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Salt Stress and Homobrassinosteroid Interactions during Germination in Barley Roots

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Abstract

Potential alleviation effects of Homobrassinosteroid (HBR) (0.5 and 1 μM HBR) on root germination, cell division and antioxidant system enzymes (superoxide dismutase and catalase) of barley (*Hordeum vulgare* L. cv. 'Hilal') roots grown under different salt concentrations (150 mM and 250 mM) were investigated during 48 and 72 h at dark with their controls. Salt applications decreased primary root lengths, seminal root lengths, number of roots from one seed, mitotic activity and induced mitotic abnormalities. In addition, salt application decreased protein content but increased enzyme activities both at 48 h and 72 h when compared to control. Roots treated with HBR enhanced root lengths and root number. HBR-treated roots showed more mitotic activity, mitotic abnormalities and significant enlargements at the root tips when compared to controls and only salt-treated samples. Salt + HBR applications stimulated root lengths and showed more mitotic activity and mitotic abnormalities when compared to only salt-treated samples. Salt application increased superoxide dismutase and catalase activities both at 48 h and 72 h. However, HBR application decreased total soluble protein content and increased enzyme activities especially at 48 h compared to control. Depending on concentration and timing, salt + HBR treatments showed varying results in total soluble protein content and enzyme activities. There was also a significant change in protein banding patterns of experimental groups separated by SDS-PAGE. These findings are expected to contribute to understanding of how HBR affect barley roots grown under different salt concentrations.

Keywords: abiotic stress, antioxidant enzyme activities, development of plant, mitotic index, plant hormone

Introduction

Salinity affects plant growth, activity of major cytosolic enzymes by disturbing intracellular potassium homeostasis, causing oxidative stress and programmed cell death, reduced nutrient uptake, metabolic toxicity, inhibition of photosynthesis, reduced CO_2 assimilation and reduced root respiration (Sairam and Srivastava, 2002; Cuin and Shabala, 2007; Chen *et al.*, 2007; Shabala, 2009; Abogadallah, 2010; Demirkiran *et al.*, 2013; Liu *et al.*, 2014).

Reactive Oxygen Species (ROS) are highly toxic and can greatly disrupt normal metabolism through membrane lipid peroxidation, DNA/RNA nicking, protein oxidation and enzyme inhibition (Tanou *et al.*, 2009). Acclimation to salinity may be achieved via enhancing the antioxidant defence system (Saha *et al.*, 2010). Plants have evolved a series of defence mechanisms to maintain the balance between the production of activated oxygen species and scavenging capacity of antioxidants. Plants possess a number of antioxidant enzymes like glutathione reductase (GR), peroxidase (POX), superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), ascorbate peroxidase and glutathione reductase for protection against the damaging effects of ROS (Bhaduri and Fulekar, 2012; Fariduddin *et al.*, 2013).

Brassinosteroids (BRs) are a special class of plant polyhydroxysteroids that play essential roles during normal plant growth and development. They bind to a small family of leucine-rich repeat receptor kinases (BRI1) at the cell surface, thus initiating an intracellular signal transduction cascade that results in the altered expression of hundreds of genes which are implicated for diverse functions including increased adaptation to various abiotic stresses such as those induced by salts, drought, low and high temperature and heavy metals (Abdullahi *et al.*, 2002; Sharma and Bhardwaj, 2007; Bajguz and Hayat, 2009; Farooq *et al.*, 2009). Some researchers have suggested that brassinosteroids promote root elongation and mitosis in a wide range in low concentrations but not high concentrations (Hu *et al.*, 2000; Howell *et al.*, 2007; Kartal *et al.*, 2009).

Hordeum vulgare L. (barley) is an important cereal crop for both malting industry and animal husbandry. It is also an excellent model organism for research in biochemistry, physiology, genetics, molecular biology and genomics. The objective of the present study was to investigate the effects

of exogenously applied 28-Homobrassinolide (HBR) on the alleviation of salt stresses on root germination, cell division and antioxidant system in barley seeds. For those purposes, barley seeds were grown under control (only water), only salt (150 mM and 250 mM), only HBR (0.5 μM and 1 μM) and salt + HBR conditions for 48 h and 72 h at dark.

Materials and methods

Plant material

Seeds of *Hordeum vulgare* L. cv. 'Hilal' were used as plant material. Four different experiment groups were prepared at 48 h and 72 h. The first group was control group. In the second group, sodium chloride (NaCl) was used for generating salt stress (150 mM and 250 mM) on barley seeds. Only HBR (0.5 μM and 1 μM) was applied in the third group. In the last group, HBR (0.5 μM and 1 μM) was applied on barley seeds supplemented with 150 mM and 250 mM salt concentrations. Seeds were placed randomly in Petri dishes (9 cm diameter) containing filter paper soaked in (a) only H_2O (control), (b) 150 mM NaCl, (c) 250 mM NaCl, (d) 0.5 μL HBR, (e) 1 μL HBR, (f) 150 mM NaCl + 0.5 μM HBR, (g) 150 mM NaCl + 1 μM HBR, (h) 250 mM NaCl + 0.5 μM HBR and (i) 250 mM NaCl + 1 μM HBR-supplemented distilled water. All cultures were kept at dark in a controlled growth chamber (26 °C). Each concentration with three separate experiments was evaluated. Totally 135 barley seeds for 48 h and 135 barley seeds for 72 h were used (5 seeds in each petri dish, three replicates for 9 different applications). 1 mg of HBR (H1267, Sigma) was dissolved in ethanol to yield a 10 mM stock solution which used for the preparation of experiments.

Growth measurements

After 48 h and 72 h, primary and seminal root lengths were measured. Root numbers from each seed were recorded. In addition, germination percent for each application was calculated.

Cytological analyses

The roots of controls, only salt treated, only HBR treated and salt + HBR treated seedlings were fixed in 1:3 acetoalcohol. The fixed root tips were hydrolysed in 1 M HCl at 60 °C for 20 min, stained with Feulgen for 1-1.5 h, smashed in a drop of 45% acetic acid and squashed (Sharma and Gupta, 1982). In squash preparations, mitosis was investigated and mitotic index (%) was calculated. About 1.000 cells were counted for each serial of treatments and control materials.

Determination of protein content and antioxidant enzyme activities

Controls and treated roots were first homogenized in a sterile mortar and pestle with liquid nitrogen, subsequently 1 ml extraction buffer (50 mM PBS [0.2 M monobasic sodium phosphate, 0.2 mM dibasic sodium phosphate pH 7.0], 0.1 mM EDTA, 4% polyvinylpyrrolidone) was used per 0.01 mg plant material. The homogenate was centrifuged at 10.500 g for 20 min at 4 °C (Pan *et al.*, 2006). Supernatant was used for protein content and enzyme activity assays. Total soluble protein contents of the enzyme extracts were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard. Characterization of protein profiles was carried out using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Supernatant for each concentration were denatured by heating at 100 °C for 5 minutes and loaded in 15% acrylamide slab gel containing 10% SDS. Run was performed at 16 mA for 30 minutes followed by 32 mA till the tracing bromophenol blue dye reached the gel bottom. Protein bands were visualized by staining the gels with 0.1% Coomassie Brilliant Blue R-250.

SOD activity was determined by measuring the ability of enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), as described by Cakmak and Marschner (1992), which measures inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. One unit of activity was determined as amount of enzyme required to inhibit the photoreduction of NBT to blue formazan by 50% and was expressed as SOD unit's mg protein^{-1}. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM Na-EDTA, 50 mM Na_2CO_3, 13 mM L-methionine, 75 μM p-nitrobluetetrazolium chloride (NBT) and 2 μM riboflavin. Reactions were carried out at 25°C, under light intensity of about 300 μmol^{-1} m^{-1} s^{-1} through 10 min.

Catalase activity was determined according to Cho *et al.* (2000), who measured the decline of the extinction of H_2O_2 at the maximum absorption at 240 nm. About 5 μl of samples were added to the reaction mixture containing 130.5 μl H_2O, 20 μl phosphate buffer (50 mM, pH 7.0) and 44.5 μl hydrogen peroxide (35%). Spectrophotometric readings were obtained after addition of enzyme extract for 120 seconds of 10 second intervals and results were presented as ΔA_{240}/min/mg protein.

Statistical analysis

All parameters were evaluated statistically by ANOVA with SPSS 16.0 program for studying the interaction of controls, only salt treated, only HBR treated and salt + HBR treated samples. Root lengths, number of roots from one seed and enzyme analyses were expressed as mean ± SD of three independent replicates. For the statistical evaluation of the results, significance was accepted at the probability level $P < 0.05$ for root lengths (Tabs. 2-3), $P > 0.1$ and $P < 0.1$ for enzyme analyses (Tab. 5).

Results and discussions

Barley seeds grown under control (only H_2O), salt stress (150 mM and 250 mM), HBR application (0.5 μM and 1 μM) and salt + HBR application showed different root lengths at 48 h and 72 h (Fig. 1 and 2, respectively). 0.5 and 1 μM HBR

Fig. 1. Morphological view of *Hordeum vulgare* L. cv. 'Hilal' seeds germinated between filter papers at dark for 48 h. Variants: 1 = control; 2 = 150 mM NaCl; 3 = 250 mM NaCl; 4 = 0.5 μM HBR; 5 = 1 μM HBR; 6 = 150 mM NaCl + 0.5 μM HBR; 7 = 150 mM NaCl + 1 μM HBR; 8 = 250 mM NaCl + 0.5 μM HBR; 9 = 250 mM NaCl + 1 μM HBR, respectively

application alleviated the negative effects of salt on barley roots (6, 7, 8, 9 in Figs. 1-2).

Germination percentage of barley seeds were recorded.

Fig. 2. Morphological view of *Hordeum vulgare* L. cv. 'Hilal' seeds germinated between filter papers at dark for 72 h. Variants: 1 = control; 2 = 150 mM NaCl; 3 = 250 mM NaCl; 4 = 0.5 μM HBR; 5 = 1 μM HBR; 6 = 150 mM NaCl + 0.5 μM HBR; 7 = 150 mM NaCl + 1 μM HBR; 8 = 250 mM NaCl + 0.5 μM HBR; 9 = 250 mM NaCl + 1 μM HBR, respectively

Germination percentage of barley seeds which were treated with salt, HBR and salt + HBR showed different results at 48 h and 72 h. Salt application decreased germination while HBR increased. High HBR concentration showed better results to alleviate salt stress on barley seeds (Tab. 1).

Increased salt concentration caused negative effect on primary root and seminal root lengths. As compared to control (1.8 ± 0.6 and 2.4 ± 0.4, 48 h and 72 h, respectively), the lengths of primary root decreased in 150 mM salt concentration (1 ± 0.4 and 1.5 ± 0.6, 48 h and 72 h, respectively) and 250 mM (0.5 ± 0.2 and 0.9 ± 0.2, 48 h and 72 h, respectively) salt concentration (Tabs. 2-3). The same result could also be seen on seminal root lengths and number of roots from one seed. On the other hand, only HBR applications (0.5 μM and 1 μM) increased all 3 parameters on barley seeds. HBR was applied on same petri dishes which were supplemented with different salt concentrations to alleviate the negative effect of salt stress on barley seeds. As a result, HBR application increased primary root lengths in 150 mM and 250 mM salt concentration especially at 48 h.

The hormone increased seminal root lengths in 150 mM salt but there was no significant change on seminal root lengths in 250 mM salt concentration at 48 h. In addition, number of emerging roots per seed in 150 mM NaCl treatment was increased with 0.5 μM and 1 μM HBR application (Tab. 2). At 72 h, primary root lengths were increased in both 150 mM and 250 mM salt concentrations. There was no significant change on seminal root lengths in 150 and 250 mM salt concentrations. On the other hand, root number was increased in both concentrations (Tab. 3).

Tab. 1. Effect of salt, HBR and salt + HBR applications on germination of barley seeds (%)

Hordeum vulgare L. cv. 'Hilal' (Treatment variants)	Germination (%)	
	48 h	72 h
Control	100	93
150 mM salt concentration	87	93
250 mM salt concentration	67	67
0.5 μM HBR	93	93
1 μM HBR	87	93
150 mM salt + 0.5 μM HBR	100	93
150 mM salt + 1 μM HBR	93	100
250 mM salt + 0.5 μM HBR	80	80
250 mM salt + 1 μM HBR	73	93

Salt application decreased primary root lengths (44.4% and 72%, 150 mM salt and 250 mM salt respectively at 48 h; 37.5% and 62.5% 150 mM salt and 250 mM salt respectively at 72 h), seminal root lengths (50% and 75%, 150 mM salt and 250 mM salt respectively at 48 h; 31.3% and 62.5% 150 mM salt and 250 mM salt respectively at 72 h) and number of roots from one seed (25% and 50%, 150 mM salt and 250 mM salt respectively at 48 h; 16% and 32% 150 mM salt and 250 mM salt respectively at 72 h) as compared to control. HBR (0.5-1 μM HBR) applications enhanced primary root lengths (22% and 38.9%, 0.5 μM HBR and 1 μM HBR respectively at 48 h; 12.5% and 8.3% 0.5 μM HBR and 1 μM HBR respectively at 72 h), seminal root lengths (17% and 33%, 0.5 μM HBR and 1 μM HBR respectively at 48 h; 6.25% in 0.5 μM HBR at 72 h) and number of roots from one seed (7% and 1.8%, 0.5 μM HBR and 1 μM HBR, respectively at 48 h; 1.6% and 6%, 0.5 μM HBR and 1 μM HBR respectively at 72 h) as compared to control at 48 h and 72 h.

Salt + HBR applications altered primary root lengths, seminal root lengths and number of roots from one seed as compared to only salt applications at 48 h and 72 h. Salt decreased but salt + HBR application enhanced primary root lengths at 48 and 72 h (20% for 150 mM + 0.5 μM and 40% for 150 mM + 1 μM; 40% for 250 mM + 0.5 μM and 20% for 250 mM + 1 μM at 48h). It could be said that high HBR concentration had positive effect on low salt stress. This differences altered at 72 h (13.3% for 150 mM + 0.5 μM and 6.7% for 150 mM + 1 μM; 11% for 250 mM + 0.5 μM and 11% for 250 mM + 1 μM). The same result was observed in seminal root lengths and root numbers at 48 h and 72 h.

Cytological analyses

Mitotic index in controls, only salt, only HBR and salt + HBR-treated barley seeds were shown in Tab. 4. Control barley roots had 20.4% and 23.6% (48 h and 72 h, respectively) of the cells in various stages of mitosis. Salt stress reduced mitotic index (8.3% and 5.6% in 150 mM salt treated and 5.5% and 3.6% in 250 mM salt treated samples for 48 h and 72 h). Moreover, mitotic index of only HBR application was higher than both control and salt treated samples. Increasing concentration of HBR alleviated salt stress effectively but their effect could be seen after long time (1 μM application showed more mitotic index for 150 mM salt and 250 mM salt application both at 48 h and 72 h). Mitotic abnormalities such as vacuole in cells, disorganized prophase, interphase and prophase in the same cells, disorganized metaphase, anaphase bridge, disorganized anaphase could be seen in only salt treated, only HBR treated and salt + HBR treated samples.

Determination of protein content and antioxidant enzyme activities

Total proteins were extracted from experimental groups after 48 h and 72 h treatment and analysed by SDS-PAGE. As visualized from SDS-PAGE, the intensity of several protein bands of molecular weight 20 kDa decreased as a result of salt, HBR and salt + HBR treatment at 48 h. HBR and salt also cause a significant change in the electrophoretic profiles of experimental groups separated by SDS-PAGE during 48 h (Fig. 3).

Protein content of all samples was increased at 72 h. High HBR application showed positive effect on low salt stress (5.7 ± 1.94, 6.48 ± 1.74, 48 h and 72 h, respectively). Protein content of 250 mM salt + 0.5 µM HBR in 72 h (6.71 ± 2) was higher than protein content of 250 mM salt + 1 µM HBR in 48 h (5.23 ± 3). This result showed that HBR application stimulated reverse effect on high salt stress during long period of time (Tab. 5).

Only salt application and only HBR application enhanced enzyme activities as compared to control. Activity of SOD in high salt treatment was reduced by high HBR concentration (0.2 ± 0.2 for 48 h, 0.29 ± 0.35 for 72 h). High HBR application decreased catalase activity in 150 mM salt + 1 µM HBR in 72 h (0.02 ± 0.01). Activities of SOD and catalase enzymes were dependent on the concentration of salt and HBR (Tab. 5).

Tab. 2. Lengths and roots number of *Hordeum vulgare* L. cv. 'Hilal' after 48 h germination between filter papers at dark

Trait	Control	150 mM salt	250 mM salt	0.5 µM HBR	1 µM HBR	150 mM salt+0.5µ M HBR	150 mM salt+1 µM HBR	250 mM salt+0.5µ M HBR	250 mM salt+1 µM HBR
Average length of primary roots (cm)	1.8 ± 0.6	1 ± 0.4	0.5 ± 0.2	2.2 ± 0.2	2.5 ± 0.4	1.2 ± 0.4	1.4 ± 0.3	0.7 ± 0.3	0.6 ± 0.3
Average length of seminal roots (cm)	1.2 ± 0.5	0.6 ± 0.3	0.3 ± 0.2	1,4 ± 0.6	1.6 ± 0.6	0.7 ± 0.4	0,8 ± 0.3	0,3 ± 0.2	0,3 ± 0.2
Number of roots from one seed	5.6 ± 0.6	4.2 ± 0.4	2.8 ± 1,5	6 ± 0.7	5.7 ± 0.7	4.4 ± 1.1	5 ± 0.7	2.7 ± 1.2	2.5 ± 1.4

Values are mean ± SD based on three replicates, P < 0.05

Tab. 3. Lengths and roots number of *Hordeum vulgare* L. cv. 'Hilal' after 72 h germination between filter papers at dark

Trait	Control	150 mM salt	250 mM salt	0.5 µM HBR	1 µM HBR	150 mM salt+0.5µ M HBR	150 mM salt+1 µM HBR	250 mM salt+0.5µ M HBR	250 mM salt+1 µM HBR
Average length of primary roots (cm)	2.4 ± 0.4	1.5 ± 0.6	0.9 ± 0.2	2.7 ± 0.5	2.6 ± 0.4	1.7 ± 0.4	1.6 ± 0.3	1 ± 0.2	1 ± 0.2
Average length of seminal roots (cm)	1.6 ± 0.6	1.1 ± 0.5	0.6 ± 0.2	1.7 ± 0.7	1.6 ± 0.6	1 ± 0.4	1 ± 0.4	0.5 ± 0.2	0,6 ± 0.2
Number of roots from one seed	6.3 ± 0.5	5.3 ± 1.6	4.3 ± 1.2	6.4 ± 0.8	6.7 ± 0.7	5.5 ± 1	5.4 ± 0.7	4.6 ± 1	4.5 ± 0.8

Values are mean ± SD based on three replicates, P < 0.05

Tab. 4. Mitotic index (1.000 cells counted) in *Hordeum vulgare* L. cv. 'Hilal' after 48 h and 72 h of germination

Mitotic index (cells/time)	Control	150 mM salt	250 mM salt	0.5 µM HBR	1 µM HBR	150 mM salt+0.5µ M HBR	150 mM salt+1µM HBR	250 mM salt+0.5µ M HBR	250 mM salt+1µM HBR
Mitotic index (%) (48 h)	20.4	8.3	5.5	25.9	20.1	13.8	18.6	16.8	20.1
Mitotic index (%) (72 h)	23.6	5.6	3.6	23	24.1	14.3	21.9	10.4	22.2

Tab. 5. Protein contents and antioxidant enzyme activities of *Hordeum vulgare* L. cv. 'Hilal' roots after 48 hours and 72 hours of germination between filter papers at dark in salt, HBR and salt + HBR application

Content/ Activity	Control	150 mM salt	250 mM salt	0.5 µM HBR	1 µM HBR	150 mM salt+0.5µM HBR	150 mM salt+1µM HBR	250 mM salt+0.5µM HBR	250 mM salt+1 µM HBR
*Protein content mg/ml (48 h)	5.9 ± 2.75	5.02 ± 2.32	4.4 ± 2.86	4.22 ± 2.3	3.55 ± 0.7	5.4 ± 2.5	5.7 ± 1.94	4.6 ± 3	5.23 ± 3
*Protein content mg/ml (72 h)	6.1 ± 1.77	5.65 ± 1.33	6.1 ± 1.78	4.86 ± 2.27	5.5 ± 1.15	5.5 ± 1.54	6.48 ± 1.74	6.71 ± 2	5.6 ± 2.29
*Superoxide dismutase activity ΔA_{560}/min/mg protein (48 h)	0.11 ± 0.09	0.21 ± 0.22	0.32 ± 0.41	0.23 ± 0.3	0.17 ± 0.17	0.18 ± 0.18	0.3 ± 0.3	0.3 ± 0.3	0.2 ± 0.2
**Superoxide dismutase activity ΔA_{560}/min/mg protein (72 h)	0.27 ± 0.38	0.33 ± 0.43	0.34 ± 0.36	0.3 ± 0.3	0.95 ± 1.43	0.42 ± 0.32	0.35 ± 0.38	0.33 ± 0.35	0.29 ± 0.35
**Catalase activity ΔA_{240}/min/mg protein (48 h)	0.06 ± 0.07	0.07 ± 0.06	0.1 ± 0.1	0.07 ± 0.02	0.13 ± 0.14	0.18 ± 0.3	0.08 ± 0.03	0.56 ± 0.87	0.06 ± 0.04
*Catalase activity ΔA_{240}/min/mg protein (72 h)	0.04 ± 0.02	0.05 ± 0.04	0.04 ± 0.03	0.04 ± 0.03	0.03 ± 0.03	0.04 ± 0.01	0.02 ± 0.01	0.05 ± 0.04	0.13 ± 0.04

Values are mean ± SD based on three replicates, *P > 0.1, ** P < 0.1.

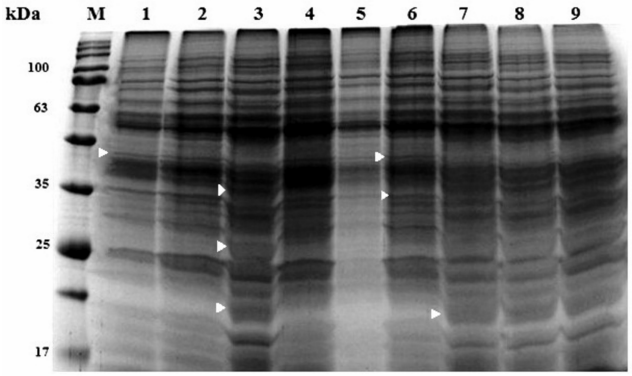

Fig. 3. Protein banding patterns of barley (*Hordeum vulgare* L. cv. 'Hilal') seeds separated by SDS-PAGE at 48 h. Variants: M = marker; 1 = control; 2 = 150 mM NaCl; 3 = 250 mM NaCl; 4 = 0.5 μM HBR; 5 = 1 μM HBR; 6 = 150 mM NaCl + 0.5 μM HBR; 7 = 150 mM NaCl + 1 μM HBR; 8 = 250 mM NaCl + 0.5 μM HBR; 9 = 250 mM NaCl + 1 μM HBR, respectively (arrows indicate polymorphic bands)

Discussion

The present study indicates the alleviation effects of HBR (0.5 μM and 1 μM) on root germination, cell division, protein content and antioxidant system enzymes (superoxide dismutase and catalase) of barley (*Hordeum vulgare* L. cv. 'Hilal') grown under different salt concentrations (150 mM and 250 mM) in 48 and 72 h. Increasing salt stress reduced root lengths, number of roots from one seed, germination percentage, mitotic activity and protein content, causing chromosomal aberrations and increased enzymatic activity when compared to controls. Only HBR application depending on the concentration and timing stimulated root lengths, number of roots from one seed and mitotic activity, decreased germination percentage, protein content and increased enzymatic activities compared to controls. When all parameters were examined, we observed HBR application on barley seeds under the salt stress alleviated the salt stress on barley seeds.

It was observed that salt stress reduced root elongation on barley and HBR application alleviated salt stress. These results are concordant with Dash and Panda (2001) who studied on *Phaseolus mungo* seeds. They concluded that a decrease in germination percentage, root length, shoot length, and fresh mass were observed with an increase in NaCl concentration (1%, 2% and 3%). Demirkiran *et al.* (2013) observed 50 mM and 100 mM salt concentration inhibited shoot growth, decreased fresh weight and protein content and increased SOD activity in a dose-dependent manner. In another study, rice seeds soaked in water or 150 mM NaCl in the presence or absence of BR were tested for germination and seed growth. When the salt solution was supplemented with BR, the inhibitory effect of salt on germination was reduced considerably. The promotion of growth by BR under salt stress conditions was associated with enhanced levels of nucleic acids and soluble proteins (Anuradha and Rao, 2001). However, some studies provided conflicting results. Ozdemir *et al.* (2004) studied with 24-epibrassinolide effects on salt-sensitive rice cultivar IR-28 grown under salt stress. They reported that 24-epiBL caused inhibition of root elongation and curvature of rice seedlings under both control and salt stress conditions. This result was also previously reported for *Arabidopsis* (Clouse *et al.*, 1993) and tomato (Dhaubhadel *et al.*, 1999). Effects of BR could depend on concentration, timing and plant parts (Houimli *et al.*, 2010). Liu *et al.* (2014) studied the effects of 24-epibrassinolide (10^{-6}, 10^{-8}, 10^{-10}, 10^{-12} M) on salt-stressed canola. They found growth promoting effects of 24-epiBL on salt-stressed shoots and roots but 10^{-10} M 24-epiBL inhibited root growth under control conditions. There are many reports showing that BRs could ameliorate plant growth under saline conditions in wheat, maize and pea (Shahbaz *et al.*, 2008; El-Khallal *et al.*, 2009; Shahid *et al.*, 2011).

Results indicated that mitotic index decreased with increasing salt concentration. However, it increased with only HBR application as compared to control. Moreover, salt + HBR application also enhanced mitotic index when compared to only salt-treated samples. Hormone application depended on mostly concentration. Howell *et al.* (2007) reported the first detailed report about the effects of 24-epibrassinolide (BL) on chromosomes and the cell cycle. They investigated the effects on the mitotic index and the growth of onion (*Allium cepa*) root tips. They concluded that different doses of BL show dissimilar results. Increasing doses of BL (0.005 ppm, 0.05 ppm and 0.5 ppm) demonstrated opposite effect on root tips as compared to controls. Low doses of BL (0.005 ppm) nearly doubled the mean root length and the number of mitosis over that of controls but differences were not statistically significant. Chromosomal aberrations were also seen in all samples except controls.

These results are supported by Tabur and Demir (2009). They studied on barley (*Hordeum vulgare* L. cv. 'Bulbul 89') and reported that plants grown on media containing 0.30, 0.35 and 0.40 M NaCl showed a significant decrease in mitotic index and higher number of chromosomal abnormalities as compared to control. On the contrary of our results, they observed the mitotic index decreased 50% approximately in 24-epibrassinolide treated samples. In another study, Tabur and Demir (2010) used 0.30, 0.35, 0.40 and 0.45 molal salt concentrations and observed that the inhibitory effect of salt stress on mitotic index of barley (*Hordeum vulgare* L. cv. 'Bulbul 89') and chromosomal aberrations enhanced with increasing salt concentration. There are also different studies about BR effects on mitosis (Miyazawa *et al.*, 2003; Aydin *et al.*, 2006; Sondhi *et al.*, 2008; Kartal *et al.*, 2009).

We also observed that only HBR decreased protein content as compared to control but salt + HBR application enhanced protein content in only salt treated samples. Bajguz (2000) reported different results. In their study, protein levels of *Chlorella vulgaris* increased, after BR application. Ozdemir *et al.* (2004) also concluded that exogenous application of 24-epiBL significantly increased soluble protein content of rice seedlings in both 24-epiBL and 24-epiBL + NaCl treated groups compared with the control group. In addition, Kartal *et al.* (2009) concluded that HBR stimulated total soluble protein content in treated materials with 0.1 μM and 0.5 μM HBR compared to control. Differences in results might have

originated from experimental materials, duration and doses of HBR hormone. Protein banding patterns of our experimental groups were also different. Parida *et al.* (2004) studied two-month-old seedlings of *Bruguiera parvifora* treated with varying levels of NaCl (100, 200 and 400 mM). As a result of SDS-PAGE analysis, they concluded that the intensity of several protein bands of molecular decreased as a result of NaCl treatment.

Enzyme activity of only salt-treated and only HBR-treated samples was enhanced compared with controls. On the contrary, salt + HBR treated samples indicated different results depending on concentrations and duration. Supporting our data, Agami (2013) studied maize and concluded that activities of antioxidant enzymes (peroxidase and catalase) in maize plants were decreased under salt stress while increased after salicylic acid (SA) or 24-epibrassinolide (EBL) application.

In another study, Talaat and Shawky (2013) stated that two wheat (*Triticum aestivum* L.) cultivars, Sids 1 and Giza 168, were grown under non-saline or saline conditions (4.7 and 9.4 dS m^{-1}) and were sprayed with 0.00, 0.05 and 0.10 mg l^{-1} 24-epibrassinolide (EBL). Antioxidative enzyme activities (POX, CAT, SOD and GR) and antioxidative molecule contents (GSH and AsA) were considerably elevated by salt stress and/or EBL treatments. In literature, there are many studies which investigate the effects of BRs mainly focusing on plant growth and antioxidant systems (Anuradha and Rao, 2001; Nunez *et al.*, 2003; Ozdemir *et al.*, 2004; Ali *et al.*, 2008; Arora *et al.*, 2008; Rady, 2011; Choudhary *et al.*, 2012). Different results from different studies could be arised from type of plants or HBR and salt concentrations and application types.

Conclusion

Data presented here is one of the first detailed analyses of alleviation effects of HBR on salt stressed barley root germination. Related to concentrations and duration, HBR improved growth parameters, mitotic index, chromosomal abnormalities and increased or decreased the level of antioxidant enzymes (superoxide dismutase and catalase) under stress and stress-free conditions. Salt stress effect barley growth and development. Our results are expected to contribute to find out alleviation of salt stress. Gene expression studies will provide inputs toward understanding how exogenous application of HBR alleviates saline stress responses at molecular level in barley.

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