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# 28-homobrassinolide induced Proteomic Responses of maize leaves under salt and cadmium stress

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**Abstract**
Cadmium stress and salt stress are the most important factors that affects maize plant growth. This particular study is related to effects of Cd and salt stress under 28-Homobrassinolide treatments on maize cultivar. Maize cultivars were exposed to different levels of Cadmium, sodium and 28-Homobrassinolide and their effect on different enzymes has been studied and reported. The activities of carbonic anhydrase (CA) and nitrate reductase (NR) are decreased in the presence of cadmium and salt stress, while increased along with HBL. The antioxidant enzyme activities, superoxide dismutase, peroxidase and catalase increased up to a certain levels under all the conditions compared to the control. Highest level of SOD was found under Cd+2µM HBL condition followed by Nacl+2µM HBL. Catalase enzymes levels were high under NaCl+ Cd+2µM HBL followed by NaCl + Cd+1µM HBL. Peroxidase enzyme and Glutathione peroxidases levels does not show significant variation compared to the control. Glutathione peroxidases were little higher under Cd +1µMHBL NaCl + 2µMHBL and compared to the control. Ascorbate peroxidase levels were low under NaCl + Cd condition and their level was increased under Cd +1µMHBL. γ-GCS and GR expressed high under NaCl+ Cd+2µM HBL and GST did not show much variation. Ascorbic acid enzyme exhibited higher levels under Cd +2µMHBL followed by NaCl +2µMHBL. GSF=H levels isgnificantly increased under NaCl+Cd +2µMHBL and NaCl +1µMHBL. Proteomic analysis states that NaCl+ $CdCl_2$ treated plants expressed a very low protein bands. While combination with HBL reported higher levels of proteins compared with the individual conditions.

**Keywords:** Salt stress, cadmium stress, 28-homobrassinolide, antioxidant enzymes and maize

## 1. Introduction
Salt stress is a genuine requirement factor for plant development and rural efficiency on a worldwide scale. Salinity mostly influences 6% of the world's aggregate land and 20% of cultivated land territory [1]. The result of plants experiencing salt stress is moderate development and even plant demise. A comprehension of plant reaction systems under salt stress would give profitable data to enhancing stress tolerance. Plant salinity reaction is a complex phenomenon including different physiological, biochemical, and molecular processes [2]. The damage incited by salt stress is controlled by a chain of quality gene expression and proteomic changes. Recognizable proof of salt pressure instigated protein-level changes is hence a critical approach for understanding the molecular mechanism of reaction to salt [3].

In developing countries cadmium (Cd) defilement in arable soils and surface water has turned out to be extreme because of inappropriate administration of waste and use of synthetic compounds containing Cd, notwithstanding salinity [4]. In arid and semi-arid regions, bio-solids might be utilized on saline soils with a specific end goal to enhance soil quality whichbrings about overwhelming metals tainting, such as, Cd [5]. In addition, soil salinity has been appeared to build Cd fixation in crops developed on soils prepared with phosphorous (P) fertilizers containing Cd [6].

In the previous two decades, phosphorous fertilizers were widely connected to these saline soils to mitigate salt stress to crops [7]. Thus, Cd content in these soils has been significantly expanded. It has been very much reported that salt and Cd stress in mix caused higher plasma film penetrability and upgraded the generation of reactive oxygen species in wheat [8]. Maize (*Zea mays* L.) is a vital food, feed, and trade trim out the world. Comprehensively become under an extensive variety of climatic conditions, is sensitive to salinity [9]. Brassinosteroids (Brs) are polyhydroxylated sterol derivatives present in all plant species. They regulate various physiological and morphological responses in plants.

One of the most important processes afected by BRs is the photosynthesis, on which the overall growth of the plant affected. BRs not only improve the growth and yield of numerous crop plants but also enhance the resistance against several abiotic stresses including pesticides and heavy metal stress [10]. 28-homobrassinolide (28-HBL) can induce plant tolerance to a variety of abiotic stresses including drought, salinity and heavy metal [11, 12]. How 28-HBL interacts within plants during stress and how plants manage their physio-biochemical environment to combat the associated changes in cells, is unknown.

## 2. Materials and Methods

### 2.1 Plant material and chemicals

The seeds of maize (*Zea mays* L.) were procured from National Seed Corporation, Hyderabad, India. 28-homobrassinolide (HBL) employed in the present study was procured from Sigma chemicals.

### 2.2 Standardization and selection of NaCl and Cadmium concentrations

To induce salt stress, NaCl salt was used. The experimental concentration was selected based on the $IC_{50}$ value using different concentrations of NaCl i.e. 50, 100, 150, and 200 mM and 150 mM was selected as workable concentration. On the basis of screening experiments with varying concentrations (0.1, 0.5, 1.0, 1.5 and 2.0 mM) of cadmium [$CdCl_2.2H_2O$], 1 mM $Cd^{2+}$ was selected based on $IC_{50}$ values where the germination and seedling growth was found inhibited substantially but not completely and Cd dose is under the safe limit (WHO, 2007).

### 2.3 Growth conditions and treatments

The seeds of maize (*Zea mays* L.) will obtained from National Seed Corporation, Hyd. The healthy seeds were sowed in peat moss trays under unheated greenhouse conditions. At the fourth leaf stage, the seedlings are transplanted in plastic pots, 20 cm in diameter. Each pot is equally filled with acid washed sand, moistened with deionized water and contained two plants. The pots are transplanted to a growth chamber adjusted to 30/24 °C, 85/60% R.H. day/night and light intensity approximately 3500 lx for a period 12 h a day. After 48 h of transplanting, the seedlings were supplemented with NaCl (150 mM) and/or $CdCl_2$ (1 mM $Cd^{2+}$) along with the nutrient solution. At 15 days after transplanting (DAT), the seedlings were sprayed with deionized water (control) or 0.5 and 1 µM 28-homobrassinolide (the stock solution of HBL is prepared by dissolving the hormone in 1 ml ethanol and final volume was maintained by double distilled water). Many studies revealed that NaCl and $Cd^{2+}$ at 150 mM and 1mM concentrations are toxic respectively and taking clue from earlier studies in the present experiment the concentrations of NaCl and $Cd^{2+}$, (150 mM and 1mM) is used. Samples collected at 30 DAT to assess antioxidant enzymes and proteomic analysis of plant leaves and $Cd^{2+}$ content of leaves.

### 2.4 Assay of the antioxidant enzymes

Fresh leaf material (1 g) was homogenized in 50 mMTris-HCl (pH 7.5) with addition of 40 mM phenyl methyl sulfonyl fluoride (PMSF) and 0.2 mM EDTA, 2% (w/v) polyvinyl pyro pyrolidone (PVPP). The extract was centrifuged at 15,000 X g for 20 min and the resultant supernatant was used for measuring the following enzyme assays. The amount of protein in the enzyme extract was calculated according to Lowry and others [13].

**2.4.1 Catalase:** (CAT, E.C.1.11.1.6.) activity was determined following Aebi (1974) [14]. The rate of $H_2O_2$ decomposition at 240 nm was measured spectrophotometrically and calculated using a molar extinction coefficient of $45.2mM^{-1}$ $cm^{-1}$. The reaction mixture consisted of 50 mM phosphate buffer, 0.1mM $H_2O_2$ and enzyme extract. One unit of catalase activity was assumed as the amount of enzyme that decomposed 1 µmol of $H_2O_2$ per mg of soluble protein per minute at 30 ºC.

**2.4.2 Peroxidase:** (POD, E.C.1.11.1.7) activity was assayed by employing the procedure of Kar and Mishra (1976) [15]. To 0.5 ml of enzyme extract, 2.5 ml of 0.1 M phosphate buffer (pH 7), 1 ml of 0.01 M pyrogallol and 1 ml of 0.005 M $H_2O_2$ were added. A blank was prepared with 0.5 ml of enzyme extract, 3.5 ml of 0.1 M phosphate buffer and 1 ml of 0.005 M $H_2O_2$. After 5 minutes of incubation at 25 ºC, the reaction was stopped by adding 1 ml of 2.5 N $H_2SO_4$. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm against a blank. The enzyme activity was expressed as Units $mg^{-1}$ protein.

**2.4.3 Superoxide dismutase:** (SOD, E.C 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of NBT (Nitrobluetetrazolium) of Beauchamp and Fridovich [16]. A 3 ml of reaction mixture contained 40 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA, 0.1 ml of enzyme extract and 2 µM riboflavin. Riboflavin was added at the end. The reaction mixture was exposed to 15 watt fluorescent tubes and the decrease in the absorbance of the reaction mixture was read at 560 nm. Fifty percent inhibition was considered as one enzyme unit.

**2.4.4 Ascorbate peroxidase:** (Ascorbate peroxidase; E.C 1.11.1.11) was assayed by the method of Nakano and Asada [17]. The reaction mixture contained 1.5 ml of 50 mM sodium phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 ml of 0.5 mM ascorbic acid, 0.5 ml 0.5 mM $H_2O_2$ and 0.5 ml of enzyme sample. The activity was recorded as the decrease in absorbance at 290 nm for 1 minute and the amount of ascorbate oxidized was calculated from the extinction coefficient of 2.6 $mM^{-1}cm^{-1}$.

**2.4.5 Glutathione reductase:** (GR; EC 1.6.4.2) activity was performed according to Jiang and Zhang [18]. The reaction mixture contained 500 µl of sodium phosphate buffer (pH 7.0), 100 µl each of 10 mM GSSG, 1 mM NADPH and 180 µl of distilled water. The reaction was started by addition of enzyme extract and NADPH oxidation was recorded as the decrease in absorbance at 340 nm for 1 min. The activity was calculated using the extinction coefficient of NADPH 6.22 $mM^{-1}cm^{-1}$.

**2.4.6 γ- Glutamate Cysteine Ligase:** (γ-GCS; EC 6.3.2.2) γ-Glutamate Cysteine Ligase was extracted and measured by the method of Ogawa [19]. Fresh material (0.1 g) was homogenized in 0.1 M HCl and immediately centrifuged at 20,000 x g for 10 min at 2 ºC. The supernatant was then used for the assay of enzyme activity. The mixture of 200 µl supernatant and 400 µl 50 mM Tris-HCl (pH 7.6) containing 0.25 mM glutamate,10 mM ATP, 1 mM dithioerythritol and 2 mM cysteine reacted at 25 ºC for 1 h. Then 600 µl phosphorus agent containing 3 mM $H_2SO_4$, distilled water, 2.5%

ammonium molybdate and 10% vitamin C was added and mixed adequately. The mixture was incubated at 45 ºC for 25 min. The mixture was cooled at room temperature after reaction finished. The absorbance at 660 nm was measured. One unit of γ-GCS activity is defined as 1 mmol cysteine-dependently generated $PO_4^{3-}$ per minute. A molar coefficient of 5.6 $mM^{-1}$ $cm^{-1}$ was used for the calculation of enzyme activity.

**2.4.7 Glutathione-S-transferase:** (GST; EC 2.5.1.18) activity was assayed according to Habig and Jakoby [20]. The reaction mixture consisted of 500 µl of 0.2 M potassium phosphate buffer (pH 7.0) 100 µl of 0.1 M 1-chloro, 2, 4-dinitrobenzene and 390 µl of distilled water. The reaction was started by the addition of enzyme extract and the increase in absorbance was measured at 340 nm for 1 min. The activity was calculated using the extinction coefficient of the conjugate 9.6 $mM^{-1}cm^{-1}$.

**2.4.8 Glutathione peroxidase:** (GSH-PX; E.C.1.11.1.9) assay was performed according to Nagalakshmi and Prasad [21]. The assay mixture contained 100 µl of each 0.2 M potassium phosphate buffer (pH 7), 10 mM $Na_2$-EDTA, 1.14 M NaCl, 10 mM GSH, 2 mM NADPH and 2.5 mM $H_2O_2$. The reaction was started by adding 5 µl of glutathione reductase (500 units/2.8 ml) and enzyme extract. The disappearance of NADPH was recorded at 340 nm for 1 min.

**2.4.9 Carbonic anhydrase activity:** The activity of carbonic anhydrase (CA) was determined following the procedure described by Dwivedi and Randhawa [22]. The leaf samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 4 ºC for 20 min. The pieces were blotted and transferred to the test tubes, containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tube was incubated at 5 ºC for 20 min. The reaction mixture was titrated against 0.05N HCl after the addition of 0.2 ml of methyl red indicator. The results were expressed as mol (CO2) $kg^{-1}$ leaf fresh mass $s^{-1}$.

**2.4.10 Nitrate reductase:** (E.C.1.6.6.1) was determined following the *in vivo* method described by Jaworski [23]. Leaves from different treatments were taken separately and were cut into small pieces. About 5gm of leaf pieces was incubated in the medium containing 1 ml of 1 M potassium nitrate, 2 ml of 0.5% Triton X-100 for 1 hour, in dark under anaerobic conditions. After one-hour, 1 ml reaction mixture was transferred to another test tube and mixed with 1 ml of 1% sulfanilamide in 2N hydrochloric acid and 1 ml of 0.2% NEEDA (N-Cl-napthylethy leneiamidedihy drochloride). 1 ml sulfanilamide and 1 ml NEEDA served as blank. The absorbance was read at 540nm in SCHIMADZU UV-1800 Spectrophotometer. Standard curve was prepared with the help of different concentrations of potassium nitrite and enzyme activity was expressed as micromoles of $NO_2$ liberated $h^{-1}$ $g^{-1}$ fresh weight.

**2.4.11 Ascorbic acid (AsA): and glutathione (GSH) levels**
Fresh leaf material (0.2 g) was homogenized in 5 ml of 5% (v/v) m-phosphoric acid. The homogenate was centrifuged at 12,000 X g for 15 min. For determination of total ascorbate, 0.1 ml supernatant and 0.5 ml of 100 mM $KH_2PO_4$ buffer (pH 7.4) containing 5 mM EDTA and 0.2 ml 10 mM DTT (dithiothreitol) were mixed and incubated at room temperature for 15 min. Then 0.2 ml 0.5% (w/v) N-ethylmaleimide was added to remove excess DTT, and then 0.8 ml 10% (w/ v) TCA, 0.8 ml 44% (v/v) o-phosphoric acid, 0.8 ml a,a´-dipyridyl in 70% (v/v) ethanol and 0.4 ml 30 g $l^{-1}$ $FeCl_3$ were added and well mixed in sequence. Ascorbic acid was assayed in a similar manner except that 0.2 ml of $ddH_2O$ was substituted for DTT. The absorbance of the mixture at 525 nm was recorded after incubation at 40 ºC for 1 h [24].
One gram of fresh leaf was homogenized with 10 ml Tris EDTA (pH 8.2) and centrifuged at 25,000 X g for 30 minutes at 4 ºC. From the homogenate 300 µl was pipetted into 1 ml of tube to which 60 µl of 25% phosphoric acid is added and kept in ice for 5 minutes, centrifuged at 25,000 X g for 30 minutes at 4 ºC. Supernatant was collected for the estimation of GSH. For measurement of GSH**,** 450 µl of cold phosphate EDTA buffer (pH 8) was added to 50 µl of supernatant and mixed thoroughly Aliquots (25 and 50 µl) was taken into 5 ml test tube and made up to 100 µl with cold glass distilled water. Phosphate EDTA buffer (1.8 ml) was added to the tube and mixed and 100 µl of OPT (O-phthalaldehyde) solution was then added and after thorough mixing, incubated at room temperature (25 ºC) for 15 minutes.

**2.5 Statistical analysis**
The results presented are the mean values of 5 replicates. The data analyses were carried out using one-way analysis of variance (ANOVA) followed by Post Hoc Test (Multiple Comparisons) using SPSS (SPSS Inc., Chicago, IL, USA). The differences were considered significant if p was ≤ 0.05. The mean values were compared and lower case letters are used in figures/table to highlight the significant differences between the treatments.

**2.6 Proteomic responses through SDS-PAGE**
**2.6.1 Protein Extraction**
Protein was extracted by method given by Jensen and Lixue [25]. Protein was extracted from leaves in protein solubilization solution (62 m MTris -HCl, pH 6.8, 10% glycerol, 2% SDS, p- mercaptoethanol and traces of bromophenol blue) then transferred to 1.5ml tube and centrifuged at 15000 rpm for 1 minute. The supernatent was transferred to a fresh tube and placed into a boiling water bath for 5 minutes.

**2.6.2 SDS-PAGE**
SDS-PAGE was done by method suggested by Laemmli (1970) [26]. It was performed on a vertical slab gel. Bromophenol blue was added to the supernatant as tracking dye to watch the movement of protein inthe gel. Leaf protein was analyzed through slab type SDS-PAGE using 10% separating gel and 5% Stacking gel. Protein Electrode buffer solution was poured into the bottom pool of the apparatus. Gel plates were placed in the apparatus carefully so as to prevent bubbles formation at the bottom of gel plated. Equal quantities of extracted protein from each sample were loaded with the micropipette into each wells of the gel. The apparatus was connected with constant electric supply. Electrophoresis was carried out at 20 mA current for 3-4 hours till the tracking dye reaches the bottom of the gel. After electrophoresis, the protein bands were visualized by staining with coomassie brilliant blue G-250 and destained with methanol, acetic acid and water (4:1:5).

**2.6.3 Gel Documentation and Analysis**
Finally gel was photographed. Molecular weight of protein bands were estimated by their relative mobility.

## 3. Results and Discussion

The exposure of maize plants to Cd or/and NaCl stress significantly decreased the activity of carbonic anhydrase (CA) and nitrate reductase (NR) (Table 1). This may be an after effect of the inhibition and/or metabolic dysfunction of the enzyme protein. Moreover, both these stress factors anyhow interfere with the structure and fluidity of the membrane as evidence from increased ELP and thus may restrict the uptake of nitrate, the inducer and substrate of the enzyme nitrate reductase resulting in decreased NR activity, the partial $CO_2$ pressure and internal $CO_2$ concentration consequently the activity of CA also decreased [27-29]. However, the application of HBL alone or in association with applied stresses elevated the activity of both NR and CA, which could be an explanation of the impact of BR on translation and/or transcription [30]. The additional reason may be the impact of HBL at the membrane to facilitate the uptake of $NO^{-3}$ and $CO_2$ to induce the synthesis of NR and CA respectively [27, 31].

The antioxidative enzymes, CAT, SOD and POD, exhibited an increasing trend in response to HBL and NaCl and/or Cd treatment (Table 2). The HBL treatment caused a significant increase in the activities of all the enzymes. However, in association with NaCl or HBL treatment, it also improved the activities of all the above mentioned enzyme activities. Maximum activities of these enzymes recorded in maize plants exposed to combined NaCl and/or Cd stress and subsequently received HBL treatment at 1 µM concentration.

NaCl and/or Cd stress decreased the GR and GSH-PX activities in maize plants. Activities of these enzymes more significantly decreased under combined stress NaCl and Cd stress. In contrast, γ-GCS, GST and Ascorbate peroxidase activities were increased under NaCl and/or Cd stress. However, foliar application of HBL to plants increased the all the enzymes i.e. γ-GCS, GST, GR, GSH-PX and Ascorbate peroxidase activities under NaCl and/or Cd stress. HBL alone treatment also increased the all the above enzyme activities (Table 4 Table 3).

In the presence of NaCl and/or Cd stress the content of Ascorbic acid was reduced considerably, whereas GSH content was increased significantly in maize (Table 5). However, the exogenous application of HBL to maize seedling under NaCl and/or Cd stress, the contents of both Ascorbic acid and GSH were improved significantly. HBL alone treatments also accounted for significant enhancement of Ascorbic acid and GSH contents in maize plants.

**Table 1:** Effect of 28-Homobrassinolide on Nitatarereductase and Carbonic anhydrase Content of maize under NaCl and /or Cd stress and stress free conditions

| Treatments | Nitatarereductase (nmol $NO_2$ $g^{-1}$ FW $s^{-1}$) | Carbonic anhydrase (nmol $CO_2$ $Kg^{-1}$ FW $s^{-1}$) |
|---|---|---|
| Control | 334 ± 1175 c | 1.4 ± 0.26 d |
| 1µM HBL | 388 ± 12.61b | 1.9 ± 0.32b |
| 2 µM HBL | 404 ± 10.45a | 2.5 ± 0.21a |
| NaCl | 211 ± 10.98f | 0.9 ± 0.08f |
| Cd | 174 ± 10.64 g | 0.7 ± 0.14f |
| NaCl + Cd | 146 ± 09.57 h | 0.5 ± 0.43 g |
| NaCl +1 µM HBL | 379 ± 11.37 b | 1.2 ± 0.21 e |
| NaCl + 2 µM HBL | 418 ± 10.37a | 1.8 ± 0.37b |
| Cd + 1 µM HBL | 301 ± 11.28de | 1.1 ± 0.42d |
| Cd + 2 µM HBL | 329 ± 12.80c | 1.5 ± 0.13c |
| NaCl + Cd+1µM HBL | 263 ± 10.96 f | 0.8 ± 0.78 |
| NaCl + Cd+2µM HBL | 317 ± 9.96d | 1.4 ± 0.58 d |

The values are means ±SE (n = 5); mean followed by the same alphabet in a column is not significantly different at p=0.05 according to Post Hoc test.

**Table 2:** Effect of 28-Homobrassinolide on the activities of CAT, POD and SOD in maize under NaCl and /or Cd stress and stress free conditions

| Treatments | CAT (mM $H_2O_2$ decomposed $g^{-1}$ FW) | POD (U $mg^{-1}$FW) | SOD (U $g^{-1}$ FW) |
|---|---|---|---|
| Control | 120.8 ± 10.47e | 46.3 ± 1.155 f | 275.8 ± 10.4h |
| 1µM HBL | 125.1 ± 10.24c | 48.9 ± 3.144f | 295.4 ± 25.3g |
| 2 µM HBL | 127.9 ± 10.81c | 51.1 ± 4.425e f | 318.4 ± 13.6f |
| NaCl | 123.8 ± 11.61cd | 55.8 ± 4.084d | 311.2 ±11.8f |
| Cd | 124.9 ± 21.87c | 58.7 ± 2.081 c | 324.4 ± 15.1f |
| NaCl+ Cd | 129.2 ± 12.08c | 59.2 ± 2.565 | 301.8 ± 10.7g |
| NaCl +1µM HBL | 125.8 ± 13.73c | 58.7 ± 1.038 c | 341.4 ± 19.1d |
| NaCl+2µM HBL | 132.4 ± 11.72b | 59.9 ± 4.074 c | 377.6 ± 18.3b |
| Cd+1µM HBL | 128.8 ± 15.78 | 61.8 ± 5.051b | 337.9 ± 22.7 d |
| Cd+2µM HBL | 134.4 ± 11.23 b | 64.9 ± 1.051a | 387.4 ± 25.1 a |
| NaCl+Cd+1µM HBL | 137.4 ± 14.68 b | 60.7 ± 1.782 b | 327.9 ± 23.2de |
| NaCl+ Cd+2µM HBL | 151.9 ± 18.57 a | 62.8 ± 1.697 b | 365.4 ± 33.8c |

The values are means ±SE (n = 5); mean followed by the same alphabet in a column is not significantly different at p=0.05 according to Post Hoc test.

**Table 3:** Effect of 28-Homobrassinolide on the activities of APX and GSH-PX in maize under NaCl and/or Cd stress and stress free conditions

| Treatments | APX (µmolAsA $mg^{-1}$protein $min^{-1}$) | GSH-PX (µmol NADPH $min^{-1}$ $mg^{-1}$ protein) |
|---|---|---|
| Control | 9.82 ±1.165h | 0.119 ±0.003e |
| 1µMHBL | 12.48 ±1.097ef | 0.126 ±0.004de |
| 2µMHBL | 13.04 ±2.436d | 0.133 ±0.005c |
| NaCl | 10.03 ±1.277g | 0.112 ±0.004e |
| Cd | 12.46 ±1.765e | 0.109 ±0.003e |
| NaCl+Cd | 12.87 ±1.574e | 0.098±0.007f |
| NaCl +1µMHBL | 11.32 ±1.581f | 0.148 ±0.005c |
| NaCl +2µMHBL | 12.39 ±2.165e | 0.185 ±0.003a |
| Cd +1µMHBL | 13.51 ±1.209c | 0.188 ±0.009a |
| Cd +2µMHBL | 15.77 ±2.106b | 0.176 ±0.007b |
| NaCl+Cd +1µMHBL | 13.89 ±1.231c | 0.121 ±0.011d |
| NaCl+Cd +2µMHBL | 16.71 ±1.234a | 0.183 ±0.007a |

The values are means ±SE (n = 5); mean followed by the same alphabet in a column is not significantly different at p=0.05 according to Post Hoc test.

**Table 4:** Effect of 28-Homobrassinolide on the activities of GST and, GR and γ-GCS Levels in maize under NaCl and/or Cd stress and stress free conditions

| Treatments | Glutathione-s-transferase (μ mole $mg^{-1}$protein $min^{-1}$) | GR (nmol NADPH $in^{-1}mg^{1}$ protein) | γ-GCS (μmol Pi $mg^{-1}$ protein $min^{-1}$) |
|---|---|---|---|
| Control | 0.668 ± 0.057f | 33.9 ± 0.72 b | 14.34 ± 0.27 g |
| 1μM HBL | 0.673 ± 0.052e | 35.6 ± 0.92 a | 15.48 ± 0.48 f |
| 2 μM HBL | 0.693 ± 0.030e | 38.7 ± 0.61 a | 15.73± 0.57 f |
| NaCl | 0.744 ± 0.085d | 24.7 ± 0.72e | 16.21 ± 0.38e |
| Cd | 0.781 ± 0.094c | 18.6 ± 0.54 g | 16.75 ± 0.44de |
| NaCl+ Cd | 0.804 ± 0.031c | 14.5 ± 0.42 h | 17.39 ± 0.58c |
| NaCl +1μM HBL | 0.737 ± 0.041d | 28.7 ± 0.28 d | 16.54 ± 0.37d |
| NaCl+2μM HBL | 0.783 ± 0.051c | 31.6 ± 0.42b | 16.96 ± 0.23d |
| Cd+1μM HBL | 0.805 ± 0.027c | 25.1 ± 0.54e | 16.91± 0.22d |
| Cd+2μM HBL | 0.858 ± 0.025a | 30.1 ± 0.86bc | 17.48 ± 0.21 c |
| NaCl+Cd+1μM HBL | 0.825 ± 0.017b | 22.4 ± 0.67f | 17.89 ± 0.37 b |
| NaCl+ Cd+2μM HBL | 0.849 ± 0.047a | 29.1 ± 0.83d | 18.11 ± 0.26a |

The values are means ±SE (n = 5); mean followed by the same alphabet in a column is not significantly different at p=0.05 according to Post Hoc test.

**Table 5:** Effect of 28-Homobrassinolide on AsA and GSH pool in maize plants under NaCl and /or Cd stress and stress free conditions

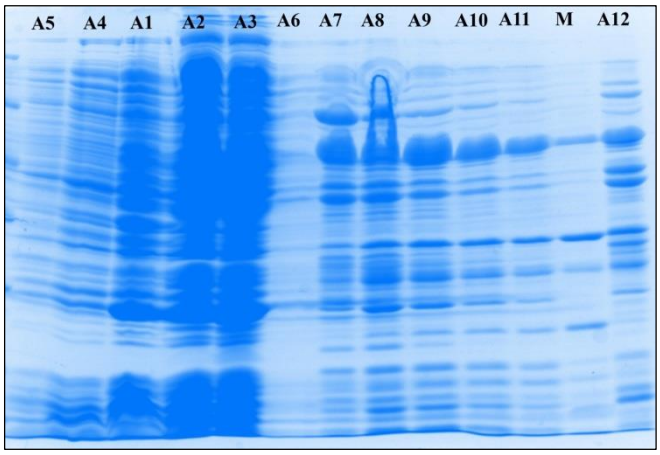
| Treatments | AsA (nmol/g DW) | GSH (nmol/g DW) |
|---|---|---|
| Control | 264.8 ± 9.4h | 744 ± 2.385d |
| 1μMHBL | 291.4 ± 11.3g | 781 ± 3.294c |
| 2μMHBL | 318.4 ± 13.7f | 796 ± 4.194c |
| NaCl | 314.2 ±11.8f | 668 ± 3.057f |
| Cd | 324.4 ± 15.1f | 673 ± 5.052e |
| NaCl + Cd | 301.8 ± 18.8g | 603 ± 5.030e |
| NaCl + 1μMHBL | 341.4 ± 19.1d | 737 ± 2.241d |
| NaCl + 2μMHBL | 377.6 ± 18.7b | 783 ± 3.751c |
| Cd + 1μMHBL | 337.9 ± 22.7 d | 805 ± 4.127c |
| Cd + 2μMHBL | 387.4 ± 25.1 a | 858 ± 4.125a |
| NaCl + Cd + 1μMHBL | 334.9 ± 23.2de | 667 ± 3.017b |
| NaCl + Cd + 2μMHBL | 357.4 ± 47.1c | 719 ± 2.847a |

The values are means ±SE (n = 5); mean followed by the same alphabet in a column is not significantly different at p=0.05 according to Post Hoc test.

### SDS Page analysis

| S. No | Sample | Code |
|---|---|---|
| 1 | Control | A1 |
| 2 | 1μM HBL | A2 |
| 3 | 2 μM HBL | A3 |
| 4 | NaCl | A4 |
| 5 | $CdCl_2$ | A5 |
| 6 | NaCl+ $CdCl_2$ | A6 |
| 7 | NaCl +1μM HBL | A7 |
| 8 | NaCl+2μM HBL | A8 |
| 9 | $CdCl_2$+1μM HBL | A9 |
| 10 | $CdCl_2$+2μM HBL | A10 |
| 11 | NaCl+ $CdCl_2$+1μM HBL | A11 |
| 12 | NaCl+ $CdCl_2$+2μM HBL | A12 |

Total protein pattern on SDS-PAGE revealed that NaCl and $CdCl_2$ dramatically altered the overall pattern of leaf protein when compared to control. In the figure it clearly shows that NaCl + $CdCl_2$ expressed a very low protein bands by the bands we can confirm that by applying NaCl + $CdCl_2$ the synthesis of polypeptides is very low as compared with all other combinations. The highest polypeptides can be seen in 1μM HBL and 2μM HBL when compared with control. By applying HBL to NaCl and $CdCl_2$ we can observe the increase in polypeptide when compared with NaCl and $CdCl_2$ individually.




Effects of Cd and Nacl along with HBL showed a varied expression of anti-oxidant enzymes. Under mixed conditions maize plants were able to cope up with the stress by both salt and cadmium. Preoteomic analysis of maize plants are able to tolerate under mixed conditions compared with individual condition alone.

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