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Application of Brassionsteroids Reverses the Inhibitory Effect of Salt Stress on Growth and Photosynthetic Activity of *Zea mays* Plants

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ABSTRACT: The present study was conducted to investigate the effect of 28-homobrassinolide (HBL) on growth and photosynthetic activity of *Zea mays* plants (30 and 60 daysold) subjected to different NaCl concentration (0, 40, 60, 80,100 mM). The parameters examined were growth (root length, shoot length, number of leaves), photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, anthocyanin and xanthophyll content) and gaseous exchange parameters (photosynthetic rate (A), stomatal conductance (gs), intercellular CO_2 concentration (Ci), transpiration rate (E) and water use efficiency (A/E). The result of present study revealed that growth and photosynthetic activity was reduced under salinity stress whereas the treatment of HBL reversed the inhibitory effects of salt stress by improving the growth and photosynthetic activity.

Keywords: 28-homobrassinolide, salt stress, maize, photosynthesis.

I. INTRODUCTION

Maize is the important cereal crop which fulfills the need of food and oil for human intake. It is also used as feed for livestock [1], throughout the world but the production and yield of this crop has been adversely affected due to salt stress as it is a major constraint to this crop. Salt stress causes worldwide huge loss in agricultural yield and production. Out of world's 5.2 billion ha of dryland, 3.6 billion ha land already suffers from the salt stress which isa foremost issue of concern [2]. The most prominent symptom of salinity stress is the plant growth reduction. Salt stress affects the plant growth and development by interfering with the normal physiological processes especially the photosynthesis [3].During salt stress, accumulation of Na^+ and Cl^- ions severely inhibits the photosynthetic enzymes, obscures the oxidation of NADPH and restoration of $NADP^+$ by affecting electron transport chain and result in production of ROS in PS1 reaction center [4]. The salinestress induced reduction of yield includes the imbalancing of evapo-transpiration rate, alteration in root density, root turgor pressure and root growth, which causes hindrance in water absorption [5]. High salt concentration impedes with the process of photosynthesis by altering the ultrastructure of the organelles, inhibited the synthesis of pigments and enzymes, reduced the rubisco activity, stomatal conductance, CO_2 availability and photosynthetic enzyme activity [6-8].

Brassinosteroids (BRs) are the plant-specific polyhydroxylated steroidal hormones which regulate a broad range of physiological processes and various aspects of plant growth and development, such as vascular system differentiation, cell division and elongation, and sex determination [9]. Among various diverse roles, BRs play important role in regulation of photosynthesis in plants. BRs application improved the photosynthetic rate under different abiotic stresses has been reported in various crops i.e. mustard [10], mung bean [11], wheat [12], eggplant [13] and cucumber [14]. Therefore the aim of present study was to investigate the effect of BRs on growth and photosynthetic efficiency of *Zea mays* plants subjected to salt stress, with a view to better understanding the role of BRs in regulation of photosynthesis.

II. MATERIAL AND METHODS

Certified seeds of *Zea mays* (var. DKC 9106) were surface sterilized with 0.03% mercuric chloride for 2 min followed by repeated rinses with sterile distilled water. Seeds were soaked in aqueous solution of HBL (10^{-10}, 10^{-8} and 10^{-6}M) for 12 hours. The field area was divided into randomised blocks.

Each block salinized with different concentrations of NaCl (0, 40, 60, 80 and 100 mM). Plants harvested after 30 days and 60 days were used for the estimation of morphological parameters, pigments and gas exchange parameters. Pigments were estimated by using double beam spectrophotometer and gas exchange characteristics were measured through Infra-Red Gas Analyzer (IRGA).

A. Growth characteristics

Plants of *Zea mays* were harvested on 30th and 60thday and their root length, shoot length and no. of leaves were recorded.

B. Gaseous exchange measurements

Photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2 concentration and water use efficiency were measured by a photosynthesis system, IRGA (LI-6400, LICOR Inc., Lincoln, NE, USA). During the measurements, photosynthetic photon flux density was set to 1000µmol m^{-2} s^{-1}, the air relative humidity was about 80-90 %, the leaf temperature was maintained at 25^{o}C and the ambient CO_2 concentration was about 400 µmol mol^{-1}. Measurement of photosynthesis was repeated in triplicate for each treatment and performed within the time period 9.00 a.m.–11.00 a.m.

C. Pigments content

(i) Chlorophyll content: Chlorophyll content was estimated according to the method given by Arnon, [15]. 1g fresh plant tissue was homogenized by using 80 % acetone (4ml). The crushed plant material was subjected to centrifugation for 20 minutes at 13,000 rpm at 4^{o}C. The supernatant was collected for the analysis of chlorophyll a, b and total chlorophyll content and the absorbance was taken spectrophotometrically at 645 and 663nm.

(ii) Carotenoid content: Carotenoid content was estimated by method of Maclachlan and Zalik, [16]. Fresh shoot tissue (1g) was homogenized in chilled pestle and mortar using% acetone. Then centrifugation was carried out for 20 minutes at 13,000 rpm. The supernatant was collected and absorbance was taken at 480 and 510nm.

(iii) Anthocyanin content: Anthocyanin content was determined by following the method given by Macinelli, [17]. 1gm fresh plant material was homogenized with 3ml of extraction mixture (methanol: water: HCl, 79:20:1). Homogenized material was centrifuged for 20 minutes at 13,000 rpm at4^{o}C and supernatant was collected for the analysis of anthocyanin content. The absorbance of the supernatant was taken at 530 and 657nm.

(iv) Xanthophyll content: Xanthophyll content was estimated by themethod purposed by Lawrence, [18]. 0.05g of dried plant material was homogenized with30 ml of extraction mixture in 100 ml of flask. Then flask was refluxed on water bath at 56^{o}C, followed by cooling. After cooling, flask containing samples were kept under dark for 1 hour. Then pipette 30ml of hexane into the flask and shake for 1 minute. After that volume was made up with 10 % sodium sulphate solution. The upper phase was collected in 50 ml volumetric flask and the volume was made up by hexane and measured spectrophotometrically at 474 nm.

III. RESULTS

Salinity had a detrimental effect on morphological parameters (root length, shoot fresh and no. of leaves) of both 30 and 60 daysold *Zea mays* plants. The observations made on 30 days morphological parameters indicated that increasing salt stress negatively affected the plant growth by decreasing root length (1.96 times), shoot length (1.49 times) and no. of leaves (1.428 times) in comparison to control. Supplementation of alone HBL at concentrations of 10^{-10}M (root length 9.2 cm, shoot length 50.50 cm and no. of leaves 6.666), 10^{-8}M (root length 9.266 cm, shoot length 55.33 cm and no. of leaves 8.0) and 10^{-6}M (root length 8.363 cm, shoot length 43.26 cm and no. of leaves 7.666) significantly improved the growth parameters. Similarly treatment of HBL in conjunction with NaCl, showed the increase (1.458 times) of root length, shoot length (2.078 times) and no. of leaves (1.657 times) under salt stress.

In 60 days old plants, similar observations were recorded. Maximum decrease of root length (2.72 times), shoot length (0.645 times) and no. of leaves (1.035 times) was observed under 100mM salt stress as compared to control. Treatment of HBL plus NaCl showed the maximum increase of root length (1.201 times) under 40mM salt stress, shoot length (1.681 times) under 80mM and no. of leaves (1.20 times) under 60 mM salt stress.

Table 1. Effect of HBL on Root length (cm), Shoot length (cm) and No. of leaves of 30 days old plant of *Zea mays* subjected to salt stress.

Treatments	Root length	Shoot length	No.of Leaves
0M	**8.233**±0.088	**38.33**±4.409	**6.666**±0.333
10^{-10}M HBL	9.200±0.115	50.50±2.291	6.666±0.333
10^{-8}M HBL	9.266±0.176	55.33±2.027	8.000±0.000
10^{-6}M HBL	8.363±0.036	43.26±3.777	7.666±0.333
40 mM NaCl	**7.200**±0.115	**36.33**±1.763	**6.000**±0.000
10^{-10}M HBL+40 mM NaCl	8.166±0.088	46.2±1.474	6.666±0.333
10^{-8}M HBL+40 mM NaCl	9.233±0.145	47.01±1.154	6.666±0.333
10^{-6}M HBL+40 mM NaCl	10.50±0.288	46.00±1.732	6.000±0.000
60 mM NaCl	**7.066**±0.066	**34.30**±2.516	**5.330**±0.333
10^{-10}M HBL+60 mM NaCl	7.533±0.202	36.33±1.763	8.833±0.333
10^{-8}M HBL+60 mM NaCl	8.166±0.166	45.66±2.027	8.000±0.000
10^{-6}M HBL+60 mM NaCl	7.4±0.264	39.33±1.453	6.000±0.000
80 mM NaCl	**4.200**±0.200	**28.33**±1.763	**5.000**±0.000
10^{-10}M HBL+80 mM NaCl	5.233±0.145	47.09±1.270	6.000±0.000
10^{-8}M HBL+80 mM NaCl	7.500±0.264	40.33±1.201	8.000±0.000
10^{-6}M HBL+80 mM NaCl	4.933±0.033	28.66±2.02	6.000±0.000
100 mM NaCl	**4.333**±0.333	**25.66**±1.763	**4.666**±0.333
10^{-10}M HBL+100 mM NaCl	6.3±0.208	53.33±0.881	8.000±0.000
10^{-8}M HBL+100 mM NaCl	8.366±0.272	32.33±1.453	6.000±0.000
10^{-6}M HBL+100 mM NaCl	5.266±0.176	40.66±0.881	6.333±0.666
	F-ratio	**F-ratio**	**F-ratio**
Treatment(28-HBL)	**32.203***	**116.49***	**3.245**
Dose (NaCl)	**13.231***	**18.137**	**32.472***
Treatment× Dose	**6.789***	**6.2607***	**5.753***

***Indicate statistically significant differences from control at p≤0.05**

Salt stress adversely affected the photosynthesis by decreasing the chlorophyll a, b, total chlorophyll, carotenoid, anthocyanin and xanthophyll content of 30 days old *Zea mays* plants. About 4.0 times decrease of chlorophyll **a** content was observed under salt stress as compared to control (Table 3). Similarly 2.17 times chlorophyll b and 3.18 times total chlorophyll content was observed to decreased under salt stress (Table 3).

On the other hand, seed presoaking treatment of HBL alone significantly enhanced the chlorophyll a, b and total chlorophyll content. Furthermore HBL along with NaCl solution enhanced the chlorophyll a (1.934 times) and chlorophyll b (1.739 times) content under 40mM salt stress and total chlorophyll content (1.782 times) under 80mM salt stress.

Table 2. Effect of HBL on Root length, Shoot length and No. of leaves of 60 days old plant of *Zea mays* subjected to salt stress.

Treatments	Root length	Shoot length	No. of leaves
0M	**16.5**±o.134	**208**±3.055	**9.66**±0.333
10^{-10}M HBL	17.3±0.234	217±0.88	10.33±0.333
10^{-8}M HBL	17.0±0.135	222±1.453	10.66±0.666
10^{-6}M HBL	17.9±0.234	216±1.453	10.33±0.333
40 mM NaCl	**12.4**±0.145	**193**±1.527	**9.333**±0.333
10^{-10}M HBL+40 mM NaCl	14.9±0.324	155±1.756	9.666±0.333
10^{-8}M HBL+40 mM NaCl	14.7±0.089	195.3±1.45	10.00±0.000
10^{-6}M HBL+40 mM NaCl	12.33±0.145	185.5±1.32	10.00±0.000
60 mM NaCl	**8.9**±0.378	**155**±0.577	**10.00**±0.000
10^{-10}M HBL+60 mM NaCl	12.33±0.284	174±1.154	12.00±0.000
10^{-8}M HBL+60 mM NaCl	13.1±0.635	229.5±1.322	10.66±0.666
10^{-6}M HBL+60 mM NaCl	11.13±0.635	216.6±1.201	11.00±0.000
80 mM NaCl	**8.2**±0.173	**138.8**±0.726	**9.666**±0.333
10^{-10}M HBL+80 mM NaCl	9.866±0.145	232.3±1.453	11.66±0.881
10^{-8}M HBL+80 mM NaCl	8.766±0.296	227.3±0.881	10.00±0.000
10^{-6}M HBL+80 mM NaCl	9.433±0.120	158.3±1.201	11.00±0.000
100 mM NaCl	**6.066**±0.066	**134.3**±3.844	**9.333**±0.333
10^{-10}M HBL+100 mM NaCl	6.366±0.072	186.8±0.927	10.66±0.666
10^{-8}M HBL+100 mM NaCl	6.6±0.208	133.1±0.600	9.333±0.666
10^{-6}M HBL+100 mM NaCl	6.166±0.088	204.6±1.453	11.00±0.000
	F-ratio	**F-ratio**	**F-ratio**
Treatment(28-HBL)	**12.20***	**16.49***	**18.245***
Dose (NaCl)	**15.23***	**11.17***	**22.472***
Treatment× Dose	**8.260***	**6.889***	**5.753***

***Indicate statistically significant differences from control at $p \leq 0.05$**

Carotenoid content was found to be decreased with increasing concentration of salt stress as compared to control. Maximum decrease in carotenoid content was observed 3.003 times under 100mM salt stress (Table 3). Furthermore presoaking treatment of HBL (10^{-8}M) plus NaCl (40mM) increased the carotenoid content 1.353 times as compared to 40 mM salt concentration only. Similar trend was observed in anthocyanin and xanthophyll content. It was decreased under salt stress in comparison to control. Salt stress decreased the anthocyanin content 1.08 times and xanthophyll content 1.45 times as compared to control**.** However supplementation of HBL along with NaCl stress improved the anthocyanin content 1.340 times under 40mM salt stress and xanthophyll content 1.60 times under 60mM salt stress.

Similar observations were recorded in 60 days old plants. Maximum decrease of Chlorophyll a, b and total chlorophyll content was observed under 100 mM salt stress. Further plants fed with HBL plus NaCl showed the maximum increase of chlorophyll a (1.610 times under 60 mM salt stress), b (2.500 times under 100 mM salt stress) and total chlorophyll content (1.382 times under 60mM salt stress) as compared to respective salt concentration only.

Similarly carotenoid (1.546 times), anthocyanin (1.683 times) and xanthophyll (1.144 times) content were also found to be decreased in comparison to control. However treatment of HBL with NaCl showed the maximum increase of carotenoid (2.130 times) and xanthophyll content (1.462 times) times) under 60mM salt stress and anthocyanin content (1.166) under 40mM salt stress.

Table 3. Effect of HBL on Chlorophyll a, b and Total Chlorophyll content, Carotenoid content, Anthocyanin content and Xanthophyll content (mg g^{-1} FW) of 30 days old seedlings of *Zea mays* subjected to salt stress.

Treatments	Chlorophyll 'a' content	Chlorophyll 'b' content	Total chlorophyll content	Carotenoid content	Anthocyanin content	Xanthophyl l content
0M	**0.064±0.008**	**0.037±0.003**	**0.102±0.008**	**1.535±0.022**	**0.202**±0.006	**10.74**±0.289
10^{-10}M HBL	0.065±0.005	0.061±0.001	0.127±0.004	1.639±0.041	0.224±0.007	11.01±0.213
10^{-8}M HBL	0.076±0.006	0.047±0.003	0.123±0.009	1.688±0.091	0.239±0.007	11.80±0.380
10^{-6}M HBL	0.068±0.006	0.048±0.003	0.116±0.006	1.619±0.058	0.223±0.004	11.18±0.181
40 mM NaCl	**0.046±0.003**	**0.023±0.001**	**0.070±0.005**	**1.391**±0.083	**0.191**±0.005	**9.156**±0.389
10^{-10}M HBL+40 mM NaCl	0.089±0.007	0.039±0.003	0.122±0.011	1.364±0.042	0.256±0.003	10.57±0.413
10^{-8}M HBL+40 mM NaCl	0.064±0.008	0.032±0.001	0.111±0.004	1.883±0.086	0.234±0.006	11.72±0.449
10^{-6}M HBL+40 mM NaCl	0.066±0.008	0.040±0.003	0.101±0.006	1.760±0.055	0.233±0.007	11.47±0.589
60 mM NaCl	**0.035±0.004**	**0.020±0.002**	**0.056±0.003**	**0.883**±0.005	**0.186**±0.003	**9.206**±0.420
10^{-10}M HBL+60 mM NaCl	0.048±0.003	0.027±0.002	0.075±0.002	1.104±±0.088	0.251±0.014	11.78±0.198
10^{-8}M HBL+60 mM NaCl	0.077±0.004	0.036±0.003	0.117±0.009	0.881±0.014	0.219±0.003	14.74±0.212
10^{-6}M HBL+60 mM NaCl	0.048±0.004	0.038±0.003	0.088±0.009	1.009±0.069	0.205±0.005	12.92±0.367
80 mM NaCl	**0.042±0.002**	**0.028±0.002**	**0.070±0.004**	**0.723**±0.054	**0.201**±0.002	**8.076**±0.279
10^{-10}M HBL+80 mM NaCl	0.044±0.003	0.023±0.003	0.071±0.017	1.222±0.101	0.224±0.005	9.580±0.640
10^{-8}M HBL+80 mM NaCl	0.084±0.004	0.039±0.002	0.123±0.003	0.709±0.057	0.219±0.006	12.28±0.953
10^{-6}M HBL+80 mM NaCl	0.053±0.002	0.038±0.002	0.091±0.002	0.867±0.026	0.225±0.002	10.50±0.584
100 mM NaCl	**0.016±0.003**	**0.017±0.002**	**0.032±0.006**	**0.511**±0.014	**0.194**±0.005	**7.380**±0.853
10^{-10}M HBL+100 mM NaCl	0.022±0.064	0.018±0.001	0.040±0.005	0.649±0.026	0.215±0.004	7.031±0.110
10^{-8}M HBL+100 mM NaCl	0.031±0.005	0.032±0.002	0.064±0.003	0.717±0.005	0.208±0.004	7.691±0.535
10^{-6}M HBL+100 mM NaCl	0.032±0.004	0.024±0.002	0.056±0.006	0.734±0.039	0.194±0.005	6.991±0.470
	F-ratio	**F-ratio**	**F-ratio**	**F-ratio**	**F-ratio**	**F-ratio**
Treatment(28-HBL)	**36.75***	**39.97***	**63.67***	**252.8***	**10.68***	**64.09***
Dose (NaCl)	**24.03***	**22.66***	**39.80***	**10.487***	**30.10***	**24.25***
Treatment × Dose	**4.346***	**5.364***	**5.863***	**8.918***	**5.353***	**5.073***

***Indicate statistically significant differences from control at $p \leq 0.05$**

Table 4. Effect of HBL on Chlorophyll a, b and Total chlorophyll content, Carotenoid content, Anthocyanin content and Xanthophyll content (mg g^{-1} FW) of 60 days old seedlings of *Zea mays* subjected to salt stress.

Treatments	Chlorophyll 'a' content	Chlorophyll 'b' content	Total chlorophyll content	Carotenoid content	Anthocyanin content	Xanthophyl l content
0M	**0.074**±0.004	**0.012**±0.001	**0.086**±0.005	**2.042**±0.014	**0.308**±0.003	**8.679**±0.773
10^{-10}M HBL	0.112±0.006	0.030±0.002	0.142±0.006	2.748±0.035	0.307±0.002	8.197±0.266
10^{-8}M HBL	0.108±0.003	0.019±0.001	0.127±0.003	3.254±0.118	0.331±0.003	13.54±1.211
10^{-6}M HBL	0.093±0.005	0.020±0.002	0.113±0.007	2.363±0.078	0.317±0.002	10.61±0.352
40 mM NaCl	**0.063**±0.002	**0.010**±0.001	**0.072**±0.001	**1.808**±0.043	**0.253**±0.002	**8.229**±0.875
10^{-10}M HBL+40 mM NaCl	0.067±0.003	0.011±0.001	0.078±0.004	2.283±0.168	0.268±0.002	10.92±0.195
10^{-8}M HBL+40 mM NaCl	0.068±0.004	0.013±0.002	0.082±0.006	2.041±0.028	0.295±0.007	10.17±0.196
10^{-6}M HBL+40 mM NaCl	0.055±0.022	0.019±0.002	0.074±0.003	2.900±0.051	0.262±0.003	10.75±0.856
60 mM NaCl	**0.059**±0.010	**0.015**±0.003	**0.081**±0.001	**1.445**±0.062	**0.232**±0.004	**9.377**±0.187
10^{-10}M HBL+60 mM NaCl	0.086±0.003	0.021±0.002	0.107±0.001	2.422±0.107	0.268±0.001	13.71±0.593
10^{-8}M HBL+60 mM NaCl	0.095±0.003	0.016±0.003	0.112±0.007	3.078±0.069	0.263±0.002	12.17±1.270
10^{-6}M HBL+60 mM NaCl	0.081±0.005	0.024±0.003	0.106±0.008	1.739±0.030	0.248±0.003	9.940±0.344
80 mM NaCl	**0.058**±0.004	**0.009**±0.001	**0.066**±0.003	**1.320**±0.062	**0.213**±0.002	**7.757**±0.324
10^{-10}M HBL+80 mM NaCl	0.077±0.002	0.014±0.002	0.092±0.006	1.599±0.029	0.245±0.003	10.73±0.578
10^{-8}M HBL+80 mM NaCl	0.093±0.003	0.015±0.003	0.108±0.006	1.825±0.054	0.222±0.003	8.688±0.398
10^{-6}M HBL+80 mM NaCl	0.066±0.004	0.016±0.048	0.081±0.007	1.669±0.030	0.225±0.002	7.794±0.319
100 mM NaCl	**0.048**±0.008	**0.010**±0.002	**0.061**±0.006	**1.458**±0.030	**0.183**±0.005	**7.567**±1.159
10^{-10}M HBL+100 mM NaCl	0.084±0.006	0.015±0.003	0.099±0.002	2.326±0.060	0.219±0.003	8.727±0.952
10^{-8}M HBL+100 mM NaCl	0.068±0.002	0.025±0.002	0.094±0.004	2.910±0.017	0.216±0.004	6.281±0.133
10^{-6}M HBL+100 mM NaCl	0.072±0.003	0.018±0.002	0.090±0.004	1.853±0.089	0.208±0.003	7.957±0.718
	F-ratio	**F-ratio**	**F-ratio**	**F-ratio**	**F-ratio**	**F-ratio**
Treatment(28-HBL)	**33.82***	**7.035***	**22.03***	**98.44***	**514.4***	**20.05***
Dose (NaCl)	**33.02***	**9.948***	**10.62***	**168.2***	**54.95***	**10.55***
Treatment × Dose	**3.175**	**2.435**	**3.565**	**27.53***	**7.142***	**7.021***

***Indicate statistically significant differences from control at p≤0.05**

Observations on gas exchange characteristics of 30 and 60 days old plants of *Zea mays* reveals that salinity stress negatively affected the photosynthetic efficiency of plants. In 30 days old plant, photosynthetic rate (1.206 times), stomatal conductance (1.941 times), transpiration rate (1.173 times) and intercellular CO_2 rate(1.784 times) was found to be decreased as compared to control. Water use efficiency was also found to decrease except in 40mM salt stress where it increased as compared to control. Application of HBL along with NaCl significantly overcome the salinity inhibited photosynthesis by increasing the photosynthetic rate (1.297), stomatal conductance (1.433 times), transpiration rate (1.336 times) and intercellular CO_2 rate (1.729 times) under salt stress. Application of HBL increased the water use efficiency under salt stress except under 40mM where it is found to decreased.

However in 60 days old plants maximum decrease of photosynthetic rate (1.457 times), transpiration rate (1.284 times) and intercellular CO_2 rate (1.360 times) was observed under 100mM salt stress whereas stomatal conductance (1.307 times) under 80 mM and water use efficiency (1.177 times) under 60mM salt stress was found to decreased as compared to control. Furthermore treatment of HBL in combination with NaCl stress showed maximum increase of photosynthetic rate (1.247 times) under 60mM salt stress, stomatal conductance (2.368 times), intercellular CO_2 rate (1.231 times), transpiration rate (1.092 times) under 40mM salt stress and water use efficiency (1.23 times) under 100mM salt stress.

V. DISCUSSION

Decrease of plant biomass was commonly induced by salinity stress [19]. Salinity can affect growth of plant in various ways. Firstly, salt reduced the water uptake ability of plants and causes the quick reduction in the growth rate [20]. Secondly salt accumulated in the leaves, leading to salt toxicity in the plants and ultimately affect the yield and biomass. Salt stress also resulted in nutritional disorders which affect the availability, absorption and transport of nutrients within the plant [21]. Nutrient deficiency as well as ion toxicity and osmotic stress are factors attributed to the deleterious effect of salinity on plant growth and productivity [22]. The other causes of growth rate reduction under salt stress involve the inadequate photosynthesis owing to stomatal closure and consequently limited carbon dioxide uptake and resulted the retarded growth [23].

In the present study, decrease of root length, shoot length and no. of leaves was observed which results in reduction of growth. However treatment of HBL significantly improved the salinity inhibited growth by increasing the root length, shoot length and no. of leaves. Application of BRs reverses the inhibitory effect of salt stress on seedling growth by enhancing the levels of nucleic acids, soluble proteins content and carbohydrate content [24]. Further BRs also overcome the salinity inhibited growth by enhancing the photosynthetic pigments, increasing the RWC and uptakement of macro and micronutrients [25, 26], which reveals the growth stimulating role of BRs under salt stress.

Photosynthesis is a good indicator of the harmful effects of salt stress as growth inhibition due to salinity, associated with inhibition of photosynthesis. Higher stomatal conductance resulted the enhanced photosynthetic rate which further increased the CO_2 diffusion into the leaves and maintain the photosynthetic efficiency. However salt stress reduced the photosynthesis by inducing the leaf stomatal closure which decreased the CO_2 availability as a result of the diffusion limitations of stomata [27, 28]. Similarly salt stress reduced the photosynthetic rate by decreasing the mesophyll conductance [29]. Stress resulted in dehydration of mesophyll cells and inhibited the basic metabolic processes of photosynthesis as well as causes reduction of plant water use efficiency and reduced the efficiency of mesophyll cells to utilize the available CO_2 [30, 31, 32, 33]. Stomatal closure minimize the loss of water through transpiration and this affects the light-harvesting and energy-conversion systems thus leading to alteration in chloroplast activity [34]. In the present study, decrease of photosynthetic rate, stomatal conductance, transpiration rate and intercellular rate were recorded under salt stress which indicated that salinity induced damage inhibited the photosynthetic efficiency (Table 5, 6) whereas BRs application increased the efficiency of photosynthesis by elevating the level of CO_2 assimilation and rubisco activity [35]. BR-induced increase of stomatal conductance was closely correlated with the improvement of net photosynthesis. BRs treatment in tomato plant under water stress enhanced the stomatal conductance, net photosynthetic rate and CO_2 intakement suggest the role of BRs in amelioration of salt stress [36].

Reduced photosynthesis under salinity stress not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also included the non-stomata factors such as photosynthetic enzymes and pigments [7]. Alteration of photosynthetic pigment biosynthesis is one of the notable effects of salt stress [37]. Salinity affected the photosynthesis by decreasing the pigments which play key role in photosynthesis. Under high salt stress, Na^+ and Cl^- ions gets accumulated in chloroplasts and affect the photosynthesis by affecting the photosynthetic electron transport, carbon metabolism or photophosphorylation [3].

Table 5. Effect of HBL on Photosynthetic rate (µmol $CO_2/m^2/s$), Stomatal conductance ($mol/m^2/s$), Transpiration rate (mmol $H_2O/m^2/s$), Intercellular CO_2rate (µmol/mol) and Water use efficiency (mmol CO_2/mol H_2O) of 30 days old plants of *Zea mays* subjected to salt stress.

Treatments	Photosynthetic rate	Stomatal condctance	Transpiration rate	Intercellular CO_2 rate	Water use efficiency
0M	**15.63**±0.589	**0.132**±0.035	**1.056**±0.026	**163**±3.511	**14.80**±0.674
10^{-10}M HBL	18.00±0.493	0.109±0.004	1.083±0.043	201.3±10.68	16.85±0.928
10^{-8}M HBL	16.26±0.317	0.197±0.004	1.133±0.029	192.6±4.055	14.35±0.158
10^{-6}M HBL	16.13±0.497	0.090±0.005	1.093±0.040	172.6±5.487	14.90±0.389
40 mM NaCl	**15.62**±0.783	**0.083**±0.008	**0.991**±0.004	**139**±5.196	**15.76**±0.754
10^{-10}M HBL+40 mM NaCl	15.7±0.416	0.105±0.003	1.068±0.037	139±1.527	14.69±0.193
10^{-8}M HBL+40 mM NaCl	15.93±0.491	0.120±0.020	1.109±0.002	153±4.933	14.35±0.424
10^{-6}M HBL+40 mM NaCl	15.36±0.554	0.126±0.008	1.102±0.006	149.3±5.487	13.92±0.444
60 mM NaCl	**13.10**±0.493	**0.090**±0.002	**0.942**±0.030	**116.6**±4.630	**13.90±0.400**
10^{-10}M HBL+60 mM NaCl	15.20±0.513	0.114±0.005	1.109±0.003	128.6±4.977	15.30±0.095
10^{-8}M HBL+60 mM NaCl	15.76±0.523	0.112±0.003	1.116±0.002	136.3±4.807	14.14±0.478
10^{-6}M HBL+60 mM NaCl	17.00±0.115	0.129±0.001	1.110±0.003	110.6±4.630	13.69±0.453
80 mM NaCl	**13.76**±0.352	**0.073**±0.004	**0.982**±0.004	**109.8**±2.682	**14.02**±0.381
10^{-10}M HBL+80 mM NaCl	15.50±0.503	0.122±0.003	0.983±0.001	125.6±3.480	15.76±0.526
10^{-8}M HBL+80 mM NaCl	16.53±0.352	0.094±0.003	0.990±0.004	116.6±5.812	16.69±0.424
10^{-6}M HBL+80 mM NaCl	14.60±0.378	0.103±0.003	0.986±**0.003**	115±5.775	14.79±0.339
100 mM NaCl	**12.96**±1.039	**0.068**±0.004	**0.900**±0.002	**91.33**±4.096	**14.39**±1.131
10^{-10}M HBL+100 mM NaCl	15.06±0.987	0.117±0.001	0.912±0.002	151.3±2.728	17.31±0.651
10^{-8}M HBL+100 mM NaCl	15.66±0.393	0.103±0.005	0.945±0.003	158±4.359	16.19±0.281
10^{-6}M HBL+100 mM NaCl	15.80±0.568	0.118±0.003	0.936±0.003	99±2.309	17.13±0.088
	F-ratio	**F-ratio**	**F-ratio**	**F-ratio**	**F-ratio**
Treatment (28-HBL)	**5.264***	**11.60***	**41.96***	**118.7**	**5.780***
Dose (NaCl)	**11.89***	**61.42***	**30.62***	**39.58***	**1.666***
Treatment × Dose	**2.547***	**9.156***	**7.687***	**8.113**	**4.757***

***Indicate statistically significant differences from control at p≤0.05**

Chlorophyll a and b constitute an important part of photosynthetic apparatus due to their role as main light capturing molecules in photosynthetic light-harvesting antennae complexes [38]. Further treatment of HBL overcomes the negative effect of salt stress on photosynthesis by enhancing the level of pigment under stress condition. BRs application improved the net photosynthesis rate by increasing the efficiency of light capturing and enhanced the pigment level by inducing transcription and translation of the enzymes involved in chlorophyll biosynthesis [39]. Application of BRs enhanced the pigment (chlorophyll and carotenoid) content in *Lycopersicon esculentum* under drought stress [40]. Similarly Janeczko *et al,* [40] also reported the enhanced pigment content in *Brassica napus* under cold stress. Thus it was concluded from present study that application of BRs overcome the salinity induced damage by improving the growth and photosynthetic activity of salt stressed maize plants.

Table 6. Effect of HBL on Photosynthetic rate(μmol $CO_2/m^2/s$), Stomatal conductance ($mol/m^2/s$), Transpiration rate (mmol $H_2O/m^2/s$), Intercellular CO_2 rate (μmol/mol), and Water use efficiency (mmol CO_2/mol H_2O) of 60 days old plants of *Zea mays* subjected to salt stress.

Treatments	Photosynthetic rate	Stomatal condctance	Transpiration rate	Intercellular CO_2 rate	Water use efficiency
0M	**18.8**±0.529	**0.085+0.003**	**1.34+0.029**	**135.6**±3.480	**13.96**±0.484
10^{-10}M HBL	23.96±1.906	0.095±0.001	1.42±0.020	142±5.291	16.82±1.260
10^{-8}M HBL	28.76±0.589	0.089±0.007	1.436±0.018	152±5.773	20.02±0.182
10^{-6}M HBL	19.70±1.069	0.085±0.005	1.406±0.021	148±4.977	13.96±0.747
40 mM NaCl	**15.63**±0.545	**0.076**±0.002	**1.30+0.026**	**125.3**±2.906	**12.18**±0.535
10^{-10}M HBL+40 mM NaCl	18.10±0.415	0.090±0.004	1.42±0.015	138±4.359	12.75±0.423
10^{-8}M HBL+40 mM NaCl	18.10±0.416	0.180±0.006	1.336±0.027	154.3±2.96	13.55±0.502
10^{-6}M HBL+40 mM NaCl	17.86±0.606	0.086±0.006	1.376±0.029	125+2.886	12.98±0.456
60 mM NaCl	**14.53**±0.393	**0.080**±0.004	**1.22**±0.018	**127.6**±2.18	**11.86**±0.496
10^{-10}M HBL+60 mM NaCl	16.96±0.520	0.091±0.007	1.286±0.017	141.3±5.206	12.78±0.692
10^{-8}M HBL+60 mM NaCl	18.13±0.393	0.113±0.007	1.403±0.008	139±5.196	12.92±0.353
10^{-6}M HBL+60 mM NaCl	17.33±0.845	0.126±0.003	1.40±0.008	123.3±4.409	12.28±0.491
80 mM NaCl	**14.36**±0.371	**0.065**±0.008	**1.12**±0.008	**118.3**±4.409	**12.78**±0.243
10^{-10}M HBL+80 mM NaCl	18.03±0.393	0.134±0.003	1.20±0.011	121.6±4.05	13.97±0.443
10^{-8}M HBL+80 mM NaCl	17.96±0.323	0.097±0.003	1.22±0.015	118±4.359	14.73±0.433
10^{-6}M HBL+80 mM NaCl	16.66±0.622	0.107±0.006	1.34±0.025	152±5.773	12.45±0.615
100 mM NaCl	**12.90**±0.608	**0.075**±0.002	**1.043**±0.029	**99.66**±4.630	**12.39**±0.787
10^{-10}M HBL+100 mM NaCl	16.23±0.440	0.087±0.004	1.256±0.017	134.6±2.666	15.48±0.693
10^{-8}M HBL+100 mM NaCl	16.90±0.519	0.131±0.004	1.05±0.020	108.3±3.756	13.45±0.502
10^{-6}M HBL+100 mM NaCl	17.90±0.577	0.066±o.028	1.166±0.012	123.2±3.817	15.35±0.613
	F-ratio	**F-ratio**	**F-ratio**	**F-ratio**	**F-ratio**
Treatment(28-HBL)	**65.06***	**10.38***	**121.53***	**22.33***	**24.35***
Dose (NaCl)	**40.81***	**49.81***	**44.90***	**12.00***	**14.81***
Treatment × Dose	**6.266***	**17.83***	**7.477***	**7.592***	**5.025***

***Indicate statistically significant differences from control at p≤0.05**

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