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DIFFERENTIAL EXPRESSION ANALYSIS OF BORON TRANSPORTERS AND SOME STRESS-RELATED GENES IN RESPONSE TO 24-EPIBRASSINOLIDE AND BORON BY SEMI-QUANTITATIVE RT-PCR IN *Arabidopsis thaliana* (L.) Heynh

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Plant steroidal hormones, brassinosteroids (BRs), promote plant developmental processes and enhance tolerance to several abiotic stresses including high boron (B) stress. To examine the possible role of BR in high B-induced stress at the transcriptional level, we investigated the response of B transporter genes (*BOR1-4*), high B-induced genes (*MATE*, *Hsp-like*), BR-induced genes (*Hsp70-4*, *Hsp90-1*) and other stress-related genes (*LTI/COR78*, *LEA4-5*) upon exogenous treatments of 24-epibrassinolide (EBL) on *Arabidopsis thaliana* (L.) Heynh exposed to high concentrations of boric acid (BA) using semi-quantitative RT-PCR. BA treatments led to down regulation of *BOR1* and *BOR3* genes in leaf and root tissues and higher concentration of EBL further decreased expression of these genes in roots. The expression of high B-induced genes was observed to be upregulated by 1 μM EBL treatment under high B stress in both tissues of the seedlings. The upregulation of BR-induced genes were clearly evident in root tissues co-treated with 1 μM EBL and BA as compared to BA alone. Higher concentration of EBL was found to be more effective in increasing expression of *LTI/COR78* gene in root and *LEA4-5* gene in shoot tissues. To our knowledge, this is

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the first report how exogenous application of EBL modulates high B stress responses at molecular level in model plant *Arabidopsis thaliana*.

Key words: Arabidopsis thaliana, boron, 24-epibrassinolide, gene expression, semi-quantitative RT-PCR, stress.

INTRODUCTION

Boron is an essential micronutrient required by the plants (WARRINGTON, 1923). In physiological conditions, B is mainly present in the form of BA [$B(OH)_3$] and a small amount of borate anion [$B(OH)_4^-$] at the physiological pH. The plant cells uptake B mostly in the form of BA (BOLANOS *et al*., 2004). The transport of BA from soil solution to root cells and xylem loading take place via three different mechanisms depending on B availability: passive transport (1), channel-mediated facilitated transport with NIP (Nodulin-like intrinsic protein) proteins (2) and active transport via B transporters (3) (HERRERA-RODRIGUEZ *et al.*, 2010). While B is needed in lower amounts, it can be toxic to plants above a certain threshold level (NABLE *et al*., 1997). B is known to have high affinity for the biomolecules with *cis*-diol groups, so notably the molecules such as ATP, NADH, NADPH and RNA having ribose entities are considered to be the primary targets. Binding of B to these molecules may disrupt cellular events such as cell division and expansion. In addition, some in vitro studies revealed that B inhibits mRNA splicing, which was suggested as one of the targets of B-toxicity (STANGOULIS and REID, 2002).

B-toxicity is a noteworthy agricultural problem around the world especially in semi-arid areas including Turkey, South Australia, Mediterranean countries, California and Chile (MIWA and FUJIWARA, 2010). Similar to other ionic stress molecules, higher levels of B cause the formation of reactive oxygen species (ROS), peroxidation of lipids and proline accumulation in plant cells (CERVILLA *et al.*, 2007). B-toxicity symptoms include inhibition of shoot and root growth, loss of leaf area, lower leaf chlorophyll content and photosynthetic rates as well as decreased lignin and suberin content (NABLE *et al.*, 1997).

Brassinosteroids (BRs), regarded as the sixth class of plant hormones, are the steroids that are essential in promoting cellular expansion, proliferation, polarization of cell membrane, differentiation of tracheary elements, male fertility and control of senescence (GRUSZKA, 2013). It was proposed that BRs can exert the physiological effects by changing the levels of some enzymes involved in differential regulation of gene expression, and affecting the membrane properties (FARIDUDDIN *et al*., 2014).

In addition to their roles on plant growth and development, BRs modulate metabolic responses to gain tolerance against various environmental stresses including salinity, drought, thermotolerance and heavy metal stress (DHAUBHADEL *et al*., 1999; DHAUBHADEL *et al*., 2002; KAGALE *et al*., 2007; ALI *et al.*, 2008; VRIET *et al.*, 2012). BRs bind to leucine-rich repeat receptor kinases (BRI1) at the cell surface to initiate signal transduction cascades that result in differential expression of numerous genes including those involved in increased adaptation to various stresses (WANG *et al*., 2012; BELKHADIR and JAILLAIS, 2015).

In our previous study, ameliorating influences of BRs against the negative effects of high B was explored in *A. thaliana* in terms of growth parameters and antioxidant system (SURGUN *et al*., 2016). Findings of the study showed that treatment of BR to the plants considerably reduced inhibitory effects of high B and in that increased biomass, pigment contents and the activities of antioxidant enzymes as well as proline content under high B conditions. The finding of this study was also supported by YUSUF *et al.* (2011), who reported similar results using *Vigna radiata* that

BRs mitigates boron toxicity. Therefore, we conclude that the phenotypic effect of BR on boron stressed plants is obvious and needs further exploration in terms of molecular mechanisms. One effective way to do is to study expression of genes that are related to BR, boron and some other stresses. Since there had been no studies in the literature that put together boron and BRs (EBL) together in terms of gene expression using semi-quantitative RT-PCR, we thought that the current study should be conducted and would likely fill the gap in the area and provide useful and new information.

MATERIALS AND METHODS

Plant material and treatments

Arabidopsis thaliana (L.) Heynh ecotype Col-0 was used in this study. Arabidopsis seedlings were grown in hydroponic culture system (SMEETS *et al*., 2008), which was further modified in our laboratory. *A. thaliana* seedlings were grown for 4 weeks in a control medium (FUJIWARA *et al*., 1992) and then seedlings were transferred to the same media containing BA (0.80 and 1.60 mM) alone or supplemented with 24-epibrassinolide (EBL) (0.01 and 1 μM). Seedlings were exposed to BA and/or EBL treatments for 60 hours. A stock solution of EBL (Sigma E1641, USA) was freshly prepared according to the procedure described by ALI *et al.* (2008). Control medium containing an equal ratio of ethanol that was used for the preparation of the EBL solution was used. Leaves (5^{th} and 6^{th}) and roots of the plants were harvested, frozen in liquid nitrogen and stored at -80 °C until RNA extraction. All experiments were maintained in a growth room at 22 ± 2 ºC under fluorescent white light (100 μmol m^{-2} s^{-1} at leaf level) with a 16-h light / 8-h dark photo period.

RNA extraction

Frozen leaf and root tissues of Arabidopsis plants were ground with liquid nitrogen to a fine powder using a mortar and pestle. Total RNA was extracted using Plant RNA Mini Prep Kit (Zymo Research, USA) as described by the manufacturer. The concentration and purity of RNA were determined by measuring absorbance at 260 and 280 nm in a spectrophotometer (Optizen Pop, Korea). RNA integrity and quality was also checked by agarose gel [0.8% agarose gels in 0.5X Tris-BA-EDTA (TBE)] electrophoresis. RNA samples were treated with DNase I (Fermentas, Germany) for 30 min to remove any traces of DNA contamination.

Semi–quantitative reverse transcriptase (RT) - polymerase chain reaction (PCR)

A two-step semi-quantitative RT-PCR method was used to measure gene expression in the leaf and root tissues of *A. thaliana*. Oligo-$(dT)_{18}$ primer was used in the first step of cDNA synthesis. Total RNA (2.4 μg) was reverse transcribed with RevertAid First Strand cDNA Synthesis Kit (Fermentas, Germany) according to the supplier's instructions. Reverse transcription was carried out in a final 20 μL reaction mixture 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) and 1 μL M-MuLV Reverse Transcriptase (200 U μL^{-1}).

RT-PCR was performed to measure gene expression of At2g47160 (*BOR1*), At3g62270 (*BOR2*), At3g06450 (*BOR3*), At1g15460 (*BOR4*), At2g04050 [one of member multidrug and toxic compound extrusion (MATE) transporter family], At5g51440 [Heat shock protein (*Hsp*)-like], At3g12580 (*Hsp70-4*), At5g52640 (*Hsp90-1*), At5g52310 [Low Temperature-Induced Protein 78 (*LTI*/*COR78*)], At5g06760 [Late-Embryogenesis Abundant Protein 4-5 (*LEA4-5*)] and *Actin2* as an

internal control. Primer sequences of At2g47160, At3g62270, At3g06450 and At1g15460 genes were obtained by personal communication with Dr. Kyoko Miwa. The rest of primers were designed using software Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) (KORESSAAR and REMM, 2007; UNTERGRASSER *et al.*, 2012). Primer sequences, expected fragment sizes and references are given in Table 1. For PCR amplification using cDNA as template, a 50 µL reaction mixture contained 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}), 1 µL of 10 pmol μL^{-1} of each primer and 4 µL of diluted (1/10) cDNA as a template. PCR conditions were 94 ºC for 3 min, 28 - 35 cycles of 94 ºC for 30 s, 48 - 52 ºC for 30 s and 72 ºC for 45 s. Each set of reactions always included negative (minus RT) and positive control. 30 µL of the PCR product for each sample was analyzed using agarose gel electrophoresis (2% agarose gels in 0.5X TBE) and stained with ethidium bromide. Gel images were digitally captured using gel documentation system (DNR MiniBis pro Bio-Imaging) and quantification of the bands was performed with the help of ImageJ version 1.48 software (National Institute of Mental Health, USA). Gene expression level of each gene was calculated by measuring band intensity of the target gene and dividing to the band intensity of *Actin2* gene. Then, gene expression value of control treatment (no boron and/or EBL application) was accepted as the normalized value of "100". Fold changes were then calculated by taking the value of control treatment as reference.

Table 1. List of primer sequences used in this study

Gene name or (Putative) Function	**Locus ID**	**Primer Sequence**	**Fragment size (bp)**	**References**
Boron transporter 1	At2g47160	F 5'-AATCTCGCAGCGGAAACG-3' R 5'-TGGAGTCGAACTTGAACTTGTC-3'	141	TAKANO *et al.*, 2005
Putative boron transporter 2	At3g62270	F 5'-CATCTCGCAGTACCGGAAGCT-3' R 5'-AGCCTTGGACTCATCTCACCT-3'	218	Personal communication
Putative boron transporter 3	At3g06450	F 5'-CATTCAATCTCAAACCGGAAGG-3' R 5'-TTCAAGCTGCTCACTTTCCTTA-3'	121	Personal communication
Boron transporter 4	At1g15460	F 5'-GGAACTGTCTTTCCGGTCGAA-3' R 5'-CTTGGGATAAATCTGGTTGCCT-3'	148	MIWA *et al.*, 2014
Multidrug and toxic compound extrusion (MATE) transporter	At2g04050	F 5'-CGTTTCCGGGTTCAGTATTT-3' R 5'-CAGGGTCTTGACCGAGAGAG-3'	200	KASAJIMA and FUJIWARA, 2007
Heat shock protein-like	At5g51440	F 5'-TCAAACCGACATGTTTCTCG-3' R 5'-TCACGTTCCAACCACGTCTA-3'	178	KASAJIMA and FUJIWARA, 2007
Heat shock protein 70-4	At3g12580	F 5'-GGAAAGTTCGAGCTCAGTGG-3' R 5'-ACCTTCCCTTGTCGTTTGTG-3'	160	This study
Heat shock protein 90-1	At5g52640	F 5'-AAACGCTCTCGAAGTTCCAA-3' R 5'-TGAGCTCACGGAGGAAGATT-3'	198	This study
Low Temperature-Induced Protein 78	At5g52310	F 5'-GAACACTCCGGTCTCTCTGC-3' R 5'-CAATCTCCGGTACTCCTCCA-3'	224	This study
Late-Embryogenesis Abundant Protein 4-5	At5g06760	F 5'-AGGCGGAGAAGATGAAGACA-3' R 5'-CATCTGATGTGTCCCAGTGC-3'	218	This study
Actin2	A3g18780	F 5'-TGCCAATCTACGAGGGTTTC-3' R 5'-TTCTCGATGGAAGAGCTGGT-3'	226	This study

Statistical analysis

Statistical analysis of the results was performed by one-way analysis of variance (ANOVA). For multiple comparisons, Tukey HSD post hoc test was used. Data are the mean ± standard error (SE) of at least four independent replicates. $P < 0.05$ was considered statistically significant. The statistical analyzes were done using the package software, SPSS version 20.0 (SPSS, USA).

RESULTS

Our aim was to investigate the anti-stress effects of BRs at the molecular level by studying expression of some selected genes in response to B, EBL and a combination of B 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 (Figure 1 and 2). Transcriptional profiling revealed that majority of the selected genes was differentially expressed in response to the treatments (Table 2 and 3).

As a result of RT-PCR analyzes, it was seen that both in leaf and root tissues mRNA levels of *BOR1* and *BOR3* exhibited similar patterns. *BOR1* gene expression level was significantly down-regulated in the leaves subjected to BA treatments as compared to untreated control. Upon application of 0.80 mM BA and 1 µM EBL together, transcript level of *BOR1* gene in the Arabidopsis leaves were detected to be decreased comparing to 0.80 mM BA alone (Figure 1a). In the roots, when compared to the control, 1 µM EBL alone or in combination with 0.80 and 1.60 mM BA led to down regulation of *BOR1* by 2.45, 3.50 and 7.60-fold, respectively (Figure 2a).

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: 0.80 mM BA, BA 2: 1.60 mM BA).

Gen name	Treatment *								
	Control	EBL 1	EBL 2	BA1	BA 1 + EBL 1	BA 1 + EBL 2	BA 2	BA 2 + EBL 1	BA 2 + EBL 2
BOR1	1.00 ± 0.00	-1.05 ± 0.01	-1.20 ± 0.02	-1.15 ± 0.02	-1.20 ± 0.04	-2.10 ± 0.04	-1.85 ± 0.03	-1.90 ± 0.01	-2.20 ± 0.02
BOR2	1.00 ± 0.00	1.00 ± 0.01	1.30 ± 0.03	1.35 ± 0.02	1.65 ± 0.03	1.60 ± 0.06	1.80 ± 0.04	2.05 ± 0.04	2.20 ± 0.05
BOR3	1.00 ± 0.00	1.00 ± 0.01	1.00 ± 0.02	-1.40 ± 0.01	-1.80 ± 0.03	-2.10 ± 0.01	-2.30 ± 0.03	-2.25 ± 0.04	-1.85 ± 0.03
BOR4	1.00 ± 0.00	1.75 ± 0.02	2.10 ± 0.01	1.75 ± 0.04	2.35 ± 0.06	2.60 ± 0.10	1.75 ± 0.02	1.85 ± 0.04	2.10 ± 0.08
MATE	1.00 ± 0.00	1.30 ± 0.10	2.00 ± 0.15	3.00 ± 0.11	3.35 ± 0.11	3.55 ± 0.11	4.80 ± 0.10	6.30 ± 0.17	5.95 ± 0.20
Hsp-like	1.00 ± 0.00	-1.15 ± 0.02	1.25 ± 0.02	1.20 ± 0.15	1.55 ± 0.11	1.90 ± 0.16	1.90 ± 0.11	2.25 ± 0.09	2.45 ± 0.11
Hsp70-4	1.00 ± 0.00	1.00 ± 0.02	1.10 ± 0.03	1.05 ± 0.04	1.10 ± 0.03	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.10 ± 0.02
Hsp90-1	1.00 ± 0.00	1.05 ± 0.01	1.05 ± 0.03	1.25 ± 0.01	1.25 ± 0.03	1.20 ± 0.01	1.30 ± 0.02	1.35 ± 0.01	1.55 ± 0.01
LTI/COR78	1.00 ± 0.00	1.20 ± 0.03	1.30 ± 0.03	1.35 ± 0.02	1.25 ± 0.04	1.50 ± 0.05	1.35 ± 0.06	1.45 ± 0.03	1.90 ± 0.02
LEA4-5	1.00 ± 0.00	1.20 ± 0.04	1.65 ± 0.03	1.70 ± 0.04	1.40 ± 0.05	2.05 ± 0.02	1.25 ± 0.02	1.45 ± 0.02	1.55 ± 0.01

*: The ratios in fold changes were calculated with respect to control (no treatment). Each value represents mean ± SE (n =4-5).

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: 0.80 mM BA, BA 2: 1.60 mM BA).

Gen name	Treatment *								
	Control	EBL 1	EBL 2	BA1	BA 1 + EBL 1	BA 1 + EBL 2	BA 2	BA 2 + EBL 1	BA 2 + EBL 2
BOR1	1.00 ± 0.00	1.15 ± 0.02	-2.45 ± 0.05	-1.35 ± 0.04	-1.35 ± 0.04	-3.50 ± 0.05	-1.50 ± 0.04	-1.70 ± 0.04	-7.60 ± 0.01
BOR2	1.00 ± 0.00	2.10 ± 0.02	2.25 ± 0.05	1.80 ± 0.10	2.15 ± 0.08	2.25 ± 0.08	2.30 ± 0.08	2.20 ± 0.06	2.40 ± 0.01
BOR3	1.00 ± 0.00	1.00 ± 0.00	-1.70 ± 0.01	-1.50 ± 0.04	-1.55 ± 0.05	-2.75 ± 0.03	-1.60 ± 0.02	-1.80 ± 0.02	- 5.00 ± 0.03
BOR4	1.00 ± 0.00	1.20 ± 0.05	-5.60 ± 0.00	1.15 ± 0.02	1.20 ± 0.04	-5.30 ± 0.00	1.35 ± 0.02	1.40 ± 0.02	**
MATE	1.00 ± 0.00	1.30 ± 0.06	2.55 ± 0.05	-2.10 ± 0.07	1.05 ± 0.06	2.40 ± 0.08	-2.45 ± 0.01	-2.50 ± 0.03	1.80 ± 0.10
Hsp-like	1.00 ± 0.00	1.75 ± 0.13	2.70 ± 0.13	1.85 ± 0.16	1.90 ± 0.16	2.50 ± 0.19	1.50 ± 0.04	1.65 ± 0.10	2.85 ± 0.17
Hsp70-4	1.00 ± 0.00	2.10 ± 0.05	3.40 ± 0.05	1.85 ± 0.02	1.75 ± 0.05	2.95 ± 0.04	2.10 ± 0.01	1.85 ± 0.07	2.90 ± 0.08
Hsp90-1	1.00 ± 0.00	1.65 ± 0.07	3.20 ± 0.07	1.80 ± 0.08	2.55 ± 0.09	3.60 ± 0.05	1.90 ± 0.04	1.95 ± 0.06	4.25 ± 0.13
LTI/COR78	1.00 ± 0.00	1.30 ± 0.06	1.65 ± 0.10	1.10 ± 0.08	1.45 ± 0.08	1.80 ± 0.10	1.10 ± 0.03	-1.45 ± 0.11	1.70 ± 0.13
LEA4-5	1.00 ± 0.00	1.20 ± 0.04	1.55 ± 0.02	1.20 ± 0.06	1.40 ± 0.02	1.45 ± 0.04	1.40 ± 0.07	1.35 ± 0.07	1.60 ± 0.08

*: The ratios in fold changes were calculated with respect to control (no treatment). Each value represents mean ± SE (n = 4-5). **: No expression.

Expression of *BOR2* gene was up-regulated in the leaves by 1.35 and 1.80-fold under the 0.80 and 1.60 mM BA treatments. However, EBL in combination with BA further increased the mRNA levels of *BOR2* (Figure 1b, Table 2). On the other hand, in the root tissues, 0.01 and 1 μM EBL treatments elevated the transcript levels of *BOR2* by 2.10 and 2.25-fold, respectively. Similar pattern of increase was also apparent in the root tissues; mRNA level of *BOR2* was up-regulated in BA-treated plants compared to the control. Also, the plants treated with 1.60 mM BA and 1 μM EBL possessed relatively higher levels of *BOR2* transcripts (Figure 2b, Table 3). The treatment with BA significantly decreased the expression level of *BOR3* gene in the leaves as compared to control (Figure 1c). Similarly, the transcript level of *BOR3* gene was down-regulated by BA treatments in the root tissues. However, 1 μM EBL alone or in combination with 0.80 and 1.60 mM BA decreased the expression levels of *BOR3* by 1.70, 2.75 and 5-fold, respectively (Figure 2c, Table 3).

The results showed that *BOR4* gene expression was induced by BA treatments (0.80 and 1.60 mM) both in leaf (1.75-fold) and root tissues (1.15 and 1.35-fold). Furthermore, EBL treatments alone or in combination with BA led to upregulation of this gene (*BOR4*) in a statistically significant manner in leaf tissues by approximately 2-fold compared to the control (Figure 1d, Table 2). In addition, levels of *BOR4* transcript were shown to be lower in the root tissues by 5.60 and 5.30-fold upon treatment with 1 μM EBL alone or in combination with 0.80 mM BA, respectively. However, no expression was detected in the root tissues treated with 1.60 mM BA and 1 μM EBL together (Figure 2d, Table 3).

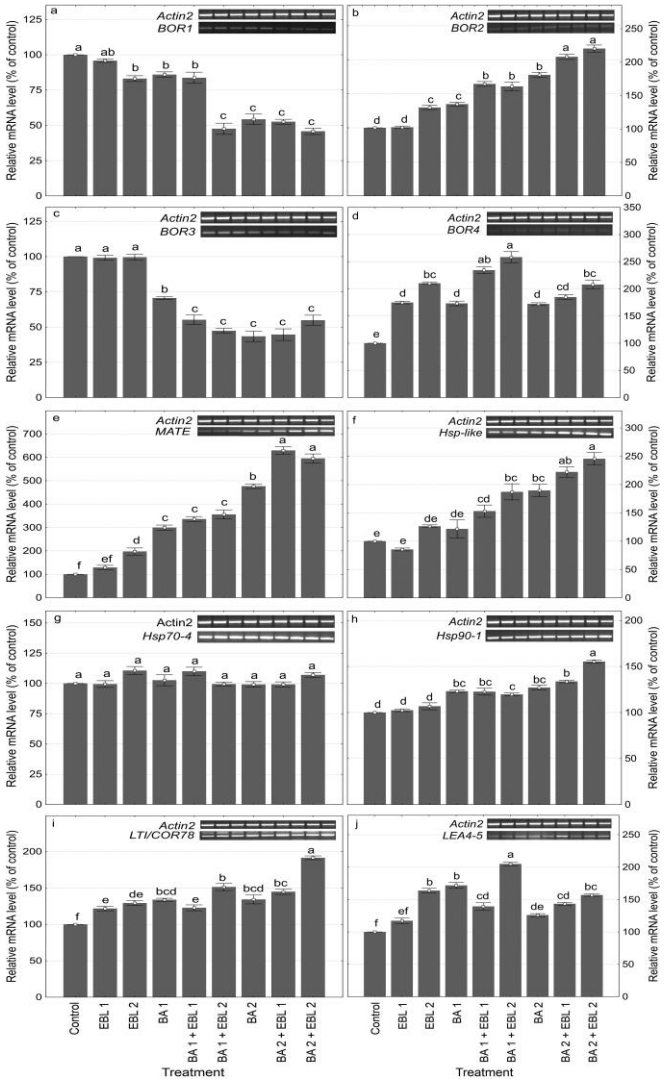

Fig. 1. Relative expression levels of the genes in leaf tissues. Effects of EBL (EBL 1: 0.01 µM EBL, EBL 2: 1 µM EBL) and/or BA (BA 1: 0.80 mM BA, BA 2: 1.60 mM BA) treatments on mRNA levels of selected genes in *A. thaliana* leaves are shown in graphs a through j. (a) *BOR1* (At2g47160), (b) *BOR2* (At3g62270), (c) *BOR3* (At3g06450), (d) *BOR4* (At1g15460), (e) *MATE* (At2g04050), (f) *Hsp-like* (At5g51440), (g) *Hsp70-4* (At3g12580), (h) *Hsp90-1* (At5g56240), (i) *LTI/COR78* (At5g52310) and (j) *LEA4-5* (At5g06760). Images are the representative results from at least four biological repeats. mRNA levels were normalized to Actin2 gene. 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.

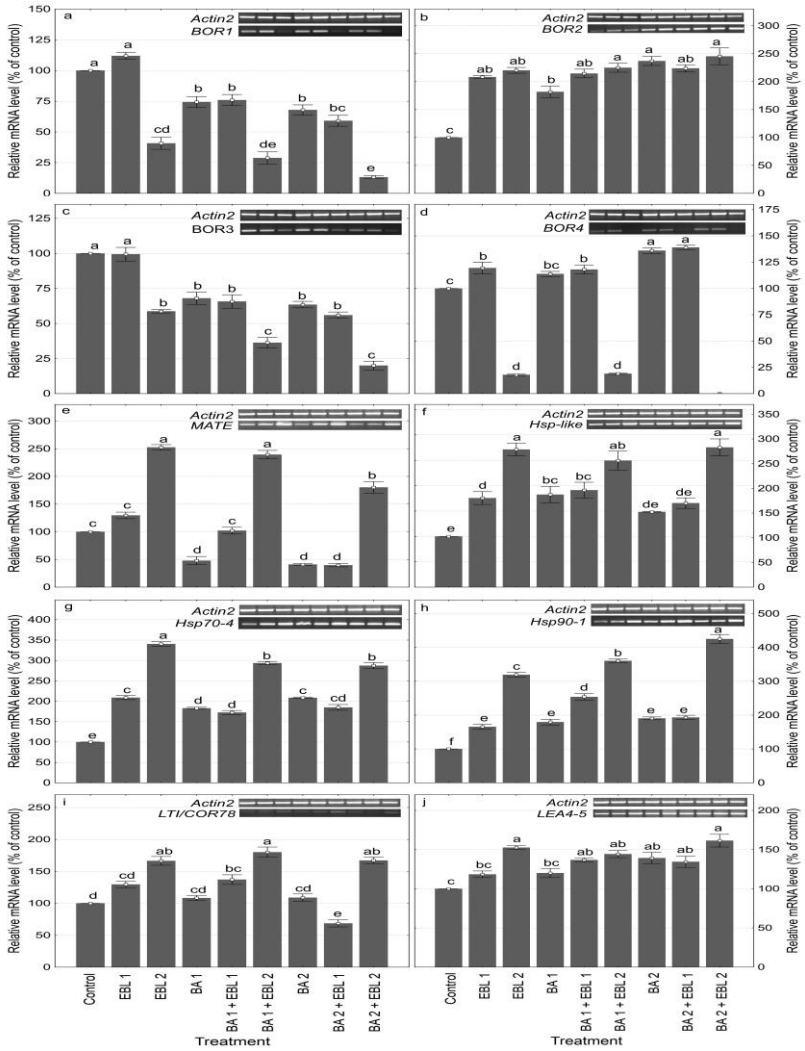

Fig. 2. Relative expression levels of the genes in root tissues. Effects of EBL (EBL 1: 0.01 µM EBL, EBL 2: 1 µM EBL) and/or BA (BA 1: 0.80 mM BA, BA 2: 1.60 mM BA) treatments on mRNA levels of selected genes in *A. thaliana* roots are shown in graphs a through j. (a) *BOR1* (At2g47160), (b) *BOR2* (At3g62270), (c) *BOR3* (At3g06450), (d) *BOR4* (At1g15460), (e) *MATE* (At2g04050), (f) *Hsp-like* (At5g51440), (g) *Hsp70-4* (At3g12580), (h) *Hsp90-1* (At5g56240), (i) *LTI/COR78* (At5g52310) and (j) *LEA4-5* (At5g06760). Images are the representative results from at least four biological repeats. mRNA levels were normalized to *Actin2* gene. 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.

Transcript levels of MATE transporter gene revealed that expression of this gene was strongly up-regulated in the leaf tissues upon treatment with 0.80 and 1.60 mM BA by 3.00 and 4.80-fold, respectively, as compared to control. Application of 1 μM EBL to the plants resulted in significant increase of mRNA levels of this gene (2-fold) in control conditions. 0.01 and 1 μM EBL combined with 0.80 mM BA elevated the transcript levels by 3.35 and 3.55-fold, respectively, compared with control. EBL treatments with 1.60 mM BA further increased the expression of MATE transporter gene compared with control and 1.60 mM BA-alone treatment in leaf tissues (Figure 1e, Table 2). In the root tissues, MATE transporter gene expression was down-regulated by 2.10 and 2.45-fold as a response to 0.80 and 1.60 mM BA treatments, respectively, in a statistically significant manner. EBL (1 μM) alone or co-treated with BA elevated transcript levels by approximately 1.80-2.55-fold (Figure 2e, Table 3).

Expression of *Hsp-like* gene was found to be increased markedly in the leaf tissues following the exposure to BA treatments. Co-treatments of BA and EBL further induced *Hsp-like* gene expression (Figure 1f). In the root tissues treated with different concentrations of BA, a significant rise in *Hsp-like* gene expression was also noticed. 0.01 and 1 μM EBL treatments alone, compared to the control, elevated the transcript levels of 1.75 and 2.70-fold, respectively. Co-treatment of 1 μM EBL with 0.80 or 1.60 mM BA up-regulated mRNA levels of *Hsp-like* by aproximately 2.50-2.85-fold as compared to control (Figure 2f, Table 3).

RT-PCR results of *Hsp70-4* gene indicated that any significant differences in the transcript levels were detected in the leaf tissues as a result of all treatments (Figure 1g). However, in Arabidopsis roots, expression of this gene was elevated around 2 fold as a result of BA treatments. Application of 1 μM EBL alone and in combination with both concentrations of BA showed induction of *Hsp70-4* gene expression by around 3-fold (Figure 2g, Table 3). For *Hsp90-1* gene, no difference or slight increase in the transcript levels in the leaf tissues was detected in all of the conditions tested in this study (Figure 1h). The experiments conducted with the root tissues showed that 1 μM EBL treatment alone or in combination with BA treatments induced the expression of *Hsp90-1* gene by 3.20 and approximately 4-fold, respectively, compared with control (Figure 2h, Table 3).

LTI/COR78 gene expression was seen to be slightly higher in the leaf tissues upon all treatments albeit at varying ratios. A relatively highest increase in the transcript levels of this gene was detected in the leaf tissues treated with 1 μM EBL and 1.60 mM BA together (Figure 1i, Table 2). For the root tissues, application of different concentrations of BA led to a slight induction in *LTI/COR78* gene expresssion but this increase was not found significant. 1 μM EBL alone or in combination with both BA concentrations caused upregulation of this gene around 1.45-fold (Figure 2i, Table 3).

BA exposure resulted in an increase of the transcript levels of *LEA4*-5 gene in a statistically significant manner in the leaf tissues when compared to the control. While the concentration of 0.01 μM EBL slightly affected the expression, 1 μM EBL has a more profound influence alone or in combination with BA treatments (Figure 1j, Table 2). On the other hand, in the root tissues, 1.60 mM BA treatment increased the expression of *LEA4-5* gene, whereas 0.80 mM BA alone did not have any significant effect. 1 μM EBL alone or in combination with 1.60 mM. BA resulted in an increase around 1.50- fold as compared to the control (Figure 2j, Table 3).

DISCUSSION

Plants have evolved various mechanisms to cope with environmental stresses. Many of these mechanisms require transcriptional activation of specific genes that are generally known as defense-related genes (LI and YI, 2012). Although the effects of BRs on stress tolerance are relatively well documented, detailed molecular mechanisms are yet to be elucidated. One of the ways to elucidate the mechanism of stress tolerance is the determination and evaluation of stress-induced changes in gene expression (KREPS *et al.*, 2002). Our approach was to investigate expression of B, EBL and stress related genes at the transcription level in leaf and root tissues of *A. thaliana* subjected to BA and EBL treatment alone or in combination.

BRs are shown to negate the detrimental effects of boron toxicity (YUSUF *et al.*, 2011). Uptake and efflux transporters are employed in plant cells to regulate B homeostasis (TAKANO *et al.*, 2008). We asked if the effect is through modulating the expression of boron transporters. Therefore, expression levels of *BOR1*, *BOR2*, *BOR3* and *BOR4* were investigated in the study. BOR1 is the first B transporter identified in *A. thaliana* (TAKANO *et al.*, 2002). BOR1 is required under B-deficient conditions to efficiently load xylem with B and translocate this element to upper part of the plant especially to young portions (NOGUCHI *et al*., 1997; TAKANO *et al*., 2001). It is reported in the literature that *BOR1* is expressed in shoots as well, but its role is not clear in regulating B distribution among the leaves. It is suggested that BOR1 may help direct B from the xylem to phloem for the delivery to young leaves (MIWA and FUJIWARA, 2010). In the present study, it was seen that *BOR1* mRNA levels were decreased in root and leaf tissues in response to increasing B concentration. Previous studies reported that in the presence of high B, BOR1 is rapidly degraded in the vacuoles following endocytosis (TAKANO *et al*., 2005). This regulation prevents B accumulation in the shoots to maintain homeostasis (KASAI *et al*., 2010). Consequently, decrease in *BOR1* gene expression is likely to be a part of this overall mechanism and the results of this study present supporting evidence. Another study revealed that application of high levels of BA represses the expression of aquaporins and nutrient transport genes in the Arabidopsis roots. One of the repressed genes was found to be *BOR1* (AQUEA *et al.*, 2012). EBL application further decreased the levels of *BOR1* mRNA in root tissues as shown by the results of this study. It is possible that EBL might provide stress tolerance against B, which might affect *BOR1* expression indirectly. Previous studies showed that *BOR3* is expressed in shoot guard cells, trichomes, root cortex as well as stigma and ovaries (PARKER and BORON, 2013). In this study, *BOR3* exhibits the same pattern of expression as *BOR1* both in leaf and root tissues in response to BA treatments. This is supported by the results obtained by MIWA *et al.* (2006), where the authors found that triple (*bor1/bor2/bor3*) and double mutants (*bor1/bor2*) have given rise to the conclusion that BOR3 may play a supporting role to BOR1 in roots. Moreover, the present study revealed that higher concentration of EBL treatment alone or together with BA resulted in further down regulation of *BOR3* expression suggesting similar involvement of BOR3 in stress tolerance. However, further investigations are needed on BOR3 transporter to explore the findings related to EBL and high B conditions.

In 2007, BOR4, a B transporter employed to provide tolerance against higher levels of B was identified (MIWA *et al*., 2007). BOR4 enables the plant to directly efflux B from roots and hence prevents the accumulation of B in xylem and growing cells. As opposed to BOR1, BOR4 is not degraded under high B concentrations and accumulated stably in the plasma membrane (MIWA *et al*., 2007). *BOR4* expression was shown to be up-regulated in both leaf and root tissues as a response to increasing levels of B, as shown by the present study. MIWA *et al.* (2014) proposed that

endogenous *BOR4* is a high B inducible gene that functions in high B tolerance. In this case, increased expression of *BOR4* may be regarded as a part of a mechanism to remove excess B from the roots. On the other hand, in the leaves, treatment with EBL alone or combination with BA resulted in higher levels of *BOR4* mRNA. MIWA and FUJIWARA (2011) reported that overexpressed *BOR4* export B from the cytoplasm to the apoplast in the leaves. Higher expression of *BOR4* in the leaves can enhance high B tolerance so that EBL can contribute to stress tolerance by affecting BOR4 levels as well in the leaves. However, co-application of BA with 1 μM EBL resulted in markedly decreased levels of *BOR4* mRNAs in the root tissues. We interpret from the data that EBL treatment masked the up-regulating effect of BA on *BOR4* mRNA levels in roots. However it is unknown how BRs modulate the expression level of *BOR4* and this needs further exploration, where appropriate mutants, various sampling times and EBL concentrations as well as new approaches such as proteomics should be integrated into the efforts. Another BOR1 paralog, BOR2, encodes an efflux B transporter localized in plasma membrane and is strongly expressed in roots. It was proposed that the function of BOR2 is to transport BA from symplast to apoplast for cross-linking of B to Rhamnogalacturonan II (RG II) in cell wall (MIWA *et al*., 2013). In this study, it was shown that *BOR2* transcript levels were increased both in leaf and root tissues in consequence of BA applications. As a result of EBL treatments, mRNA levels of *BOR2* further increased in the investigated tissues. *BOR2* and *BOR4* expression patterns were similar in the leaf tissues. It is possible that in addition to its role in RG II biosynthesis, BOR2 as well is likely to be implicated in B tolerance.

MATE and *Hsp-like* genes were selected based on their high level expression under high boron conditions as shown by microarray analysis (KASAJIMA and FUJIWARA, 2007). The objective in selecting these genes was: (1) to provide as a control in our study whether BA treatment would increase the expression of these genes in the experimental system we used and (2) to see whether EBL treatment affects further the expression of these genes as additive or antagonistic. The studies using plants and some fungi have revealed that accumulation of numerous harmful and toxic substances in the vacuoles is carried out by secondary transport systems, which belong to multidrug and toxic compound extrusion (MATE) transporter family (DIENER *et al*., 2001; OMOTE *et al*., 2006). ROGERS and GUERINOT (2002) showed that mutation in one of the alleles of *FRD3* gene (named *frd3-3*), a member of MATE family, led to accumulation of two fold excess iron and zinc as well as four fold excess manganese in shoots indicating the role of the MATE protein in metal transport. Moreover, as a result of several studies, the proteins belonging to MATE family were found to be involved in transport of the metals (LIU *et al*., 2009) and toxins (DIENER *et al*., 2001) for detoxification. In the present study, transcripts of MATE transporter gene were found at higher levels in BA-treated leaf tissues when compared to untreated ones. Also, it was observed that B treatment of the plants caused decreased levels of mRNA of MATE transporter gene in roots. Investigation of expression of more genes belonging to MATE family following the exposure to BA may reveal a possible relationship between MATE transporters and B. Expression of the MATE transporter gene, which is implicated in internal transport and detoxification, was found to be significantly up-regulated both in leaf and tissues as a result of EBL treatment. It may be possible that exogenous EBL treatment helps plant survive stress condition via elevating expression of stress related genes such as MATE.

Under almost all stress conditions, it is reported that expressions of heat-shock proteins (Hsps) are expected to be up-regulated (TOMBULOGLU *et al*., 2012). The function of the heat shock proteins known as stress proteins is to prevent the separation of oligomeric complexes,

denaturation of the polypeptides or refolding of the denatured proteins under various environmental stresses. The presence of denatured proteins in the cell induces the synthesis of Hsp proteins (AL-WHAIBI, 2011). In this study, we showed that mRNA levels of *Hsp-like* gene encoding a heat shock protein (Hsp23.5), localized in mitochondria were up-regulated in response to BA treatment both in leaf and root tissues. PARK *et al.* (2013) conducted a study using two types of cabbage plants that are sensitive or tolerant to heat and reported that this gene is among the up-regulated genes under heat stress. Furthermore, LI and YI (2012) reported that expression of this gene was also up-regulated in *A. thaliana* under sulphur dioxide stress. We showed in this study that EBL treatment of the high concentration (1 μM) markedly increased the transcript levels of *Hsp-like* compared to the control. All taken together, these results provide detailed information regarding the possible role of EBL in stress amelioration. It is, therefore, likely that *Hsp-like* gene may be one of the candidates involved in stress response and tolerance provided indirectly by BR under high B stress.

Differentially expressed genes regulated by BRs were determined in several microarray studies (GODA *et al*., 2002). Among stress-related genes induced by BRs, *Hsp70-4* and *Hsp90-1* genes were repeatedly shown to be up-regulated in the studies (YUXIN *et al*., 2001; MUSSIG *et al*., 2002; MUSSIG and ALTMANN, 2003); therefore these genes were selected in the current study. Other researchers investigated the expression of these genes using northern and RT-PCR analyzes under various stress conditions such as heat, cold, drought and salinity (DHAUBHADEL *et al*., 1999; DHAUBHADEL *et al*., 2002; KAGALE *et al*., 2007; DIVI and KRISHNA 2010). No study was present until this one that investigated the expression of these genes under boron stress. Hsp70s proteins have a general role as chaperons and are implicated in regulation of expression of stress-related genes. Hsp90s play significant roles in signal transduction pathways regulated by steroid hormones, regulation of cell cycle and protein localization (PARK *et al*., 2013). It was shown by SHIGETA *et al.* (2015) reported that Hsp90s is involved in BR signaling, where two transcription factors (BES1 and BZR1) function to control a number of BR responsive genes. In the present study, BA treatments caused up-regulation of expression of both genes in roots with respect to the control plants. It was evident that higher concentration of EBL induced significantly mRNA levels of *Hsp70-4* and *Hsp90-1* genes. Similar results were obtained from different studies as well. YUXIN *et al.* (2001) identified four genes which are regulated by BRs and involved in stress response. Two of the genes were found to be *Hsp81* and *Hsp82* belonging to *Hsp90* family. Some other results by DHAUBHADEL *et al.* (1999), where the accumulation of four classes of Hsp (Hsp100s, Hsp90s, Hsp70s and sHsps) was increased dramatically in *Brassica napus* and tomato, supported the notion that BRs play effective roles in increasing the tolerance of the plants against heat stress. Same researchers investigated in a different study the synthesis of Hsp proteins in vivo and in vitro in *Brassica napus* treated with EBL. It was pointed out that EBL affects the translational mechanism, because the levels of initiation factors (elFs) and elongation factors (eEFs) differed dramatically among the plants treated with or without EBL (DHAUBHADEL *et al.*, 2002). KAGALE *et al.* (2007) showed that EBL treatments boosted the tolerance of *A. thaliana* and *B. napus* plants against drought, cold and high salt stress. *Hsp90* was one of the genes with higher level of upregulation under BR treatment. All these evidence and our results demonstrate that BRs are important in enhancing stress tolerance of the plants. A slight difference in the transcript size of the *Hsp70-4* and *Hsp90-1* between treated and untreated leaf tissues was also noted in replicate experiments. SINGLA *et al.* (1997) stated that Hsp synthesis is dependent upon cell/tissue type and development/differentiation stage in terms of quantity and quality.

KREPS *et al.* (2002) carried out global gene expression studies, where the effect of salt, cold and osmotic stress was investigated in *A. thaliana*. *LTI/COR78* and *LEA4-5* genes were two of the genes that were highly expressed in response to all three stress types. However, these genes are also regulated by abscisic acid (ABA) and it was found that exogenous ABA treatment induced the expression of these genes as shown by several studies (DALAL *et al*., 2009; OKAMOTO *et al*., 2012). Studies have indicated that BRs and ABA can coregulate the expression of hundreds of genes which result in inter-hormonal interactions in controlling many developmental processes and mediating plant stress responses (EPHRITIKHINE *et al*., 1999; NEMHAUSER *et al*., 2006; VRIET *et al*., 2012). For these reasons, in this study, we investigated the effect of BA and EBL treatments on the expression of these genes. We showed that upon BA treatment, mRNA levels of *LTI/COR78* gene were up-regulated in leaf tissues in response to BA. However we did not detect significant difference in expression level of *LTI/COR78* gene in roots in response. KREPS *et al.* (2002) showed that level of *LTI/COR78* expression was higher at 3 hour after the exposure to stress in both root and leaf tissues. Later at the 27th hour, while the transcript levels in the roots returned to the same levels with the control, expression in the leaf tissues was still kept at higher levels. In our study, the fact that the sampling was done at the 60th hour explains the results regarding expression levels in the roots. Higher concentration EBL (1 μM) treatment elevated expression of *LTI/COR78* gene in both leaf and root tissues.

As a result of genome analyzes, it was demonstrated that *LEA4-5* is an important member of *LEA* genes. LEA proteins act like chaperons to protect the cells from unsuitable environment in freezing periods with lack of water, to prevent misfolding of secondary and tertiary structure of proteins, and to circumvent protein denaturation causing enzyme inhibition (REYES *et al*., 2008; BIES-ETHEVE *et al*., 2008). We also showed for the first time that BA as well caused upregulation of expression of this gene in both leaf and root tissues of *A. thaliana.* Upon EBL application, it was observed that mRNA levels of *LEA4-5* were further increased particularly in leaf tissues. The increase in the transcript levels of ABA-responsive genes (*LTI/COR78* and *LEA4-5*) in EBL-treated tissues suggests that stress tolerance provided by BRs may occur through the interaction present in the pathways of the phytohormones such as ABA, ethylene and salicylic acid (DIVI *et al*., 2010). BAJGUZ (2009) found that BRs can enhance the endogenous ABA content in *Chlorella vulgaris* subjected to short-term heat stress. Similarly, according to DIVI *et al.* (2015), BR increases endogenous ABA levels, activating a stress response regulon to contribute the tolerance against heat in *A. thaliana*. All these results provide further clues toward an uncharted mechanism, where BRs and ABA are involved in a complex crosstalk, which requires further exploration.

In conclusion, detecting changes in gene expression is sometimes a good starting point to obtain clues in clarifying detailed mechanisms as well as finding gene function. Therefore, transcript profiling of subset of genes was investigated in this study to assess the effect of BR in Arabidopsis leaf and root tissues subjected to different B levels. The semi-quantitative RT-PCR results showed that EBL treatments caused differential expression of B transporter genes. Nevertheless, further investigation is needed to decipher the molecular mechanisms responsible for the effects of BRs on B transporters. Furthermore, this study showed that EBL in general positively regulated expression of stress related genes under high B stress. Higher concentration of EBL was found to be more effective and gene expression changes in roots were more pronounced. Future studies could involve the use of mutants, transgenic plants and proteomics approaches to understand the effect of BRs on stress tolerance by identifying more genes and components of pathways.

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REFERENCES

AL-WHAİBİ, M.H (2011): Plant-heat-shock proteins: a mini review. J King Saud University Sci *23*: 139-150.

ALI, B., S.A. HASAN, S. HAYAT, Q. HAYAT, S. YADAV, Q, FARIDUDDIN, A. AHMAD (2008): A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). Environ Exp Bot *62*: 153-159.

AQUEA, F., F. FEDERICI, C. MOSCOSO, A. VEGA, P. JULLIAN, J. HASELOFF, P. ARCE-JOHNSON (2012): A molecular framework for the inhibition of Arabidopsis root growth in response to boron toxicity. Plant Cell Environ *35*: 719-734.

BAJGUZ, A (2009): Brassinosteroid enhanced the level of abscisic acid in *Chlorella vulgaris* subjected to short-term heat stress. J Plant Physiol *166*: 882-886.

BELKHADIR, Y., Y. JAILLAIS (2015): The molecular circuitry of brassinosteroid signaling. New Phytologist *206*: 522-540.

BIES-ETHEVE, N., P. GAUBIER-COMELLA, A. DEBURES, E. LASSERRE, E. JOBET, M. RAYNAL, M. DELSENY (2008): Inventory, evolution and expression profiling diversity of the LEA (Late Embryogenesis Abundant) protein gene family in *Arabidopsis thaliana*. Plant Mol Biol *67*: 107-112.

BOLANOS, L., K. LUKASZEWSKI, I. BONILLA, D. BLEVINS (2004): Why boron? Plant Physiol Biochem *42*: 907-912.

CERVILLA, L.M., B. BLASCO, J.J.L. ROMERO, J.M. RUIZ (2007). Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. Ann Bot *100*: 747-756.

DALAL, M., D. TAYAL, V. CHINNUSAMY, K.C. BANSAL (2009): Abiotic stress and ABA-inducible Group 4 LEA from *Brassica napus* plays a key role in salt and drought tolerance. J Biotechnol *139*: 137-145.

DHAUBHADEL, S., S. CHAUDHARY, K.F. DOBINSON, P. KRISHNA (1999): Treatment with 24-epibrassinolide, a brassinosteroid, increases the basic thermotolerance of *Brassica napus* and tomato seedlings. Plant Mol Biol *40*: 333-342.

DHAUBHADEL, S., K.S. BROWNING, D.R. GALLIE, P. KRISHNA (2002): Brassinosteroid functions to protect the translational machinery and heat-shock protein synthesis following thermal stress. Plant J *29*: 681-691.

DIENER, A.C., R.A. GAXIOLA, G.R. FINK (2001): Arabidopsis *ALF5*, a multidrug efflux transporter gene family member, confers resistance to toxins. Plant Cell *13*: 1625-1638.

DIVI, U.K., T. RAHMAN, P. KRISHNA (2010).: Brassinosteroid-mediated stress tolerance in Arabidopsis shows interactions with abscisic acid, ethylene and salicylic acid pathways. BMC Plant Biol *10*: 151-165.

DIVI, U.K., T. RAHMAN, P. KRISHNA (2015): Gene expression and functional analyses in brassinosteroid-mediated stress tolerance. Plant Biotechnol J doi:10.1111/pbi.12396.

EPHRITIKHINE, G., M. FELLNER, C. VANNINI, D. LAPOUS, H. BARBIER-BRYGOO (1999): The sax1 dwarf mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. Plant J *18*: 303-314.

FARIDUDDIN, Q., M. YUSUF, I. AHMAD, A. AHMAD (2014): Brassinosteroids and their role in response of plants to abiotic stresses. Biol Plant *58*(1): 9-17.

FUJIWARA, T., M.Y. HIRAI, M. CHINO, Y. KOMEDA, S. NAITO (1992): Effects of sulfur nutrition on expression of the soybean seed storage protein genes in transgenic petunia. Plant Physiol *99*: 263-268.

GODA, H., Y. SHIMADA, T. ASAMI, S. FUJIOKA, S. YOSHIDA (2002): Microarray analysis of brassinosteroid-regulated genes in Arabidopsis. Plant Physiol *130*: 1319-1334.

GRUSZKA, D (2013): The brassinosteroid signaling pathway-new key players and interconnections with other signaling networks crucial for plant development and stress tolerance. Int J Mol Sci *14*: 8740-8774.

HERRERA-RODRIGUEZ, M.B., A. GONZALEZ-FONTES, J. REXACH, J.J. CAMACHO-CRISTOBAL, J.M. MALDONADO, M.T. NAVARRO-GOCHICOA (2010): Role of boron in vascular plants and response mechanism to boron stress. Plant Stress *4*: 115-122.

KAGALE, S., U.K. DIVI, J.E. KROCHKO, W.A. KELLER, P. KRISHNA (2007): Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. Planta *225*: 353-364.

KASAI, K., J. TAKANO, K. MIWA, A. TOYODA, T. FUJIWARA (2010): High boron-induced ubiquitination regulates vacuolar sorting of the BOR1 borate transporter in *Arabidopsis thaliana*. J Biol Chem *286*: 6175-6183.

KASAJIMA, I., T. FUJIWARA (2007): Identification of novel *Arabidopsis thaliana* genes which are indeced by high levels of boron. Plant Biotechnol *24*: 344-360.

KORESSAAR, T., M. REMM (2007): Enhancements and modifications of primer design program Primer3. Bioinformatics *23*(10):1289-1291.

KREPS, J.A., Y.J. WU, H.S. CHANG, T. ZHU, X. WANG, J.F. HARPER (2002): Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol *130*: 2129-2141.

LI, L., H. YI (2012): Differential expression of Arabidopsis defense-related genes in response to sulfur dioxide. Chemosphere *87*: 718-724.

LIU, J.P., J.V. MAGALHAES, J. SHAFF, L.V. KOCHIAN (2009): Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. Plant J *57*: 389-399.

MIWA, K., J. TAKANO, M. SEKI, K. SHINOZAKI, T. FUJIWARA (2006): Roles of BOR3-5 in boron transport in *Arabidopsis thaliana*. Plant Cell Physiol *47* :154 (Abstract).

MIWA, K., J. TAKANO, H. OMORI, M. SEKI, K. SHINOZAKI, T. FUJIWARA (2007): Plants tolerant of high boron levels. Science *318*: 141.

MIWA, K., T. FUJIWARA (2010): Boron transport in plants: co-ordinated regulation of transporters. Ann Bot *105*: 1103-1108.

MIWA, K., T. FUJIWARA (2011): Role of overexpressed BOR4, a boron exporter, in tolerance to high level of boron in shoots. Soil Sci Plant Nutr *57*: 558-565.

MIWA, K., S. WAKUTA, S. TAKADA, K. IDE, J. TAKANO, S. NAITO, H. OMORI, T. MATSUNAGA, T. FUJIWARA (2013). Roles of BOR2, a boron exporter, in cross linking of rhamnogalacturonan II and root elongation under boron limitation in Arabidopsis. Plant Physiol *163*: 1699-1709.

MIWA, K., I, AIBARA, T. FUJIWARA (2014): *Arabidopsis thaliana* BOR4 is up-regulated under high boron conditions and confers tolerance to high boron. Soil Sci Plant Nutr *60*: 349-355.

MUSSİG, C., S. FİSCHER, T. ALTMANN (2002): Brassinosteroid-regulated gene expression. Plant Physiol *129*: 1241-1251.

MUSSIG, C., T. ALTMANN (2003): Genomic brassinosteroid effects. J Plant Growth Regul *22*: 313-324.

NOGUCHI, K., M. YASUMORI, T. IMAI, S. NAITO, T. MATSUNAGA, H. ODA, H. HAYASHI, M. CHINO, T. FUJIWARA (1997): *bor1-1*, an *Arabidopsis thaliana* mutant that requires a high level of boron. Plant Physiol *115*: 901-906.

NABLE, R.O., G.S. BANUELOS, J.G. PAULL (1997): Boron toxicity. Plant Soil *193*: 181-198.

NEMHAUSER, J.L., F. HONG, J. CHORY (2006): Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell *126*(3): 467-475.

OKAMOTO, M., Y. TSUBOI, H. GODA, T. YOSHIZUMI, Y. SHIMADA, T. HIRAYAMA (2012): Multiple hormone treatment revealed novel cooperative relationships between abscisic acid and biotic stress hormones in cultured cells. Plant Biotechnol *29*: 19-34.

OMOTE, H., M. HIASA, T. MATSUMOTO, M. OTSUKA, Y. MORIYAMA (2006): The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. Trends Pharmacol Sci *27*: 587-593.

PARK, H.J., W.Y. JUNG, S.S. LEE, J.H. SONG, S.Y. KWON, H. KIM, H.S. CHO (2013): Use of heat stress responsive gene expression levels for early selection of heat tolerant cabbage (*Brassica oleracea* L.). Int J Mol Sci *14*: 11871-11894.

PARKER, M.D., W.F. BORON (2013): The divergence, actions, roles, and relatives of sodium-coupled bicarbonate transporters. Physiol Rev *93*: 803-959.

ROGERS, E.E., M.L. GUERINOT (2002): FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in Arabidopsis. Plant Cell *14*: 1787-1799.

REYES, J.L., F. CAMPOS, H. WEI, R. ARORA, Y.I. YANG, D.T. KARLSON, A.A. COVARRUBIAS (2008): Functional dissection of hydrophilins during in vitro freeze protection. Plant Cell Environ *31*: 1781-1790.

SHIGETA, T., Y. ZAIZEN, Y. SUGIMOTO, Y. NAKAMURA, T. MATSUO, S. OKAMOTO (2015): Heat shock protein 90 acts in brassinosteroid signaling through interaction with BES1/BZR1 transcription factor. J Plant Physiol *178*:69-73.

SINGLA, S.L., A. PAREEK, A. GROVER (1997): Yeast HSP104 homologue rice HSP110 is developmentally and stress-regulated. Plant Sci *125*: 211-219.

SMEETS, K., J. RUYTINX, F. VAN BELLEGHEM, B. SEMANE, D. LIN, J. VANGRONSVELD, A. CUYPERS (2008): Critical evaluation and statistical validation of a hydroponic culture system for *Arabidopsis thaliana*. Plant Physiol Biochem *46*: 212-218.

STANGOULIS, J.C.R., R.J. REID (2002): Boron toxicity in plants and animals. In: Goldbach HE, Rerkasem B, Wimmer MA, Brown PH, Bell R, editors. Boron in plant and animal nutrition. Kluwer Academic, New York, pp. 227-241.

SURGUN, Y., B. ÇÖL, B. BÜRÜN (2016): 24-Epibrassinolide ameliorates the effects of boron toxicity on *Arabidopsis thaliana* (L.) Heynh by activating an antioxidant system and decreasing boron accumulation. Acta Physiologiae Plantarum *38* (3): 71.

TAKANO, J., M. YAMAGMI, K. NOGUCHI, H. HAYASHI, T. FUJIWARA (2001): Preferential translocation of boron to young leaves in *Arabidopsis thaliana* regulated by the BOR1 gene. Soil Sci Plant Nutr *47*: 345-347.

TAKANO, J., K. NOGUCHI, M. YASUMORI, M. KOBAYASHI, Z. GAJDOS, K. MIWA, H. HAYASHI, T. YONEYAMA, T. FUJIWARA (2002): Arabidopsis boron transporter for xylem loading. Nature *420*: 337-340.

TAKANO, J., K. MIWA, L. YUAN, N. VON WIREN, T. FUJIWARA (2005): Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. PNAS *102*: 12276-12281.

TAKANO, J., K. MIWA, T. FUJIWARA (2008): Boron transport mechanisms: collaboration of channels and transporters. Trends Plant Sci *13*: 451-457.

TOMBULOGLU, H., N. SEMIZOGLU, S. SAKCALI, G. KEKEC (2012): Boron induced expression of some stress-related genes in tomato. Chemosphere *86*: 433-438.

UNTERGRASSER, A., I. CUTCUTACHE, T. KORESSAAR, J. YE, B.C. FAIRCLOTH, M. REMM, S.G. ROZEN (2012): Primer3 - new capabilities and interfaces. Nucleic Acids Res *40*(15): e115

WANG, Z.Y., M.Y. BAI, E. OH, J.Y. ZHU (2012): Brassinosteroid signaling network and regulation of photomorphogenesis. Annu Rev Genet *46*: 701-724.

WARRINGTON, K (1923): The effect of BA and borax on the broad bean and certain other plants. Ann Bot *37*: 629-672.

VRIET, C., E. RUSSINOVA, C. REUZEAUA (2012): Boosting crop yields with plant steroids. Plant Cell *24*: 842-857.

YUSUF, M., Q. FARIDUDDIN, A. AHMAD (2011): 28-Homobrassinolide mitigates boron induced toxicity through enhanced antioxidant system in *Vigna radiata* plants. Chemosphere *85*(10): 1574-1584.

YUXIN, H., W. ZHENGKE, W. YONGHONG, B. FANG, L. NING, P. ZHENHUA, L. JIAYANG (2001): Identification of brassinosteroid responsive genes in Arabidopsis by cDNA array. Sci China Ser C: Life Sci 44: 637-643.

DIFERENCIJALNA ANALIZA EKSPRESIJE BORON TRANSPORTERA I NEKI GENI VEZANI ZA ODGOVOR NA STRES IZAZVAN 24-EPIBRASINOLIDOM I BOROM KORIŠĆENJEM SEMI-KVANTATIVNOG RT-PCR KOD *Arabidopsis thaliana* (L.) Heynh

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Izvod

Biljni steroidni hormoni, brasikosteroidi (BRs), pokreću proces razvoja biljaka i održavaju tolerantnost na neke abiotičke stresove, uljučujići stress od visoke koncetracijebora (B). U cilju ispitivanja moguće uloge bora u indukovanju stresa na nivou transkripcije vršena su ispittivanja odgovora B transporter gena (*BOR1-4*), visoko B-indukovani geni (*MATE*, *Hsp-like*), BR-indukovani geni (*Hsp70-4*, *Hsp90-1*) i drugi geni vezani za stres (*LTI/COR78*, *LEA4-5*) kroz egzogeni tretman sa 24-epibrassinolide (EBL) na (L.) Heynh, izloženoj visokim koncentracijama borne kiseline (BA) primenom semi-kvantitativnog RT-PCR. Tretmaan bornom kiselinom je doveo do smanjenja regulacije *BOR1* i *BOR3* gena u tkivima lista i korena a viša koncentracija je dalje smanjivala ekspresiju oviih gena u korenu. Ekspresija gena indukovana visokim sadržajem bora (Borna kiselina) je zapažena kao apregulisana bri 1 μM EBL tretmanom visokim B stresom u oba tkiva klijanaca. Apregulacija gena indukovana borom je jasno evidentirana u tkivu krena, kotretiranog sa 1 μM EBL i bornom kiselinom u poređenju samo sa bornom kiselinomas. Više koncentracije EBL je bila efektivnija u povećanju ekspresije *LTI/COR78* gena u korenu i *LEA4-5* gena u nadzemnom delu tkivu biljke. Prema literaturnim podacima ovo su prvi rezultati kako egzogena primena EBL modulira visok odgovor na stres borom na molekularnom nivou kod *Arabidopsis thaliana*.

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