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Research Article

Effect of 28-Homobrassinolide on photosynthesis and carbohydrate content of Maize under salt and cadmium stress

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Abstract: This study was aimed to find the effects of 28-homobrassinolide (28-HBL) on maize seedlings subjected to the combined stress of cadmium and salinity (180 mM), either alone and supplemented with 28-HBL treatments (1 and 2µM). NaCl and Cd stress alone and combined stress plants exhibited a decrease in net photosynthetic rate and quantum efficiency of PSII. However exogenous application of HBL to NaCl and/or Cd stressed plants increased the photosynthetic rate and its related attributes (Gs, Ci and E). Chlorophyll fluorescence values were significantly decreased by Cd treatments in comparison with the control suggesting induction of photo inhibition. BR could protect photosystem II from salt and Cd induced oxidative damage and improve chlorophyll fluorescence parameters and increase the absorption of light energy and electron transfer. HBL promotes the activity of Calvin cycle enzymes and also regulates the carbohydrate metabolism in maize under NaCl and/or Cd stress. NaCl or/and Cd stress significantly elevated the levels of glucose and fructose, whereas sucrose and starch levels were considerably decreased in comparison to control. Increase in glucose and fructose levels were accompanied by significant increase in sucrose breakdown enzymes i.e. AI and SS under Cd or/and NaCl stress, the degradation of sucrose to glucose and fructose was increased, whereas its biosynthesis was inhibited under NaCl or/and Cd stress. Accumulation of glucose, sucrose, and fructose during stressed conditions plays a very important role in carbon storage, osmotic regulation, and homeostasis, as well as scavenging of free radicals. These maintain the osmotic potential as well as involve in redox reactions and contribute in maintaining the structures of macromolecules and membranes. HBL alone applications also accounted for significant increase in the carbohydrate fractions in maize plants, which leads to the up regulation of sucrose metabolism and biosynthesis enzymes.

Key words: Maize, 28-Homobrassinolide, photosynthetic pigments, Calvin Cycle Enzymes, Carbohydrate Metabolism, salt stress, cadmium stress

Introduction

Salt stress and heavy metal stress have major effect on plant growth and development. Individual as well as combined stress conditions can cause imbalances in the homeostasis of the cell due to the overproduction of reactive oxygen species (ROS). ROS cause membrane deterioration, lipid peroxidation and DNA modifications that lead to irreparable metabolic and structural dysfunction and end in cell death (Mittler, 2002). Brassinosteroids (BRs) are a group of naturally occurring plant steroid hormones that can induce plant tolerance to various plant stresses and play a crucial role in cell division, vascular differentiation, male fertility, timing senescence, leaf development, gene expression, protein synthesis, photosynthesis (Clouse and Sasse, 1998; Krishna, 2003). Moreover, BRs regulate the expression of hundreds of genes, affect the activity of numerous metabolic pathways, and help to control overall developmental programs leading to morphogenesis. On the other hand, the potential application of BRs in agriculture to improve growth and yield under various stress conditions including drought, salinity, extreme temperatures, and heavy metal (Cd, Cu, Al, and Ni) toxicity, is of immense significance as these stresses severely hamper the normal metabolism of plants. Keeping in mind the multifaceted role of BRs, an attempt has been made to cover the various aspects mediated by BRs particularly under stress conditions and a possible mechanism of action of BRs has also been suggested. How 28-HBL interacts within plants during a combined stress of extreme temperature and salinity and how plants manage their physio-biochemical environment to combat the associated changes in cells, is unknown. Overall growth of the plant relies on the photosynthesis. BRs are found capable of preventing the loss of photosynthetic pigments either by activating or inducing the synthesis of enzymes involved in chlorophyll biosynthesis. BRs play important role in maintaining PS II efficiency by stabilizing D1 protein (Siddiqui, 2018). It overcomes the stomatal limitations and elevates the efficiency of photosynthetic carbon fixation. BRs also act at various levels of light and dark reactions leading to enhanced carbohydrate synthesis. Therefore, it becomes important to focus and collect information related to various effects of BRs on photosynthesis and its related attributes. The present study deals with the effect of BRs on photosynthesis under normal as well as salt and cadmium stressful conditions. This study was aimed to find the effects of 28-homobrassinolide (28-HBL) on maize seedlings subjected to the

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combined stress of cadmium and salinity (180 mM), either alone and supplemented with 28-HBL treatments (1 and 2µM).

BRs are effective in increasing photosynthesis, particularly the capacity of CO_2 assimilation in the Calvin cycle, which was mainly attributed to an increase in the initial activity of Rubisco (Yu *et al.,* 2004). Two field grown faba bean cultivars (*Vicia faba* L) - Sakha 1 and Giza 40 have been accounted for marked enhancement of total carbohydrates and plant growth after the foliar spraying with 28-HBL and 24-EBL (Talaat and Abdallah, 2010). Foliar application of 24-epibrassinolide increased the total soluble sugars in wheat cv. Tokra plants (Janeczlo *et al.,* 2010). Foliar spraying of EBL maintains the stability of cellular membrane and positively improve the carbohydrate metabolism in cucumber seedlings (*Cucumis sativus* L. cv. Jinyou No. 4) growing under salinity stress [80 mM Ca $(NO_3)_2$]. Exogenous application of EBL enhanced the enzyme activities of sucrose phosphate synthase, sucrose synthase and acid invertase; thus, raising the level of sucrose, fructose and total soluble sugars exposed to salinity stress (Yuan *et al.,* 2014). Jiang *et al.,* (2012) reported that HBL and H_2O_2 increased contents of total soluble sugar, sucrose, hexose, and starch, followed by enhanced activities of sugar metabolism such as sucrose phosphate synthase, sucrose synthase, and invertase in *Cucumis sativus*. Supplementation of 1 µg L^{-1} 24-EBL to the nutrient solution caused an increase in the concentration of total soluble sugars and sucrose in the roots of *Cucumis sativus* L. seedlings grown under hypoxia stress (Kang *et al.,* 2009). Enhancement of photosynthetic rate accompanied with elevated carbohydrate accumulation in coleus was observed by Swamy and Rao, (2011).

Materials and Methods

Plant Material

The seeds of maize (*Zea mays* L.var. DHM 109) were procured from National Seed Corporation, Hyderabad, India. To understand the impact of Brassinosteroids on the performance of maize plants under salinity and cadmium stress conditions, two experimental approaches were adopted. They were

I. Seed germination and seedling growth studies
II. Whole Plant studies.

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. Based on screening experiments with varying concentrations (0.1, 0.5, 1.0, 1.5 and 2.0mM) 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).

Hormone preparation and concentration selection

The stock solution of HBL was prepared by dissolving the required quantity of HBL in 5 ml of ethanol, in a 100-ml volumetric flask and the final volume was made up to the mark by using double-distilled water (DDW) containing 0.05 % Tween-20. The working concentration of HBL (1.0, or 2.0 µM) were prepared by diluting stock with double distilled water. The steroid concentrations were selected based on preliminary experiment, performed using 0.1, 0.25, 0.5, 1.0, 2.0, 3.0, or 4.0 µM of HBL and the concentrations of 1.0, or 2.0 µM HBL were selected based on significant growth stimulation. The treatment details are as follows: Control (no salt, cadmium and HBL), HBL: 1.0 and 2.0 µM (no salt and Cd), Nacl, Cadmium, Nacl+Cd, Nacl+1µMHBL, Nacl+2µMHBL, Cd+1µMHBL, Cd+2µMHBL, acl+Cd+1µMHBL, Nacl+Cd+2µMHBL.

Determination of photosynthetic pigments

Chlorophyll pigments were extracted and estimated according to the method of Arnon, (1949). 200 mg of fresh leaf material was taken in a clean mortar and homogenized with pestle using 80% (v/v) acetone. The green slurry was centrifuged at 4000 rpm for 10 minutes. The supernatant was transferred to a 25 ml volumetric flask. The residual pigments were re-extracted using small amounts of 80% acetone and centrifuged. The supernatant was transferred to the volumetric flask. The extraction was repeated till complete white residue was obtained. The combined chlorophyll extracts were made up to 25 ml with 80% acetone. The optical density was recorded at 645 nm, 663 nm and 480 nm against 80% (v/v) acetone as blank in UV-Visible Spectrometer (SCHIMADZU UV-1800, Japan).

The amount of pigments present in the pigment extract was determined employing the following formulae:

Chlorophyll 'a' =
$[(OD\ 663 \times 12.7) - (OD\ 645 \times 2.69)] \times V / (1000 \times W)$
Chlorophyll 'b' =
$[(OD\ 663 \times 22.9) - (OD\ 645 \times 4.68)] \times V / (1000 \times W)$
Total chlorophylls =
$[(OD\ 663 \times 20.2) - (OD\ 645 \times 8.02)] \times V / (1000 \times W)$
Carotenoids =
$(1000\ OD\ 480 - 3.27\ (Chl\ a) - 104\ (Chl\ b)) / 227$

Where,
V-volume of the pigment extract; W -weight of the leaf material in grams.
Chlorophyll and carotenoid contents were expressed in mg g/fresh weight.

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Gas exchange measurements

Different leaf gas exchange parameters such as net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (Gs) and internal CO_2 concentration (Ci) were measured with the portable CIRAS-1 infrared gas analyzer equipment. These measurements were carried out on the middle part of the youngest (fully opened second leaf), which avoided the leaf vein. The measurements were conducted from during 8:30 to 10 am., during this time the curtain of the greenhouse was shut down to avoid effects of different light conditions. The device parameters were set to: molar flow of air per unit leaf area 407.62 m mol lol^{-1} 1 m^{-2} s^{-1}, water vapour pressure into leaf chamber was 3.4 mbar, photosynthetically active radiation (PAR) at leaf surface was up to 1201 mol photons m^{-2} s^{-1}, ambient temperature was 36.39 - 40.98 °C, ambient CO_2 concentration was 399.3 mol^{-1} CO_2 and relative humidity (RH) was 52.32 %.

Chlorophyll fluorescence: Chlorophyll fluorescence parameters were determined using a PAM-2500 chlorophyll fluorescence analyser (WALZ, Germany) between 9:00 and 12:00. After a 20 min dark adaptation period, the maximal photochemical efficiency of PSII Fv/Fm, quantum efficiency of PSII (ϕ_{PSII}) and Photochemical quenching (q_P) were determined. The cuvette of the gas exchange system was modified to accept the fibre optic of the fluorimeter at a 60° angle without significantly interfering with PPFD distribution at the leaf surface. Minimal fluorescence (F_0) was measured under a weak pulse of modulating light over a 0.8 s period, and maximal fluorescence (F_m) was induced by a saturating pulse of light (8000 µmol m^{-2}s^{-1}) applied over 0.8s. The maximal quantum efficiency of PSII was determined as F_v/F_m, where F_v is the difference between F_0 and F_m An actinic light source (600 µmol m^{-2} s^{-1}) was then applied to achieve steady-state photosynthesis and to obtain F_s (steady-state fluorescence yield), after which a second saturation pulse was applied for 0.7s to obtain F'_m (light-adapted maximum fluorescence). Fluorescence parameters were calculated by the FMS-2, based on the dark-adapted and light adapted fluorescence measurements. The quantum efficiency of PSII (ϕ_{PSII}) and the efficiency of excitation capture by open PSII centers were calculated as $(F_m–F_s)/F_m$ and F_v/F_m, respectively. Photochemical quenching (q_P) was calculated as $(F'_m–F_s)/(F'_m–F_0)$.

Calvin Cycle Enzymes

Carbonic anhydrase activity: The activity of carbonic anhydrase (CA) was determined following the procedure described by (Dwivedi and Randhawa, 1974). The leaf samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 4 °C 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°C 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 (CO_2) kg^{-1} leaf FW s^{-1}.

Ribulose-1,5-bisphosphate carboxylase

(RuBPcase: EC.4.1.1.39) Extraction was done as described by (Makino *et al.,* 1988). RuBPcase was activated for 20 min at 0°C after preparation of the supernatant in the activation medium that contained 75 mM Hepes–KOH at pH 7.5, 10 mM $MgCl_2$ and 10 mM $NaHCO_3$. The reaction mixture contained 100 mM bicine at pH 8.2, 5 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM creatine phosphate, 1 mM ATP-2 Na, 0.1 mM NADH, 0.3 mM RuBP, 10 units of phosphocreatine kinase, 10 units of glyceraldehydes-3-phosphate dehydrogenase and 10 units of phosphoglycerate kinase, as described by Sawada *et al.,* (1990). The enzymatic activities were corrected for the decrease in absorbance at 340 nm in a control assay medium prepared without ribulose bisphosphate at 25°C.

Phosphoenol-pyruvate carboxylase

(PEPcase: EC 4.1.1.31) Leaf material (0.1 gm) was homogenized in a buffer containing 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10% (v/v) glycerol, 1 mM EDTA, 14 mM b-mercapto-ethanol, 1 mM PMSF and 10 µg ml^{-1} leupeptin. The homogenate was centrifuged at 13,000g for 15 min and the supernatant was again centrifuged at 100,000g for 30 min. The soluble extracted proteins were dialyzed against the same homogenization buffer and were used for PEPcase activity assays directly. PEPcase was determined by coupling its activity to malate dehydrogenase-catalyzed NADH oxidation in 1.5 ml final volume of a standard buffer containing 100 mM Tris–HCl (pH 8.0), 5 mM $MgCl_2$, 2.5 mM PEP, 0.2 mM NADH, 10 mM $NaHCO_3$, and 15 µg ml^{-1} MDH. NADH oxidation was determined spectrophotometrically at 340 nm at 25°C. Assays were initiated by adding aliquots of the protein extracts (Gonzalez *et al.,* 1998).

Carbohydrate Fractions

Recent fully expanded leaves were harvested and homogenized in 70% (v/v) ethanol and used for soluble sugar analysis. The residue left after extracting soluble sugars was used for determination of starch content. Ethanol homogenate (2.5 ml) was taken into centrifuge tubes. The tubes were kept in a boiling water bath for 5 minutes. After cooling, the contents were centrifuged at 4,000 rpm for 10 minutes. The supernatant was collected. The residue was re-extracted with 5 ml of 70% (v/v) ethanol and was centrifuged again. This procedure was repeated 3

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times. The ethanol supernatant were pooled and made up to 10 ml. This was used for the estimation of total sugars and reducing sugars.

Estimation of total sugars

Total sugars were estimated according to the method of (Yoshida *et al.,* 1976). 5 ml of alcohol extract was evaporated to dryness in a clean beaker in a water bath at 60 ºC. The lipids and pigments were removed by washing the evaporated residue repeatedly with diethyl ether. Then the residue was dissolved in 5 ml of 40 % (v/v) ethanol. This was used for the estimation of total sugars by anthrone reagent.

Anthrone reagent: 200 mg of anthrone dissolved in 100 ml of concentrated sulphuric acid. One ml of extract was taken and to it 5 ml of anthrone reagent was added. The tubes were heated for 7½ minutes in a boiling water bath. The tubes were cooled and the intensity of brown colour developed was recorded at 630 nm in UV Visible Spectrometer (SCHIMADZU UV-1800, Japan) using blank. The blank consisted of 1 ml of 40 % (v/v) ethanol and 5 ml of anthrone reagent. The total sugars were estimated as D-glucose equivalents. The amount of glucose was found out from a glucose standard curve. The amount of total sugars was expressed as mg g^{-1}fr.wt.

Estimation of reducing sugars

Reducing sugars were determined according to (Nelson, 1944) method. Nelson reagent was used for the estimation of reducing sugars (Glucose and Fructose, using standard graphs).

Nelson reagent: Nelson reagent was prepared by mixing reagents A and B prior to their use as follows:

Reagent A: 2.5 g of sodium carbonate, 2.5 g of sodium potassium tartarate, 2 g of sodium bicarbonate and 400 mg of copper sulphate were dissolved in distilled water and then the volume was made up to 100 ml in a volumetric flask with distilled water.

Reagent B: Reagent B contains solution 1 and 2.

Solution 1: 2.5g of ammonium molybdate was dissolved in 90 ml of distilled water and to this 2.1 ml of concentrated sulphuric acid was added.

Solution 2: 300 mg of sodium arsenate was dissolved in 7.9 ml of distilled water.

Just before use, solution 1 and solution 2 were mixed and heated gently to obtain light yellowish Reagent B.

One ml of Nelson reagent A was added to 1 ml of the sample. A blank was prepared with 1 ml of 70% ethanol instead of sample and 1 ml of reagent A. The colour of the mixture turns to light green. The contents were heated in a water bath for 15 minutes till the green color disappears. It was cooled to room temperature and to this 1 ml of Nelson reagent B was added. Soon after the addition of Nelson reagent B, the mixture turned to thick blue color. The contents were diluted by adding 5 ml of distilled water. The absorbance was recorded in at 550 nm against the blank in UV Visible Spectrometer (SCHIMADZU UV-1800, Japan).

Estimation of Non-reducing sugars

The amount of non-reducing sugars was calculated by the following formula as given by Loomis and Shull, 1937)**:**

Non-reducing sugars =

(total sugars – free reducing sugars) ×0.95

The amount of non-reducing sugars was expressed as glucose equivalents in terms of mg g^{-1} fresh weight.

Estimation of Starch

Starch was estimated from the residue left after alcohol extraction of the sugar by employing the method of (Mc. Cready *et al.,* 1950). The starch was solubilized from the residue for 1 hour with 5 ml of 52% perchloric acid. The contents were centrifuged at 3000 rpm for 15 minutes. The supernatant was collected. 1 ml of perchloric acid extract was diluted to 3 ml with distilled water. To this 5 ml of freshly prepared anthrone reagent was added. The mixture was heated in a water bath for 7 ½ minutes at 100ºC. The contents were cooled and were thoroughly shaken. The absorbance of the contents was measured at 630 nm in a UV Visible Spectrometer (SCHIMADZU UV-1800, Japan) against blank, which was made without the starch extract. The amount of glucose was calculated from a standard curve prepared by using known amount of glucose. The starch content was calculated by multiplying the glucose equivalents present in the sample with 0.9. The content of starch was expressed as mg g^{-1} fresh weight.

Carbohydrate Metabolism Enzymes

Extraction of Sucrose phosphate synthase (*EC*2.4.1.14; SPS) and sucrose synthase (*EC* 2.4.1.13; SS) enzymes: About 1 g of liquid nitrogen frozen fresh maize leaf tissue was ground on ice using mortar and pestle in 5 mL grinding buffer containing 50 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 0.25% BSA, 5 mM dithiothreitol, and 0.05% (v/v) Triton X-100 according to Lowell *et al.,* (1989). The extract was filtered and centrifuged for 1 min at 10000 g. Crude extract was desalted on 2 ml Sephadex G25 columns equilibrated with the grinding buffer. The amount of protein in the enzyme extract was determined by Bradford method (Bradford, 1976).

SPS activity was determined by measuring sucrose-6-phosphate produced from the substrates, UDP-glucose (uridine diphospho-glucose) and fructose 6-phosphate at 37°C by the method of Hubbard *et al.,* 1989). Approximately 20 µl of crude enzyme was incubated in a reaction mixture contained 50 mM HEPES NaOH (pH 7.5), 15 mM $MgCl_2$, 1 mM

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EDTA, 5 mM NaF, 6 mM UDP glucose, 4 mM fructose- 6 phosphate, 20 mM glucose- 6 phosphate. Reaction mixtures were incubated for 30 min at 37 °C and the reaction was terminated by the addition of 100 µl of 5 N NaOH. Unreacted fructose or fructose- 6 phosphate were destroyed by placing the tubes in boiling water for 10 min. After cooling, 1 ml of 0.14% anthrone in 13.8 M H_2SO_4 was added and incubated at 40 °C in a water bath for 20 min. Colour development of cooled solutions was measured at 620 nm and the SPS activity was calculated.

SS was assayed in both the synthetic and degradative directions with the method of Lowell *et al.,* 1989). Reaction mixtures (70 µl) for SS synthetic directions contained 80 mM HEPES (pH 8.5), 5 mM KCN, 5 mM NaF, 100 mM fructose, 15 mM UDPG, and 20 µl desalted extract. The reaction mixture was incubated for 15 min at 30°C and was terminated by boiling for 1 min. Other assays used identical conditions to those for SPS. The mixtures (490 µl) used for sucrose cleavage contained 80 mM MES (pH 5.5), 5 mM NaF, 100 mM sucrose, and 5 mM UDP. Reactions proceeded for 30 min at 30 °C and were terminated by the addition of 490 µl DNS reagent. Tubes were heated in boiling water for 5 min. After cooling, colour development was measured at 520 nm.

Extraction of acid invertase (EC 3.2.1.26; AI):
About 1 g of liquid nitrogen frozen fresh maize leaf tissue was homogenized in 3 volumes of extraction buffer containing 50 mM HEPES -NaOH (pH 7.5), 0.5 mm Na EDTA, 2.5 mm DTT, 3 mM diethyl dithiocarbonic acid, 0.5% (w/v) BSA, and 1% (w/v) insoluble PVP. After centrifugation at 1 8,000g for 30 min, supernatants were dialyzed for about 16 h against 25 mM Hepes-NaOH (pH 7.5) and 0.25 mM Na-EDTA and used as the crude soluble enzyme extract.

AI activity was measured by monitoring the formation of reducing groups in a reaction mixture at 30°C**.** Approximately, 50µl of crude enzyme was incubated with 4% sucrose in 50 mM sodium acetate buffer (pH 4.5). The reaction mixture was incubated at 30 °C for 15 min. Reducing groups formed in the reaction mixture were measured by the 3,6-dinitrophthalic acid procedure (Endo *et al.,* 1990). One unit of enzyme activity was denned as the amount of enzyme that hydrolyzes 1 µmol of sucrose in 1 min under the conditions of the reaction.

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.

Results

Effect of HBL treatment on Photosynthetic pigments
Maize plants showed the sign of chlorosis to NaCl (150 mM) and Cd (100 µM) stress after 2 weeks. The chlorophyll a (by 43.5 and 50 %), chlorophyll b (by 21 and 24.15%) and carotenoids (by 28.6 and 22.87%) levels were decreased drastically under NaCl and Cd alone stresses in comparison to control respectively. The toxic effect was more pronounced under the combined stress conditions compared to their individual ones. However, NaCl and/or Cd stressed plants supplemented with HBL, the inhibitory effect of NaCl and/or Cd on the photosynthetic pigments was alleviated. Application of HBL to stressed plants exhibited significant ($p \leq 0.005$) improvement in chlorophyll a, chlorophyll b, and carotenoids at 2µM concentration. Moreover, plants from HBL alone treatments showed a marked increase in chlorophyll a (25.8%), chlorophyll b (25.8%), and carotenoids levels (13.5%) over the control at 2µM concentration. The effect of HBL on the chlorophyll a, b and carotenoid contents of maize plants grown under NaCl and/or Cd stress presented in Table 1.

Effect of HBL treatment on photosynthetic efficiency and gas exchange parameters in response to NaCl and/or Cd stress
Photosynthetic rate (*Pn*): Plants grown under NaCl and Cd alone stresses showed a significant decrease in net *Pn* by 42.1 and 51% compared to control. Under the combined NaCl+Cd stress the decrease was more severe (67.35%) than that of NaCl and Cd alone stresses compared with unstressed control. However, application of HBL to stressed plants improved the process of *Pn* markedly. The *Pn* increased by 55.5, 55.4 and 100% in NaCl and/or Cd stressed plants receiving at 2µM concentration of HBL over the stressed control. Plants sprayed with HBL alone treatment improved the *Pn* by 15.54 % (at 1µM of HBL) and 26 % (at 2µM of HBL) in comparison to the unstressed control (Fig.1A).

Stomatal conductance (g_S): Under NaCl and/or Cd stress stomatal conductance (g_S) also decreased by 22.1, 31.8 and 45.3 % respectively over the control (Fig.1B). However, supplementation of HBL to NaCl and/or Cd stressed plants able to increase the g_S significantly by 21.4, 28.6 and 51.1 % over the respective stress controls. HBL application to NaCl stressed plants, the g_S was restored near to the control. Unstressed plants exhibited the significant increase in g_S by 39.7 % upon HBL alone application at 2µM concentration.

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Intracellular CO_2 concentration (Ci): A significant reduction in intracellular CO_2 concentration (Ci) was noted for NaCl and Cd stressed plants when compared with untreated controls. The maximum decrease in Ci, was observed in combined NaCl+ Cd stress (by 63 %) followed by Cd (by 52.8 %) and NaCl (by 32.7 %) alone stresses. Application of HBL to NaCl and/or Cd stressed plants significantly improved the Ci over their respective stressed controls. Application of HBL alone treatment produced no significant improvement in Ci value in comparison to control (Fig. 1C).

Transpiration rate (E): Compared with control, transpiration rate (E) was decreased by 32.2, 46.3 and 64.1 % following NaCl and/or Cd treatments respectively. Follow up treatment of HBL to NaCl and/or Cd stressed plants able to increase the E, significantly over the stressed controls. Application of HBL to combined NaCl+Cd stressed plants showed a more significant improvement in E value (73.4%) when compared with NaCl+Cd stressed plants. Plants exhibited a significant increase in E value treated with 2µM HBL alone over the control (Fig. 1D).

Effect of HBL treatment on the changes in chlorophyll fluorescence parameters in response to NaCl and/or Cd stress

A significant decrease in *Fv*/*Fm*, ΦPSII, and qP, in NaCl and/or Cd stressed plants was observed when compared with untreated control plants. The maximum decrease in *Fv*/*Fm*, ΦPSII, and qP was observed in combined NaCl+ Cd stress (by 71.1, 47 and 31.96 %) followed by Cd alone (by 50.9,22 and 22.2 %) and NaCl (by 41.5, 17 and 9.5 %) alone stress. Exogenous application of HBL to NaCl and/or Cd stressed plants able increase the *Fv*/*Fm* and ΦPSII over their respective stressed control at 2µM concentration. However, there was no significant increase in qP in HBL treated NaCl stressed plants compared with NaCl stressed plants. Plants receiving the HBL alone treatments also induced the *Fv*/*Fm*, ΦPSII, and qP levels. There was a marginal increase in *Fv*/*Fm* was observed upon HBL treatment. However, ΦPSII, (32.9 %) and qP (13.5%) levels showed a significant increase over the unstressed control at 2µM concentration of HBL (Fig. 2A-C).

Influence of HBL on the enzyme activities of calvin cycle under NaCl and/or Cd stresses in maize plants

Carbonic anhydrase (CA) activity: The CA activity was declined by 35.7, 50 and 64.28% following NaCl and/or Cd stress treatments as compared to the control. (Fig. 3A). However, exogenous application of HBL completely nullified the toxic effects of NaCl and/or Cd stress. Supplementation of HBL restored the CA activity to normal level at 2 µM concentration. HBL treatment accounted for the maximum increments in CA activity in NaCl and/or Cd stressed plants by 100, 114.2 and 180% compared with stressed control. Plants receiving HBL alone treatments also registered a marked enhancement in CA activity. The most significant increase (78.5%) in CA activity was found in plants treated with HBL alone at 2µM concentration.

Ribulose 1, 5-bisphosphate carboxylase (RuBPcase) activity: When plants subjected to the NaCl and Cd alone stresses, RuBPcase activity declined significantly (by 33.8 and 29.1 % respectively) compared with control. But the activity was most significantly decreased by 91.7 %, under the combined NaCl+Cd stress. However, the supplementation of HBL negated the toxic effects of NaCl and/or Cd stress on RuBPcase activity in comparison to stressed control. Stressed plants exhibited the significant enhancement in RuBPcase activity over the stressed control at 2µM HBL concentration. HBL alone treatments marginally enhanced the RuBPcase activity (Fig. 3B).

Phosphoenolpyruvate carboxylase (PEPcase) activity: Maize plants stressed with NaCl and/or Cd stresses showed significant decrease in PEPcase by 41.5, 50.9 and 71.1 % respectively compared with control. Exogenous application of HBL to stressed plants rescued the PEPcase activity to normal levels. 2µM HBL concentration registered the most significant enhancement in NaCl and/or Cd stressed plants, which were 61.2, 74.9 and 178.4 % respectively compared with stress control. It was also observed that maize plants from HBL alone treatments recorded the significant increased activity at 2µM concentration (Fig. 3C).

Effect of HBL on Carbohydrate metabolism of maize plants under NaCl and/or Cd stresses

Sucrose Phosphate Synthase (SPS) activity: NaCl and Cd alone combined NaCl+Cd stress had significant effect on SPS activity in maize. SPS activity was reduced under NaCl and/or Cd stress by 21.3, 29.63 and 38.96% compared with control respectively (Fig. 12 A). However, application of HBL restored the SPS activity near to the normal level. It was noticed that upon HBL treatment, SPA activity was increased by 28.2, 46.37 and 55.58 % in comparison to treated controls. It was noteworthy that HBL alone treatment recorded the 25.5 % improvement in SPS activity compared with control (Fig. 4A).

Sucrose synthase (SS) activity: SS activity was significantly ($p<0.05$) higher in NaCl and/or Cd stressed plants over the control. The maximum increase in SS activity was observed in Cd alone stress (by 64.43 %) followed by combined NaCl+ Cd stress (by 48.52 and NaCl (by 38.46 %) alone stress. The activity was further considerably increased upon HBL supplementation to stressed

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plants compared to treated control. It was more significant (by 27.55%) in combined NaCl+ Cd stressed plants at 2µM HBL concentration compared with stress control. HBL alone also strongly enhanced the SS activity by 29.77 and 39.7% at 1µM and 2µM concentration respectively over untreated control (Fig. 4B).

Acid invertase (AI) activity: Maize plants under NaCl and/or Cd stress exhibited increase (44.14, 71.3 and 85 %) in AI activity when compared with control. However, HBL supplementation to plants under NaCl and/or Cd stress further enhanced the AI activity. Maximum AI activity was recorded at 2µM HBL by 36.36, 32.6 and 37.43% compared with treated control respectively. In HBL alone treated seedlings also, there was appreciable increase in AI activity. Treatment of HBL alone showed significant increase in APX activity (by 39.4% at 2µM concentration) (Fig. 4C).

Effect of HBL on the levels of carbohydrate fractions in maize under NaCl and/or Cd stress- and stress-free conditions

Cellular sucrose and starch levels were significantly ($p<0.05$) declined in plants grown under NaCl and/or Cd alone stress conditions compared to control (Fig. 5A & B). Cd alone treatment more significantly decreased the sucrose levels by 54.7 % than NaCl alone and combined NaCl+Cd stress. However, application of HBL to stressed plants negated the toxic effects of NaCl and/or Cd stress on sucrose and starch content. Stressed plants receiving the HBL treatment showed a remarkable enhancement in sucrose content by 40, 54.7 and 49.8% respectively over the stressed control plants. Similarly, starch content was increased by 39.6, 20.8 and 33 % in NaCl and/or Cd stressed plants upon HBL treatment at 2µM concentration compared with stressed control. Plants showed a significant improvement in sucrose and starch levels in response to HBL alone treatments. At 2µM concentration of HBL showed significant enhancement in sucrose and starch levels (by 26.12 and 18.73 %; $p<0.05$) in comparison to control. Exposure of NaCl and/or Cd stress resulted in significant ($p<0.05$) accumulation of glucose content compared to control. Plants under combined NaCl + Cd stress showed more significant increase (57.14%) in glucose concentration (Fig. 5C). Foliar spray of HBL to stressed plants further improved the glucose content. At 2µM concentration of HBL, glucose content was increased more significantly by 31.9, 28.38 and 28.67 % under NaCl and/or Cd stress conditions compared to stress control respectively. HBL alone treatments also resulted in significant increase in glucose concentration (by 24.73 % at 1µM and 39.72 % at 2µM).

A significant accumulation of fructose content was noticed in maize plants growing under NaCl and/or Cd stress conditions (Fig. 5D). Plants showed more significant increase (by 53.13 %) in fructose levels in Cd alone stressed plants that NaCl and combined NaCl+Cd stressed plants. Application of HBL treatments to stressed plants further improved the fructose accumulation by 16.7, 13.55 and 24.81 % reactively in comparison to stressed controls. Plants receiving the HBL treatment also accounted for the considerable improvement in fructose levels (22.1% at 2µM concentration).

Table 1. Effect of HBL on photosynthetic pigment content of maize under NaCl and /or Cd stress and stress-free conditions.

Treatments	(mg g $^{-1}$ FW)		
	Chlorophyll a	Chlorophyll b	Carotenoids
Control	1.768 ±0.417b	0.954 ±0.059b	0.717 ±0.051c
1µM HBL	1.684 ±0.357c	0.957 ±0.037b	0.758 ±0.066b
2µM HBL	1.931 ±0.421a	1.201 ±0.089a	0.814 ±0.086 a
NaCl	0.998 ±0.219f	0.753 ±0.081f	0.512 ±0.067gh
Cd	0.879 ±0.325g	0.724 ±0.074f	0.553 ±0.087 f
NaCl+Cd	0.745 ±0.214 h	0.685 ±0.092 g	0.444 ±0.072i
NaCl +1µM HBL	1.216 ±0.448 e	0.893 ±0.064e	0.689 ±0.077 d
NaCl +2µM HBL	1.687 ±0.075c	0.915 ±0.073 d	0.709 ±0.087c
Cd +1µM HBL	0.929 ±0.284f	0.931 ±0.054 c	0.685 ±0.064 d
Cd +2µM HBL	1.378 ±0.378 e	0.871 ±0.094 e	0.691 ±0.077cd
NaCl+Cd +1µM HBL	0.934 ±0.412 f	0.738 ±0.092 f	0.524 ±0.072 g
NaCl+Cd +2µM HBL	1.418 ±0.235 d	0.948 ±0.083c	0.656 ±0.021e

The values are expressed as means ±SE (n = 5); mean followed by the same alphabet in a column is not significantly different at $p \leq 0.05$ according to Post Hoc test. FW: Fresh Weight; HBL: 28-Homobrassinolide.

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Figure 1 A & B: Effect of exogenous HBL on Net Photosynthetic Rate (Pn) (A) and Stomatal Conductance of (Gs) (B) of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test.

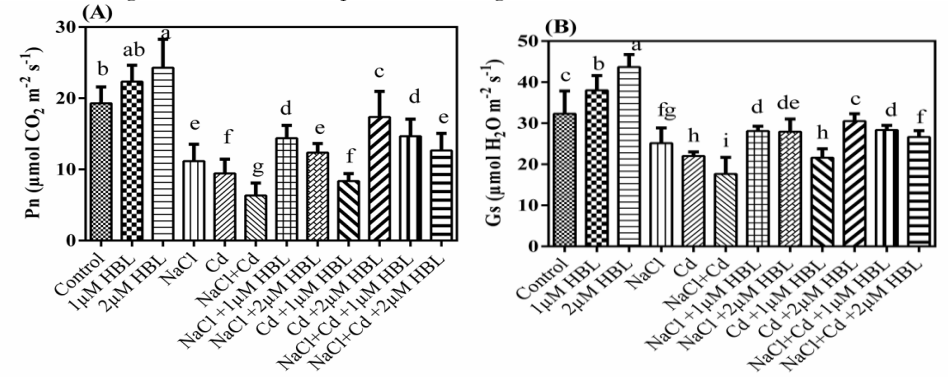

Figure 1 C & D: Effect of exogenous HBL on Transpiration Rate (E) (C) and Intercellular CO_2 Concentration (*Ci*) (D) of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test.

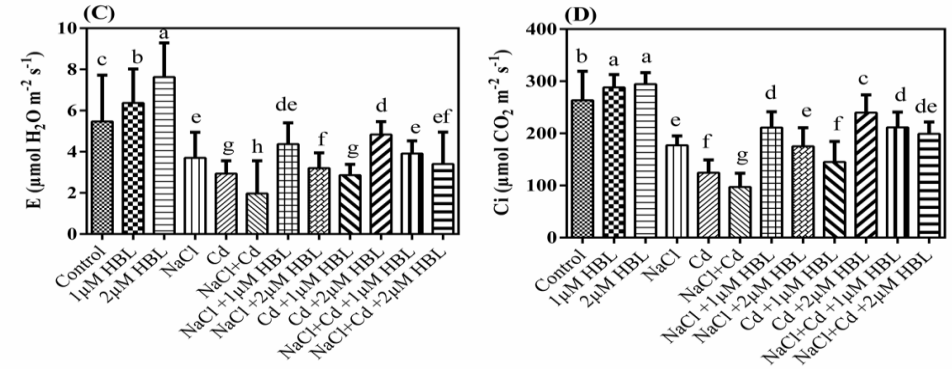

Figure 2: Effect of HBL on Maximal Photochemical Efficiency of PSII (Fv/Fm) (A), Quantum Efficiency of PSII Photochemistry (ΦPSII) (B) and Photochemical Quenching Coefficient (*qP*) (C) of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test.

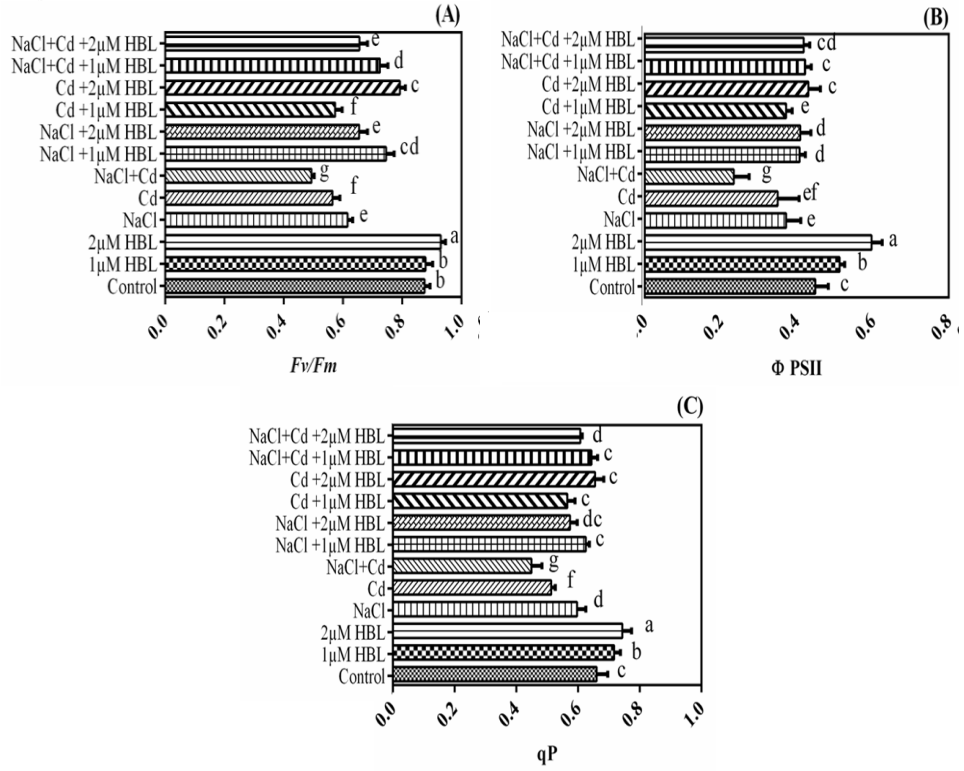

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Figure 3 A: Effect of HBL on Carbonic Anhydrase (CA) activity of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test.
Figure 3 B: Effect of HBL on Ribulose-1,5-bisphosphate Carboxylase (RuBP case) activity of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test.
Figure 3 C Effect of HBL on Phosphoenol-Pyruvate Carboxylase (PEP case) activity of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test

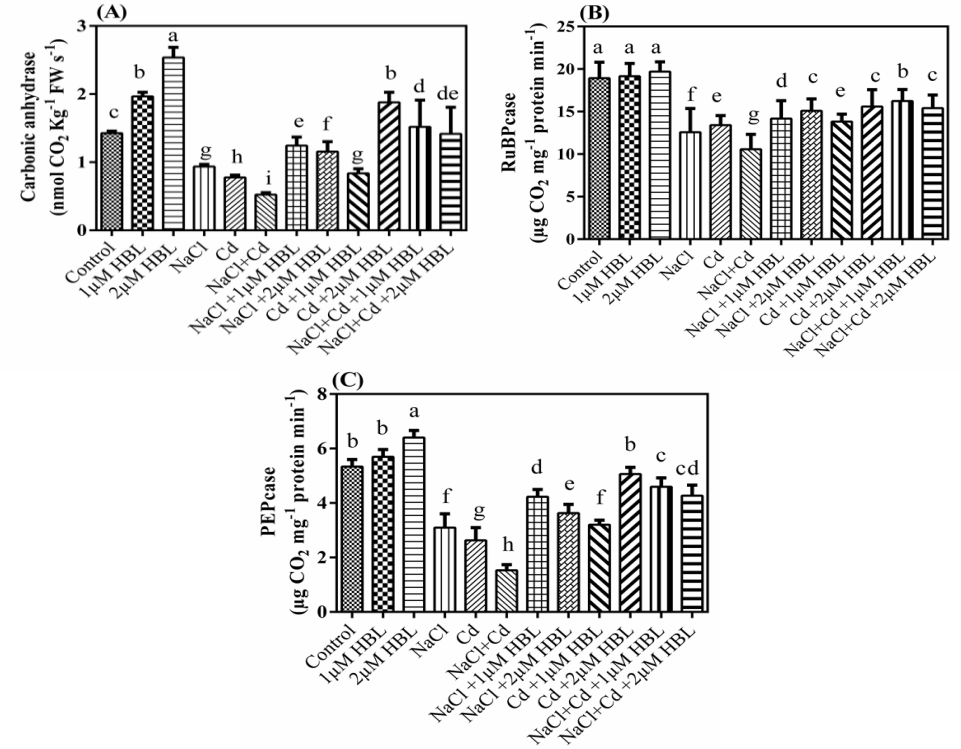
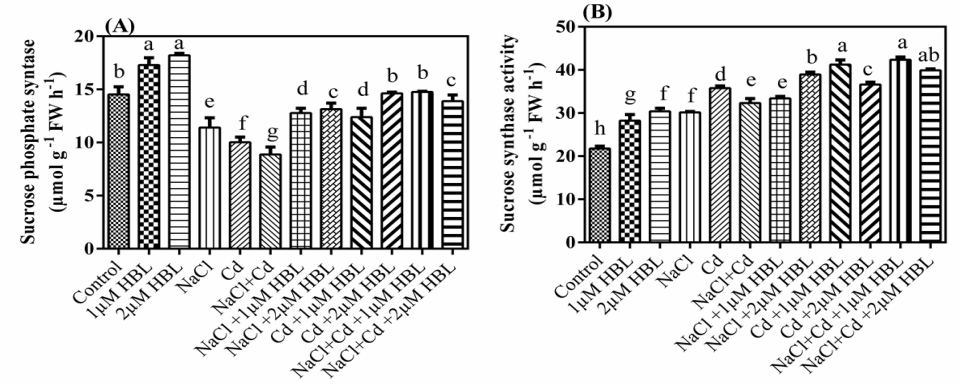


Figure 4 A: Effect of HBL on Sucrose Phosphate Synthase (SPS) activity of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test.
Figure 4 B: Effect of HBL on Sucrose Synthase (SS) activity of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test
Figure 4 C: Effect of HBL on Acid Invertase (AI) activity of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test



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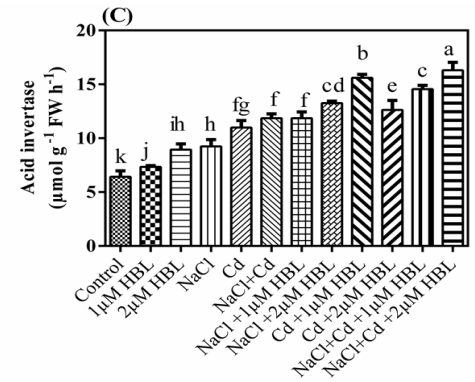

Figure 5 A & B: Effect of HBL on Sucrose (A) and Starch (B) content of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant differences at $p \leq 0.05$ according to Post Hoc Test

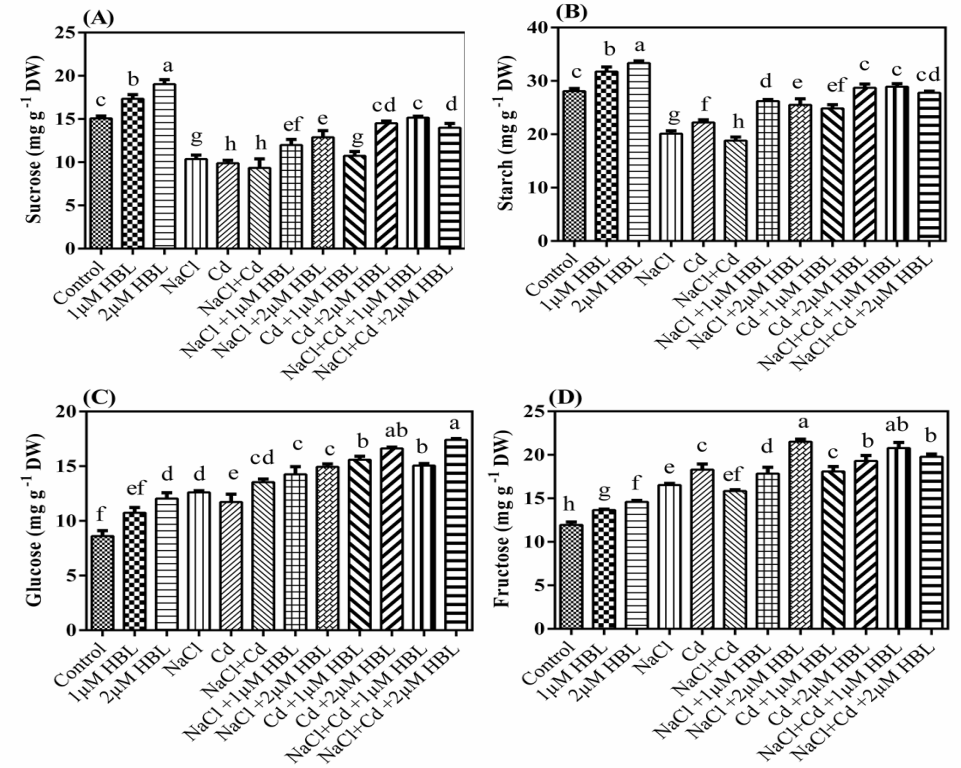

Figure 5 C & D: Effect of HBL on accumulation of Glucose (C) and Fructose (D) of maize plants under NaCl and/or Cd stress. Vertical bars represent means ±SE (n = 5); Different letters on the top of bars denotes significant difference at p≤0.05 according to Post Hoc Test.

Discussion

Photosynthesis is the most important process by which green plants convert solar energy to chemical energy in the form of organic compounds synthesized by the fixation of atmospheric carbon dioxide. In higher plants abiotic stresses like drought, salinity, heavy metal toxicity, and high light, confer serious damage on the photosynthetic machinery (Lu *et al.,* 2000; Tanyolac *et al.,* 2007; Akther *et al.,* 2015; Gururani *et al.,* 2015). Our results revealed that plants exposed to NaCl and Cd alone stress exhibited a slowdown of the net photosynthetic rate (*Pn*) that was accomplished by a significant decrease in stomatal conductance (*Gs*), intercellular CO_2 concentration (*Ci*), and transpiration rate (*E*) in maize plants (Fig. 1A-D).

The decrease in above gas exchange parameters were exacerbate under combined NaCl+Cd stress (Fig. 1A-D). It has shown that phenanthrene (PHE) and Cd stress inhibit *P*n and *g*s in tomato. It was also observed that PHE+Cd co-contamination could inhibit *P*n, but the value of *P*n was slightly higher than with Cd alone and lowers than with PHE alone (Ahammed *et al.,* 2012). Our results are corroborating with the findings of Wei *et al.,* (2007) who found that decrease of P_n was significantly correlated with G_s, E, SPAD value and F_v/F_m under alone and combined effect of Cd and NaCl stress in soybean genotypes. Similarly, Ali *et al.,* (2011) found that synergistic interaction of chromium and salinity stress markedly decreased

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the photosynthetic rate and stomatal conductance in barley genotypes.

However exogenous application of HBL to NaCl and/or Cd stressed plants increased the Pn and its related attributes (Gs, Ci and E). Moreover, HBL alone treatments also further enhanced the net photosynthetic capacity of maize plants (Fig.1A-D). Similarly, foliar application of 24-EBL and 28-HBL increased P, g, C, E, Fv/Fm and PSII of the salt stressed chickpea plants (Wani *et al.,* 2017; Jiang *et al.,* 2017) showed that exogenous application of selenium (1µM) alleviated the damage to chloroplast ultra-structure and enhanced the net photosynthetic rate in maize plants.

When seedlings of *Vigna radiata* were exposed to salt stress experienced a significant decrease in the levels of photosynthetic rate, SPAD chlorophyll and fluorescence but when these seedlings were treated with HBL as a follow-up treatment, the damage was partially overcome (Hayat *et al.,* 2010). However, the treatment with HBL alone gave maximum values for photosynthesis. It has been shown that gas exchange parameters such as Pn, gs, Ci, and E decreased in cotton plants under Cd stress. However, follow up treatment of glycine betaine led to protection of photosynthesis against Cd and further improved the Pn, gs, Ci, and E in cotton (Farooq *et al.,* 2016).

Photosystem II (PSII) is considered to be the most susceptible and primary site of the photosynthetic apparatus injury during stress (Sharkey *et al.,* 2010; Picorel *et al.,* 2017). Injury to PSII can lead to a change in chlorophyll fluorescence. Therefore, chlorophyll fluorescence can be used as a powerful and reliable non-invasive method to assess changes in the function of PSII and to examine the primary photosynthetic processes under environmental stress conditions (Oguntimehin *et al.,* 2010). In our results, maximum quantum efficiency of PSII photochemistry (Fv/Fm), qP and Φ_{PSII} decreased (Fig.a2 A-C). Fv /Fm represent the maximum efficiency of PSII primary photochemistry capacity. A decrease in *F*v/*F*m ration indicates the negative effects of NaCl and/or Cd stress on the photochemical reactions which may affect the efficiency of PSII photochemistry by blocking electron transport.

It was also noticed that the decrease in the quantum efficiency of PSII (ΦPSII) (the proportion of light absorbed by chlorophyll in PSII) and qP (the proportion of open PSII reaction centers) in maize plants subjected to NaCl and Cd stress alone and combined stress (Fig. 10 A). These observations suggest that a possible photoinhibition in the PSII might be connected to the high percentage of PSII closed reaction centers resulted in decreased excitation energy reaching PSII reaction centers (Li *et al.,* 2012; Liu *et al.,* 2014) and changes in the pigment–protein complexes of thylakoid membranes and further reduced the capacity of PSII to re-oxidize QA (Misra *et al.,* 2001; Huang *et al.,* 2013; Wang *et al.,* 2017).

It is evident that under salt stress excess Cl^- disrupt the oxygen-evolving complex of PSII and affect the performance of PSII (Kawakami *et al.,* 2009; Killi and Haworth, 2017). Inactivation of part of Photosystem II reaction centres and their transformation into excitation quenching forms as well as disturbed electron transport in the oxygen-evolving complex). The Cd and NaCl block the electron transfer from the primary acceptor plastoquinone (QA) to the secondary acceptor plastoquinone (QB) at the acceptor site of PSII leading to decreased Fv/Fm values (Mehta *et al.,* 2010).

Our results are consistent with Liu *et al.,* (2014) who observed that significant variations of chlorophyll fluorescence values upon Cd treatment. Fv/Fm, qP and Φ_{PSII} were significantly decreased by Cd treatments in comparison with the control suggesting induction of photoinhibition. Recently, Paunov *et al.,* (2018) reposted that Cd and Zn metals disturbed photosynthetic electron transport processes which led to suppression of the efficiency of energy transformation in Photosystem II in durum wheat. Similar results have been observed in maize plants under salt stress in maize (Wang *et al.,* 2017) and under chromium and NaCl stress in barley (Ali *et al.,* 2013).

However, supplementation of HBL as a follow up treatment alleviated toxic effects of NaCl and/or Cd stress on PSII in maize plants (Fig.2A). HBL application alone and with NaCl and/or Cd increased the Fv/Fm, qP and Φ_{PSII} in maize (Fig.2 A-C). The protective effects of BR could protect photosystem II from salt and Cd induced oxidative damage and improve chlorophyll fluorescence parameters and increase the absorption of light energy and electron transfer as reflected by increased values of the Fv/Fm, qP and Φ_{PSII} in maize. These results are consistent with Li *et al.,* (2012) who studied the effect of BR on drought-stressed *Chorispora bungeana*. Under drought stress levels of Chl a and Chl b decreased resulted in chlorosis. However, EBL supplementation negated the negative effects of drought and improved the chlorophyll content, Fv/Fm value and ΦPSII. Similarly, brassinosteroids protected PSII and maintained the integrity of thylakoid membrane under salt stress in *Solanum melongena* (Wu *et al.,* 2012). Ahammed *et al.,* (2013) observed that Fv/Fm, chlorophyll content, Pn and their related parameters (Gs, Ci, WUE, and E) were decreased in tomato exposed to Cd and phenanthrene stress. EBR application alleviated the oxidative damage of these pollutants by enhancing the photosynthesis and photochemistry of PSII. Chilling stress

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depressed the Fv/Fm, chlorophyll content, PN and their related parameters (Gs, Ci, WUE, and E), while the application of HBL improved the Chl content, PN and their related parameters and the Fv/Fm ratio in stressed as well as in the non-stressed plants (Fariduddin *et al.,* 2011). With increasing concentrations of salt *Brassica juncea* plants exhibited a decrease in various photosynthetic attributes (P, Gs, Ci, and E) and quantum efficiency of PSII (Fv/Fm and ΦSII).

Recently, Yusuf *et al.,* (2017) observed that the presence of excess aluminum and salt individually and in combination significantly decreased the rate of net photosynthesis and maximum quantum yield of PS II (Fv/Fm) along with the activities of Rubisco in both *Triticum aestivum* cultivar LOK-1 (Salt tolerant) and 502 (Salt-sensitive). However, seed soaking, and foliar mode of EBL supplementation significantly countered the damage caused by the combined stress of Al and salt through enhanced photosynthesis rate, maximum quantum yield of PS II (Fv/Fm) and activity of Rubisco. The plants of *Vigna radiata* c.v. T-44 exposed to high temperature and/or NaCl exhibited a significant decline in growth, photosynthetic parameters and a maximum quantum yield of PSII.

Further to know the effect of HBL on CO_2 fixation in plants grown in the soil amended with NaCl and/or Cd, we have measured the activities of carbonic anhydrase (CA), ribulose 1,5-bisphosphate carboxylase (RuBPcase) and phosphoenolpyruvate carboxylase (PEPcase). CA, an enzyme responsible for conversion of HCO^{3-} to CO_2 for the RuBPcase reaction and conversion of CO_2 to HCO^{3-} for PEPcase reaction (Yusuf *et al.,* 2011).

Our experimental data showed that maize grown in soil amended with Cd and/or NaCl significantly lowered the activities of CA, RuBPcase and PEPcase (Fig.3A-C). Similarly, Wani *et al.,* (2017) observed the decreased activities of CA and RuBPcase in chickpea cultivars under Cd and/or NaCl alone and combined stress. The reported decrease in the activity of CA and RuBPcase by Cd is also in agreement with others (Qian *et al.,* 2009; Guo *et al.,* 2016) while salinity with Sobhanian *et al.,* 2010 and Guo *et al.,* 2016). It has been reported that, both Cd and NaCl stress show the negative effects on CO_2 fixation by decreasing the activities of CA, RuBPcase and PEPcase (Qian *et al.,* 2009). This may be an after effect of the inhibition and/or metabolic dysfunction of the enzyme protein.

Salt stress has been shown to alter protein expression and content of key photosynthetic enzyme, RuBPcase and CA results in decline in photosynthesis during stress conditions (Sobhanian *et al.,* 2010; Liu *et al.,* 2011; Killi and Haworth 2017). Cd hampers calvin cycle by slowing down activity of various enzymes hence resulting in decreased photosynthesis (Ying *et al.,* 2010). Cd has also been known to show inhibitory effect on various enzymes such as RuBPcase, PEPcase, aldolase, fructose-6-phosphate kinase, fructose-1,6-bisphosphatase, $NADP^{+}$-glyceraldehyde-3-phosphate dehydrogenase and carbonic anhydrase (Mobin and Khan, 2007; Latif, 2008; Parmar *et al.,* 2013). It has been shown that Cd^{2+} ions lower the activity of RuBPcase and damage its structure by substituting for Mg^{2+} ions, which are important cofactors of carboxylation reactions, and may also shift RuBPcase activity towards oxygenation reactions (Siedlecka *et al.,* 1998; Kranteva *et al.,* 2008). However, foliar application of HBL enhanced the activity of RuBPcase (Fig. 2B), the major enzyme of calvin cycle and activity of CA, enzyme responsible for increasing the availability of CO_2 for rubisco binding. These observations corroborate with increased Pn efficiency, and increased sucrose, soluble sugars and starch contents in maize under Cd and/or NaCl stress (Fig.1A; Fig.5 A-D).

In consistence with these observations, Zhang *et al.,* (2008) reported that EBL application increased the quantum efficiency of PSII and the activities of RuBPcase and PEPcase under drought stress in soybean resulted in improved photosynthesis. BR treatment increased CO_2 assimilation through regulating the expression of Rubisco sub units and other calvin cycle enzymes. BR, increase the sucrose, soluble sugars and starch contents occurs subsequently with an increase in activities of sucrose phosphate synthase (SPS), sucrose synthase (SS) and acid invertase (AI) (Yu *et al.,* 2004). Xia *et al.,* (2009) showed that expression levels of Rubisco large sub-unit (rbcL) and other photosynthetic genes were down regulated in the presence of Brz, a BR synthesis inhibitor. In addition, EBL application enhanced the expression of calvin cycles genes, which increased the carboxylation efficiency of Ribisco in *Cucumis sativus*. *Analysis of Arabidopsis* BR insensitive or deficient mutants (*bri1-116*) showed the enlarged thylakoids, and smaller photosystem II super complexes strongly inhibited oxygen evolution led to reduced photosystem II quantum yield compared to the wild plants, suggesting that an optimal BR level is required for the normal thylakoid structure and function. A high BR level promotes photosynthesis mainly through regulating the nonstomatal factors in the transgenic tomato plants overexpressing *Dwarf*, a BR biosynthetic gene that encodes the *CYP85A*. However, the reduction in photosynthetic rate in BR biosynthetic mutant d^{im} was accompanied with the reduced stomatal conductance and intercellular CO_2 concentration, indicating that the CO_2 diffusion through stomata is at least one of the targets through which BR regulates photosynthetic rate (Li *et al.,* 2016)

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Recently, it has been observed that BR biosynthetic mutant *d* ^{im} inhibited in vivo carboxylation efficiency of Rubisco (Vc, max) and regeneration rate of RuBPcase (Jmax). However, over expressing Dwarf, a BR biosynthetic gene that encodes a CYP85A1, significantly increased Vc, max and Jmax, indicating that BR regulates the activity of RuBisCO mainly through increasing the activation state (Li *et al.,* 2016). A high BR level promotes photosynthesis mainly through regulating the non-stomatal factors in the transgenic tomato plants over expressing *Dwarf*, a BR biosynthetic gene that encodes the CYP85A. However, the reduction in photosynthetic rate in BR biosynthetic mutant *d* ^{im} was accompanied with the reduced stomatal conductance and intercellular CO_2 concentration, indicating that the CO_2 diffusion through stomata is at least one of the targets through which BR regulates photosynthetic rate (Li *et al.,* 2016)

Our data suggest that foliar application of HBL protect the photosynthetic machinery of plants against Cd and/or NaCl-stresses (Fig.1&2). Thus the enhanced quantum yield of photosystem II and CO_2 assimilation rate could be attributed to the role of BRs on photosynthetic apparatus; it seems that BRs affect the net photosynthetic rate by enhancing the activity of RuBPcase, the major enzyme of calvin cycle and activity of CA, enzyme responsible for increasing the availability of CO_2 for Rubisco binding. Increase in the E by BRs increases the CO_2 intake. Apart from it, BRs increases the light-capturing efficiency of plants and chlorophyll content which ultimately results in increasing the net photosynthetic rate (Siddiqui *et al.,* 2018).

HBL regulates the carbohydrate metabolism in maize under NaCl and/or Cd stress

Abiotic stresses such as mineral toxicity, salinity, water deficiency and heat stress adversely affect the carbohydrate metabolism in plants (Devi *et al.,* 2007; Das *et al.,* 2018). In many plant species, accumulation of soluble sugars occurs to counteract stressful environment through osmotic alterations (Rosa *et al.,* 2009; Neeta and Shitole, 2010). Sucrose is primary end product of photosynthesis and is major form of translocated carbon (Singh *et al.,* 2015) whereas starch comprises the temporary reserve form of carbon which gets finally stored in the grains (Ruan, 2014). The enzyme sucrose phosphate synthase (SPS) catalyses sucrose biosynthesis in the plant tissues whereas sucrose synthase (SS) and acid invertase (AI) involved in sucrose-cleavage in vivo and translocating the assimilates to diverse pathways in plant storage cells (Rosa *et al.,* 2009; Ruan, 2014).

In the present study, the results show that NaCl or/and Cd stress significantly elevated the levels of glucose and fructose, whereas sucrose and starch levels were considerably decreased in maize plants in comparison to control (Fig.5C&D). Increase in glucose and fructose levels were accompanied by significant increase in sucrose breakdown enzymes i.e. AI and SS under Cd or/and NaCl stress (Fig.4B&C). NaCl and Cd alone and combined NaCl+Cd stresses were significantly inhibited the sucrose biosynthesis by decreasing the SPS activity (Fig.4A). This may indicate the degradation of sucrose to glucose and fructose was increased, whereas its biosynthesis was inhibited under NaCl or/and Cd stress.

The present study also showed a decrease in starch contents under NaCl or/and Cd stress which may occur due to starch degradation, or reduced synthesis of starch in order to counteract NaCl or/and Cd stress (Fig. 6A&B). Accumulation of glucose, sucrose, and fructose during stressed conditions plays a very important role in carbon storage, osmotic regulation, and homeostasis, as well as scavenging of free radicals (Singh *et al.,* 2015). These maintain the osmotic potential as well as involve in redox reactions and contribute in maintaining the structures of macromolecules and membranes (Singh *et al.,* 2015). Findings of present study are in coherence with the observations of Gengmao *et al.,* (2014), where carbohydrates were reported to increase in *Salvia miltiorrhiza* plants under NaCl toxicity. Similarly, elevated levels of glucose, fructose and sucrose were observed in *Brassica juncea* plants under Cd toxicity (Kapoor *et al.,* 2016). Glucose and fructose are involved in maintaining osmotic potential and scavenging free radicals in *Oryza sativa* (Pattanagul and Thitisaksakul, 2008). Furthermore, soluble sugars are also involved in ROS anabolism and catabolism, such as the oxidative pentose phosphate pathway associated with ROS scavenging (Couée *et al.,* 2006).

The exogenous application of HBL further enhanced the glucose and fructose levels in NaCl or/and Cd stressed plants (Fig.1B&C). Moreover, the decrease in sucrose and starch fractions in NaCl or/and Cd stressed plants was significantly restored by 28-HBL treatment. HBL alone applications also accounted for significant increase in the carbohydrate fractions in maize plants (Fig.1 B&C). In the present study, starch level was increased by HBL in maize leaves under NaCl or/and Cd stress (Figs-1B). Assimilated CO_2 during photosynthesis can be used immediately or stored as starch, and both newly fixed and stored carbon are essential for maintaining export from mature leaves to roots. Rahman and Krishna, (2010) demonstrated the EBL treatment induced the starch biosynthesis by up-regulating the related gene expression. The increased carbohydrate pools may attribute to the up regulation of sucrose metabolism and biosynthesis enzymes by HBL treatment. In agreement with this, a BR-deficient *Arabidopsis* mutant has been found to have decreased starch and sucrose contents, and reduced activities of invertase as compared with the wild type (SchluÈter *et al.,* 2002). Yu *et al.,* (2004)

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reported that application of 24-EBL significantly increased in Rubisco activity and in the sucrose, soluble sugars and, starch contents followed by substantial increases in sucrose phosphate synthase, sucrose synthase and acid invertase in cucumber plants grown in a greenhouse.

The observed increase in the carbohydrate fractions in maize due to HBL application might have been due to increased net photosynthetic rate or its role in regulation of photosynthesis by sugar-signal-induced feedback regulation. It is likely that increased photosynthetic CO_2 assimilation provided more carbohydrate for metabolism and export to sink. Sink strength could be stimulated due to direct effects of enhanced substrate availability, and also through stimulation of the expression of genes encoding enzymes involved in the carbohydrate metabolism. According to our results, it can be concluded that HBL alters the profile and content of sugars in plants, which might stimulate the sugar signal under stress conditions.

Conclusion

The present study shows that maize plants under cadmium and salt stress, photosynthetic activity was reduced by effecting enzymes associated with it. But 28-homobassinolide application increased photosynthetic activity and carbohydrate content even under stress condition. Exogenous application of HBL promotes the growth and development of maize plant under different stress conditions. Further research is required for the detailed analysis

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