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(*Cucumis sativus* L.) through various levels of 28-
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Regulation of Growth and Physiological traits of Cucumber (*Cucumis sativus* L.) through various levels of 28-Homobrassinolide under salt stress conditions

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Regulation of Growth and Physiological traits of Cucumber (*Cucumis sativus* L.) through various levels of 28-Homobrassinolide under salt stress conditions

ABSTRACT

Brassinoids are plant steroidal hormones which are ubiquitous in plant kingdom and regulate a wide range of physiological and stress responses. In a plastic tunnel, pot bioassay experiment, three levels of homobrassinolide (HBL) at 1, 3 and 5 μ M were tested on growth and physiological attributes of cucumber cultivars Jinyou 1[#] (salt sensitive) and Changchun Mici (salt tolerant), grown at various salt stress conditions (60 and 120 mM). Cucumber plants of both cultivars subjected to salt stress exhibited reduced growth attributes and altered antioxidant enzymes at 60 and 120 mM. However, the deleterious effects of salt stress were partially improved by HBL. The foliar application of HBL enhanced shoot and root fresh and dry weight, chlorophyll content at 60 mM and 120 mM NaCl stress in cv Jinyou 1[#] and cv Changchun Mici, respectively. HBL application also altered the antioxidant enzyme levels by increasing Super oxide dismutase (SOD), Peroxidase (POD) levels and decreased the Malondialdehyde content (MDA) under 60 and 120 mM stressed plants in both cultivars. Overall results showed that HBL could enhance growth attributes, chlorophyll content and antioxidant enzymes for both of the cultivars under mild and high NaCl stress.

Key words: 28-Homobrassinolide; Salt stress; Chlorophyll; Antioxidant enzymes, Plant physiology

Running Head: 28-Homobrassinolide attenuates salt stress in cucumber

INTRODUCTION

Cucumber (*Cucumis sativus* L.) a member of family *cucurbitaceae*, is one of the most important economic vegetables in the world (Liu et al. 2016) and is classified as salt sensitive (Stepien and Klobus 2006). It is grown in many countries throughout the world, however China is the main producer with the production of 54.3 million tons (<http://faostat.fao.org/>). Cucumber is consumed as fresh vegetable, pickled or cooked, as well as, it is also utilized in variety of cosmetics preparations and applications. However, its production is considerably altered due to biotic and abiotic stresses, among which salinity is major constrains against its production (Melorose et al. 2015).

Salinity is a major abiotic stress which acutely affects crop productivity (Ahmad et al. 2013) through osmotic stress, alter metabolism and disrupts plant physiology by accumulation of osmolytes, ionic toxicity (Munns 2002), protein dysfunction (Janicka-Russak et al. 2013), causes electrolyte leakage and eventually death of a tissue (Ali et al. 2008). Salinity induced oxidative stress, triggers the production of reactive oxygen species which causes a severe damage to plant cells. Therefore, the role of plant antioxidant enzymes super oxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) etc. have a key role in stress tolerance (Niu et al. 2016). These antioxidant enzymes are used to assess the severity of stress in plants (Mittler 2002).

Various physiological and agronomic practices (Hayat et al. 2010b) are applied to minimize salinity effect. The effective way to overcome this situation is to introduce salt tolerant cultivars (Khan et al. 2006; Yildirim et al. 2008) which have limitations due to lack of reliable traits during selection. However, due to genetic characteristics of these crops and unfavorable

conditions, the growth of such tolerant cultivars is at question. Therefore, natural and synthetic growth regulators are applied to plants under stress to improve their productivity (Baninasab and Baghbanha 2013). Salt tolerant plants adopt various morpho-anatomical, physiological and biochemical strategies to reduce damage and loss. Tolerant plants adjust osmotic stress by triggering the production of various osmolytes (free proline, glycine betaine, low molecular weight sugars) and maintain their turgor (Maqsood et al. 2008). These reducing and non-reducing sugars, free proline etc helps tolerant plants to avoid fatal damage and maintain turgor under salt and various abiotic stresses (Ahmad et al. 2013). So far significant progress has been made in understanding the fact that plant growth regulators results in a wide range of metabolic and physiological responses in plants which affect plant growth and development. Salicylic acid, jasmonic acid, abscisic acid, ethylene, auxin, gibberellins, cytokinins and brassinosteroids mechanisms have been studied under biotic and abiotic stress (Hayat et al. 2010a; An and Mou 2011; Baninasab and Baghbanha 2013). Several reports have shown that brassinoids increased stress tolerance under salinity (Fariduddin et al. 2014), high temperature (Hayat et al. 2010b; Niu et al. 2016) and heavy metal stress (Ali et al. 2008). However, among brassinosteroids, 28-homobrassinolide compound mechanism still needs to be studied under different salinity stress levels.

Brassinoids (BRs) are steroidal compounds occur in free form and conjugated to sugars and fatty acids. So far 70 BRs have been isolated from plants (Bajguz and Hayat 2009). BRs have a critical role in elongation and differentiation of cells and vascular tissue, pollen tube growth, proton pump activation, nucleic acid and protein synthesis, regulation of gene expression, flowering time, seeds production, yield (Divi and Krishna 2009; Hayat et al. 2010b) and also in enhancing stress tolerance by activation of antioxidant enzymes in plants under heavy metal

stress (Ali et al. 2008), salt stress (Özdemir et al. 2004; Hayat et al. 2010b; Fariduddin et al. 2014), temperature stress (Niu et al. 2016) and oxidative stress (Cao et al. 2005).

Studies have showed that brassinoids play a vital role in development and adaptation of plants under salt stress by increasing the antioxidant enzymes and decreasing the Malondialdehyde (MDA) levels under stress conditions in rice (Sharma et al. 2013). They also have a role in smooth germination process. The germination of BR biosynthetic mutants in *Arabidopsis* proved that BR signaling is necessary during germination process (Steber and McCourt 2001). Leaves treated with BRs have a high electron transport in photosynthesis II, increased activity of rubisco, soluble sugars and carbon dioxide assimilation in cucumber (Yu et al. 2004), activated antioxidant enzymes and photosynthetic activity in brassica under copper stress (Fariduddin et al. 2009), increased free proline content and decreased lipid peroxidation level under the presence of salinity and cadmium in wheat (Hayat et al. 2014). This study evaluates the role of foliar applied HBL in changes of antioxidant system and tolerance of two cucumber cultivars exposed to mild and high salinity stress and to dissect how far salt tolerant mechanisms along with HBL cope with adverse situation of salts.

MATERIALS AND METHODS

Plant Material

This study was conducted at Northwest A&F University, Yangling, Shaanxi, China, under the plastic tunnel. Two cultivars of cucumber (*Cucumis sativus*, L.) i.e., Jinyou 1[#] (salt sensitive) and Changchun Mici (salt tolerance) were used in this experiment. The healthy seeds selected and surface sterilized with 1% sodium hypochlorite solution followed by repeated washings with distilled water prior to sowing in plastic trays filled with organic substrate. Twenty days after

sowing (DAS) seedlings were transplanted to the pots (30 x 30 cm). Each pot contains 6 kg of soil, perlite and organic matter mixture (2:1:1 v/v) and were randomly placed inside the plastic tunnel.

28-Homobrassinolide Treatments

28-homobrassinolide (HBL, $C_{29}H_{50}O_6$ molecular weight of 494.70) was procured from Shanghai China. HBL stock solution was prepared initially by dissolving it in a little amount of 99.9% Ethanol as solvent and the concentrations 1, 3 and 5 μM were prepared by dilution and 0.02% of Tween 20 (polyoxyethylenesorbitan monolaurate) was added as a surfactant to enhance chemical absorption. Plants were sprayed with different levels of HBL the day after transplantation. The spray was performed on the lower and upper leaf surface, as the absorption by lower leaf surface is effective (Hajihashemi et al. 2007). Control plants were sprayed with distilled water with the same amount of 0.02% Tween20. Handheld sprayer was used for spraying the plants. Plants from each treatment received 100 ml of spray (Ekinici et al. 2012).

Salt Treatments

Stock solution was made for salt treatments (0, 60 and 120 mM) and the treatments were made by dilution factor. The salt free treatments were irrigated with tap water having electrical conductivity value 1.31 ds m^{-1} . The plants were irrigated with saline solutions till EC values reaches 7.13 and 11.46 ds m^{-1} . One hundred and eighty pots were used for each cultivar with five pots for each treatment. Treatments were from T1 to T12 for both cultivars Jinyou 1[#] and Changchun Mici, T1 plants having no treatments (control), T2 plants having 1 μM HBL only, T3 plants having 3 μM HBL only, T4 plants having 5 μM HBL only, T5 plants having 60 mM NaCl only, T6 plants having 60 mM salt + 1 μM HBL, T7 plants having 60 mM salt + 3 μM HBL, T8

plants having 60 mM salt + 5 μ M HBL, T9 plants having 120 mM NaCl only, T10 plants having 120 mM salt + 1 μ M HBL, T11 plants having 120 mM +3 μ M HBL and T12 plants having 120 mM salt + 5 μ M HBL. To maintain the salinity level after transplantation, all pots were irrigated with respective salt treatments up to field capacity. After 30 days of treatments plants samples were collected to measure chlorophyll content and antioxidant enzymes activity, superoxide dismutase (SOD), Peroxidase (POD) and MDA content.

Growth Variables

Thirty days after treatments, plants were harvested to measure growth variables for each replicate. Shoot/Root length were measured with measuring tape and shoot/root, fresh/dry weights per plant were measured.

Chlorophyll Content

The chlorophyll content was determined from fresh cucumber leaves samples of 0.2 g in 25 ml of 80% acetone and were placed at room temperature for 48 hrs in dark. The absorbance of the extract was recorded on spectrophotometer (UV-3802, UNICO, MDN, USA) on 665 and 645 nm wave length (Wellburn 1994).

Preparation of crude enzyme for enzymes and MDA content.

The crude extract for the determination of antioxidant enzymes, (Gao 2006) method was followed with some modifications. Leaf samples (0.5 g) were placed in pre-chilled mortar and pestle and crushed having 6 ml of 0.05 M phosphate buffer (pH 7.8). The entire mixture was centrifuge at 10,000 x g for 20 min and 4°C. The supernatant obtained was used to performed the enzymes activities and MDA content for each treatment in triplicate.

Determination of SOD, POD and MDA content.

Total SOD activity (superoxide dismutase) was assessed by the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) (Gao 2006). The reaction mixture consists 1.5 ml 0.05 M phosphate buffer (pH 7.8), 0.3 ml 0.1 mmol. L⁻¹ EDTA-Na₂, 0.3 ml 0.75 mmol. L⁻¹ NBT, 0.3 ml 0.02 mmol. L⁻¹ riboflavin, 0.3 ml 0.13 mol. L⁻¹ methionine, 0.25 ml distilled water and 0.05 ml of enzymatic extract in a total volume of 3 ml reaction mixture. After exposure of fluorescent light (800 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$) for 10-20 min, the color change absorbance was noted at the 560 nm wavelength. SOD activity was determined as 50 % inhibition of the NBT reduction caused by the superoxides generated from the reaction of photo-reduced riboflavin and oxygen. The total SOD activity was expressed in units per gram of fresh leaves ($\text{u}\cdot\text{g}^{-1}\text{Fw}$).

POD (Peroxidase) activity was determined through guaiacol method (Bestwick et al. 1998). The reaction mixture used consisted of 50 mL 0.05 M phosphate buffer (pH7.8), 19 μL 30% H₂O₂ (v/v) and 28 micro liter guaiacol. The enzyme extract 0.5 mL was added and a total of 3.0 mL of the reaction mixture solution was placed into a cuvette. The increase in absorbance was recorded at 470 nm wavelength at 30 s intervals for 3 mins. The results were presented as D470 per minute per gram of fresh leaves ($\text{U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$).

The MDA (Malondialdehyde) content was measured through thiobarbituric acid (TBA) reaction method (Vos et al. 1991). 1.5 mL of the extract super- natant was mixed with 2 mL 0.6% (w/v) TBA solution dissolved in 5% (v/v) trichloroacetic acid (TCA) was heated in boiling water for 10 min and then cooled to allow the flocculate to settle down. The supernatant was used for the spectrophotometric determination of MDA at 450 and 532 nm wavelength and subtracted from

the absorbance at 600 nm. The MDA content was expressed as the amount of substance per gram of fresh leaves ($\text{nmol} \cdot \text{g}^{-1} \text{Fw}$).

Treatments were arranged in a split plot design with three replicates. The collected data were subjected to analysis of variance (ANOVA) and means were separated according to least significant difference (LSD) at 0.05 level of probability using SPSS statistical program.

RESULTS

Growth attributes

The elevated NaCl concentrations in soil significantly reduced plant growth in cucumber plants. The effect of NaCl at 120 mM showed more deleterious effect in decreasing shoots fresh and dry weight and root fresh and dry weights as compared to control. However, the foliar application of HBL significantly enhanced the growth variables with and without NaCl conditions. HBL ($3 \mu\text{M}$) (table 1) enhanced shoot fresh and dry weight and root fresh and dry weight at 60 mM NaCl, while HBL ($1 \mu\text{M}$) showed best results at 120 mM NaCl for shoot fresh and dry weight and HBL ($3 \mu\text{M}$) for root fresh and dry weight in cv Jinyou 1[#]. Moreover, HBL ($3 \mu\text{M}$) enhanced shoot fresh and dry weight and root fresh and dry weight at both 60mM and 120mM NaCl whereas shoot fresh weight was elevated at 60 mM NaCl in cv Changchun Mici. HBL ($1 \mu\text{M}$) increased shoot fresh weight and root dry weight at 60 mM and 120 mM salt respectively.

Shoot and root length showed a drastic decrease in increasing NaCl concentrations. However, HBL applications showed pronounced results in both cultivars. As compared to their respective controls (table 1), HBL ($1 \mu\text{M}$) showed best results by increasing shoot length by (15.15%) and (18.48%) in cv Jinyou 1[#], while, (13.35%) and (16.20%) in cv Changchun Mici at 60 mM and 120 mM NaCl respectively. The highest root length was observed in plants foliar sprayed with

HBL (3 μ M) at 60 mM and (1 μ M) at 120 mM in cv Jinyou 1[#], while (1 μ M) showed best results in both stresses for cv Changchun Mici.

Chlorophyll

Results shows that chlorophyll (chl) content was altered with increasing salinity concentrations as it is known that under stress conditions metabolic activity is lowered and chlorophyll content is damaged. Foliar applications of HBL had an aided effect under salt conditions. HBL (1 μ M) expressed pronounced results in ameliorating chl a, b and total chl (Table 3 and 4) under 60 mM and 120 mM NaCl concentrations in cv Jinyou 1[#] and Changchun Mici respectively.

Antioxidant enzymes (SOD, POD) and MDA

The antioxidant enzymes activity and MDA content were perturbed in plants which were grown under salt conditions. However, application of HBL under salt conditions enhanced the antioxidant enzymes and lowered the MDA levels. Maximum activity level of superoxide dismutase (Fig 1A) was observed in plants supplemented with HBL (1 μ M) slightly followed by HBL (3 μ M) under 60 mM NaCl, while the lowest findings were observed under 120 mM NaCl in cv Jinyou 1[#]. Fig 1 (B) reveals that highest activity level for SOD was noted in plants having exogenous spray of HBL (1 μ M) under 60 mM NaCl and best results under high salt was observed in plants sprayed with HBL (3 μ M) in cv Changchun Mici. The cv Changchun Mici showed higher activity in all treatments as compared to cv Jin you 1[#] which shows its resistant nature.

Salinity levels have altered plant peroxidase activity which demonstrates that plants were under high oxidative stress. It was significantly enhanced by the application of HBL under stress conditions. The foliar application of HBL showed highest peroxidase activity in plants under

saline stress. The plants supplied with HBL (1 μ M) showed highest peroxidase levels under 60mM NaCl, while it showed best results under 120mM NaCl in cv Jinyou 1[#] (Fig 1A) and in cv Changchun Mici (Fig 1B) at 60 and 120 mM NaCl concentrations.

Results show that with increasing salinity levels results in increased MDA content in plants, indicating that plants were under osmotic stress. However, the foliar applications of plants with HBL under stress conditions ameliorated its effect. The foliar application of HBL (1 μ M) shows overwhelming results in cv Jinyou 1[#] (Fig 1A) and in cv Changchun Mici (Fig 1B) at 60 and 120 mM NaCl concentrations over their respective controls.

DISCUSSION

Our findings revealed that NaCl in soil causes acute decline in shoot and root growth, its biomass and chlorophyll content in both salt sensitive and tolerant cultivars of cucumber. The decrease in growth under salinity is due to the fact that it causes hyper ionic and hyperosmotic stress (Mahajan et al. 2005; Sacala et al. 2008) resulting in lack of water absorption, ionic imbalance, nutritional deficiency and ultimately disrupted metabolic activity (Karlidag et al. 2011). Our results were in accordance with (Ghoulam et al. 2002; Özdemir et al. 2004; Fariduddin et al. 2014) who showed a drastic decline in growth parameters in sugar beet, cucumber and rice cultivars. Salinity is one of the major environmental factor which confine agricultural productivity more drastically (Pardo 2010) and has a deep impact in regions where saline water is used for irrigation. Therefore, efforts towards increasing tolerant crop bears a significant importance in agriculture sector and the potential in increasing overall yield (Türkan and Demiral 2009). Previous research findings confirm that BRs can enhance tolerance in different species under various abiotic stress (Bajguz and Hayat 2009) such as water, nickel, cold and salt

stress (Ali et al. 2008; Yuan et al. 2010; Fariduddin et al. 2011, 2014; Hayat et al. 2014) in tomato, brassica and cucumber respectively. In our study, the HBL significantly enhanced physiological and morphological parameters under 60mM and 120mM NaCl. The increase in weight of cucumber plants, foliar sprayed with BRs may be due to the positive effect on accumulation of various osmolytes (Zeng et al. 2010; Balal 2014), cell division and cell enlargement (Hu et al. 2000). BRs control a wide range of biological activities which enhances stress tolerance of plants, cell elongation, genetically enhancing BR biosynthesis (Divi and Krishna 2009) regulates growth, biomass and metabolism (Yuan et al. 2012). Exogenous application of BRs elevates growth and plant biomass under stress conditions, thus, our results were in coherence with (Hayat et al. 2012; Yuan et al. 2012; Alyemeni et al. 2013; Sharma et al. 2013). Increasing levels of salinity shows a decline in chlorophyll content (Sharma et al. 2013). The decrease in chlorophyll content under stress conditions can be attributed to the increase activity of chlorophyll degrading enzyme chlorophyllase (Misra et al. 1997; Ali et al. 2008) while, exogenous applications of BRs have an ameliorating role in chlorophyll content under various stresses. BRs enhances chlorophyll content under salinity stress (Mahajan et al. 2005; Karlidag et al. 2011; Ahmad et al. 2013; Alyemeni et al. 2013). Chlorophyll's are one of the important and abundant pigments involved in several photosynthesis functions. They are active in absorbing light and transferring it with high efficiency (Mizoguchi et al. 2012). In our findings the chlorophyll content was increased under HBL applications which might be due to the fact that HBL accelerates synthesis of specific enzymes which are responsible for chlorophyll synthesis (Fariduddin et al. 2014). The increase in chlorophyll content was also observed in treatments where HBL was applied without stress (Fariduddin et al. 2011).

In our findings cucumber plants subjected to salinity elevates the antioxidant enzymes however, the foliar application of HBL also had a significant effect on enhancing the antioxidant levels in absence and presence of salinity stress. Therefore, the maximum values for antioxidant enzymes were noted in plants having foliar spray of HBL with or without NaCl. An increase was observed in MDA levels of cucumber plants under salt and it was significantly lowered in HBL treatments. MDA levels under heat stress were elevated while BR treatments reduced its level in *L. chinenses* (Niu et al. 2016). Salinity and other environmental factors induces oxidative stress in plants (Grant 2012), which may be due to the fact that salinity induced oxidative stress results in decreased availability of atmospheric CO₂ because of stomatal closure and decreased consumption of NADPH by Calvin cycle. The environmental stresses can boost the production of reactive oxygen species as compared to production in normal metabolic processes (Mittler 2006). These ROS mainly includes hydrogen peroxide (H₂O₂), superoxide (O₂⁻) and hydroxyl radicals (OH⁻) (Zeng et al. 2010). The super oxide radicals (O₂⁻) are produced by reduction during the process of Mehler reaction and initiates chain reactions to produce more harmful single oxygen radicles (Türkan and Demiral 2009). Plants have natural endogenous defense system where they overcome the over production of reactive oxygen species through enzymatic (superoxide dismutase, peroxidase, catalase etc.) and non-enzymatic antioxidants (glutathione, proline, carotenoids, tocopherols etc.) (Melorose et al. 2015). On the basis of physiological, molecular and genetic approaches the enhancement of antioxidants under BRs was due to the enhance expression of DET2 gene which elevated the resistance of oxidative stress in Arabidopsis (Cao et al. 2005). BRs regulates elevated reactive oxygen species through antioxidant enzyme system (Niu et al. 2016). The superoxide dismutase catalyzes the conversion of O₂⁻ to H₂O₂ and is ultimately catalysis to H₂O by peroxidase enzyme. It is also the first line of

defense in encountering O_2^- radicle (Alscher et al. 2002). Peroxidase reduces H_2O_2 using reductants like phenolic compounds, ascorbate and guaiacol while, Catalase convert H_2O_2 to H_2O and O_2 (Ali et al. 2008). Our results were in correspondence with those (Özdemir et al. 2004; Ali et al. 2008; Yuan et al. 2012; Fariduddin et al. 2014) which stated that the antioxidant enzymes activity decreases as effect of stress increases while exogenous BRs shows increase in activity with or without stress.

CONCLUSION

Based on current findings, we hereby conclude that HBL can attenuate the effect of salt stress on the subjected cucumber plants. Particularly, 1 μ M of HBL can enhance cucumber growth attributes, leaf chlorophyll contents, and antioxidant enzymes levels both not only in the presence of salt stress but also, in the plants grown under normal conditions which allow us to postulate its future aspects of study. In the future, studies will be carried out to elaborate the mechanism of action of these HBL on a more pronounced and sophisticated platform so as to figure out a standardized dose for cucumber growth under various salt stress conditions.

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Table 1. 28-Homobrassinolide effect on length (cm), fresh and dry weight (g) of cucumber cv Jinyou 1[#] and Changchun Mici under NaCl.

Cultivar	Salt levels (mM)	HBL levels (μM)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Jinyou 1 [#]	0	0	90.73±0.81 <i>ab</i>	43.07±0.87 <i>a</i>	69.42±3.65 <i>ab</i>	9.68±0.18 <i>ab</i>	10.7±0.35 <i>a</i>	1.2±0.08 <i>a</i>
		1	91.81±0.59 <i>a</i>	43.33±0.51 <i>a</i>	71.13±4.21 <i>a</i>	9.97±0.34 <i>a</i>	9.89±0.41 <i>bc</i>	1.12±0.04 <i>a</i>
		3	90.09±0.43 <i>b</i>	42.61±0.92 <i>a</i>	72.23±4.56 <i>a</i>	9.82±0.20 <i>a</i>	10.63±0.28 <i>ab</i>	1.11±0.06 <i>a</i>
		5	90.07±0.42 <i>b</i>	3.27±0.45 <i>a</i>	70.23±0.94 <i>ab</i>	9.87±0.32 <i>a</i>	9.81±0.11 <i>c</i>	1.1±0.01 <i>a</i>
		60	81.89±1.14 <i>e</i>	35.38±0.75 <i>c</i>	54.21±1.28 <i>de</i>	6.37±0.18 <i>d</i>	7.76±0.21 <i>ef</i>	0.81±0.01 <i>bc</i>
	60	1	84.85±0.88 <i>c</i>	37.93±1.14 <i>b</i>	62.82±0.63 <i>bc</i>	7.29±0.15 <i>c</i>	9.06±0.17 <i>cd</i>	0.89±0.02 <i>bc</i>
		3	83.86±0.87 <i>cd</i>	38.69±1.22 <i>b</i>	66.55±1.08 <i>ab</i>	7.81±0.20 <i>b</i>	9.37±0.1 <i>c</i>	0.90±0.07 <i>b</i>
		5	83.01±0.87 <i>de</i>	37.59±1.24 <i>b</i>	8.71±0.62 <i>cd</i>	7.12±0.21 <i>c</i>	8.56±0.56 <i>d</i>	0.84±0.04 <i>bc</i>
		120	76.84±0.97 <i>g</i>	30.80±0.30 <i>e</i>	50.07±1.02 <i>e</i>	4.68±0.28 <i>g</i>	6.79±0.16 <i>f</i>	0.49±0.04 <i>e</i>
		1	81.51±2.02 <i>e</i>	32.80±0.29 <i>cd</i>	56.37±0.54 <i>cd</i>	5.66±0.30 <i>de</i>	8.01±0.37 <i>de</i>	0.63±0.01 <i>d</i>
	120	3	79.00±1.54 <i>f</i>	32.32±0.09 <i>de</i>	53.25±0.88 <i>de</i>	5.29±0.20 <i>ef</i>	8.15±0.18 <i>de</i>	0.75±0.04 <i>bc</i>
		5	78.03±1.07 <i>fg</i>	31.79±0.22 <i>de</i>	51.30±1.73 <i>de</i>	5.01±0.13 <i>fg</i>	7.27±0.37 <i>f</i>	0.73±0.04 <i>cd</i>
CCMC	0	0	90.68±0.49 <i>b</i>	46.18±1.03 <i>ab</i>	84.94±1.98 <i>a</i>	9.36±0.23 <i>ab</i>	16.13±0.34 <i>a</i>	1.77±0.22 <i>a</i>
		1	91.54±0.48 <i>a</i>	46.14±0.5 <i>ab</i>	85.07±0.86 <i>a</i>	9.18±0.18 <i>ab</i>	15.91±0.41 <i>ab</i>	1.75±0.1 <i>a</i>
		3	90.87±0.42 <i>ab</i>	46.62±0.84 <i>a</i>	84.24±0.77 <i>a</i>	9.51±0.20 <i>a</i>	16.25±0.13 <i>a</i>	1.60±0.02 <i>ab</i>
		5	90.18±0.10 <i>b</i>	45.61±0.32 <i>b</i>	83.83±0.94 <i>a</i>	9.33±0.15 <i>ab</i>	15.83±0.13 <i>ab</i>	1.63±0.12 <i>ab</i>
		60	84.58±0.63 <i>e</i>	39.43±0.70 <i>d</i>	68.27±1.05 <i>de</i>	6.89±0.3 <i>ef</i>	13.16±0.29 <i>de</i>	1.18±0.1 <i>cd</i>
	60	1	86.65±0.27 <i>c</i>	42.40±0.60 <i>c</i>	76.91±1.08 <i>bc</i>	8.33±0.4 <i>abc</i>	14.81±0.22 <i>bc</i>	1.59±0.01 <i>ab</i>
		3	86.08±0.21 <i>cd</i>	42.05±0.48 <i>c</i>	78.37±2.91 <i>b</i>	8.65±0.14 <i>abc</i>	14.95±0.19 <i>bc</i>	1.54±0.02 <i>ab</i>
		5	85.56±0.22 <i>d</i>	41.67±0.27 <i>c</i>	74.65±2.33 <i>bc</i>	8.14±0.23 <i>bcd</i>	14.5±0.63 <i>c</i>	1.51±0.01 <i>ab</i>
		120	80.96±0.37 <i>g</i>	34.93±0.55 <i>f</i>	66.55±0.4 <i>e</i>	6.51±0.77 <i>f</i>	12.8±0.15 <i>e</i>	1.03±0.08 <i>d</i>
	120	1	83.79±0.44 <i>e</i>	36.88±0.56 <i>de</i>	74.23±1.49 <i>bc</i>	7.36±0.45 <i>cde</i>	14±0.47 <i>cd</i>	1.34±0.02 <i>bc</i>
		3	82.57±0.1 <i>f</i>	36.51±0.55 <i>e</i>	74.15±1.27 <i>bc</i>	7.66±0.65 <i>cde</i>	14.16±0.27 <i>cd</i>	1.33±0.07 <i>bc</i>
		5	81.90±0.12 <i>f</i>	36.12±0.46 <i>e</i>	72.42±0.77 <i>cd</i>	6.92±0.14 <i>def</i>	13.28±0.36 <i>de</i>	1.20±0.02 <i>cd</i>

Note: Means within a column not sharing a lowercased italic letter differ significantly at the P<0.05 level.

Table 2. 28-Homobrassinolide effect on chlorophyll a, b and a+b (mg g⁻¹ FW) of cucumber cv Jinyou 1# and Changchun Mici under NaCl.

Cultivar	Salt level (mM)	HBL level (μM)	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Chlorophyll a+b
Jinyou 1 [#]	0	0	13.51±0.37 <i>a</i>	2.89±0.27 <i>b</i>	16.4±0.1 <i>ab</i>
		1	14.11±0.5 <i>a</i>	3.97±0.15 <i>a</i>	18.08±0.65 <i>a</i>
		3	14.01±0.39 <i>a</i>	2.91±0.13 <i>b</i>	16.92±0.44 <i>ab</i>
		5	13.78±0.41 <i>a</i>	2.71±0.21 <i>b</i>	16.49±0.56 <i>ab</i>
	60	0	9.57±0.65 <i>e</i>	1.53±0.15 <i>ef</i>	11.10±0.81 <i>f</i>
		1	13.09±0.39 <i>ab</i>	2.65±0.1 <i>bc</i>	15.74±0.45 <i>bc</i>
		3	11.82±0.13 <i>bc</i>	2.64±0.06 <i>bc</i>	14.46±0.19 <i>cd</i>
		5	11.17±0.30 <i>cd</i>	2.51±0.16 <i>bcd</i>	13.68±0.47 <i>de</i>
	120	0	9.51±0.54 <i>e</i>	1.22±0.26 <i>f</i>	10.73±0.33 <i>f</i>
		1	11.28±0.29 <i>cd</i>	2.43±0.12 <i>bcd</i>	13.72±0.41 <i>de</i>
		3	10.24±0.59 <i>de</i>	1.99±0.37 <i>cde</i>	12.23±0.95 <i>ef</i>
		5	10.11±0.26 <i>de</i>	1.92±0.39 <i>de</i>	12.03±0.27 <i>f</i>
CCMC	0	0	16.14±0.41 <i>ab</i>	3.67±0.19 <i>b</i>	19.81±0.38 <i>b</i>
		1	16.47±0.50 <i>a</i>	4.75±0.24 <i>a</i>	21.22±0.73 <i>a</i>
		3	16.37±0.39 <i>ab</i>	3.69±0.1 <i>b</i>	20.07±0.48 <i>b</i>
		5	15.89±0.37 <i>ab</i>	3.49±0.13 <i>b</i>	19.38±0.31 <i>bc</i>
	60	0	13.01±0.42 <i>d</i>	2.31±0.19 <i>ef</i>	15.31±0.41 <i>fg</i>
		1	15.32±0.49 <i>b</i>	3.43±0.05 <i>bc</i>	18.75±0.55 <i>cd</i>
		3	14.34±0.23 <i>c</i>	3.41±0.09 <i>bc</i>	17.76±0.22 <i>de</i>
		5	14.16±0.14 <i>c</i>	3.29±0.13 <i>bcd</i>	17.45±0.23 <i>e</i>
	120	0	11.01±0.29 <i>f</i>	2.01±0.22 <i>f</i>	13.01±0.11 <i>h</i>
		1	12.47±0.14 <i>de</i>	3.21±0.06 <i>bcd</i>	15.69±0.17 <i>f</i>
		3	12.04±0.11 <i>de</i>	2.76±0.42 <i>cde</i>	14.81±0.43 <i>fg</i>
		5	11.72±0.12 <i>ef</i>	2.7±0.34 <i>ef</i>	14.42±0.26 <i>g</i>

Note: Means within a column not sharing a lowercased italic letter differ significantly at the P<0.05 level.

Figure 1. 28-Homobrassinolide ameliorates enzymatic activities and attenuates lipid peroxidation level of cucumber under NaCl. A represents cucumber cultivar Jinyou 1[#] while, B represents cultivar Changchun Mici, (SOD) Superoxide dismutase activity, (POD) Peroxidase activity and (MDA) Malondialdehyde content. Bars represents means and standard errors of biological triplicates. Data were significantly different at P=0.05 using LSD.