DOI: 10.1007/s11099-014-0052-7 PHOTOSYNTHETICA 52 (3): 464-474, 2014

24-epibrassinolide and/or putrescine trigger physiological and biochemical responses for the salt stress mitigation in *Cucumis sativus* L.

Q. FARIDUDDIN+, B.A. MIR, M. YUSUF, and A. AHMAD

Plant Physiology and Biochemistry Section, Department of Botany, Aligarh Muslim University, Aligarh-202002, India

Abstract

Brassinosteroids (BRs) and polyamines, well-established growth regulators, play a key role in abiotic stress response in plants. In the present study, we examined the role of 24-epibrassinolide (EBL, an active BR) and/or putrescine (Put) in the salt-induced stress in cucumber. The 15-d-old plants were exposed to 100 mM NaCl and they were subsequently treated by exogenous EBL and/or Put. The salt stress reduced significantly plant growth and gas-exchange parameters, and increased proline content and electrolyte leakage in the leaves. Toxic effects induced by salt stress were completely overcome by the combination of EBL and Put. EBL and/or Put treatments improved the growth parameters of the NaCl-treated plants, such as shoot length, root length, fresh and dry mass. Our data also indicated that applications of EBL and Put upregulated the activities of the antioxidant enzymes, such as catalase, peroxidase, and superoxide dismutase under salt stress.

Additional key words: brassinosteroids; *Cucumis sativus*; polyamines; salt stress.

Introduction

Salinity stress is a major limiting factor for sustainable productivity, especially that of glycophytic plants, such as cucumber and tomato. A high salt content in the soil affects the soil porosity, decreases the soil water potential (Hopkins and Huner 2009), and it also affects the physiology of plants at the cellular as well as the whole-plant level (Murphy and Durako 2003). Plant growth is affected by osmotic stress–specific ion toxicity, ion imbalance, and oxidative stress generated by salt stress (Li *et al.* 2010). It affects severely various morphological, physiological, and biochemical processes (Koca *et al.* 2007) such as photosynthesis, accumulation of low-molecular mass compounds, such as proline and glycine betaine (Mutlu and Bozcuk 2005, Yusuf *et al.* 2008); and protein (Unni and Rao 2001) and lipid metabolisms (Parida and Das 2005). An excessive amount of sodium ions in the cells causes also the inhibition of enzymes, such as those for nitrogen metabolism and carbon fixation (Soussi *et al.* 1998, 1999). Ionic imbalance induced by salt stress causes high Na^+ concentration in plants and it also influences the uptake of other ions, particularly K^+ and Ca^{2+}. The accumulation of toxic amounts of salts leads to the hyperosmotic stress in plants that ultimately stimulates the production of reactive oxygen species (Ahmad *et al.* 2009), dehydration, and turgor loss in cells and tissues (Mittler 2002). Both the dehydration of cells and the high Na^+/K^+ ratio due to the accumulation of high amounts of Na^+ inactivate enzymes and affect metabolic processes of the plants (Ashraf 2004).

BRs form a group of steroidal lactones, ubiquitously distributed in plant kingdom. They regulate various developmental and physiological processes including cell elongation, morphogenesis, and tissue differentiation (Gudesblat and Russinova 2011). BRs also confer both biotic and abiotic stress tolerance in plants (Bajguz *et al*. 2011, Gudesblat and Russinova 2011). Molecular and signal transduction studies have established that the pleiotropic effects of BRs result partly from the interactions with other phytohormones (Divi *et al*. 2010). BRs are extensively used to strengthen a plant defence system by enhancing the activities and contents of enzymic and nonenzymic antioxidants against various abiotic stresses

Received 12 March 2013, *accepted* 8 November 2013.
+Corresponding author; phone: +91 9412172134, fax: +91-571-2702016, email: qazi_farid@yahoo.com
Abbreviations: BR – brassinosteroid; CA – carbonic anhydrase; CAT – catalase; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DDW – double distilled water; DM – dry mass; EBL – epibrassinolide; EL – electrolyte leakage; FM – fresh mass; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; MDA – malondialdehyde; NR – nitrate reductase; PA – polyamine; POX – peroxidase; Put – putrescine; P_N – net photosynthetic rate; SOD – superoxide dismutase; SPAD – soil and plant analysis development; WUE – water-use efficiency.
Acknowledgements: The authors gratefully acknowledge the financial support provided by University Grants Commission New Delhi, India for funding this work in the form of U.G.C Major Research Project [File No.39-437/2010 (SR)].

including heavy metal (Anuradha and Rao 2007, Fariduddin *et al.* 2009a), drought (Fariduddin *et al.* 2009b), and salt stress (Hayat *et al.* 2012). Application of EBL improved seed germination, free proline content, and activities of antioxidative enzymes (Anuradha and Rao 2003). Among BRs, EBL has been extensively used to ameliorate the harmful effects of abiotic stress in plants (Ali *et al.* 2008, Bajguz 2010, 2011). EBL improved photosynthetic capacity and decreased oxidative damage in *Vigna radiata* exposed to different levels of Ni stress (Yusuf *et al.* 2012).

Polyamines (PAs) are small, aliphatic, nitrogenous compounds with universal distribution; they play an important role in cellular processes, such as embryogenesis, cell division, growth and development, ethylene biosynthesis, and senescence (Bouchereau *et al.* 1999, Thomas and Thomas 2001, Groppa and Benavides 2008). Applications of PAs provide protection against a large number of biotic and abiotic stresses (Bouchereau *et al.* 1999, Yang *et al.* 2007, Choudhary *et al.* 2012). PAs are thus considered to be an essential component of plant defence mechanisms (Hussain *et al.* 2011).

The latest advancements in plant hormonal interactions have shown a possible involvement of plant growth regulators in the management of various abiotic stresses (Hayat *et al.* 2010a, 2012). Besides the study of Choudhary *et al.* (2011, 2012), scant information is available on the interactive effects of BRs and PAs in the mitigation of abiotic stress. Keeping in view the diverse role of BRs and PAs, the present study was designed with the aim to assess the comparative effect of EBL and Put against salinity stress.

Materials and methods

EBL and Put preparation: The stock solutions of EBL or Put were prepared by dissolving the required quantity of EBL or Put in 5 ml of ethanol, in 100 ml volumetric flasks. Tween-20 (5 mL) was added and the final volume was made up to the mark by using double distilled water (DDW). The desired concentrations of EBL or Put were prepared by the dilution of stock solution with DDW.

Plant material and treatment*:* Seeds of cucumber (*Cucumis sativus* L. cv. Summer best) were purchased from *National Seed Corporation Ltd*., Pusa, New Delhi, India. Healthy seeds of uniform size were surface-sterilized with 0.05% (w/v) NaClO solution followed by repeated washings with deionised water. The surface-sterilized seeds were sown in acid-washed sand, moistened with deionised water in plastic pots (25 cm^3). These seeds were allowed to germinate and the resulting seedlings were grown in a climate chamber [14-h light period, PAR of 200 μmol(photon) m^{-2} s^{-1}, 25ºC, 60% relative air humidity) for 30 d. The pots were supplied with full nutrient solution (Hewitt 1966) from 7 d stage of growth onwards. To maintain the sufficient nutrients, the supply of nutrient solution was maintained throughout the whole experiment. After 15 d, the plants were exposed to 100 mM NaCl and sprayed simultaneously with EBL (10^{-8} M) and/or Put (10^{-3} M). The control set was sprayed with equal amount of DDW. A total number of 40 pots were arranged for 8 treatments with 5 replicates each, having 3 plants per pot in a simple randomized block design. The plants were harvested after 30 d of growth.

Plant growth: Harvested plants (one plant from each replicate) were removed together with the sand and dipped in a beaker filled with tap water. The adhering soil particles were removed while ensuring the integrity of the roots. The plants were blotted on paper and the lengths of roots and shoots were measured; then fresh mass (FM) was recorded. The roots and shoots were dried in an oven at 80ºC for 72 h and weighed to note their dry mass (DM). Leaf area of randomly selected leaves from each treatment was determined by tracing the outline of the leaf on the graph sheet and counting the number of squares covered by the leaf.

SPAD chlorophyll (Chl): The Chl content was measured by SPAD Chl meter (*SPAD-502*, *Konica, Minolta Sensing, Inc*., Japan) in the intact leaves of the plants (one plant from each replicate).

Leaf gas-exchange parameters: The rate of photosynthesis (P_N) and related parameters, *i.e.,* stomatal conductance (g_s), water-use efficiency (WUE), and intercellular CO_2 concentration (C_i) were measured using portable photosynthetic system *(LI-COR 6400-40*, *LI-COR*, Lincoln, NE, USA). The measurements were made on the uppermost, fully expanded leaves (usually the third leaf from the top) attached to the plant (the same plant that was used for SPAD value of Chl) between 11:00 and 13:00 h under clear sunlight. The atmospheric conditions during measurements were: PAR of 1,016 ± 6 μmol(photon) m^{-2} s^{-1}, relative air humidity 60 ± 3%, atmospheric temperature 22 ± 1ºC, and atmospheric CO_2 of 360 μmol mol^{-1}. The ratio of atmospheric CO_2 to C_i was constant.

Maximum quantum yield of PSII: The Chl fluorescence [*i.e*., maximum photochemical efficiency of PSII (F_v/F_m)] was measured on the upper surface of the intact leaves of the plants (the same as those used for the gas-exchange measurements) using portable photosynthesis system (*LI-COR-6400*, *LI-COR*, Lincoln NE, USA). The minimal fluorescence level (F_0) was determined by modulated light, which was sufficiently low (< 1 μmol m^{-2} s^{-1}) not to induce any significant variable fluorescence. The maximal fluorescence (F_m) was determined by a 0.8-s saturation pulse at 4,200 μmol m^{-2} s^{-1} on dark-adapted leaves. The sampled leaf was dark-adapted for 30 min prior to measurement of F_v/F_m.

Nitrate reductase and carbonic anhydrase activities: Nitrate reductase (NR, E.C. 1.6.6.1) activity was measured following the method of Jaworski (1971). Fresh leaf samples were cut into small pieces and transferred to plastic vials containing phosphate buffer (pH 7.5) followed by addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30ºC, for 2 h followed by the addition of N-1-naphthylethylenediamine dihydrochloride and sulphanilamide. Absorbance at 540 nm was determined by spectrophotometer (*Spectronic 20D, Milton Roy*, USA) and it was compared with that of the calibration curve. The NR activity was expressed in [nmol(NO_2) g^{-1}(FM) s^{-1}].

Activity of carbonic anhydrase (CA, E.C. 4.2.1.1) was determined following the procedure described by Dwivedi and Randhawa (1974). Leaf samples were cut into small pieces and suspended in cysteine hydrochloride. Samples were incubated at 4ºC for 20 min. The pieces were blotted and transferred to a test tube containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tube was incubated at 5ºC for 20 min. The reaction mixture was titrated against 0.5 N HCl after the addition of few drops of methyl red as indicator. The results were expressed as [mol(CO_2) kg^{-1}(FM) s^{-1}] by using the formula:

$$\text{CA activity} = V \times 22 \times N/W,$$

where V – volume of HCl used; 22 – equivalent mass of CO_2; N – normality of HCl used; W – fresh mass of leaf tissue used.

Leaf proline content: The proline content in fresh leaf samples (one plant per replicate) was determined by the method of Bates *et al.* (1973). Samples were extracted in sulfosalicylic acid. An equal volumes of glacial acetic acid and ninhydrin solutions were added to the extract. The sample was heated at 100ºC, to which 5 ml of toluene was added. The absorbance of the aspired layer was read at 520 nm on a spectrophotometer (*Spectronic 20D, Milton Roy*, USA).

Electrolyte leakage (EL): Total inorganic ions leaked out of the leaf were estimated by the method described by Sullivan and Ross (1979). Twenty leaf discs were collected in a boiling test tube containing 10 ml of DDW, and electrical conductivity was measured (EC_a) by conductivity meter (*Eutech Instruments PC-700, Thermo Fisher Scientific*, Singapore). The tubes were heated at 45ºC and 55ºC for 30 min in water bath, and electrical conductivity was measured (EC_b) each time. Later, the contents were again boiled at 100ºC for 10 min, and electrical conductivity was again recorded (EC_c). The electrolyte leakage was calculated using the formula:

$$EL\ [\%] = [(EC_b - EC_a)/(EC_c)] \times 100$$

Antioxidant enzymes: For the assay of antioxidant enzymes, the leaf tissue (0.5 g) was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone. The homogenate was centrifuged at 27,600 × *g* for 10 min at 4ºC and the supernatant was used as source of enzymes.

Peroxidase (POX, E.C. 1.11.1.7) and catalase (CAT, EC 1.11.1.6) were assayed following the procedure described by Chance and Maehly (1956). CAT was estimated by titrating the reaction mixture, consisting of phosphate buffer (pH 6.8), 0.1 M H_2O_2, enzyme extract and 2% H_2SO_4, against 0.1 N $KMnO_4$ solution, to find the residual H_2O_2 until a purple color persists for at least 15 s. Similarly, a control set was maintained where the enzyme activity was stopped by the addition of H_2SO_4 prior to the addition of enzyme extract. The reaction mixture for POX consisted of pyragallol, phosphate buffer (pH 6.8), 1% H_2O_2, and enzyme extract. Change in absorbance due to catalytic conversion of pyragallol to purpurogallin, was noted at an interval of 20 s for 2 min, at 420 nm on a spectrophotometer (*Spectronic 20D, Milton Roy*, USA). A control set was prepared by using DDW instead of enzyme extract. The activity of superoxide dismutase (SOD, E.C. 1.15.11) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium following the method of Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM nitroblue tetrazolium, 2 mM riboflavin, 0.1 mM EDTA, and 0–50 µL of the enzyme extract and it was placed under 15 W fluorescent lamp. The reaction was started by switching on the light and was allowed to run for 10 min. The reaction was stopped by switching off the light and nonilluminated reaction mixture was used as blank. The absorbance was measured at 560 nm on a spectrophotometer *(Spectronic 20D, Milton Roy*, USA) and the SOD activity was expressed as [unit g^{-1}(FM)]. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of nitroblue tetrazolium photoreduction.

Statistical analysis: The experiment was conducted according to simple randomized block design. Each treatment was replicated five times. Data were statistically analysed for analysis of variance (*ANOVA*) using *SPSS* software, *version 17 for Windows* (*SPSS*, Chicago, IL, USA). Least significant difference (LSD) was calculated to separate the means.

Results

Growth characteristics: A significant reduction in all the growth parameters was observed in plants exposed to salt stress (Fig. 1). The shoot length, root length, FM, DM, and leaf area decreased by 31.8, 41.2, 47.3, 41.1, and 32.4%, respectively, compared with their respective controls (Fig. 1). Plants treated with EBL or EBL+Put overcame completely the inhibitory effects generated by NaCl stress. The plants treated with EBL alone showed enhancement of all growth parameters, such as shoot length, root length, FM, DM, and leaf area by 38, 32.2, 38.9, 47.3, and 25.2 %, respectively, when compared with their respective controls.

Chl content and Chl fluorescence: The stress generated by NaCl decreased the SPAD Chl content and F_v/F_m by 28.8 and 19.2%, respectively, compared with the respective controls (Figs. 1*F,* 2*E*). Treatment with EBL and/or Put improved significantly both Chl content and F_v/F_m.

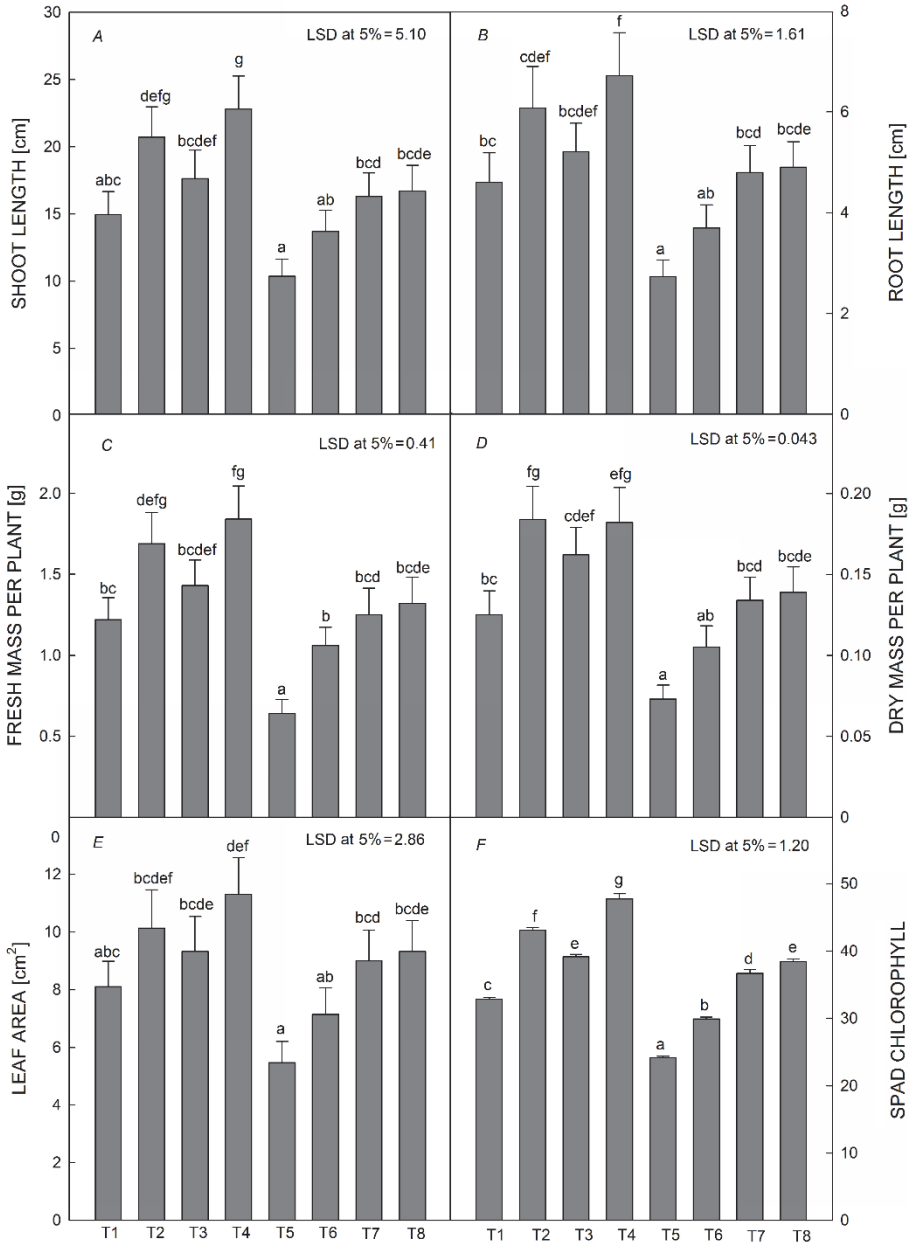

Fig. 1. Effect of 24-epibrassinolide (EBL; 10^{-8} M) and/or putrescine (Put; 10^{-3} M) on the NaCl (100 mM) induced changes in the (*A*) shoot length, (*B*) root length, (*C*) fresh mass per plant, (*D*) dry mass per plant, (*E*) leaf area, and (*F*) SPAD chlorophyll content of *Cucumis sativus* L. plants 35 days after sowing. T1 – control; T2 – EBL; T3 – Put; T4 – EBL+Put; T5 – NaCl; T6 – NaCl+Put; T7 – NaCl+EBL; T8 – NaCl+EBL+Put.

SPAD values increased in the plants treated with EBL+Put, EBL, or Put by 43.2, 31.4, and 19.3%, respectively, whereas F_v/F_m increased by 32.3, 20.1, and 15.2% compared with the respective control. The NaCl-induced negative effects were removed by treatment with EBL or EBL+Put.

EL: Plants exposed to salt stress induced greater EL (32.1%) as compared with the controls. However, the exogenous application of EBL alone or together with Put in nonstressed plants reduced the EL by 15.4 and 22.7%, respectively, as compared with the controls (Fig. 2*F*). Moreover, the application of Put to the plants exposed to NaCl (100 mM) partially restored the leakage of ions.

Impacts of EBL and Put on gas-exchange parameters: The exogenous application of EBL to nonstressed plants increased significantly the P_N, g_s, C_i, and WUE by 35.6, 35.6, 29.2, and 36.7%, respectively, compared with the

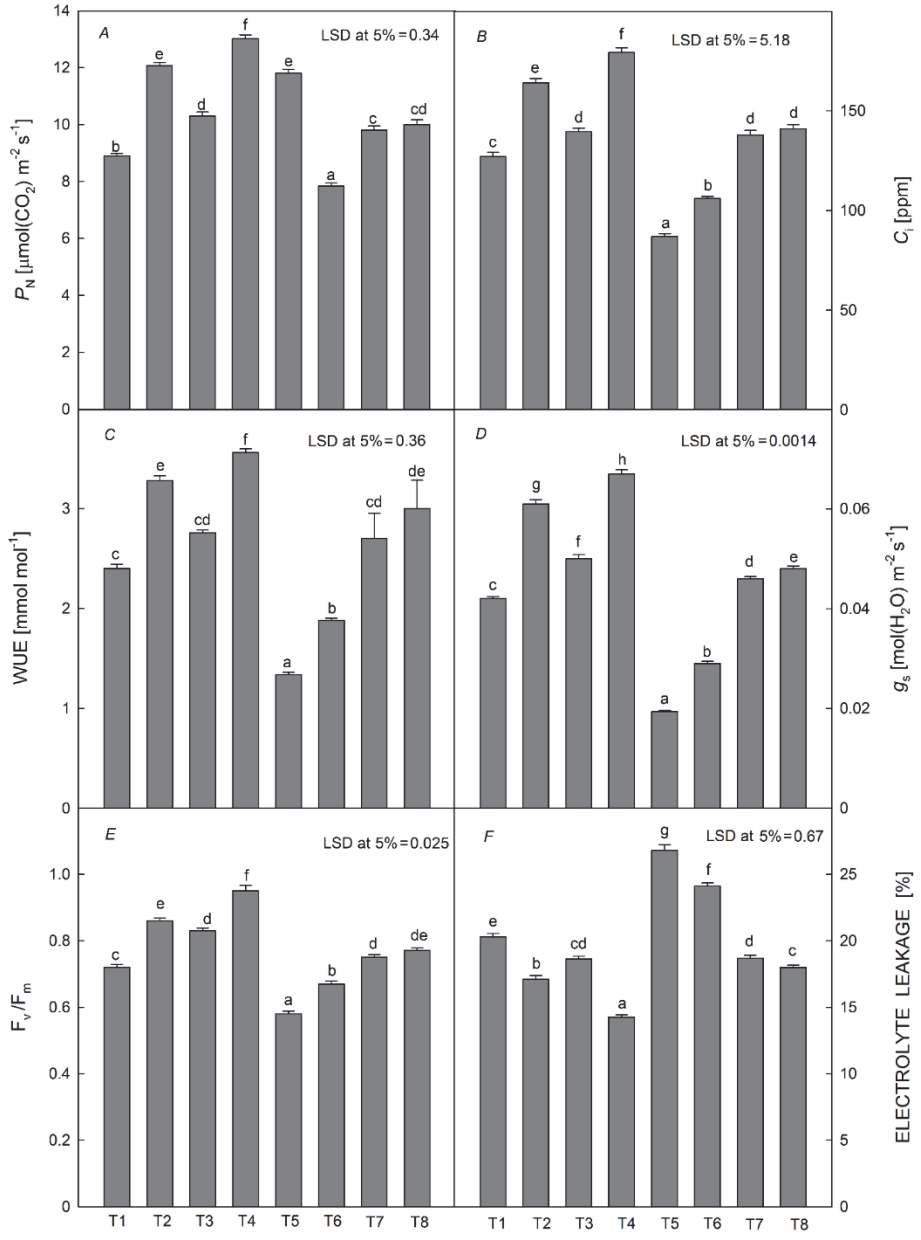

Fig. 2. Effect of 24-epibrassinolide (EBL; 10^{-8} M) and/or putrescine (Put; 10^{-3} M) on the NaCl (100 mM) induced changes in the (*A*) net photosynthetic rate (P_N), (*B*) intercellular CO_2 concentration (C_i), (*C*) water-use efficiency (WUE), (*D*) stomatal conductance (g_s), (*E*) maximum quantum yield (F_v/F_m), and (*F*) electrolyte leakage (EL) of *Cucumis sativus* L. plants 35 days after sowing. T1 – control; T2 – EBL; T3 – Put; T4 – EBL+Put; T5 – NaCl; T6 – NaCl+Put; T7 – NaCl+EBL; T8 – NaCl+EBL+Put.

respective control (Fig. 2*A*,*B*,*C*,*D*). NaCl proved to be toxic and reduced significantly the values of these parameters by 32.8, 35.7, 31.3, and 43.8%, respectively, compared with the respective control. However, the plants treated by both EBL and Put showed maximum values in the gas-exchange parameters (P_N, g_s, C_i, and WUE) and the values increased by 46.3% (P_N), 61.3% (g_s), 41.2% (C_i), and 48.4% (WUE). The plants treated with Put (10^{-3} M) alone showed higher values of P_N, g_s, C_i and WUE by 15.8, 21.3, 9.9, and 15.3%, respectively, as compared with their controls (Fig. 2).

NR and CA activities: The plants treated with either EBL or Put possessed higher activity of NR and CA than the respective control (Fig. 3*A*,*B*). The NR activity increased by 43.0 and 21.0% and CA activity by 33.3 and 14.8%,

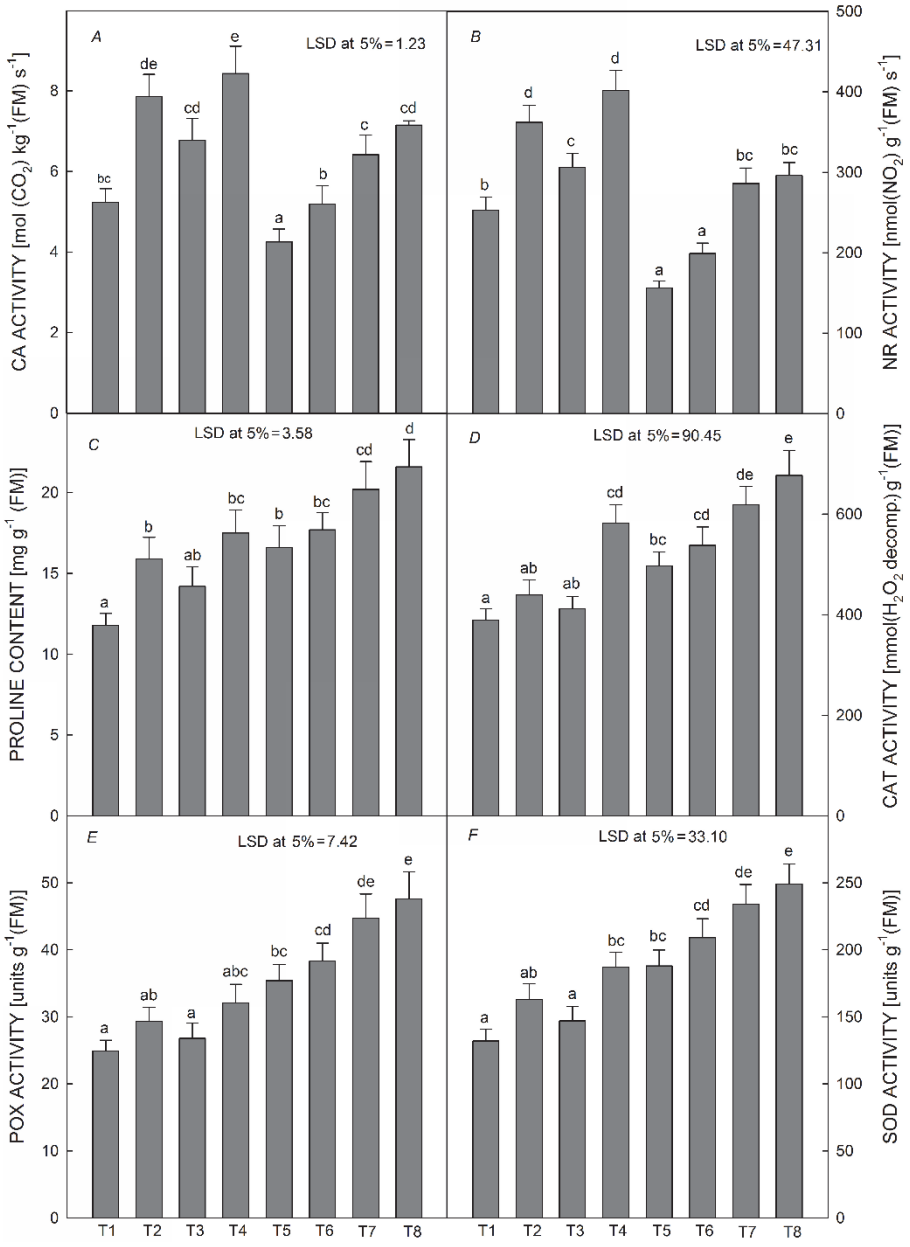

Fig. 3. Effect of 24-epibrassinolide (EBL; 10^{-8} M) and/or putrescine (Put; 10^{-3} M) on the NaCl (100 mM) induced changes in the (*A*) carbonic anhydrase (CA) activity, (*B*) nitrate reductase (NR) activity, (*C*) proline content, (*D*) catalase (CAT) activity, (*E*) peroxidase (POX) activity, and (*F*) superoxide dismutase (SOD) activity of *Cucumis sativus* L. plants 35 days after sowing. T1 – control; T2 – EBL; T3 – Put; T4 – EBL+Put; T5 – NaCl; T6 – NaCl+Put; T7 – NaCl+EBL; T8 – NaCl+EBL+Put.

respectively, compared with the respective control. However, the NaCl-stressed plants treated by both EBL+Put improved significantly the NR and CA activities by 17 and 15.9% as compared with the nonstressed controls (Fig. 3*A*,*B*).

Proline content: The plants exposed to stress were found to have higher proline content than the control plants (Fig. 3*C*). Proline content in response to NaCl was 41.3% higher. However, EBL and/or Put treatment of the salt-stressed plants had an additive effect on the proline content. EBL, Put or both EBL+Put increased the proline content by 35.3, 20.3, and 47.8%, respectively, over the control. The plants exposed to salt stress and subsequently treated with EBL+Put were found to have the highest content of proline.

Antioxidant enzymes: The activities of CAT, POX, and SOD were enhanced significantly by 27.8, 42.3, and 37% in the plants exposed to salt stress. The plants exposed separately to EBL showed higher activities of CAT, POX and SOD by 12.8, 17.8, and 24%, respectively; whereas, Put alone stimulated the activities of these enzymes by 5.8, 7.9, and 11%, respectively, as compared with the respective controls. Furthermore, the maximal activities of CAT, POX, and SOD were found in the plants exposed to stress and consequently treated with both EBL and Put.

Discussion

Salinity limits crop production in arid and semiarid regions, where soil salt content is naturally high and precipitation can be insufficient to drain away salt from soil (Zhao *et al.* 2007). In the present study, all growth parameters were significantly lowered in the plants exposed to salinity stress. Our results were in agreement with that of Ghoulam *et al.* (2002), who showed marked reduction in growth parameters of sugar beet subjected to NaCl stress. Growth inhibition by salinity has been attributed to the disturbance in water and osmotic potential, toxicity of excessive Na^+ and Cl^-, disturbance in the accumulation of nutrients, disruption in the structure and the activity of the enzymes, damage in cell organelles and plasma membrane, disturbances in photosynthesis, respiration, and protein synthesis (Feng *et al.* 2002, Munns 2005). However, the subsequent application of EBL and/or Put was beneficial to sustain the growth and to reduce deleterious effects of salt stress (Fig. 1). The stressed plants treated with EBL or Put increased their FM and DM as well as root and shoot length and leaf area, compared with those grown without EBL or Put (Fig. 1). A similar increase by EBL treatment has been reported by Ali *et al.* (2008). BRs (Zeng *et al.* 2010) and PAs (Benavides *et al*. 1997, Duan *et al.* 2008) are known to improve the growth of root and/or shoot in various plant groups. The increase in the growth parameters caused by BR is due to the involvement of BR-regulated genes in plant growth and development, such as cell wall modification, cytoskeleton formation, and hormone synthesis (Vert *et al.* 2005). Furthermore, the involvement of PAs, in wide array of plant processes, such as DNA replication, transcription of genes, cell division, and root growth (Tiburcio *et al.* 2002, Bais and Ravishankar 2002), are responsible for the growth improvement. The enhanced tolerance to salt stress may be due to involvement of PAs in the maintenance of tonoplast H^+-ATPase, proton-translocating inorganic pyrophosphatases (H^+-PPase), and Na^+/H^+ transporter (Zhao and Qin 2004, Liu *et al.* 2006). Similarly, BR-treated plants also exhibited the improved growth parameters in our experiment (Fig. 1). It could be mainly due to activated cell division and cellular enlargement induced by the BR application (Hu *et al.* 2000) or due to the upregulation of xyloglucan transferase/hydrolase (cell wall loosening enzyme) and its enhanced gene expression or by activating the H^+-ATPase activity (Sun *et al.* 2005). It is interesting to observe that co-application of EBL and Put showed more pronounced effects on the improvement of growth parameters under salt stress, perhaps due to their synergistic or additive effects. The synergistic effects of PAs and BRs were also shown by Choudhary *et al.* (2012).

In addition to the primary effects, salinity inevitably leads to oxidative stress through an increase in ROS, such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) (Zheng *et al.* 2009). It is now widely accepted that these cytotoxic ROS are responsible for various stress-induced damages to macromolecules and ultimately to cellular structure (Amor *et al.* 2006, Sekmen *et al.* 2012). ROS detoxification by enzymatic (SOD, CAT, and POX) antioxidants is the effective defence mechanism against oxidative damage in plants (Verslues *et al.* 2006, Khan and Panda 2008). In the present study, we observed the enhanced activities of CAT, POX, and SOD, as well as that of proline content in the plants exposed to NaCl stress with or without EBL and/or Put treatments (Fig. 3*C,D,E,F*). The enhancement in the activities of antioxidative enzymes by BRs is a gene-regulated phenomenon. The expression of POX-encoding genes, *ATP2* and *ATP24a*, has been demonstrated by Goda *et al.* (2002) to be regulated by BRs in *Arabidopsis*. Since the activity of POX is activated by various environmental stresses, it is possible that BRs might mediate the detoxification of ROS (Andre *et al.* 2010). Similarly, the application of Put had been also shown to ameliorate NaCl stress in chickpea plants through elevating the activities of CAT, glutathione peroxidase, glutathione reductase, and SOD (Sheokand *et al.* 2008). Our results validated the synergistic interactions of EBL and Put for amelioration of the oxidative stress generated by NaCl. The same results were also obtained by Choudhary *et al.* (2011) in *Raphanus sativus* under copper stress. Salt stress damages

the photosynthetic machinery at multiple levels, such as pigment content, structure and function of thylakoids, electron transport and enzymes, stomata functioning, and gas exchange (Geissler *et al.* 2009). Salinity causes a decrease in Chl content *via* the acceleration of Chl degradation or inhibition of its biosynthesis (Xu *et al.* 2000). The effect of these processes resulted in the decreased SPAD value of Chl as it was found in our study (Fig. 1*F*). Similar decrease in Chl content was observed by Hayat *et al.* (2012). However, the subsequent treatment by EBL and/or Put improved the SPAD values. In the present study, EBL fed to nonstressed plants increased the Chl content; it was also found by others (Yu *et al.* 2004, Ali *et al.* 2008). The possible reason is that EBL-induced transcription and/or translation involves the expression of specific genes responsible for synthesis of enzymes determining Chl synthesis (Bajguz 2007). Moreover, Put treatment also increased the SPAD value. Krishnamurthy (1991) reported that Put also increased the Chl content in rice. It could be due to the elevated concentration of Mg^{2+} (Lakra *et al.* 2006), which is essential for Chl synthesis.

Salt stress caused the stomata closure (Bethkey and Drew 1992), therefore, it decreased partial CO_2 pressure and thus C_i, g_s, and consequently CA activity (Ali *et al.* 2008). Besides this, salinity impairs photosynthesis and the photosynthetic electron transport chain (Sudhir and Murthy 2004). All these impaired events finally resulted in a severe decline in P_N (Fig. 2*A*). The damage caused by salt stress could be also attributed to water stress or a kind of physiological drought generated by NaCl (Perez-López *et al.* 2009, Belkheiri and Mulas 2013) as evident from the decrease in WUE in our present study. Decrease in all gas-exchange parameters (P_N, g_s, C_i, and WUE) due to salinity has been also reported in *B. juncea* L. (Yusuf *et al.* 2011), and *Vigna radiata* (Hayat *et al.* 2010b). Other reasons for the decrease in P_N under NaCl are faster senescence and changes in enzyme activities induced by dysfunction of proteins and negative feedback by reduced sink activity (Iyengar and Reddy 1996). BRs are known to activate the enzyme Rubisco (Yu *et al.* 2004) and CA (Fariduddin *et al.* 2011), possibly by accelerating the CO_2 assimilatory rate (Sinha *et al.* 1993) through the expression of specific genes (Khripach *et al.* 1999) and by improved water relations, such as increase of relative water content and water uptake (Ali *et al.* 2005). It could lead to the increase in WUE, g_s, and finally the P_N (Fig. 2). However, treatment of EBL and/or Put improved the gas exchange parameters. Furthermore, BRs can also modify the membrane structure/stability under stress conditions (Hamada 1986). It was evident from the values of EL in our experiment (Fig. 2*F*). BRs have also a positive impact on Rubisco activity (Yu *et al.* 2004). Therefore, the plants treated with EBL, both in presence and absence of stress, exhibited the higher P_N and related attributes. Moreover, PAs play a role in retarding the loss of D1, D2, and cytochrome *f* from the thylakoid membranes as well as large subunits of Rubisco and Chl from the leaf tissue (Besford *et al.* 1993). This could increase P_N in the present study. Put application improved the CA activity by its involvement at the level of transcription and/or translation (Cohen 1998) that might generate a significant impact on CA activity (Fig. 3*A*).

Salinity might increase the turnover of D2 protein of PSII leading to a decrease in F_v/F_m as observed in the present study (Fig. 2*E*). Similarly, such a decrease was also observed in *Triticum aestivum* (Shahbaz *et al.* 2008) and in *Vigna radiata* (Hayat *et al.* 2010b) exposed to salinity. However, treatment of EBL and/or Put improved the values of F_v/F_m in stressed plants indicating that EBL and Put helped in the protection of PSII against overexcitation under stress that is often associated with decrease in photosynthetic electron transport activities (Subhan and Murthy 2001, Talaat *et al.* 2012) due to inhibition of PSII activity (Kao *et al.* 2003). Shahbaz *et al.* (2008) also reported that BRs improved the quantum yield of PSII under salt stress in wheat. Moreover, Put treatment also improved the values of F_v/F_m indicating Put plays a positive role in protecting the PSII machinery. These results are in agreement with that of Subhan and Murthy (2001), who reported that PAs protected the activities of the whole chain of electron transport, PSI and PSII. All these results suggest that Put application reduced the sensitivity of *Cucumis sativus* to NaCl stress similarly as in our study.

Plants exposed to salinity exhibited the significantly lowered activity of NR (Fig. 3*B*). This might be an after effect of the inhibition and/or metabolic dysfunction of NR (Hopkins and Huner 2009). Moreover, stress factors interfere with the structure and fluidity of the membrane (Alia-Mohanty and Saradhi 1992, Karim *et al.* 1999) as evident from the increase in EL after the exposure to salt stress (Fig. 2*F*). It might restrict the uptake of nitrate, the inducer and substrate for the NR (Campbell 1999) resulting in the decreased NR activity (Fig. 3*B*). However, treatment of EBL and/or Put to both stressed and nonstressed plants enhanced the activity of NR, which could be explained, *e.g.,* by the impact of BRs (Khripach *et al.* 2000) and PAs (Shi *et al.* 2008) on translation and/or transcription. Another reason may be impact of EBL on membrane anion channels (Zhang *et al.* 2005) to facilitate the uptake of nitrate. Moreover, increased activity of NR by Put is attributed to the fact that Put is involved in increasing the transcript levels of NR and its cofactor-binding domain genes, thereby stimulating the activities of NR and nitrate reduction (Shi *et al.* 2008). Furthermore, Rosales *et al.* (2012) proposed that PAs could participate in the regulation of NR activity in a dual manner. At short time, PAs inhibit the NR activity by increasing NO prodution (a signalling molecule involved in the inhibition of NR activity) and 14-3-3 protein-master regulator of many signal transduction cascades and their interaction with NR (Athwal and Huber 2002). At longer time, PAs modulate the association of 14-3-3 proteins with the H^+-ATPase, thus activating NR activity, and this action could prevail over the effect of the increased NO

concentration.

The role of BRs and PAs in restoring growth has been shown independently in several studies (Verma and Mishra 2005, Bajguz and Hayat 2009, Rady 2011, Choudhary *et al.* 2011, 2012). It is apparent that both plant growth regulators (BRs and PAs) crosstalk to induce defensive genes countering stress conditions.

Conclusion: Salt stress decreased the growth, physiological, and biochemical parameters in *C. sativus*. Co-application of EBL and Put was more effective in alleviation of NaCl stress than their individual impacts. Our study confirmed further that the judicious application of EBL and Put augmented their stress-ameliorating effects.

References

Ali, B., Hayat, S., Ahmad, A.: Response of germinating seeds of *Cicer arietinum* to 28-homobrassinolide and/or potassium. – Gen. Appl. Plant Physiol. **31**: 55-63, 2005.

Ali, B., Hayat, S., Fariduddin, Q., Ahmad, A.: 24-Epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. – Chemosphere **72**: 1387-1392, 2008.

Alia, Mohanty, P., Saradhi, P.P.: Effect of sodium chloride on primary photochemical activities in cotyledonary leaves of *Brassica juncea*. – Biochem. Physiol. Pfl. **188**: 1-12, 1992.

Ahmad, P., Jaleel, C.A., Azooz, M.M., Nabi, G.: Generation of ROS and non enzymatic antioxidants during abiotic stress in plants. – Bot. Res. Int. **2**: 11-20, 2009.

Amor, N.B., Jiménez, A., Megdiche, W. *et al*.: Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. – Physiol. Plantarum **126**: 446-457, 2006.

Andre, C.M., Lorondelle, Y., Evers, D.: Dietary antioxidants and oxidative stress from a human and plant perspective: a review. – Curr. Nutr. Food Sci. **6**: 2-12, 2010.

Anuradha, S., Rao, S.S.R.: Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth and improved photosynthetic pigment levels and nitrate reductase activity. – Plant Growth Regul. **40**: 29-32, 2003.

Anuradha, S., Rao, S.S.R.: The effect of brassinosteroids on radish (*Raphanus sativus* L.) seedlings growing under cadmium stress. – Plant Soil Environ. **53**: 465-472, 2007.

Arndt, S.K., Arampatsis, C., Foetzki, A. *et al*.: Contrasting patterns of leaf solute accumulation and salt adaptation in four phreatophytic desert plants in a hyperarid desert with saline groundwater. – J. Arid Environ. **59**: 259-270, 2004.

Ashraf, M.: Some important physiological selection criteria for salt tolerance in plants. – Flora **199**: 361-376, 2004.

Athwal, G.S., Huber, S.C.: Divalent cations and polyamines bind to loop 8 of 14-3-3 proteins, modulating their interaction with phosphorylated nitrate reductase. – Plant J. **29**: 119-129, 2002.

Bais, H.P., Ravishankar, G.A.: Role of polyamines in the ontogeny of plants and their biotechnological applications. – Plant Cell Tiss. Org. **69**: 1-34, 2002.

Bajguz, A.: Metabolism of brassinosteroids in plants. – Plant Physiol. Biochem. **45**: 95-107, 2007.

Bajguz, A., Hayat, S.: Effect of brassinosteroids on the plant responses to environmental stresses. – Plant Physiol. Biochem. **47**: 1-8. 2009.

Bajguz, A.: An enhancing effect of exogenous brassinolide on the growth and antioxidant activity in *Chlorella vulgaris* cultures under heavy metals stress. – Environ. Exp. Bot. **68**: 175-179, 2010.

Bajguz, A.: Suppression of *Chlorella vulgaris* growth by cadmium, lead, and copper stress and its restoration by endogenous brassinolide. – Arch. Environ. Con. Tox. **60**: 406-416, 2011.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water-stress studies. – Plant Soil **39**: 205-207, 1973.

Beauchamp, C., Fridovich, I.: Superoxide dismutase improved assays and an assay applicable to acrylamide gels. – Anal. Biochem. **44**: 276-286, 1971.

Belkheiri, O., Mulas, M.: The effects of salt stress on growth, water relations and ion accumulation in two halophyte *Atriplex* species. – Environ. Exp. Bot. **86**: 17-28, 2013.

Benavides, M.P., Aizencang, G., Tomaro, M.L.: Polyamines in *Helianthus annuus* L. during germination under salt stress. – J. Plant Growth Regul. **16**: 205-211, 1997.

Besford, R.T., Richardson, C.M., Campos, J.L., Tiburcio, A.F.: Effect of polyamines on stabilization of molecular complexes in thylakoid membranes of osmotically stressed oat leaves. – Planta **189**: 201-206, 1993.

Bethke, P.C., Drew, M.C.: Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of *Capsicum annum* during progressive exposure to NaCl salinity. – Plant Physiol. **99**: 219-226, 1992.

Bouchereau, A., Aziz, A., Larher, F., Martin-Tanguy, J.: Polyamines and environmental challenges: Recent development. – Plant Sci. **140**: 103-125, 1999.

Campbell, H.W.: Nitrate reductase structure, function and regulation bridging the gap between biochemistry and physiology. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 277-303, 1999.

Chance, B., Maehly, A.C.: Assay of catalase and peroxidase – In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol.2. Pp. 764-775. Academic Press., New York 1955.

Choudhary, S.P., Kanwar, M., Bhardwaj, R., Gupta, B.D., Gupta, R.K.: Epibrassinolide ameliorates Cr(VI) stress via influencing the levels of indole-3-acetic acid, abscisic acid, polyamines and antioxidant system of radish seedlings. – Chemosphere **84**: 592-600, 2011.

Choudhary, S.P., Kanwar, M., Bhardwaj, R., Yu, J.Q., Tran, L.S.P.: Chromium stress mitigation by polyamine-brassinosteroid application involves phytohormonal and physiological strategies in *Raphanus sativus* L. – PLoS ONE: DOI: 10.1371/journal.pone.0033210, 2012.

Clouse, S.D.: The molecular intersection of brassinosteroids regulated growth and flowering in *Arabidopsis*. – Proc. Natl. Acad. Sci. USA **105**: 7345-7346, 2008.

Cohen, S.S.: A Guide to the Polyamines. Pp. 595. Oxford University Press, New York 1998.

Divi, U.K., Rahman, T., Krishna, P.: Brassinosteroid-mediated stress tolerance in *Arabidopsis* shows interactions with abscisic acid, ethylene and salicylic acid pathways. – BMC Plant Biol. **10**: 151-164, 2010.

Duan, J.J., Li, J., Guo, S.R., Kang, Y.Y.: Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. – J. Plant Physiol. **165**: 1620-1635, 2008.

Dwivedi, R.S., Randhawa, N.S.: Evaluation of a rapid test for the hidden hunger of zinc in plants. – Plant Soil **40**: 445-451, 1974.

Fariduddin, Q., Khanam, S., Hasan, S.A. *et al*.: Effect of 28-homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of *Brassica juncea* L. – Acta Physiol. Plant. **31**: 889-897, 2009b.

Fariduddin, Q., Yusuf, M., Chalkoo, S., Hayat, S., Ahmad, A.: 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system in the presence of chilling stress. – Photosynthetica **49**: 55-64, 2011.

Fariduddin, Q., Yusuf, M., Hayat, S., Ahmad, A.: Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in *Brassica juncea* plants exposed to different levels of copper. – Environ. Expt. Bot. **66**: 418-424, 2009a.

Feng, G., Zhang, F.S., Li, X.L. *et al*.: Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. – Mycorrhiza **12**: 185-190, 2002.

Geissler, N., Hussin, S., Koyro, H.W.: Interactive effects of NaCl salinity and elevated atmospheric CO_2 concentration on growth, photosynthesis, water relations and chemical composition of the potential cash crop halophyte *Aster tripolium* L. – Environ. Exp. Bot. **65**: 220-231, 2009.

Ghoulam, C., Foursy, A., Fares, K.: Effect of salt stress on growth, inorganic ions and proline accumulation in relation of osmotic adjustment in five sugar beet cultivars. – Environ. Exp. Bot. **47**: 39-50, 2002.

Goda, H., Shimada, Y., Asami, T., Fujioka, S., Yoshida, S.: Microarray analysis of brassinosteroid-regulated genes in *Arabidopsis*. – Plant Physiol. **130**: 1319-1334, 2002.

Groppa, M.D., Benavides, M.P.: Polyamines and abiotic stress: recent advances. – Amino Acids **34**: 35-45, 2008.

Gudesblat, G.E., Russinova, E.: Plants grow on brassinosteroids. – Curr. Opinion Plant Biol. **14**: 530-537, 2011.

Hamada, K.: Brassinolide in crop cultivation. – In: MacGregor, P. (ed.): Plant Growth Regulators in Agriculture. Pp. 190–196. Food and Fertilizer Technology Centre for the Asian and Pacific Region, Taiwan 1986.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J.: Plant cellular and molecular responses to high salinity. – Annu. Rev. Plant Phys. **51**: 463-499, 2000.

Hayat, S., Hasan, S.A., Hayat, Q., Ahmad, A.: Brassinosteroids protect *Lycopersicon esculentum* from cadmium toxicity applied as shotgun approach. – Protoplasma **239**: 3-14, 2010a.

Hayat, S., Hasan, S.A., Yusuf, M., Hayat, Q., Ahmad, A.: Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiata*. – Environ. Exp. Bot. **69**: 105-112, 2010b.

Hayat, S., Maheshwari, P., Wani, A.S. *et al*.: Comparative effect of 28-homobrassinolide and salicylic acid in the amelioration of NaCl stress in *Brassica juncea* L. – Plant Physiol. Biochem. **53**: 61-68, 2012.

Hopkins, W.G., Huner, N.P.A. (ed.): Responses of plants to environmental stresses. – In: Introduction to Plant Physiology (4th ed.). Pp. 223-239. J. Wiley & Sons Inc., Hoboken 2009.

Hu, Y.X., Bao, F., Li, J.Y: Promotive effect of brassinosteroids on cell division involves a distinct *CycD3*-induction pathway in *Arabidopsis*. – Plant J. **24**: 693-701, 2000.

Hussain, S.S., Ali, M., Ahmad, M., Siddique, K.H.M.: Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. – Biotechnol. Adv. **29**: 300-311, 2011.

Iyengar, E.R.R., Reddy, M.P.: Photosynthesis in high salt-tolerant plants. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 897-910. Marcel Dekker, Baten Rose 1996.

Jaworski, E.G.: Nitrate reductase assay in intact plant tissues. – Biochem. Bioph. Res. Co. **43**: 1274-1279, 1971.

Kao, W.Y., Tsai, T.T., Shih, C.N.: Photosynthetic gas exchange and chlorophyll a fluorescence of three wild soybean species in response to NaCl treatments. – Photosynthetica **41**: 415-419, 2003.

Karim, M.A., Fracheboud, Y., Stamp, P.: Photosynthetic activity of developing leaves of *Zea mays* is less affected by heat stress than that of developed leaves. – Physiol. Plantarum **105**: 685-693, 1999.

Khan, M.H., Panda, S.K.: Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. – Acta Physiol. Plant. **30**: 81-89, 2008.

Khripach, V., Zhabinskii, V., Groot, A.E.: Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. – Ann. Bot. **86**: 441-447, 2000.

Khripach, V.A., Zhabinskii, V.N., de Groot, A.E.: Brassinosteroids in Nature. – In: Khripach, V.A., Zhabinskii, V.N., de Groot, A.E. (ed.): Brassinosteroids: A New Class of Plant Hormones. Pp. 301-324. Academic Press, San Diego 1999.

Koca, H., Bor, M., Özdemir, F., Türkan, I.: The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. – Environ. Exp. Bot. **60**: 344-351, 2007.

Krishnamurthy, R.: Amelioration of salinity effects in salt tolerance rice by foliar applications of putrescine. – Plant Cell Physiol. **32**: 699-703, 1991.

Lakra, N., Mishra, S.N., Singh, D.B., Tomar, P.C.: Exogenous putrescine effect on cation concentration in leaf of *Brassica juncea* seedlings subjected to Cd and Pb along with salinity stress. – J. Environ. Biol. **27**: 263-269, 2006.

Li, R.L., Shi, F,C., Fukuda, K.: Interactive effects of various salt and alkali stresses on growth, organic solutes, and cation accumulation in a halophyte *Spartina alterniflora* (Poaceae). – Environ. Exp. Bot. **68**: 66-74, 2010.

Liu, J., Zhou, Y.F., Zhang, W.H., Liu, Y.L.: [Effects of exogenous polyamines on chloroplast-bound polymine content and photosynthesis of corn suffering salt stress.] – Acta Bot. Boreali-Occidentalia Sin. **26**: 254-258, 2006. [In Chinese]

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. – Trends Plant Sci. **7**: 405-410, 2002.

Munns, R.: Genes and salt tolerance: bringing them together. – New Phytol. **167**: 645-663, 2005.

Murphy, L.R., Kinsley, S.T., Durako, M.J.: Physiological effects of short-term salinity changes on *Ruppia maritima*. – Aquat. Bot. **75**: 293-309, 2003.

Mutlu, F., Bozcuk, S.: Effects of salinity on the contents of polyamines and some other compounds in sunflower plants differing in salt tolerance. – Russ. J. Plant Phys. **52**: 29-34, 2005.

Parida, A.K., Das, A.B.: Salt tolerance and salinity effects on plants: a review. – Ecotoxicol. Environ. Saf. **60**: 324-349, 2005.

Pérez-López, U., Robredo, A., Lacuesta, M., Mena-Petite, A., Muñoz-Rueda, A.; The impact of salt stress on the water status of barley plants is partially mitigated by elevated CO_2. – Environ. Exp. Bot. **66**: 463-470, 2009.

Rady, M.M.: Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. – Sci.

Hortic.-Amsterdam **129**: 232-237, 2011.
Rosales, E.P., Iannone, M.F., Groppa, M.D., Benavides, M.P.: Polyamines modulates nitrate reductase activity in wheat leaves: involvement of nitric oxide. – Amino Acids **42**: 857-865, 2012.
Sanita, di Toppi, L., Gabbrielli, R.: Response to cadmium in higher plants. – Environ. Exp. Bot. **41**: 105-130, 1999.
Sasse, J.M.: Physiological action of brassinosteroids: An update. – J. Plant Growth Regul. **22**: 276-288, 2003.
Schutzendubell, A., Polle, A.: Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. – J. Exp. Bot. **53**: 1351-1365, 2002.
Sekmen, A.H., Turkan, I., Tanyolac, Z.O., Ozfidan, C., Dinc, A.: Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. – Environ. Exp. Bot. **77**: 63-76, 2012.
Shahbaz, M., Ashraf, M., Athar, H.R.: Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? – Plant Growth Regul. **55**: 51-64, 2008.
Sheokand, S., Kumari, A., Sawhney, V.: Effect of nitric oxide and putrescine on antioxidative responses under NaCl stress in chickpea plants. – Physiol. Mol. Biol. Plant. **14**: 355-362, 2008.
Shi, K., Ding, X.T., Dong, D.K., Zhou, Y.H., Yu, J.Q.: Putrescine enhancement of tolerance to root-zone hypoxia in *Cucumis sativus*: a role for increased nitrate reduction. – Funct. Plant Biol. **35**: 337-345, 2008.
Sinha, S.K., Srivastava, H.S., Tripathi, R.D.: Influence of some growth regulators and cations on inhibition of chlorophyll biosynthesis by lead in maize. – Bull. Environ. Contamin. Toxicol. **51**: 241-246, 1993.
Soussi, M., Lluch, C., Ocana, A.: Comparative study of nitrogen fixation and carbon metabolism in two chickpea (*Cicer arietinum* L.) cultivars under salt stress. – J. Exp. Bot. **50**: 1701-1708, 1999.
Soussi, M., Ocana, A., Lluch, C.: Effect of salt stress on growth, photosynthesis and nitrogen fixation in chickpea (*Cicer arietinum* L.). – J. Exp. Bot. **14**: 1329-1337, 1998.
Subhan, D., Murthy, S.D.S.: Effect of polyamines on chlorophyll and protein content, photochemical activity, and energy transfer in detached wheat leaves during dark incubation. – Biol. Plantarum **44**: 529-533, 2001.
Sudhir, P., Murthy, S.D.S.: Effects of salt stress on basic processes of photosynthesis. – Photosynthetica **42**: 481-486, 2004.
Sullivan, C.Y., Ross, W.M.: Selection for drought and heat tolerance in grain sorghum. – In: Mussel, H., Staples, R.C. (ed.): Stress Physiology in Crop Plants. Pp. 263-281. J. Wiley & Sons, New York 1979.
Sun, Y., Veerabomma, S., Abdel-Mageed, H.A., Fokar, M., Asami, T.: Brassinosteroid regulates fiber development on cultured cotton ovules. – Plant Cell Physiol. **46**: 1384-1391, 2005.
Talaat, N.B., Shawky, B.T.: 24-Epibrassinolide ameliorates the saline stress and improves the productivity of wheat (*Triticum aestivum* L.). – Environ. Exp. Bot. **82**: 80-88, 2012.
Tassoni, A., Franceschetti, M., Bagni, N.: Polyamines and salt stress response and tolerance in *Arabidopsis thaliana* flowers. – Plant Physiol. Bioch. **46**: 607-613, 2008.
Thomas, T., Thomas, T.J.: Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. – Cell Mol. Life Sci. **58**: 244-258, 2001.
Tiburcio, A.F., Altabella, T., Masgrau, C.: Polyamines. – In: Bisseling, T., Schell, J. (ed.): New Developments in Plant Hormone Research. Pp. 38-85. Springer-Verlag, New York 2002.
Unni, S., Rao, K.K.: Protein and lipopolysac-charide profiles of a salt-sensitive *Rhizobium* sp. and its exopolysaccharide-deficient mutant. – Soil Biol. Biochem. **33**: 111-115, 2001.
Verma, S., Mishra, S.N.: Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. – J. Plant Physiol. **162**: 669-677, 2005.
Verslues, P.E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., Zhu, J.K.: Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. – Plant J. **45**: 523-539, 2006.
Vert, G., Nemhauser, J.L., Geldner, N., Hong, F., Chory, J.: Molecular mechanisms of steroid hormone signaling in plants. – Annu. Rev. Cell Dev. Biol. **21**: 177-201, 2005.
Xu, X.M., Ye, H.C., Li, G.F.: [Progress in research of plant tolerance to saline stress.] – Chin. J. Appl. Environ. Biol. **6**: 379-387, 2000. [in Chinese]
Yang, J.C., Zhang, J.H., Liu, K., Wang, Z.Q., Liu, L.J.: Involvement of polyamines in the drought resistance of rice. – J. Exp. Bot. **58**: 1545-1555, 2007.
Yu, J.Q., Huang, L.F., Hu, W.H. *et al.*: A role of brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. – J. Exp. Bot. **55**: 1135-1143, 2004.
Yusuf, M., Fariduddin, Q., Ahmad. A.: 24-Epibrassinolide modulates growth, nodulation, antioxidant system, and osmolyte in tolerant and sensitive varieties of *Vigna radiata* under different levels of nickel: A shotgun approach. – Plant Physiol. Biochem. **57**: 143-153, 2012.
Yusuf, M., Hasan, S.A., Ali, B. *et al.*: Effect of salicylic acid on salinity induced changes in *Brassica juncea.* – J. Integ. Plant Biol. **50**: 1-4, 2008.
Zeng, H., Tang, Q., Hua, X.: Arabidopsis brassinosteroid mutants det2-1 and bin2-1 display altered salt tolerance. – J. Plant Growth Regul. **29**: 44-52, 2010.
Zhang, Z., Ramirez, J., Reboutier, D. *et al.*: Brassinosteroids regulate plasma membrane anion channels in addition to proton pumps during expansion of *Arabidopsis thaliana* cells. – Plant Cell Physiol. **46**: 1494-1504, 2005.
Zhao, F.G., Qin, P.: Protective effect of exogenous polyamines on root tonoplast function against salt stress in barley seedlings. – Plant Growth Regul. **42**: 97-103, 2004.
Zhao, J., Ren, W., Zhi, D., Wang, L., Xia, G.: Arabidopsis DREB1A/CBF3 bestowed transgenic tall rescue increased tolerance to drought stress. – Plant Cell Rep. **26**: 1521-1528, 2007.
Zheng, C., Jiang, D., Liu, F. *et al.*: Effects of salt and water logging stresses and their combination on leaf photosynthesis, chloroplast ATP synthesis, and antioxidant capacity in wheat. – Plant Sci. **176**: 575-582, 2009.