thaliana* L., brassinosteroid, boron, cell cycle, cell wall, gene expression.

INTRODUCTION

The phytohormones, brassinosteroids (BRs), constitute a specific class of plant steroids [1]. BRs are effective in enhancing tolerance to various stress factors in addition to their regulatory effects on plant growth and development [1, 2]. BRs' promotive effects on growth involve cell division and elongation and these effects were first shown in the bean second-internode bioassay [3]. Rescue of the dwarf phenotypes of BR-deficient mutants by BR application showed that BRs are an important class of plant hormones responsible for the normal plant development [2, 4]. Thus it was concluded that the most remarkable and early-recognized characteristic of BRs are their promotive effects on cell elongation and proliferation [5].

Cell elongation is critical for the growth and differentiation of plant organs and it is controlled by coordination of cell wall mechanical properties, cell hydraulics, biochemical processes and gene expression [2]. Regulation and synthesis of the wall-modifying enzymes, such as xyloglucan endotransgly-cosylase/hydrolases (XTHs), glucanases, expansins, sucrose synthase and cellulose synthase, constitute the obvious target of hormones responsible for cell elongation [2]. During cell elongation, BRs affect the mechanical properties of cell walls, via genomic and non-genomic pathways [6]. BR-regulated genes that promote cell elongation were identified in genetic and global microarray studies. These identified genes were found related with cell wall modifications, cellulose biosynthesis, ion and water transport, and cytoskeleton rearrangements [7]. Besides, it was revealed that many genes in this classification regulate expressions, which are important for cell elongation, through BR signal transduction way by binding directly to BRI1, the BR receptor [2]. On the other hand, BRs were shown to promote wall loosening in soybean epicotyls [8] and hypocotyls of *Brassica chinensis* and *Cucurbita maxima* [9, 10] by biophysical measurements.

Another factor that is essential for cell elongation is the control of microtubule organization. Microtubule orientation helps to align cellulose microfibrils during synthesis, and transversely arranged microfibrils in the cell wall are required for normal cell elongation. Microscopic analysis results demonstrated that BR-deficient Arabidopsis mutants have very few microtubules and these align parallel to each other. Research results revealed that BRs allow longitudinal growth by affecting re-configuration of microtubules and providing cross orientation [11]. In addition to alterations in cell wall properties, BRs were found effective on transport of water via aquaporins and on vacuolar H^+- ATPase activity, both of which are associated with cell elongation [12]. In recent years, comprehensive studies of fluorescence lifetime spectroscopy, confocal microscopy and electrophysiology led to the conclusion that BRs promote membrane hyperpolarization and rapid cell elongation through BR-modulated interaction of BRI1 and ATPase in the plasma membrane [13].

In addition to cell elongation, BRs promote cell division and proliferation [14, 15]. BRs were shown to stimulate cell division in parenchyma cells of *Helianthus tuberous* [16] and protoplasts of Chinese cabbage and petunia, in the presence of auxin and cytokinin [17, 18]. ChIP-chip analysis results indicated that in addition to

CycD3 (a protein involved in the regulation of G1/S transition in the cell cycle), other cyclins and cyclin-dependent kinases are regulated by BRs [2, 18].

Boron (B) is an essential element for plants which take up boron from soil in the form of boric acid (BA) [19]. Boron is known to be essential for structural component of the rhamnogalacturonan II complex in cell wall [20]. Like many other elements, B becomes toxic for plant at high concentrations. Most typical symptom seen in the plants exposed to high B concentrations is decrease or inhibition of growth and development. B toxicity triggers the formation of reactive oxygen species and affects many different physiological processes, such as inhibition of germination, loss of leaf area, lower leaf chlorophyll content and photosynthesis rates [21]. BA and borate have the ability to form strong complexes with important biological compounds containing two hydroxyl groups in *cis*-configuration such as ribose, ATP, NADH, NADPH [22]. B interferes with transcription and/or translation mechanism by binding to ribose [19]. In addition, it was accounted that high B induces the expression of several genes in roots and rosette leaves of Arabidopsis. Part of these genes is constituted of encoding transcription factors. This result might support the putative role of B as a cellular signal capable of interaction with transcription factors [23].

To examine the possible role of BR in high B-induced stress at the transcriptional level, we investigated the response of cell cycle (*CycD3;1*) and cell wall-related (*TCH4* and *KOR*) genes upon exogenous treatments of 24-Epibrassinolide (EBL) on *Arabidopsis thaliana* exposed to high concentrations of BA using semi-quantitative RT-PCR.

MATERIALS AND METHODS

Seeds of *Arabidopsis thaliana* (L.) Heynh ecotype Col-0 were provided from Antwerpen University, Belgium. Seedlings were grown in hydroponic culture [24] which was further modified in our laboratory. *A. thaliana* seedlings were grown for 4 weeks in a control medium [25] and then transferred to the same media containing boric acid (BA) (50 or 100 mg L^{-1}) and/or 24-Epibrassinolide (EBL) (0.01 or 1 µM) and incubated for 60 hours. Leaves (5^{th} and 6^{th}) and roots of the plants were harvested after 60 hours, frozen in liquid nitrogen and stored at -80°C until RNA extraction. All plants were grown under controlled environmental conditions at 22±2ºC under fluorescent white light (100 µmol m^{-2} s^{-1} at leaf level) with a 16-h light / 8-h dark regime.

Total RNA was isolated from 100-300 mg of a frozen leaf and root tissues of Arabidopsis plants ground with mortar and pestle in liquid nitrogen until a fine powder appeared. The total RNA was extracted using Plant RNA Mini Prep Kit from Zymo Research according to manufacturer's instructions. RNAs were quantified spectrophotometrically at 260 nm (Optizen Pop, Korea), and integrity and quality was estimated after migration on a 0.8% agarose gel. RNA samples were treated with RNase free DNase I (Fermentas, Germany) at 37°C for 30 min and DNase I was inactivated by heating for 10 min at 65°C. cDNA synthesis were carried out using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Germany) according to the supplier's instructions. For conversion of total RNA to cDNA, a 20 µL reaction mixture was prepared containing 1 µL oligo-$(dT)_{18}$ primer (100 µM), 4 µL 5X Reaction buffer (250 mM Tris-HCl pH 8.3, 250 mM KCI, 20 mM $MgCl_2$, 50 mM DTT), 1 µL RiboLock RNase Inhibitor (20 U μL^{-1}), 2 µL dNTP mix (10 mM), 1 µL M-MuLV Reverse Transcriptase (200 U μL^{-1}) and 2.4 µg of total RNA. cDNA was stored at -20°C until used.

RT-PCR was performed to measure gene expression of At4g34160 [Cyclin-D3-1 (*CycD3;1*)], At5g57560 [Xyloglucan Endotransglucosylase/hydrolase protein 22 (*TCH4*)], At5g49720 [Endo-1,4-beta-D-glucanase (*KOR*)] and *Actin2* as an internal control. Primers were designed using software Primer3 [26, 27] available on-line at http://bioinfo.ut.ee/primer3-0.4.0/. The primer sequences used and the predicted amplification product sizes are indicated in Table 1. Polymerase chain reactions were performed on an Eppendorf Mastercycler Gradient PCR machine (Mastercycler Gradient, Eppendorf, Germany) using 4 µL of diluted (1/10) cDNA as a template, 5 µL 10X Taq buffer (100 mM Tris-HCl pH 8.8, 500 mM KCl, 0.8% Nonident P40), 1 µL dNTP mix (10 mM), 3.5 µL $MgCl_2$ (25 mM), 0.5 µL Taq DNA Polymerase (5 U μL^{-1}) and 1 µL of 10 pmol μL^{-1} of each primer in a 50 µL volume. The PCR programme initially started with a 95 ºC denaturation for 3 min, followed by 35 cycles of 94ºC/30 s, 50ºC/30 s and 72ºC/ 45 s. Each set of reactions always included negative (minus RT) and positive control. The PCR products (30 µL) was loaded on 2% agarose gels in 0.5X TBE buffer (0.5X) and stained with ethidium bromide (10 µg/mL). The gel images were digitally captured using gel documentation system (DNR MiniBis pro Bio-Imaging, Israel) and signal intensity of the stained bands were measured with the help of ImageJ ver 1.48 software (National Institute of Mental Health, USA) available on-line at http://imagej.nih.gov/ij/. Relative gene expression was determined semi-quantitatively by calculating the ratio of the density values of PCR bands for the specific genes with respect to the internal control.

The data were analyzed statistically using the package software, SPSS ver 20.0 (SPSS, USA). Statistical analysis of the results was performed by one-way analysis of variance (ANOVA). Data are the mean ± standard error (SE) of at least four independent replicates. Mean values were statistically compared by Tukey HSD post hoc test at $P < 0.05$ level using different letters.

RESULTS

Semi-quantitative RT-PCR results in leaf tissue

Applications of BA and/or EBL to *Arabidopsis thaliana* seedlings for 60 hours caused alterations in *CycD3;1* expression in leaf tissue at very low ratios and these changes were not considered statistically significant as compared to untreated control (Figure 1 and Table 2).

The treatment with 50 and 100 mg L^{-1} BA caused statistically significant increase in *TCH4* gene expression when compared to the control (Table 2). While treatment of 0.01 µM EBL alone did not change mRNA level of *TCH4* in leaf tissue, 1 µM EBL treatment enhanced expression statistically significant with respect to control (Figure 1). 50 mg L^{-1} BA application enhanced transcript levels of *TCH4* by approximately 1.35-fold with respect to control; whereas co-treatments of EBL (0.01 and 1 µM) and 50 mg L^{-1} BA did not change transcript level of *TCH4* gene compared to 50 mg L^{-1} BA application alone (Figure 1 and Table 2). 1 µM EBL application carried out with 100 mg L^{-1} BA increased mRNA level in comparison with 100 mg L^{-1} BA, however, this increase did not have statistical significance (Figure 1).

Table 1. List of primer sequences used in this study

Gene name or (putative) function	Locus ID	Primer sequence	Expected fragment size (bp)	References
Cyclin-D3-1 (*CycD3;1*)	At4g34160	F-TCGTTGAACAGTCCAAGCTG	182	This study
		R-TGCAAAATCGGCTTCTTCTT		
Xyloglucan endotransglucosylase/ hydrolase protein 22 (*TCH4*)	At5g57560	F-CAAGAACATGGAGTCTCTAGGCAC	135	This study
		R-GTGAAAGGAGCTTTAGACCAATCG		
Endo-1,4-beta-D-glucanase (*KOR*)	At5g49720	F-GTCCAACGGAGCAGAAGAAG	240	This study
		R-TTGGCAATTTCCCAGATTTC		
Actin2	At3g18780	F-TGCCAATCTACGAGGGTTTC	226	This study
		R-TTCTCGATGGAAGAGCTGGT		

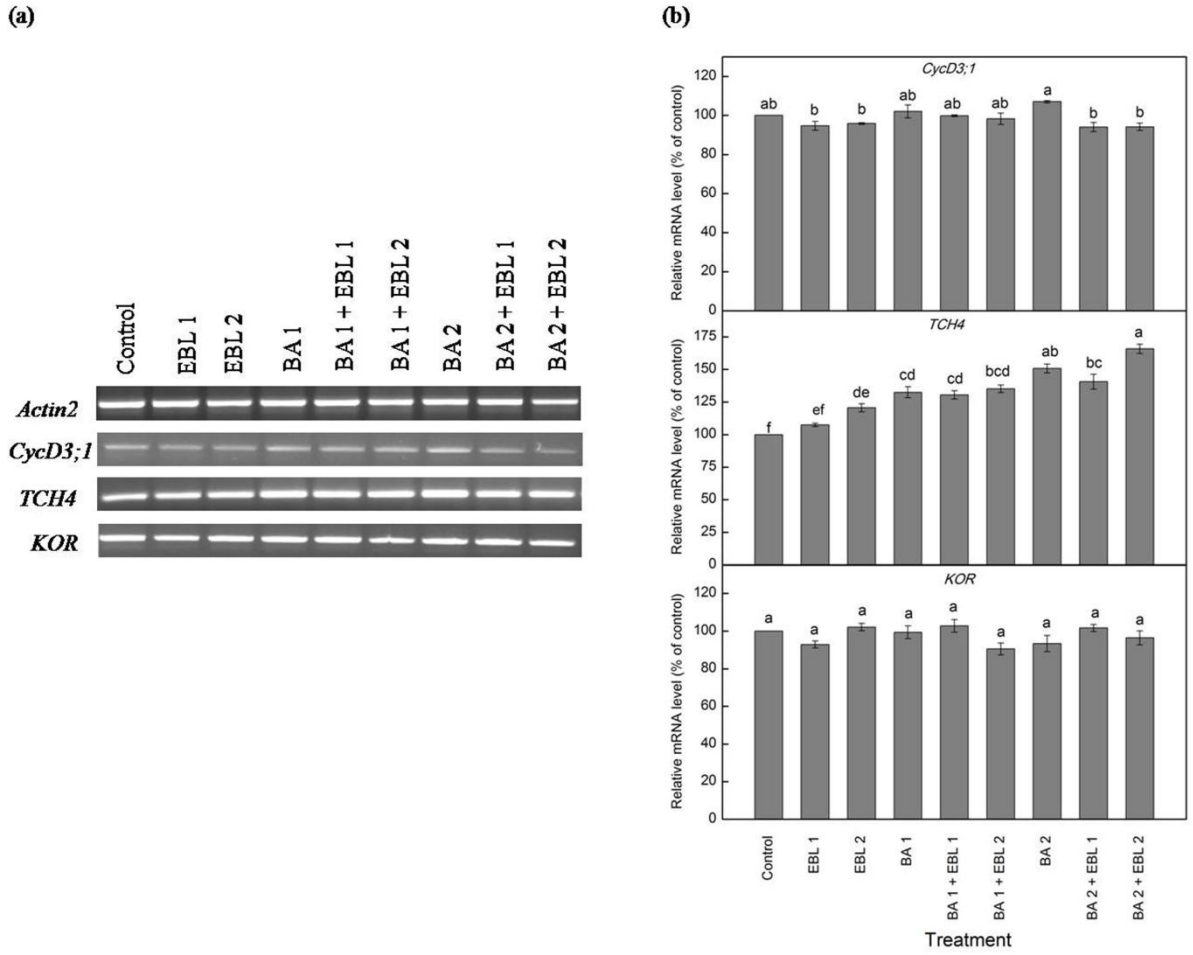

Figure 1. Semi-quantitative RT-PCR analysis of *CycD3;1*, *TCH4* and *KOR* genes in leaf tissues. Effects of EBL (**EBL 1**: 0.01 µM EBL, **EBL 2**: 1 µM EBL) and/or BA (**BA 1**: 50 mg L^{-1} BA, **BA 2**: 100 mg L^{-1} BA) treatments on mRNA levels of selected genes in *A. thaliana* leaves. *Actin2* was used as internal control. **(a)** Displayed are images of representative four independent experiment results of selected genes, **(b)** The intensity of each band was quantified using ImageJ software. Relative transcription levels were calculated 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 $P < 0.05$ according to Tukey HSD test.

KOR gene expression was seen to be slightly changes (increases or decreases) in the leaf tissues upon all treatments albeit at varying ratios but these alterations were not found to be statistically significant (Figure 1 and Table 2).

Semi-quantitative RT-PCR results in root tissue

In the roots, when compared to the control, 50 and 100 mg L^{-1} BA treatments led to down regulation of *CycD3;1* by 1.35 and 1.80-fold, respectively (Figure 2 and Table 3). Expression of *CycD3;1* gene was significantly up-regulated in the roots by 1.35-fold under the low concentration EBL (0.1 µM) treatments whereas high concentration EBL (1 µM) application caused decrease at similar ratio compared to control (Figure 2). 0.01 µM EBL alone or in combination with BA (50 and 100 mg L^{-1}) increased the expression levels of *CycD3;1* in root tissue compared to control. When high concentration EBL (1 µM) application is performed alone or with BA (50 and 100 mg L^{-1}), it caused significant decreases (1.35, 2.70 and 6.30, respectively) in mRNA level of *CycD3;1* gene with respect to control (Figure 2 and Table 3).

Table 2. Fold changes of mRNA levels in the leaves subjected to BA and/or EBL treatments with respect to control (**EBL 1**: 0.01 µM EBL, **EBL 2**: 1 µM EBL, **BA 1**: 50 mg L^{-1} BA, **BA 2**: 100 mg L^{-1} BA)

Gen name	Treatment [a]								
	Control	**EBL 1**	**EBL 2**	**BA1**	**BA 1 + EBL 1**	**BA 1 + EBL 2**	**BA 2**	**BA 2 + EBL 1**	**BA 2 + EBL 2**
CycD3;1	1.00 ± 0,00	-1.05 ± 0.02	-1.05 ± 0.00	1.00 ± 0.04	1.00 ± 0.05	1.00 ± 0.03	1.05 ± 0.00	-1.05 ± 0.02	-1.15 ± 0.01
TCH4	1.00 ± 0,00	1.10 ± 0.01	1.20 ± 0.03	1.35 ± 0.04	1.30 ± 0.03	1.35 ± 0.02	1.50 ± 0.03	1.40 ± 0.05	1.65 ± 0.03
KOR	1.00 ± 0,00	-1.10 ± 0.01	1.05 ± 0.01	1.00 ± 0.03	1.00 ± 0.03	-1.10 ± 0.03	-1.10 ± 0.04	1.00 ± 0.04	-1.05 ± 0.03

[a]: The ratios in fold changes were calculated with respect to control (no treatment). Each value represents mean ± SE (n =4)

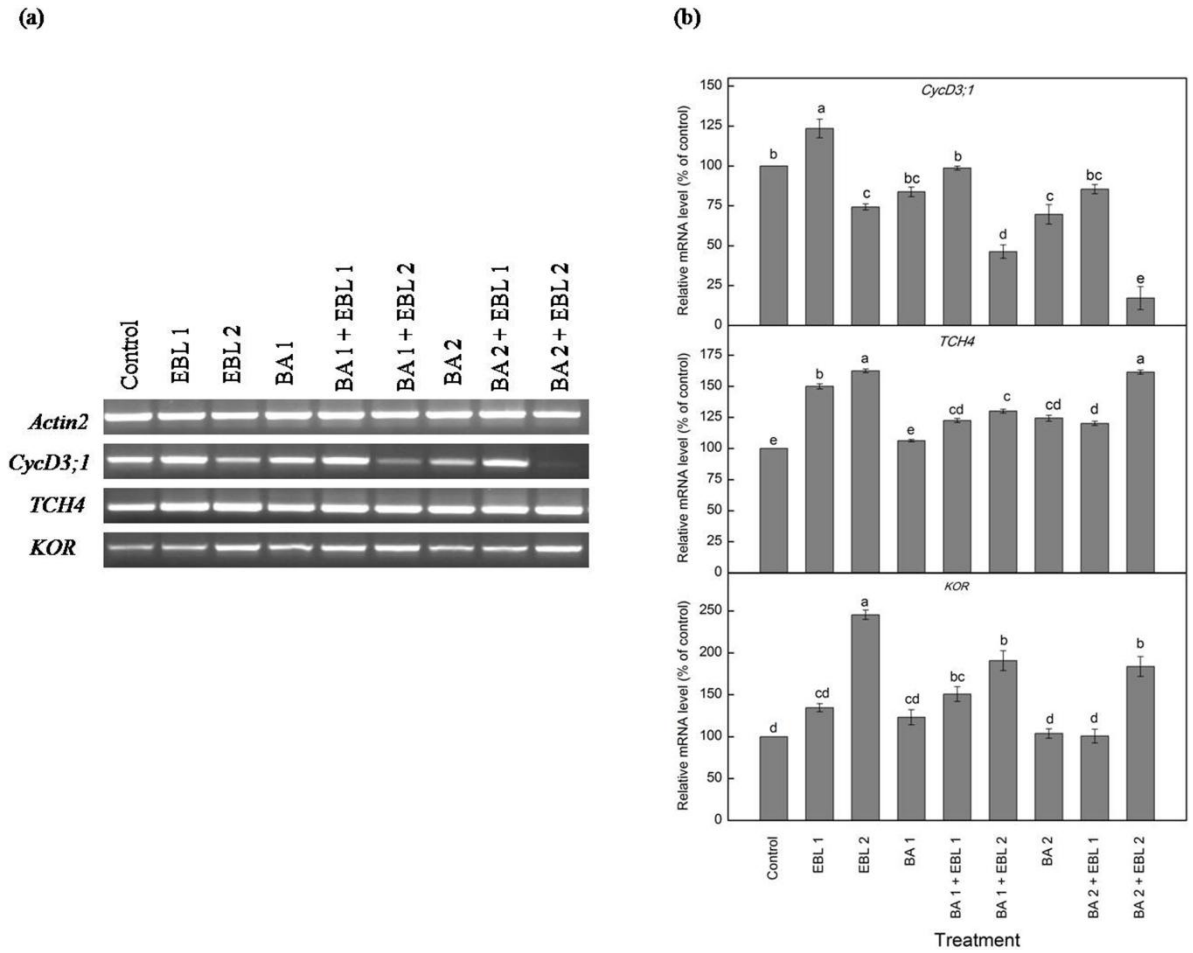

Figure 2. Semi-quantitative RT-PCR analysis of *CycD3;1*, *TCH4* and *KOR* genes in root tissues. Effects of EBL (**EBL 1**: 0.01 µM EBL, **EBL 2**: 1 µM EBL) and/or BA (**BA 1**: 50 mg L^{-1} BA, **BA 2**: 100 mg L^{-1} BA) treatments on mRNA levels of selected genes in *A. thaliana* roots. *Actin2* was used as internal control. **(a)** Displayed are images of representative four independent experiment results of selected genes, **(b)** The intensity of each band was quantified using ImageJ software. Relative transcription levels were calculated 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 $P < 0.05$ according to Tukey HSD test.

Table 3. Fold changes of mRNA levels in the roots subjected to BA and/or EBL treatments with respect to control (**EBL 1**: 0.01 µM EBL, **EBL 2**: 1 µM EBL, **BA 1**: 50 mg L^{-1} BA, **BA 2**: 100 mg L^{-1} BA)

Gen name	Treatment [a]								
	Control	**EBL 1**	**EBL 2**	**BA1**	**BA 1 + EBL 1**	**BA 1 + EBL 2**	**BA 2**	**BA 2 + EBL 1**	**BA 2 + EBL 2**
CycD3; 1	1.00 ± 0.00	1.35 ± 0.01	-1.35 ± 0.01	-1.20 ± 0.03	1.00 ± 0.00	-2.70 ± 0.02	-1.80 ± 0.03	-1.35 ± 0.00	-6.30 ± 0.04
TCH4	1.00 ± 0.00	1.50 ± 0.05	1.65 ± 0.01	1.10 ± 0.02	1.20 ± 0.02	1.30 ± 0.01	1.35 ± 0.05	1.30 ± 0.05	1.70 ± 0.05
KOR	1.00 ± 0.00	1.35 ± 0.04	2.45 ± 0.05	1.25 ± 0.08	1.50 ± 0.08	1.90 ± 0.11	1.05 ± 0.05	1.00 ± 0.08	1.85 ± 0.11

[a]: The ratios in fold changes were calculated with respect to control (no treatment). Each value represents mean ± SE (n =4)

0.01 and 1 μM EBL applications significantly elevated the transcript levels of *TCH4* by 1.50 and 1.65-fold respectively, compared with control (Table 3). Both EBL treatments with 50 mg L^{-1} BA increased mRNA level of *TCH4* gene at statistically significant compared to 50 mg L^{-1} BA application alone (Figure 2). As 100 mg L^{-1} BA application alone increased transcript level of *TCH4* gene, it led to an increase in expression at the 1.70-fold compared to control when applied with 1 μM EBL (Figure 2 and Table 3).

Different concentrations of BA did not change expression level of *KOR* gene in root tissue (Figure 2 and Table 3). 0.01 μM EBL applications alone or with BA increased mRNA level of *KOR* gene at low ratios or did not change with respect to control with the exception of the combination treatment of 50 mg L^{-1} BA and 0.01 μM EBL (Figure 2). 1 μM EBL treatments alone or with BA elevated the transcript levels of 2.45, 1.90 and 1.85-fold respectively, compared to the control (Figure 2 and Table 3).

DISCUSSION

In the present study, our aim was to investigate the anti-stress effects of BRs at the molecular level by studying expression of cell cycle (*CycD3;1*) and cell wall-related (*TCH4* and *KOR*) genes in response to BA, EBL and a combination of BA and EBL. Expression patterns of the genes were monitored by semi-quantitative RT-PCR in the leaf and root tissues of *A. thaliana* seedlings exposed to different concentrations of BA and/or EBL.

As a result of the studies, three types of cyclin homologues were identified in plants and specified as *CycA*, *CycB*, *CycD*. *CycA* and *CycB* act in a cell-cycle dependent manner during the G2/M transition. However, *CycD* genes show a cell-cycle-independent expression pattern and function as mediators of internal and environmental stimuli to drive cell division [18]. In our study, expression pattern of *CycD3;1* gene which is one of the plant D-type cyclin genes, were examined. Treatment of BA and/or EBL did not cause changes in the expression level of *CycD3;1* gene in leaf tissue but high concentrations of BA decreased the expression level of *CycD3;1* gene in the root tissue. Aquea et al. [28] demonstrated that high BA concentrations regulate cell cycle in the root tissue and cause alterations in the expression level of key cell cycle genes. For this purpose, they investigated expression levels of key cell cycle regulators; cyclin-dependent kinases (*CDKA1*, *CDKB1;1* and *CDKB2;1*) and cyclins (*CycA1;1*, *CycA2*, *CycB1;1* and *CycD3;1*) and expressions were found to decrease after 12 hours of BA treatment. These results suggest that following 12 hour BA treatment, cell cycle progression is repressed and they are in support of our findings. In our study, low EBL concentration alone or combined with BA enhanced the expression level of *CycD3;1* in the root tissue, compared to the control or boric acid treatments alone. In another study, suspension cultures of Arabidopsis *det2* mutants were treated with EBL at different concentrations and dose-specific enhancement in transcript level of *CycD3;1* were observed after 4 hours of treatment [18]. Enhancement in *CycD3;1* gene expression after EBL treatment, raised the question of whether the promotive effect of BRs on cell division might be attained via the gene *CycD3;1*. Findings of the studies obtained by the researchers led to the conclusion that inductive effects of BRs on cell cycle progression are attained through the enhancement in the expression of *CycD3;1* gene [18]. In another study performed using BR-insensitive *bri1-116* mutant, expression levels of cell division markers (*CYCB1;1*, *ICK2/KRP2* and *KNOLLE*) were studied. They determined the important effect of *CycD3;1* gene on cell division and elongation and its relation with BRs as a result of overexpression of *CycD3;1* gene in *bri1-116* mutant [29].

In our study, *CycD3;1* gene expression was demonstrated to enhance with the treatments of low EBL concentration but decrease gradually with the treatments of high EBL concentration. Similarly, different studies showed that low BR concentrations promote root development, whereas high concentrations inhibit [30]. BR level was found to be lower in the root compared to the other organs. This finding reveals that the roots need less BR than the leaves and inflorescences, and supports the effects of BRs on root development. However, it was stated that different findings of BRs' effects on root development might result of the changes of culture conditions (light or darkness), treatment duration and concentration [31]. Mussig et al. [32] indicated that high BR concentration inhibit root development in wild-type plants. Researchers concluded that the threshold value of concentration that promotes or inhibits root development is variable depending on the biological activity of BR applied. It was proposed that the inhibition in roots after EBL treatment might depend on ethylene, because the auxins, which are a class of hormones similar to BRs, enhance the level of ethylene via promoting the biosynthesis of ethylene in root and ethylene inhibits root growth [33, 34].

Goda et al. [35] using the DNA microarray technique, investigated BR-regulated genes in wild-type and BR-deficient *det2* mutants of Arabidopsis. In the end of the study, part of the BR-regulated genes was demonstrated to be in relation with cell elongation and cell wall organization. One of the genes of this group is *TCH4* gene and it encodes xyloglucanendotransglucosylase /hydrolase protein 22 in *Arabidopsis thaliana*. In our study, mRNA level of *TCH4* gene increased as a result of BA treatments in root and leaf tissues. Expression of *TCH4* gene in Arabidopsis was revealed to be regulated rapidly in response to environmental variables (touch, darkness, heat stress) and changes in expression level of *TCH4* gene directly cause modifications in cell wall properties and structure [36]. In the study, we applied BA as a stress factor and increase in expression was observed. This is a new finding for BA differently from other stress factors. It was proposed that regulation of the genes that encode cell wall modifying enzymes is important for regulating the morphogenetic response of plant to environment. It was stated that the reason of regulation is to alter cell wall properties in order that the plant adapts rapidly to environmental conditions [36]. In our study, EBL treatments, alone or in combination with BA, increased *TCH4* gene expression in root and leaf tissue. In other studies, BR treatments also increase transcript levels of different genes defined as homologues of *TCH4* gene. For example, it was determined that *LeBR1* gene in *Lycopersicon esculentum* [37] and *OsXTR1* and *OsXTR3* genes in *Oryza sativa* [38] increase expression levels as a result of BR treatments. Goda et al. [35] determined that brassinolide treatment induce the expression of *TCH4* gene in Arabidopsis mutant. They concluded that similarity of the expression pattern of brassinolide-induced genes and early auxin-induced genes led to the conclusion that BRs promote cell elongation inducing the genes related with auxin. Additionally, microarray analysis results supported

the potential mechanism of interaction between BRs and auxin. Xu et al. [36] showed that auxin and brassinosteroid hormones have an effect on *TCH4* gene expression in addition to environmental inducers. The question of how do such different (two) inducers (stress and EBL) produce a common molecular response was explained as there might be distinct signal pathways in control of *TCH4* gene.

Endo-1.4-beta-D-glucanase (EGs), hydrolyze 1.4-beta bonds and studies proved that EGs take part in different developmental processes such as leaf abscission, fruit maturation and cell proliferation. A member of endo-1.4-beta-D glucanase family, membrane-bound protein KORRIGAN (KOR) plays a core role in cell wall elongation [39, 40]. It was determined that *kor1-1* mutants, which were obtained by mutations in the promoter area of *KOR* gene, show extreme dwarfism and create significant variance in the cell wall architecture [40]. In our study, responses of *KOR* gene, which was revealed to be regulated by BRs [41], to treatments of BA and/or EBL, were examined and treatments generated alterations in the expression level of *KOR* gene at lower ratios in leaf tissue. However, EBL treatments (1 µM) caused significant increase in expression level of *KOR* gene in the root tissue as compared to the control. Regulation of *KOR* gene by BR was determined with global gene expression studies but no studies on expression under stress have been encountered in the literature. In this respect, this finding was introduced for the first time. Increase in expression after EBL treatments prove BRs' positive roles in cell elongation and cell proliferation. In a study by Nicol et al. [39], it was determined that mRNA level of *KOR* gene does not change in the mutants of auxin, gibberellin and ethylene hormones, whereas *KOR* mRNA level find three times lower in BR-deficient mutant *det2* compared to wild-type. In addition to this, exogenous brassinolide treatment partially rescued hypocotyl growth and caused an increase in transcript level of *KOR*. Decrease in *KOR* transcript level in BR-deficient *det2* mutant then increase following a BR treatment showed the potential mechanism between *KOR* and BR.

In conclusion, despite the fact that BRs' anti-stress effects under different stress conditions were known physiologically, detailed molecular mechanisms are yet to be elucidated. Detecting alterations in gene expressions constitute important starting points for understanding of mechanisms and researching the functions of genes. Our approach was to investigate expression of some BR-regulated cell cycle and cell wall-related genes at the transcription level in leaf and root tissues of *A. thaliana* subjected to BA and EBL treatment alone or in combination. The semi-quantitative RT-PCR results showed that root was the more responsive tissue than leaf. BRs had different effects on expression patterns of the researched genes depending on the applied concentration.

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