TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2015) 39: 540-549

doi:10.3906/biy-1501-28

Antioxidant activity of heme oxygenase 1 in *Brassica juncea* (L.) Czern. (Indian mustard) under salt stress

Khushbu VERMA[1], Shubham DIXIT[1], Gyan Singh SHEKHAWAT[2], Afroz ALAM[1,*]
[1]Department of Bioscience and Biotechnology, Banasthali University, Banasthali, Rajasthan, India
[2]Department of Botany, JNV University, Jodhpur, Rajasthan, India

Received: 11.01.2015 • **Accepted/Published Online:** 23.03.2015 • **Printed:** 30.07.2015

Abstract: Antioxidant enzymes play a protective role in plants under oxidative stress. The present study explores the role of antioxidant system heme oxygenase1 (HO1) in *Brassica juncea* (L.) Czern. in salt stress conditions. *B. juncea* seedlings were treated with different NaCl concentrations (0–200 mM) for 5 days. Salt stress elicited the highest response at 150 mM NaCl concentration; at this level, gene expression of HO1 was also upregulated. Apart from HO1, ascorbate peroxidase (APX), peroxidase (POD), and glutathione reductase (GR) also showed their highest activity at 150 mM NaCl concentration. The study shows that HO1 is indispensable in *B. juncea* for overcoming the oxidative stress caused by salinity. Understanding the intricacy of the defense network, including HO1, against salt stress will be useful for future research into developing salt-tolerant varieties.

Key words: Abiotic stress, antioxidant, *Brassica juncea*, heme oxygenase, salt stress

1. Introduction

Abiotic stress is a global agricultural issue that limits plant growth and yield. Amongst abiotic stresses, salt stress leads to significant crop losses worldwide (Greenway and Munns, 1980). Although natural salts are present in soil, their increased concentration affects plant growth adversely. Rao et al. (2008) reported that salinity affects about 932 million hectares of land globally. In plant cells under salt stress, Na^+ and Cl^- ions accumulate in cytoplasm and lower the external water potential, resulting in turgor loss (Binzel et al., 1988). It creates an imbalance of cellular ions that results in ion toxicity, or osmotic stress, followed by significant oxidative stress (Gosset et al., 1996; Goemez et al., 1999; Hasegawa et al., 2000). Together, these factors lead to decreased plant growth, development, and survival. Elevated amounts of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl ($OH^{\cdot}$) radicals can critically disturb cellular homeostasis and standard metabolism through oxidative damage to protein, lipids, and nucleic acid (Baxter et al., 2007).

Plants elicit a molecular response to prevent oxidative damage due to ROS production and adjust to the oxidative stress (Baxter et al., 2007; Demirkaya, 2014; Yaycılı and Alikamanoğlu, 2012). They have developed a multifaceted antioxidant defense with enzymatic molecules, including ascorbate peroxidase (APX, EC 1.11.1.11), glutathione reductase (GR, E.C. 1.6.4.2), and peroxidase (POD, EC 1.11.1.7). All these molecules play an important role in scavenging ROS formed during oxidative damage (Erdal and Çakırlar, 2014; Petrić et al., 2014).

Recently, the role of heme oxygenase (HO, EC 1.14.99.3) has been recognized among plant defenses as a catalyst for the oxidation of heme to biliverdin IXα (BV), CO, and Fe^{+2} (Shekhawat and Verma, 2010; Shekhawat et al., 2011). BV and billirubin (BR) have antioxidant characteristics in plants (Noriega et al., 2004; Balestrasse et al., 2005, 2008) and animals, respectively (Ortiz de Montellano et al., 2001; Noriega et al., 2003). The role of HO is also noticeable in phytochrome-chromophore synthesis (Terry et al., 1993; Verma and Shekhawat, 2013). In plants, HO1 (co-localized in chloroplast to mitochondria) is a major stress-response protein among HO isoforms (HO1, HO2, HO3, and HO4) (Xu et al., 2011; Jin et al., 2011; Dixit et al., 2013) and acts as an antioxidant under different abiotic stresses such as UV-B stress (Yannarelli et al., 2006) and cadmium stress (Matsumoto et al., 2004; Cui et al., 2011; Fu et al., 2011). Recent studies in plants demonstrated that HO contributes to alleviating the effects of salt stress. These studies found that the activity of HO was induced due to oxidative stress under salinity conditions (Zilli et al., 2008, 2009; Xie et al., 2008, 2011; Wei et al., 2013).

* Correspondence: afrozalamsafvi@gmail.com

The present study was carried out in *B. juncea* with the aim of acquiring improved knowledge of the effect of salt stress on the antioxidant defense system. We observed the behavior of antioxidant enzymes (APX, GR, and POD) along with HO1 to elucidate the role of HO1 in plant tolerance of salt-stress conditions.

2. Materials and methods

2.1. Plant material, salt (NaCl) treatment, and experimental design

B. juncea (L.) Czern. cv. Bio 902 (Indian mustard) seeds were collected from Krishi Vigyan Kendra (Agricultural Science Center), Banasthali University, Rajasthan, for the present study. The seeds were sterilized in 0.5% sodium hypochlorite for 10 min and rinsed with distilled water. After that, seeds were germinated in a petri dish containing filter paper soaked in distilled water at 30 °C under dark conditions. After 3 days, uniformly germinated seedlings were taken and transferred to plastic pots containing half-strength Hoagland's nutrient solution and allowed to grow in a thermostatically controlled culture room kept at 25 ± 2 °C and 500 μmol^{-2} s^{-1} with a 16-h photoperiod. Fifteen-day-old acclimatized seedlings of *Brassica juncea* were treated with different NaCl concentrations (0, 50, 100, 150, 170, and 200 mM) for 5 days. The control plants were maintained on half-strength Hoagland's medium devoid of salt treatment.

2.2. Physiological parameters

Physiological parameters were calculated as root elongation rate and growth rate by measuring root length and fresh weight before and after salt treatment.

Root elongation rate (cm day^{-1}): mean final longest RL – mean initial longest RL/Δt ($t_2 - t_1$);

(RL: root length; t_1: last day of treatment; t_2: initial day of treatment).

$$\text{Growth rate (FW g day}^{-1}) = \frac{\ln W_2 - \ln W_1}{T_2 - T_1};$$

(W_1: fresh weight recorded initially; W_2: final recorded fresh weight; T_1: duration before treatment; T_2: duration after treatment).

2.3. Determination of chlorophyll content

Fresh leaves of *B. juncea* seedlings were collected and processed for determination of pigments using the method of Arnon (1949). Leaves (0.1 g) were ground in cold conditions using 80% chilled acetone in the dark; centrifugation of the homogenate was done at 10,000 × *g* for 10 min at 4 °C. Absorbance of the supernatant was measured at 645, 652, and 663 nm.

2.4. Determination of lipid peroxidation

Lipid peroxidation was measured in the root, leaves, and shoots through recording malondialdehyde (MDA) levels using the method of De Vos et al. (1989). Plant tissue was extracted in 10 mL of 0.25% (w/v) 2-thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The extract was heated at 95 °C for 30 min followed by rapid cooling on ice. After centrifugation at 3000 × *g* for 10 min, the absorbance of supernatant was taken at 532 nm and 600 nm (extinction coefficient: 155 mM^{-1} cm^{-1}).

2.5. Histochemical localization of H_2O_2

H_2O_2 content in leaf and root was detected by the procedure of Zhou et al. (2007). Leaves and root parts were chopped and dipped into vacuum-infiltrated DAB (3,3' diaminobenzidine)-HCl solution (1 mg mL^{-1}, pH 3.8) for 8 h under light at 25 °C and treated with different concentrations of NaCl in the nutrient solution (half strength) for 24 h. For removal of chlorophyll, leaves were incubated in boiling ethanol (95%) for 10 min.

2.6. Determination of H_2O_2 content

H_2O_2 content was determined in root, leaf, and shoot samples by using the method of Alexieva et al. (2001). The reaction mixture contained 0.5 mL of 0.1% (TCA), supernatant of plant extract, 2 mL reagent (1 M KI w/v in fresh double-distilled water), and 0.5 mL of 100 mM phosphate (pH 6.8) buffer. The reaction mixture was incubated for 1 h in the dark, and absorbance was taken at 390 nm.

2.7. Antioxidant enzyme preparations and assays

For preparation of enzyme extracts, tissue was extracted under ice-cold conditions in 3 mL of extraction buffer containing 0.05% triton-X 100, 1 mM EDTA, 1 mM ascorbate, and 2% PVP in 50 mM phosphate buffer (pH 7). Homogenate was centrifuged at 14,000 rpm for 20 min and stored at –20 °C for further enzyme assays.

APX activity was determined in a reaction mixture by taking the absorbance at 290 nm; the mixture contained 2.8 mL of phosphate buffer with 0.5 mM ascorbic acid, 0.1 mL of H_2O_2, and 0.1 mL enzyme extract (Chen and Asada, 1989).

GR was assayed by the procedure of Smith et al. (1988). The reaction mixture contained 0.1 M phosphate buffer (pH 7.5), 0.5 mM EDTA, 0.75 mM 5-5'-dithiobis (2-nitrobenzoic acid), 1 mM GSSG, and 0.1 mM NADPH; absorbance was recorded for 5 min at 412 nm (extinction coefficient 6.2 mM^{-1} cm^{-1}).

POD assay was carried out by the method of Putter (1974). The reaction mixture contained enzyme extract, 50 mM potassium phosphate buffer (pH 7.0), 3.4 mM guaiacol, and 0.9 mM H_2O_2. POD activity was determined by monitoring the formation of tetraguaiacol from the initial substrate guaiacol in the presence of H_2O_2 at 436 nm (extinction coefficient: 6.39 mM^{-1} cm^{-1}).

2.8. Heme oxygenase (HO) assay

The HO assay was carried out by the method of Balestrasse et al. (2005). Plant tissue (0.3 g) was homogenized in 4

volumes of ice-cold 0.25 M sucrose solution containing 0.2 mM EDTA, 1 mM phenylmethyl sulfonyl fluoride, and 50 mM potassium phosphate buffer (pH 7.4). Homogenate was centrifuged with 20,000 × *g* for 20 min, and supernatant was used for the assay. The reaction mixture contained 10 mM potassium phosphate buffer (pH 7.4), 60 nM NADPH, 250 µL of HO extract (0.5 mg of protein), and 200 nM hemin in a final volume of 500 µL. Samples were incubated at 37 °C for 60 min, and absorbance was recorded at 650 nm. Activity of HO was determined by measuring the formation of biliverdin (extinction coefficient: 6.25 mM^{-1} cm^{-1}).

2.9. Isolation of RNA and semiquantitative RT-PCR

Total RNA was extracted from 500 mg of plant tissue by grinding in liquid nitrogen followed by treatment of TRIzol reagent. RNA was then treated with RNase-free DNase I (Promega). Subsequently, cDNA was synthesized in a 20 µL reaction using 2.5 U of RT enhancer and Verso Enzyme Mix (Genei) and l M oligo (dT) primer. RT-PCR reactions were carried out using different parts of *B. juncea*. PCR reactions were performed using 10 pmol of each oligonucleotide primer, 2 µL of cDNA, and 1 U of Taq polymerase (Genei) in a 20 µL reaction volume. Primers used were as follows: for *Brassica juncea* HO1 (accession number: JX275832.1), forward (5'-ATGGCTTACTCAGCTCCCATATCTCCATCCCT-3') and reverse (5'- TTAAGCTTTGAAGCAAATAAGAG -3'), amplifying an 811-bp fragment; for actin (accession number: AF111812), forward (5'-CTGGAATGGTGAAGGCTGGGTT-3') and reverse (5'-CGGAGGATAGCGTGAGGAAGAG-3), amplifying a 293 bp. For standardization of the results, the relative profusion of actin was analyzed and used as the internal standard. The number of cycles of PCR was standardized for each gene to obtain visible bands on agarose gels. After standardization with actin, 2 µL of the reverse-transcribed material was amplified using a gene-specific primer pair through PCR; 1.2% ethidium bromide stained gels were checked, and the level of HO1 mRNA in different plant parts was analyzed to check the expression level.

2.10. Statistical analysis

All experiments were done in triplicate (n = 3). Values in the text, table, and figures indicate mean values ± SD. Differences between control and treatments were statistically examined by t-test, and the level of significance was $P < 0.05$.

3. Results

3.1. Effect of salt stress on physiological parameters

In the present study, the *B. juncea* root elongation rate decreased significantly ($P < 0.05$) with increasing salt concentrations (Table). Growth rate was also significantly reduced ($P < 0.05$) with increases in salt concentration.

3.2. Effect of salt stress on chlorophyll content, lipid peroxidation, and H_2O_2

It was observed that Chl a and total chlorophyll decreased significantly ($P < 0.05$), except at 100 mM NaCl. A significant decline in Chl b was observed, except at 100 and 150 mM NaCl. Decreases in Chl a, Chl b, and total chlorophyll were observed at 200 mM NaCl, about 1.8-fold in comparison to the control (Figure 1a).

Under salt stress, the MDA content significantly ($P < 0.05$) increased in leaf, root, and shoot samples of *B. juncea*. Exceptionally high MDA content was observed in the leaf, root, and shoot samples (82.4%, 72.5%, and 50.5%, respectively) at 200 mM NaCl in comparison to control plants (Figure 1b).

Higher H_2O_2 concentration was observed in the roots and leaves in comparison to shoot samples of *B. juncea* under salinity stress (Figure 1c). Elevated levels of H_2O_2 were recorded at 50 mM NaCl in the root sample. In the leaf sample, it increased ($P < 0.05$) significantly in comparison to the control leaf at 50 and 200 mM NaCl (1.5- and 1.9-fold, respectively). Histochemical detection

Table. Changes in growth parameters in *Brassica juncea* at different NaCl concentrations.

Salt (NaCl) concentration (mM)	Root elongation rate (cm/day)	Plant growth rate (g fresh weight/day)
Control	1.53 ± 0.15	0.064 ± 0.001
50 mM	1.06 ± 0.05*	0.051 ± 0.001*
100 mM	0.8 ± 0.17*	0.039 ± 0.0005*
150 mM	0.46 ± 0.15*	0.026 ± 0.004*
170 mM	0.2 ± 0.1*	0.017 ± 0.004*
200 mM	0.06 ± 0.05*	0.007 ± 0.002*

Data presented are mean ± SD (n = 3); *significant mean difference from control at $P < 0.05$ according to t-test.

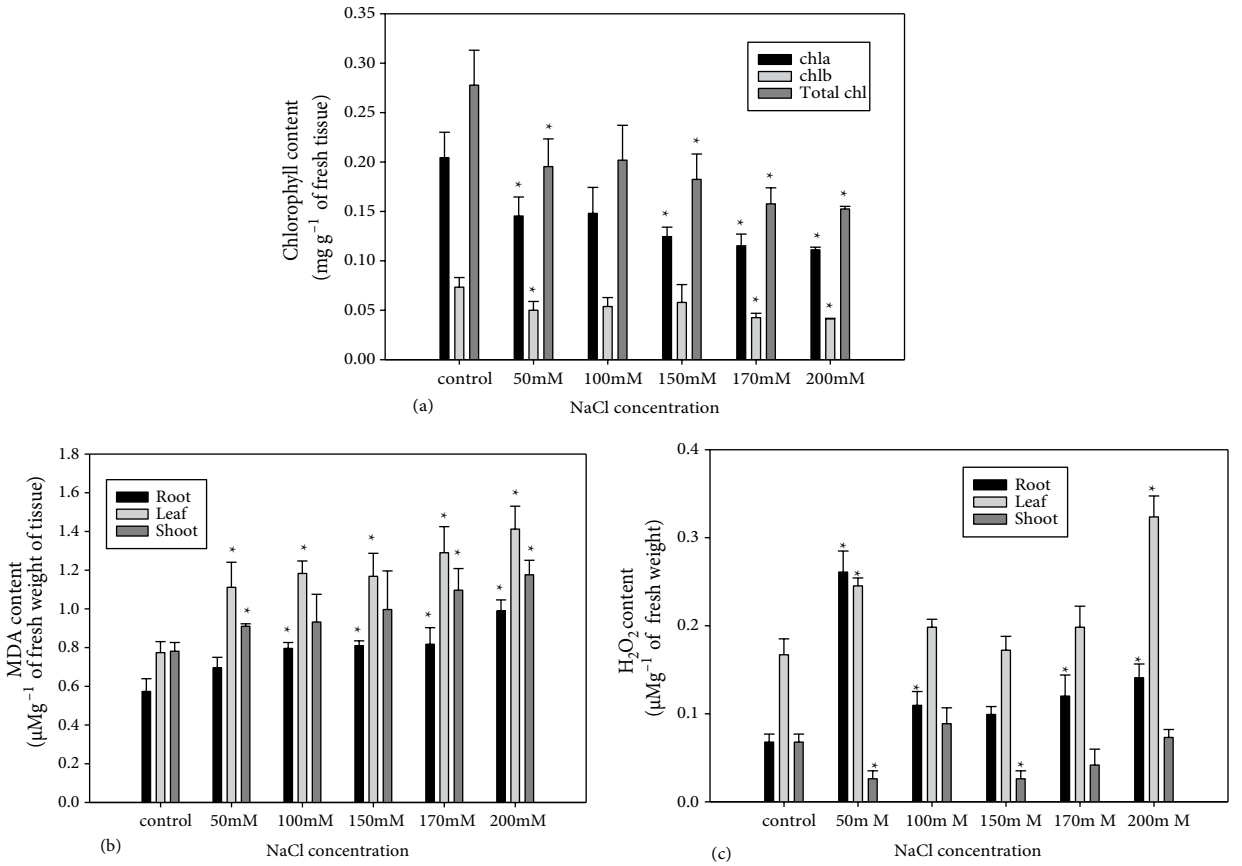

Figure 1. Shows (a) chlorophyll content, (b) MDA content level, and (c) H_2O_2 content in *Brassica juncea* treated with NaCl. Seedlings treated with 0–200 mM salt (NaCl) concentration for 5 days. Bars show SD, and data points marked with asterisks indicate that mean values are significantly different between treatments and control (*P < 0.05).

of H_2O_2 by DAB staining showed intense staining of the leaves of *B. juncea* treated with different concentrations of NaCl as compared to the leaves of the control plants. Roots treated with 50 mM NaCl took on dark brown stains in comparison to roots of control which were lighter brown in pigmentation (Figures 2a and 2b). The results obtained from histochemical localization were similar and supportive of the biochemical detection of H_2O_2.

3.3. Response of usual antioxidants (APX, POD, and GR) to salt stress

In root and leaf samples, APX activity significantly ($P < 0.05$) increased up to 150 mM NaCl, after which the activity decreased. Lower levels of APX activity were observed in shoots in comparison to leaves and roots (Figure 3).

In leaf tissue, GR level was significantly ($P < 0.05$) higher than in the control at all concentrations (Figure 4a). The activity of GR was highest at 150 mM NaCl in the roots, leaves, and shoots. In shoot samples, GR activity significantly decreased at 100 mM NaCl.

Higher POD activity was observed in the roots in comparison to the leaves and shoots (Figure 4b). The highest increase in POD activity (2.46-fold) in comparison to the control was observed in root samples at 150 mM NaCl.

3.4. Heme oxygenase activity

Increase in HO activity was higher in root samples in comparison to leaf and shoot samples at all concentrations (Figure 5a). In root and leaf tissue, HO significantly ($P < 0.05$) increased up to 150 mM NaCl. At 150 mM NaCl concentration, an increase of about 2- and 3.78-fold was observed in the root and leaf samples, respectively, compared with the control. HO activity declined at 170 and 200 mM NaCl in comparison to 150 mM NaCl. In shoot samples, no significant change in HO activity was observed.

3.5. HO1 response of *B. juncea* at transcriptional level

The expression of HO1 mRNA was observed at 150 and 170 mM salt treatment (NaCl) after 5 days. Semiquantitative RT-PCR results show that HO1 gene was induced in roots at 150 mM and to a lesser extent at 170 mM (Figure 5b). In leaf samples, HO1 mRNA levels increased at 150 mM NaCl (Figure 5c). The transcript level was higher in roots leaves.

Figure 2. Shows histochemical detection of H_2O_2 in leaves and roots of *Brassica juncea* treated with different NaCl concentrations. (a) Leaves from control and NaCl-treated seedlings stained for H_2O_2 (DAB) detection and (b) roots from control and NaCl-treated seedlings stained for H_2O_2 (DAB) detection.

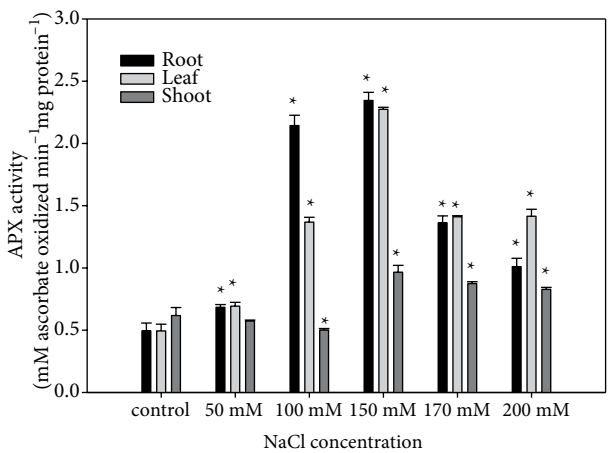

Figure 3. Shows changes in APX activity in *Brassica juncea* treated with NaCl (0–200 mM concentrations). Values are means of three replicates, and bars show SD. Data points marked with asterisks indicate that mean values are significantly different between treatments and control (*$P < 0.05$).

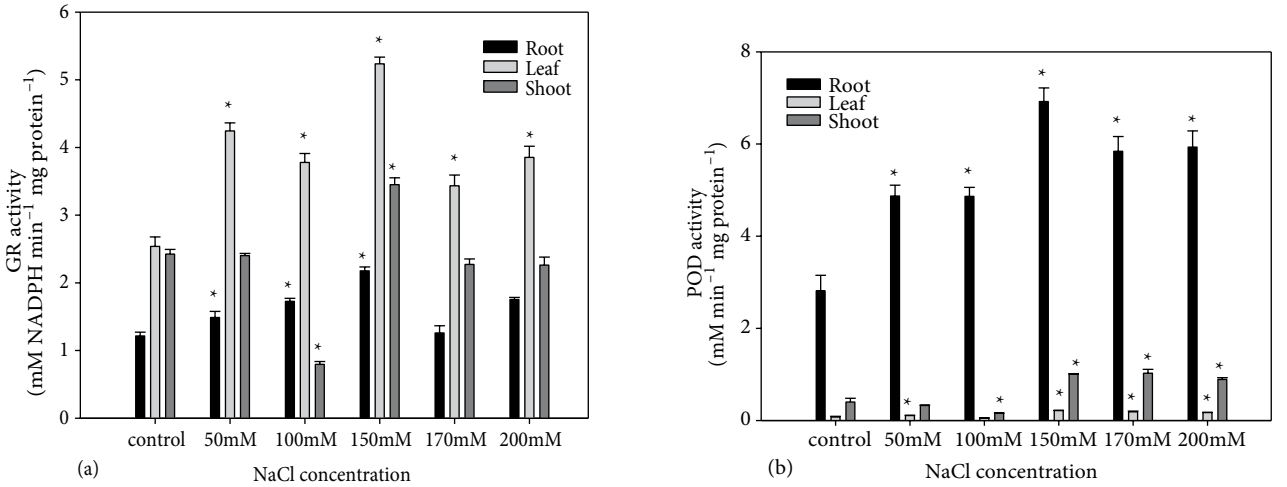

Figure 4. Shows (a) changes in activity of GR and (b) changes in POD activity in *Brassica juncea* under NaCl (0–200 mM concentrations). Values are means of three replicates, and bars show SD. Data points marked with asterisks indicate that mean values are significantly different between treatments and control (*P < 0.05).

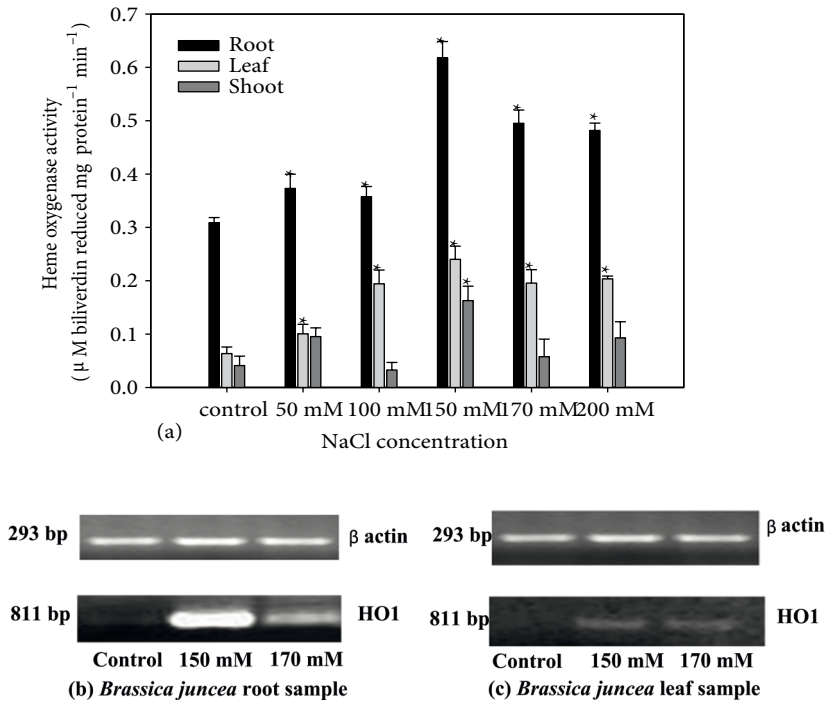

Figure 5. Shows (a) changes in heme oxygenase (HO) activity in *Brassica juncea* treated with NaCl (0–200 mM concentrations). Values are means of three replicates, and bars show SD. Data points marked with asterisks indicate that mean values are significantly different between treatments and control (*P < 0.05). HO1 gene expression in (b) *Brassica juncea* root and (c) leaf (at control, 150, and 170 mM concentrations) checked by semiquantitative RT-PCR. Actin band shows confirmation of equal loading of cDNA and RT efficiency.

4. Discussion

Salt-stress tolerance in plants is an interrelated upshot of morphological, physiological, and biochemical processes (Ashraf, 1993). Salt stress decreases a plant's ability to absorb water, which causes stunted growth with metabolic changes identical to water stress (Munns, 2002; Bybordi et al., 2010c). The development and yield of economically important crops is affected by salt stress; it negatively affects yield by impacting the water and nutritional balance of the plant (Taffouo et al., 2006: Khan et al., 2007). The high osmotic potential and toxicity of Na^+ and Cl^- ions limits water and nutrient availability in plant roots (Al-Karaki, 1997; Bybordi et al., 2010a). The present study revealed that an increase in NaCl concentrations has an adverse effect on the growth of *B. juncea* seedlings. Root elongation and growth rates decreased with increases in salt concentration. A decline in the plant growth rate is due to decreased photosynthesis by partial stomatal closure and a fall in water uptake. Hydrolysis of food reserves from storage tissue is restricted causing a disturbance in the translocation of nutrients to the growing axis (Demiral and Turkan, 2005; Eyidogan and Oz, 2007; Khan and Panda, 2008).

Katsuhara et al. (2005) confirmed that lipid peroxidation is a marker of membrane damage under salt stress. It was calculated as the amount of MDA produced due to peroxidation of polyunsaturated fatty acids in the membrane. Salt stress induces ion leakage, causing severe damage to membrane integrity. ROS generated during leaf photosynthesis or respiration can affect membrane integrity, which leads to lipid peroxidation (Savoure et al., 1999). Several reports confirm enhancement of lipid peroxidation under salt stress (Khan and Panda, 2002, 2008; Panda and Khan, 2003). Root tissue showed better ROS scavenging and prevention of membrane damage than leaf tissue, which indicates higher levels of SOD enzyme in root tissue (Abogadallah, 2010).

In the present study, the H_2O_2 level increased at 50 mM NaCl concentration, but a decrease was observed at 150 mM NaCl in roots, leaves, and shoots. However, H_2O_2 concentration increased again at NaCl concentrations above 150 mM in roots, leaves, and shoots. Reduction in H_2O_2 levels may be caused by scavenging through APX, because at this concentration APX activity was highest in leaf and root samples. The results are in accordance with those observed by in situ H_2O_2 localization. Similarly, in chickpea, Eyidogan and Oz (2007) reported an increase in APX activity with decreasing H_2O_2 content. Zilli et al. (2009) carried out histochemical analysis of H_2O_2 in soybean leaves under salt-stress conditions. They reported a decrease in H_2O_2 levels at 100 mM, while the activity of other antioxidant enzymes including HO1 was highest. The lowest activity in these enzymes was reported along with an increase in H_2O_2 at 200 mM NaCl. H_2O_2 participates in the signal transduction required for the response of antioxidant enzymes to overcome the effects of stress (Vranova et al., 2002; Zilli et al., 2009). In the present study, antioxidant enzyme activity decreased at 170 and 200 mM concentrations in comparison to 150 mM samples. Due to the decrease in antioxidant enzyme activity at high concentrations of NaCl, H_2O_2 accumulation increased in all parts of the plant.

A decline in chlorophyll content was observed under salt stress. This is in agreement with Khan (2004), Panda and Khan (2009), Abdelkader et al. (2007), and Zilli et al. (2009). These authors reported on different plants and found that their respective object plants, grown under increasing salinity, had a reduced chlorophyll build-up.

Under salt-stress conditions antioxidant enzyme activity exhibited different patterns. The activity of APX in root, leaf, and shoot samples was higher than in the control. High APX activity was observed at 150 mM NaCl, and at this concentration H_2O_2 levels were low. The results suggest APX is a chief enzyme involved in H_2O_2 scavenging to overcome oxidative stress. Overexpression of APX has been reported in plant systems under salt stress (Savoure et al., 1999; Kawasaki et al., 2001).

GR is actively engaged in the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) and maintenance of a high GSH/GSSG ratio. POD is the enzyme that scavenges H_2O_2 produced during dismutation of $O_2^{\bullet-}$ catalyzed by SOD. The highest activity of these enzymes was recorded at 150 mM NaCl in both leaf and root samples. Increased POD activity caused by salinity is well recognized (Gosset et al., 1994; Sudhakar et al., 2001; Lin and Kao, 2002). As reviewed by Abogadallah (2010), POD, APX, and GR impose tight controls on H_2O_2 concentration. In the current study, all antioxidant enzymes (APX, GR, and POD) were lowest at the highest concentration of salt (200 mM), possibly because of deactivation. The inhibition of enzyme activities at higher concentrations of stress could be credited to ROS-induced changes such as DNA damage, transductional modifications, protein fragmentation, and increased vulnerability to proteolysis (Smirnoff, 1998).

Previous studies showed that HO1 plays an important part in antioxidant defense against different abiotic stresses (Balestrasse et al., 2005, 2008; Noriega et al., 2004; Yannarelli et al., 2006). Zilli et al. (2009) reported that HO1 is induced under salt stress in soybean leaves. Roots are the first organ of the plant to face salt stress from medium or due to translocation of HO1 from its origin (chloroplast) to the roots due to high levels of ROS, and roots showed the highest induction of HO1 activity among studied *B. juncea* parts. At 200 mM NaCl, HO1 activity decreased in comparison to 150 mM NaCl. As mentioned previously,

Zilli et al. (2009) also reported a decrease in the activity of HO1 at higher concentrations of NaCl. At 150 mM NaCl, very high activity of some antioxidant enzymes (APX, GR, and POD), along with HO1, was found in different parts of the plant. Based on earlier results, HO1 may work within a group of antioxidant enzymes that create the defense machinery for the plant's survival. Findings of the present study indicate a basic plant response to salt stress via antioxidant enzymes APX, GR, and POD that is common to most stress-response pathways and a specific response of HO1 that was previously unknown in *B. juncea*. As APX, GR, and POD and HO1 became unregulated, the H_2O_2 level was overwhelmed. Activation of HO1 shows that this enzyme has a leading role in the defense system against salt stress. This study increases our understanding of the complexity of the defense network, including HO1, against salt stress, and this will be helpful for future research into developing salt-tolerant varieties.

Acknowledgment
The authors are grateful to Professor Aditya Shastri, Vice Chancellor and Professor Vinay Sharma, Dean, Faculty of Science and Technology, Banasthali University, Rajasthan, India, for their kind support for this research work.

References

Abdelkader AF, Aronsson H, Sundqvist CS (2007). High salt stress in wheat leaves causes retardation of chlorophyll accumulation due to a limited rate of protochlorophyllide formation. Physiol Plantarum 130: 157–166.

Abogadallah GM (2010). Antioxidant defense under salt stress. Plant Signal Behav 5: 369–374.

AI-Karaki GN (1997). Barley response to salt stress at varied levels of phosphorous. J Plant Nutr 20: 1635–1643.

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24: 1337–1344.

Arnon DI (1949). Copper enzymes in isolated chloroplasts: polyphenol oxidases in *Beta vulgaris*. Plant Physiol 24: 1–15.

Ashraf M (1993). Effect of sodium chloride on water relations and some organic osmotica in arid zone plant species *Melilotus indica* (L.) All. Der Tropenlandwirt 94: 95–102.

Balestrasse KB, Noriega GO, Batlle A, Tomaro ML (2005). Involvement of hemeoxygenase as antioxidant defense in soybean nodules. Free Radical Res 39: 145–151.

Balestrasse KB, Yannarelli GG, Noriega GO, Batlle A, Tomaro ML (2008). Heme oxygenase and catalase gene expression in nodules and roots of soybean plants subjected to cadmium stress. Biometals 21: 433–441.

Baxter CJ, Redestig H, Schauer N, Repsilber D, Patile KR, Nielsen J, Selbig J, Liu J, Frenie AR, Sweetlove LJ (2007). The metabolic response to heterotrophic Arabidopsis cells to oxidative stress. Plant Physiol 143: 312–325.

Binzel ML, Hess FD, Bressan RA, Hasegawa PM (1988). Intracellular compartmentation of ions in salt adapted tobacco cells. Plant Physiol 86: 607–614.

Bybordi A, Tabatabaei SJ, Ahmadev A (2010a). Effect of salinity on the growth and peroxidase and IAA oxidase activities in canola. J Food Agric Environ 8: 109–112.

Bybordi A, Tabatabaei SJ, Ahmadev A (2010c). Effects of salinity on fatty acid composition of canola (*Brassica napus* L.). J Food Agric Environ 8: 113–115.

Chen GX, Asada K (1989). Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol 30: 987–998.

Cui W, Fu G, Wu H, Shen W (2011). Cadmium-induced heme oxygenase-1 gene expression is associated with the depletion of glutathione in the roots of *Medicago sativa*. Biometals 24: 93–103.

De Vos CHR, Schat H, Vooijs R, Ernst WHO (1989). Copper-induced damage to the permeability barrier in roots of *Silene cucubalus*. J Plant Physiol 135: 164–179.

Demiral T, Turkan I (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ Exp Bot 53: 247–257.

Demirkaya M (2014). Improvement in tolerance to salt stress during tomato cultivation. Turk J Biol 38: 193–199.

Dixit S, Verma K, Shekhawat GS (2013). In vitro evaluation of mitochondrial–chloroplast subcellular localization of heme oxygenase1 (HO1) in *Glycine max*. Protoplasma 251: 671–675.

Erdal SC, Çakırlar H (2014). Impact of salt stress on photosystem II efficiency and antioxidant enzyme activities of safflower (*Carthamus tinctorius* L.) cultivars. Turk J Biol 38: 549–560.

Eyidogan F, Oz MT (2007). Effect of salinity on antioxidant responses of chickpea seedlings. Acta Physiol Plant 29: 485–493.

Fu G, Zhang L, Cui W, Wang Y, Shen W, Ren Y, Zheng T (2011). Induction of heme oxygenase-1 with β-CD-hemin complex mitigates cadmium–induced oxidative damage in the roots of *Medigo sativa*. Plant Soil 345: 271–285.

Goemez JM, Hernandez JA, Jiménez A, del Río LA, Sevill F (1999). Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Radical Res 31: S11–S18.

Gosset DR, Banks SW, Millhollon EP, Lucas MC (1996). Antioxidant response to NaCl stress in a control and an NaCl-tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. Plant Physiol 112: 803–809.

Gosset DR, Millhollon EP, Lucas MC (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci 34: 706–714.

Greenway H, Munns R (1980). Mechanism of salt tolerance in nonhalophytes. Ann Rev Plant Physiol 1: 149–190.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annu Rev Plant Phys 51: 463–499.

Jin QJ, Yuan XX, Cui WT, Han B, Feng JF, Xu S, Shen WB (2011). Isolation and characterization of a heme oxygenase-1 gene from Chinese cabbage. Mol Biotechnol 50: 8–17.

Katsuhara M, Otsuka T, Ezaki B (2005). Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase, but this reduction of lipid peroxides is not enough for a recovery of root growth in Arabidopsis. Plant Sci 169: 369–373.

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ (2001). Gene expression profiles during the initial phase of salt stress in rice. Plant Cell 13: 889–905.

Khan MA, Qayyum A, Noor E (2007). Assessment of wheat genotypes for salinity tolerance. In: Proceedings of the 8th African Crop Science Conference, El-Minia, Egypt.

Khan MH, Panda SK (2002). Induction of oxidative stress in roots of *Oryza sativa* L. in response to salt stress. Biol Plantarum 45: 625–627.

Khan MH, Panda SK (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol Plant 30: 81–89.

Khan NA (2004). NaCl-inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. Bio Plantarum 47: 437–440.

Lin CC, Kao CH (2002). Osmotic stress-induced changes in cell wall peroxidase activity and hydrogen peroxide level in roots of rice seedlings. Plant Growth Regul 37: 177–183.

Matsumoto F, Obayashi T, Sasaki-Sekimoto Y, Ohta H, Takamiya K-I, Masuda T (2004). Gene expression profiling of the tetrapyrrole metabolic pathway in Arabidopsis with a mini-array system. Plant Physiol 135: 2379–2391.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell Environ 25: 239–250.

Noriega GO, Balestrasse KB, Batlle A, Tomaro ML (2004). Heme oxygenase exerts a protective role against oxidative stress in soybean leaves. Biochem Bioph Res Co 323: 1003–1008.

Noriega GO, Tomaro ML, Batlle AMC (2003). Bilirubin is highly effective in preventing in vivo δ-aminolevulinic acid-induced oxidative cell damage. Biochim Biophys Acta 1638: 173–178.

Ortiz de Mentellano PR, Wilks A (2001). Heme oxygenase structure and mechanism. Adv Inorg Chem 51: 359–407.

Panda SK, Khan MH (2003). Salt stress influences lipid peroxidation and antioxidants in the leaf of an indica rice (*Oryza sativa* L). Physiol Mol Biol Plants 9: 273–278.

Panda SK, Khan MH (2009). Growth, oxidative damage and antioxidant responses in greengram (*Vigna radiata* L.) under short-term salinity stress and its recovery. J Agron Crop Sci 195: 442–454.

Petrić M, Jevremović S, Trifunović M, Tadić V, Milošević S, Subotić A (2014). Activity of antioxidant enzymes during induction of morphogenesis of *Fritillaria meleagris* in bulb scale culture. Turk J Biol 38: 328–338.

Polle A, Eiblmeier M, Sheppard L, Murray M (1997). Responses of antioxidative enzymes to elevated CO_2 in leaves of beech (*Fagus sylvatica* L.) seedlings grown under a range of nutrient regimes. Plant Cell Environ 20: 1317–1321.

Putter J (1974). Peroxidase. In: Bergemeyer HU, editor. Methods of Enzymatic Analysis. London, UK: Academic Press, pp. 685–690.

Rao PS, Mishra B, Gupta SR, Rathore A (2008). Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. Plant Breeding 127: 256–261.

Savoure A, Thorin D, Davey M, Hua XJ, Mauro S, Montagu MV, Inze D, Verbruggen N (1999). NaCl and $CuZnSO_4$ treatments trigger distinct oxidative defence mechanism in *Nicotiana plumbaginifolia* L. Plant Cell Environ 22: 387–396.

Shekhawat GS, Verma K (2010). Haem oxygenase (HO): an overlooked enzyme of plant metabolism and defence. J Exp Bot 61: 2255–2270.

Shekhawat GS, Dixit S, Verma K, Nasybullina EI, Kosmachevskaya OV, Topunov AF (2011). Heme oxygenase: enzyme with functional diversity. J Stress Physiol Biochem 7: 88–94.

Smirnoff N (1998). Plant resistance to environmental stress. Curr Opin Biotech 9: 214–219.

Smith IK, Vierhgeller TL, Throne CA (1988). Assay of glutathione reductase in crude tissue homogenate using 5, 5'-dithiobis (2-nitrobenzoic acid). Anal Biochem 175: 408–413.

Sudhakar C, Lakshmi A, Giridarakumar S (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci 161: 613–169.

Taffouo VD, Kenne M, Wamba-Fotsop O, Sameza ML, Ndomou M, Amougou A (2006). Salinity effects on growth, ionic distribution and water content in salt-tolerant species *Gossypium hirsutum* (Malvaceae). J Cam Acad Sci 6: 167–174.

Terry MJ, Wahleithner JA, Lagarias JC (1993). Biosynthesis of the plant photoreceptor phytochrome. Arch Biochem Biophys 306: 1–15.

Verma K, Shekhawat GS (2013). Phytochrome-chromophore biosynthesis and chloroplast development: possible role and regulation of HO. In: Moliere A, Vigneron E, editors. New Developments in Chromophore Research. Hauppauge, NY, USA: Nova Science Publishers, pp. 267–280.

Vranova E, Inze D, Breusegem FV (2002). Signal transduction during oxidative stress. J Exp Bot 53: 1227–1236.

Wei M-N, Chao Y-Y, Kao CH (2013). NaCl-induced heme oxygenase in roots of rice seedlings is mediated through hydrogen peroxide. Plant Growth Regul 69: 209–214.

Xie YJ, Ling TF, Han Y, Liu KL, Zheng QS, Huang LQ, Yuan XX, He ZY, Hu B, Fang L et al. (2008). Carbon monoxide enhances salt tolerance by nitric oxide-mediated maintenance of ion homeostasis and up-regulation of antioxidant defence in wheat seedling roots. Plant Cell Environ 31: 1864–1881.

Xie Y-J, Xu S, Han B, Wu M-Z, Yuan X-X, Han Y, Gu Q, Xu D-K, Yang Q, Shen WB (2011). Evidence of Arabidopsis salt acclimation induced by up-regulation of HY1 and the regulatory role of RbohD-derived reactive oxygen species synthesis. Plant J 66: 280–292.

Xu D-K, Jin Q-J, Xie Y-J, Liu Y-H, Lin Y-T, Shen W-P, Zhou Y-J (2011). Characterization of a wheat heme oxygenase-1 gene and its responses to different abiotic stresses. Int J Mol Sci 12: 7692–7707.

Yannarelli GG, Noriega GO, Batlle A, Tomaro ML (2006). Heme oxygenase up regulation in ultraviolet-B irradiated soybean plants involves reactive oxygen species. Planta 224: 1154–1162.

Yaycılı O, Alikamanoğlu S (2012). Induction of salt-tolerant potato (*Solanum tuberosum* L.) mutants with gamma irradiation and characterization of genetic variations via RAPD-PCR analysis. Turk J Biol 36: 405–412.

Zhou ZS, Huang SQ, Guo K, Mehta SK, Zhang PC, Yang ZM (2007). Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. J Inorg Biochem 101: 1–9.

Zilli CG, Balestrasse KB, Yannarelli GG, Polizio AH, Santa-Cruz DM, Tomaro ML (2008). Heme oxygenase upregulation under salt stress protects nitrogen metabolism in nodules of soybean plants. Environ Exp Bot 64: 83–89.

Zilli CG, Santa-Cruz DM, Yannarelli CG, Noriega GO, Tomaro ML, Balestrasse KB (2009). Heme oxygenase contributes to alleviate salinity damage in *Glycine max* L. leaves. Int J Cell Biol 2009: 1–9.