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# Protective Roles of Brassinolide on Tomato Seedlings under Drought Stress

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**ABSTRACT:** Tomato(Lycopersicum esculentum L.) was used to study the role of 24-epibrassinolide (24-EBL) in protection from drought stress. In this experiment, when the third leaf of tomato plants appeared, 24-epibrassinolide was sprayed at 0.01 and 1 µM concentrations for 3 days alternately. Then three levels of drought stress (control, 3 and 5 days withholding water) were applied. Thereafter, Analysis was conducted on the contents of leaf dry and fresh weight, water content, chlorophylls, reduced and total carbohydrates, ethylene and the leakage of electrolyte. Also the activities of lypoxygenase (LOX) and superoxide dismutase (SOD) and SOD isozymes expression levels in leaves. The result showed that Reduction in weight, chlorophylls, reduced and total carbohydrates content in drought condition probably due to the effect of drought on chlorophyll synthesis and degradation and in turn on photosynthesis . Also drought-induced increase in lypoxygenase activity caused an increase in ion leakage. However, alleviation in ion leakage, lypoxygenase activity and ethylene production in plants treated with concentration of 24-EBL under drought stress was observed. This could be because of the positive effect BR on stability membrane. Under the drought treatment, application of BR significantly increased leaf weight and water, the contents of chlorophyll and carbohydrates, and the activity of SOD and the expression of SOD isozymes in the leaves. Therefore, probably our results showed that 24-EBL could decline oxidative damage caused by drought stress and that BR plays an important role in protection of tomato plants from drought stress by enhancing the activities or expression level of protective enzymes in the leaves.

***Key words****:* Drought; Brassinolide; Tomato plant Lepoxygenase; Ethylene

***Abbreviations:*** BRs–brassinosteroids; ROS–reactive oxygen species; 24-EBL– 24epibrassinolide; SOD – superoxide dismutase

## INTRODUCTION

Drought is one of the most important abiotic stress factors that limit plant growth and ecosystem production around the world (12). It is estimated that the percentage of droughty terrestrial areas will redouble by the end of 21st century. Under selective pressures imposed by adverse environmental conditions, plants have evolved both fast stress responses (such as stomatal closure) and longer-term adaptations, which include modulation of metabolism (24,35, 37). Also, upon exposure to drought stress, plants respond at the whole-plant, cellular and molecular levels (14,27). At the whole-plant level, the effect of drought stress is usually perceived as a decrease in photosynthesis and growth, which is associated with alterations in carbon and nitrogen metabolisms (31,8,9). At cellular level, drought stress often leads to the accumulation of reactive oxygen species (ROS). Excessive ROS production can cause oxidative stress to the photosynthetic apparatus and seriously impair the normal function of cells and decrease in antioxidative capacity of the cells (3,1,32). The enhanced amount of ROS can be viewed as a threat to the cell, but they can also act as secondary messengers involved in the stress signal transduction pathway (19,34). The capability of scavenging ROS and reducing their damaging effects may correlate with the drought tolerance of plants (18,33).

The mechanism adopted by the plants for drought tolerance includes certain secondary metabolites and PGR's. They include ABA, Ethylene, Auxin, Jasmonic acid and plant steroids. Among various compounds exploited to alleviate plant stress, the brassinosteroids (BRs) are recognized as group of phytohormones that regulate plant growth and productivity. These steroidal compounds occur in free form and conjugated to sugars and fatty acids. Till this date, up to 70 BRs have been isolated from plants. However, brassinolide (BL), 24 epibrassinolide (EBL)

and 28 homobrassinolide (HBL) are three bioactive BRs being the most widely used in physiological studies (28). Ameliorative roles of BRs have been recognized in plants subjected to various biotic and abiotic stresses (15,7). Exogenous application of BRs increased tolerance to low and/or high temperature stress (7) and heavy metal stress (23), drought stress [9,8,19), salinity (2) and waterlogging (7). The ability of BRs to induce tolerance in plants to a broad spectrum of stress agents, results largely from the interaction with other phytohormones (7).

Although numerous reports have confirmed the potential of plant hormones to synergistically improve crop performance under abnormal environmental conditions, very little information is available on the presence of BRs in Lycopersicum esculentum.L. under drought stress. The study presented here was designed with an aim to assess the effect of EBL against soil drought based on the analysis of various growth and oxidative and antioxidative parameters in °, and to evaluate the significance of changes in these features for plant performance during soil drying.

## MATERIALS AND METHODS

### *Hormone preparation*

The stock solutions of EBL was prepared by dissolving the required quantity of EBL in 5 ml of ethanol, in 100 ml volumetric flask. 5 mL of surfactant “Tween-20” was added to it and final volume was made by using double distilled water (DDW). The EBL desired concentrations was prepared by the dilution of stock solution with DDW.

### *Growth of plants and experimental design*

The healthy seeds of Tomato (Lycopersicon esculentum, Var. Tomba (BB204) were surface-sterilized with 0.01% mercuric chloride solution followed by the repeated washing with DDW in order to remove adhering solution of mercuric chloride. The seeds were grown in trays of compost until the seeds germinated. When the second leaf appeared, the seedlings were transferred to plastic pots with a 11-cm diameter containing sand, loam and peat (2:1:1) in a greenhouse. The seedlings were irrigated with water once a day and with Hoagland’s solution (pH 6.7) once a week (on soil media around the root) to prevent mineral deficiency. Then the tomato plants with 3 fully expanded leaves, were left to a growth chamber at a day/night temperature of 26/18°C, 16/8 hour (light/dark) photoperiod and 6000 lux light intensity for 5 day. After the adaptation period in the growth chamber, 24-epibrassinolide (Sigma chemicals, USA) was sprayed at 0.01 and 1 µM concentrations for 3 days alternately. Then three levels of water stress (control, 3 days and5 days withholding water) were applied. After treatment, the third leaf of plants was harvested. The harvested leaves were rapidly frozen in liquid nitrogen and stored at -80°C for biochemical analysis.

### *Leaf fresh and dry weight and relative water content measurements*

After treatments (24epiBL and withholding water), FW of leaves were measured. For DW
Determination at first the samples were oven dried at 80°C for 48-72 h. For leaf water content assay, tomato leaves were excised, and their fresh weight immediately determined (FW). After floating in deionised water at 4 °C overnight in the dark, the leaves were blotted and their turgid weight (TW) was determined. Finally, they were dried in an oven at 80 °C overnight and weighed to determine dry weight (DW). The relative water content (RWC) was calculated as follows:

$$RWC = [(FW - DW)/(TW - DW)] \times 100.$$

### *Biochemical analysis*

### *Photosynthetic pigments assay*

The amount of chlorophylla,b and total were determined according to the method of Arnon (1949)(4). Leaf samples (0.25 g) were homogenized in acetone 80%). Extract centrifuged at 3,000×g and absorbance was recorded at 646.8 nm and 663.2 nm for chlorophylls assay by a UV-Visible spectrophotometer (Cary50, Germany). Pigment content was calculated according to the following formulae:

$$Chla = (12.25\ A\ 663.2 - 2.79\ A), Chlb = (21.21\ A\ 646.8 - 5.1\ A), Totalchl = Chla + Chlb$$

### *Carbohydrates assay*

Freshly harvested leaves were immediately frozen in liquid nitrogen and extracted three times for 30 min each in 3 ml 80% (v:v) ethanol at 80°C. The extracts were cleared by centrifugation, evaporated and dissolved in sterile water. Total soluble sugars were measured according to Hodge and Hodfreiter(1962)(26) and reducing sugars were measured as described by Somogy (41).

***Electrolyte leakage***

Total inorganic ions leaked out of the leaf were estimated by the method described by Ben Hamed (10). Twenty leaf discs were taken in a boiling test tube containing 10 mL of DDW, and electrical conductivity was measured (EC a ). The tubes were heated at 45 °C and 55 °C for 30 min in water bath, and electrical conductivity was measured (EC b ) each time. Later, the contents were again boiled at 100 °C for 10 min, and electrical conductivity was again recorded (EC c ). The electrolyte leakage was calculated using the formula:
Electrolyte Leakage% = (EC b - ECc / EC c a) ×100

***Ethylene Determination***

The rates of ethylene production in detached tomato leaves desiccated were determined by enclosing 2 gram of leaves in tubes closed with Subaseal with 2-3 ml of H2O for maintaining relative humidity. Ethylene was allowed to accumulate for a 30-min period. Intercellular ethylene was extracted by the vacuum method as described by wright (43). The concentration of ethylene produced by leaves was quantified using a gas chromatograph (6890N Network GC System) and a flame ionization detector (FID). Ethylene in the gas was identified by comparing its retention time and co-chromatography with authentic standards (0.9 ± 0.1 L L−1 of ethylene in nitrogen) Calculations of ethylene production are expressed as µl/gr fresh wt. hr.

***Lypoxygenase activity assay(LOX)(EC 1.13.11.12)***

Fresh samples (0.5 g) were homogenized by homogenizer in 5 mL of 100 mM potassium phosphate buffer (pH 7.6) containing 1 mM EDTA-Na2 and PVPP (polyvinylpolypyrrolidone) 1%. The homogenized samples were centrifuged at 10,000g for 5 min. The supernatant was used as crude enzyme extract in LOX enzyme analyse. All colorimetric measurements were made at 20°C using a spectrophotometer (Shimadzu UV/Vis 1201). Lypoxygenase activity was determined by the oxidation of the substrate linoleic acid, and changes in absorbance determined at 550 nm (Borrell et al., 1997)(11).

***Assay of superoxide dismutase activity(SOD,EC 1.15.1.1)***

Frozen leaf samples (0.5 g) were used for enzyme extraction. Samples were ground in 2 ml of 50 mM phosphate buffer (pH 7.2) using pre-chilled mortar and pestle. The phosphate buffer contained 1 mM EDTA, 1 mM PMSF and 1% PVP40. Then the extract was centrifuged at 4°C at 17,000g for 10 min. The supernatant was used for measurements of enzyme activity.

A photochemical method published by Giannopolitis and Reis (1977)(22) was used to determine superoxide dismutase activity. The reaction solution (3 ml) contained 50 µM NBT, 1.3 µM riboflavin, 13 mM methionine, 75 nM EDTA, 50 mM phosphate buffer (pH 7.8), and 20-50 µl of the enzyme extract. The test tubes containing the reaction solution were irradiated under light (15 fluorescent lamps) for 15 min. The absorbance of the irradiated solution was read at 560 nm using a spectrophotometer (Cary 50). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% ρ-nitro blue tetrazolium chloride (NBT) photoreduction.

***SOD Isozymes expression***

The crude enzyme solution (0.1 mL) and 0.1 mL 40% sucrose solution were put into a finger tube, and the tube was shaken thoroughly for isozyme analysis. The polyacrylamide vertical slab gel was used for electrophoresis. The stacking gel concentration was 3.5%, and the resolving gel concentrations were 10% for SOD. The added sample was 40 µL per well. The electrophoresis was conducted in the Tris-glycine buffer in a 4˚C refrigerator for 8–9 hours, under the current intensities at 1 mA/well for stacking gel and 2 mA/well for resolving gel. After electrophoresis, the gels were stained with NBT for SOD.

***Statistical analysis***

The experiment was conducted according to completely randomized design. Each treatment was replicated four times. Data was statistically analyzed by analysis of variance (ANOVA) using SPSS software version 17 for window (SPSS, Chicago, IL, USA). Least significant difference (LSD) was calculated to separate the means. Data were statistically analyzed by one-way analysis of variance using SPSS and the means were separated by Duncan's multiple range test at 0.05 probability level.

## RESULTS AND DISCUSSION

***Effects of BR on leaf Dry and Fresh weight and water content***

Water deficit decreased the dry and fresh weight of the leaf. However, 24epibressinolide at the tested concentrations (0.01 and 1 µM) significantly increased leaf fresh and dry matter under normal and stress

conditions, especially fresh eight(Fig. 1A, B). Leaf dry matter increased by 20% and 50% under mild and severe drought stress at 1 µM BR treatment, respectively. 1 µM BR treatment increased fresh weight by 30% and 50% under normal and drought conditions, respectively.Drought stress markedly increased relative leaf water content (RWC). This increase was significantly elevated by pretreatment with BR at both levels of stress (Fig. 1C).

***Effects of BR on Chlorophylls and carbohydrates contents***

Drought regime caused a significant decrease in total chlorophyll. However, 42-EBL pretreatment had a more obvious effect on these parameters compared to control(Fig. 2A, B, C). Under drought stress, especially 1 µM BR, significantly enhanced chlorophylls content.

carbohydrates content were influenced by 24-epibrassinolide treatment under normal growth conditions, but it decreased significantly under severe drought stress (Fig. 2D,E). However, an increase in Reduced and total carbohydrates contents was found in EBR treated plants under mild and severe stress but BR couldn't elevate these parameters till control level. .

***Effects of BR on Ion leakage and lypoxygenase activity***

Electrolyte leakage increased significantly under drought stress, and nearly doubled

in severe drought treatment (Fig. 3B). Both levels of BR treatment decreased significantly electrolyte leakage under both normal and stress conditions. Similarly, the lepoxygenas activity was reduced in plants treated with 24-epiBL under drought stress, especially in severe stress was nearly halved (Fig. 3A). It was obvious that BR treatment decreased lipid peroxidation in plants.

***Effects of BR on ethylene production***

The effects of BR on ethylene production in the leaves of tomato seedling varied significantly with the intensity of drought stress (Fig. 3C). In three days stress, the ethylene levels in the leaves of seedlings treated by 0.01 and 1 µM BR were 50% and 70% lower than that in the drought stress respectively. However, in control condition the ethylene content in leaves of seedlings treated by BR was higher than that in the control both under 0.01 and 1 µM concentration of 24-EBL. (Fig. 3C). The results suggest that BR can regulate the ethylene production in tomato seedlings under drought stress, but the regulating effects varied with the concentration.

***Effects of BR on activity and isozyme expression of SOD***

SOD is the key enzyme scavenging active oxygen free radicals in plants, and its activity is directly correlated with the antioxidative capability of plants. Drought stress increased significantly the activity of SOD at both levels of stress. Exogenous application of BR in drought stressed plants enhanced the activity of enzyme at 0.01 and 1 µM. However, there was no significant difference between both of concentrations of BR applied under drought stress (Fig. 4). The results suggest that BR can increase the activity of antioxidant enzymes in tomato seedlings.

Three SOD isoforms were visualized on activity staining gels (Fig. 4). But the width and brightness of the native bands were different. For example, Intensity of activity staining of SOD increased especially under BR and stress condition and it was higher than that of well-watered controls. The highest intensity in isoforms was observed in SOD2 when BR was applied. Also gels stained revealed one new band under drought stress and BR treatment (Fig. 4). The results above suggest that under high temperature stress BR not only affects the activities of SOD but also induces the new native bands of isozyme mentioned above emerging and then protect rice seedlings.

Plants grown in the soil amended with water deficit exhibited a significant decline in all the growth parameters compared to the control plants. Drought decreased the dry and fresh weight of the tomato leaf. However, 24-EBL at the tested concentrations (0.01 and 1 µM) significantly increased leaf weight and water content. Especially at 1 µM of 24-EBL, Leaf fresh weight increased under normal and stress conditions by30% and 60% as compared to untreated plants respectively (Fig. 1). A decrease in plant growth was observed under drought stress (46). These results are in agreement with that of Li et al. who marked Brassinosteroids, especially 24-EBL caused an increase in plant biomass (8,31). Zhang and coworkers (2008) found that application of BRs could partially alleviate the detrimental effects of water stress on growth of soybean through improving antioxidant system and promoting dry weight accumulation(45). Also it was reported that promotion of growth in stressed seedlings of Robinia (31), tomato (1) and wheat (40) under stress conditions might be related to enhance levels of nucleic acids, soluble proteins and photosynthesis. It has been proposed that this growth inhibition caused by drought could partly be due to the shortage of energy because processes involved in drought damage repair on membrane or proteins are energy consuming (30,38). Similarly, leaves of BR treated plant possessed more surface area which could mainly be an expression of activated cell division and cellular enlargement induced by the BR application

(15,19). Reduction in leaf water content in the leaves of plants under drought was observed in the present study (Fig. 1). The decrease in RWC is mainly due to the increased osmotic potential in the surrounding medium and decreased water absorption (42,13). It could be also attributed to the increased electrolyte leakage and LOX activity in the leaves subjected to drought stress as observed in the present study (Fig. 3). However, treatment of 24-EBL to stressed and non-stressed plants improved the values for RWC.

BR probably generated such a response due to its involvement in ATPase pump activation(29) and also due to the decrease in electrolyte leakage and LOX activity across the membrane. There are many reports of drought damage to photosynthetic machinery at multiple levels such as pigment content, stomatal functioning and gaseous exchange, structure and function of thylakoids, electron transport and enzymes (9,21). Drought causes a decrease in chlorophyll content through chlorophyll biosynthesis inhibition or acceleration of its degradation (37). Therefore, On the basis of our finding, 24-EBL fed to non-stressed and stressd plants increased the chlorophyll content in this study is supported also by other workers (2,1,24). The possible reason is the BL-induced impact on transcription and/or translation (6) by involving the expression of specific genes responsible for synthesis of enzymes determining chlorophyll synthesis.

Production and scavenging of the free radicals in plants are in a dynamic balance under normal conditions (34,15) but the balance will be broken if stress happens, then free radicals will increase sharply and the plant will be harmed. As a natural course plants exposed to stress produce large quantities of reactive oxygen species (27,39) that may oxidize proteins, lipids and nucleic acids resulting in abnormalities at the level of cell (33,16) In order to counteract these reactive oxygen species, plants induce the synthesis of antioxidant metabolites (ascorbate, glutathione etc.) and enzymes (superoxide dismutase, catalase etc.) that neutralize the toxic effects of ROS generated through stress. In this experiment, BR treatment could increase the activity of protective enzyme (SOD) in the leaves under drought stress (Fig. 4), suggesting that it was beneficial to scavenge free radicals in plants and to decrease the ion leakage, thereby ensuring the integrity of membrane structure and function. The expression of SOD isozymes under drought stress was not the same as that of non-stressed isozymes. The treatment of BR under drought could enhance the expression of SOD isozymes. (Fig4). The role of BRs in restoring abiotically constrained growth has been independently shown in several studies and also reviewed (8,9,1,24,45). Our study has shown that application of this hormone ameliorates the stress-properties at the physiological level. The results suggest that BR treatment can change the activity of SOD under high drought stress, as well as the composition of its isozymes. The effects of BR on the expression of SOD isozymes under drought stress may be due to that the regulation of BR on gene expression.

Summarizing all the results above, we conclude that BR plays an important role in protecting the tomato against drought stress. It can increase the activity of SOD and enhance the expression of its isozymes, scavenge the free radicals which increase in the stress conditions, stabilize membrane structure, decrease the leakage of electrolyte and alleviate the injury of water deficit on chlorophyll, thereby keep tomato seedlings in normal physiological function under drought. This experiment confirmed that application of BR could enhance the drought tolerance of the plants, alleviate the harm of high drought on tomato effectively. With the advantages of simple operation and instant effects, BR might be applied widely in agriculture production.

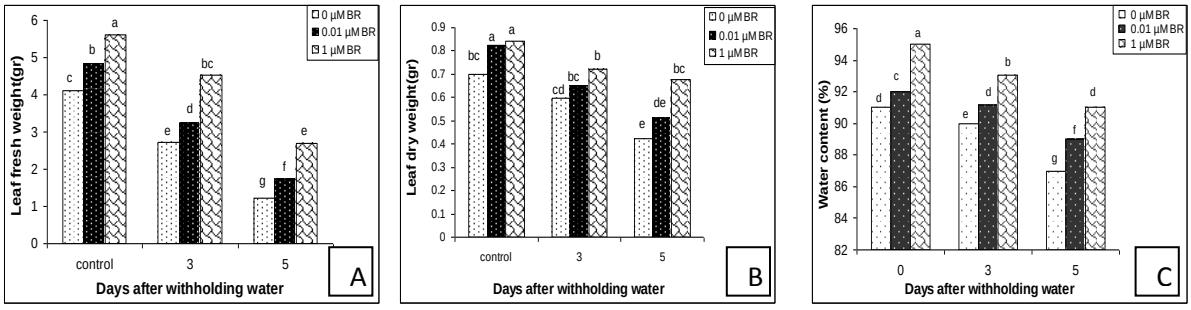


Figure 1. The effects of BR and drought stress on the leaf fresh weight(A), leaf dry weight(B), relative water content(C) in Lycopersicon esculentum L. plants. Values are means of four replicates and SEM significant differences are at P<0.05 according to Duncan's test.

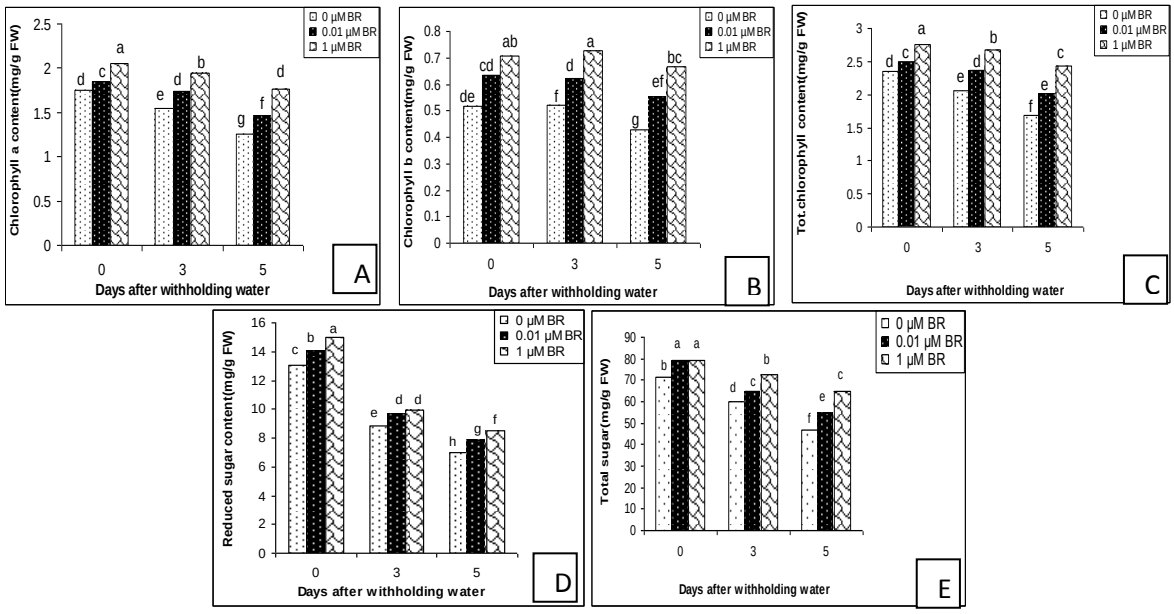


Figure. 2. The effects of BR and drought stress on the leaf chlorophyll a(A), chlorophyll b(B), total chlorophyll(C),reduced carbohydrate(D) and total carbohydrate(E) content in Lycopersicon esculentum L. plants. Values are means of four replicates and SEM significant differences are at P<0.05 according to Duncan's test.

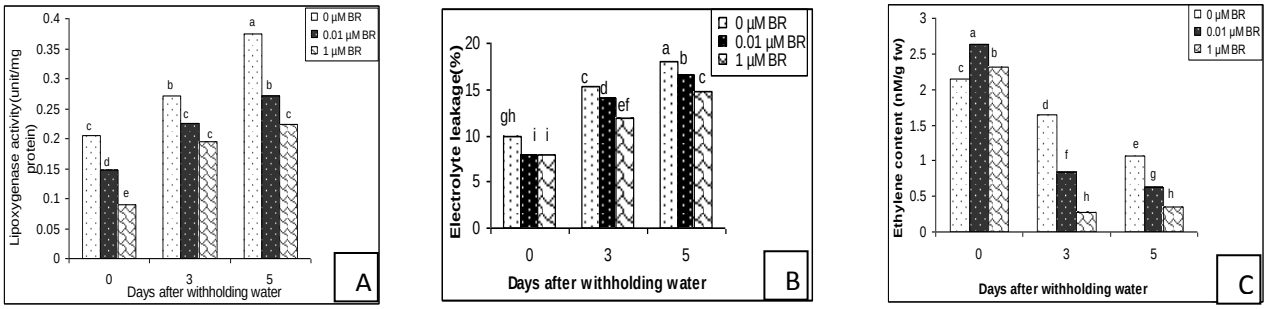


Figure 3. The effects of BR and drought stress on the leaf lypoxygenase activity(A), electrolyte leakage (B) and ethylene content(C) in Lycopersicon esculentum L. plants. Values are means of four replicates and SEM significant differences are at P<0.05 according to Duncan's test.

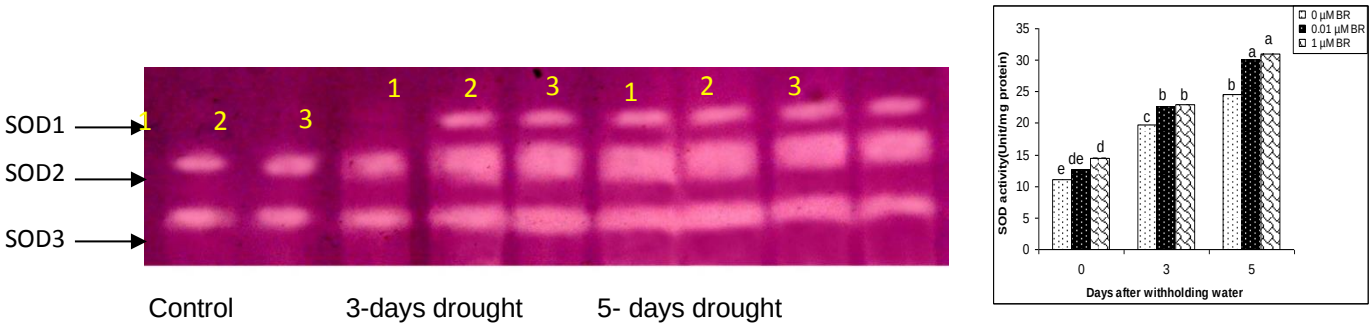


Figure 4. The effects of BR and drought stress on the leaf SOD activity and SOD isozymes in Lycopersicon esculentum L. plants. Values are means of four replicates and SEM significant differences are at P<0.05 according to Duncan's test. Numbers of 1-3 indicate concentrations of 0,0.01 I µM 24-EBL.

## REFERENCES


Ahammed GJ, Yuan HL, Ogweno JO, Zhou YH, Xia XJ, Mao WH, Shi K, Yu JQ.2012. Brassinosteroid alleviates phenanthrene and pyrene phytotoxicity by increasing detoxification activity and photosynthesis in tomato. Chemosphere 86: 546–555

Ali B, Hayat S, Ahmad A.2007. 28-homobrassinolide ameliorates the saline stress in chickpea (Cicer arietinum L. Environ. Exp. Bot. 59: 217-223.

Aranjuelo I, Molero G, Erice G, Avice JC, Nogués S.2010. Plant physiology and proteomics reveals the leaf response to drought in alfalfa (Medicago sativa L.). J. Exp. Bot. 62 111-123.

Arnon D. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24, 1–15

Ashraf M, Akram NA, Arteca RN, Foolad MR.2010. The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Crit. Rev. Plant Sci. 29: 162-190.

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

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

Behnamnia M*, Kalantari2 Kh M , Rezanejad2 F.2009. Exogenous application of brassinosteroid alleviates drought-induced oxidativestress in Lycopersicon esculentum L. General and Applied Plant Physiology 35 (1–2): 22–34

Behnamnia M, Kalantari KhM, Ziaie J.2009. The effects of brassinosteroid on the induction of biochemical changes in Lycopersicon esculentum L. under drought stress.Turk J Bot 33: 417-428.

Ben Hamed K, Castagna A, Salem E, Ranieri A, Abdelly C.2007. Sea fennel (Crithmum maritimum L.) under salinity conditions: a comparison of leaf and root antioxidant responses. Plant Growth Regul. 53: 185-194.

Borrell A, Carbonelli L, Farras R, Puig-Parellada P, A. Tiburcio E.1997. Polyamines inhibit lipid peroxidation in senescing oat leaves. Physiol. Plant. 99: 385-390

Boyer JS.1982. Plant productivity and environment.Science 218:443–8.

Chartzoulakis K, Psarras G, Vemmos S, Loupassaki M, Bertaki M.2006. Response of two olive cultivars to salt stress and potassium supplement. J. Plant Nutr. 29: 2063-2078.

Chaves MM, Flexas J, Pinheiro C.2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103 551-560.

Clouse SD, Sasse JM.1998. Brassinosteroids: essential regulators of plant growth and development. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49: 427-451.

Di Toppi LS, Gabbrielli R.1999. Response to cadmium in higher plants. Environ.Exp. Bot. 41: 105-130.

Dietz KJ, Fannschmidt TP.2011. Novel regulators in photosynthetic redox control of plant metabolism and gene expression. Plant Physiol. 155:1477-1485.

Faize M, Burgos L, Faize L, Piqueras A, Nicolas E, Barba-Espin G, Clemente-Moreno MJ, Alcobendas R, Artlip T, Hernandez JA.2011. Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. J. Exp. Bot. 62 :2599-2613.

Fariduddin Q, Khanam S, Hasan SA, Ali B, Hayat S, Ahmad A.2009a. Effect of 28homobrassinolide on the drought stress-induced changes in photosynthesis and antioxidant system of Brassica juncea L. Acta Physiol. Plant 31: 889-897.

Foyer CH, Noctor G.2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid. Redox Signaling 11: 861-905.

Geissler N, Hussin S, Koyro HW.2009. Interactive effects of NaCl salinity and elevated atmospheric CO concentration on growth, photosynthesis, water relations and chemical composition of the potential cash crop halophyte Astertripolium L. Environ. Exp. Bot. 65 :220-231.

Giannopolitis CN, Ries SK. 1977. Superoxide dismutase. I. occurrence in higher plants. Plant Physiol 59: 309-314.

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

Hayat Sh, Maheshwari, ShafiWani A, Irfan M, Alyemeni MN, l Ahmad A.2012. Comparative effect of 28 homobrassinolide and salicylic acid in the amelioration of NaCl stress in Brassica juncea L. Plant Physiology and Biochemistry 53 : 61-68

Health RL, Packer L.1969. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys 125: 189-198.

Hodge JE, Hodfreiter BR.1962. Determination of reducing sugars and carbohydrates, in: R.L. Wilster, M.L. Wolfrom (Eds.), Methods in Carbohydrate Chemistry, 1, Academic Press, New York. 380–394.

Jaleel CA, Gopi R, Manivannan P, Panneerselvam R.2007. Antioxidative Potentials as a Protective Mechanism in Catharanthus roseus (L.) G.Don. Plants under Salinity Stress. Turk J Bot 31: 245-251.

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

Khripach VA, Zhabinskii VN, Khripach NB.2003. New practical aspects of brassinosteroids and results of their 10 year agricultural use in Russia and Balarus, in: S. Hayat, A. Ahmad (Eds.), Brassinosteroids: Bioactivity and Crop Productivity, Kluwer Academic Publisher, The Netherlands pp. 189-230.

Kleinkopf GE, Wallace A.1974. Physiological basis for salt tolerance in Tamarix ramosissima. Plant Sci. Lett. 3: 157-160.

Lawlor DW, Cornic G.2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 25: 275-294.

Li KR, Wang HH, Han G, Wang QJ, Fan J.2008. Effects of brassinolide on the survival, growth and drought resistance of Robinia pseudoacacia seedlings under water-stress. New Forests 35: 255-266.

Mahdavian K, GHorbanli M, Kalantari Kh M.2008. The Effects of Ultraviolet Radiation on the Contents of Chlorophyll, Flavonoid, Anthocyanin and Proline in Capsicum annuum L. Turk J Bot 32:25-33.

Munir N, Aftab F.2009. The role of polyethylene glycol (PEG) pretreatment in improving sugarcane's salt (NaCl) tolerance. Turk J Bot 33: 407-415.

Namdjoyan Sh, Namdjoyan Sh, Kermanian H.2012. Induction of phytochelatin and responses of antioxidants under cadmium stress in saffl ower (Carthamus tinctorius) seedlings. Turk J Bot 36: 495-502.

Novikova GV, Moshkov IE, Los DA.2007. Protein sensors and transducers of cold and osmotic stress in cyanobacteria and plants. J Mol Biol. 41:427–37.
Reddy MP, Vora AB.1986. Changes in pigment composition, hill reaction activity and saccharide metabolism in bajra (Pennisetum typhoides S&H) leaves under NaCl salinity. Photosynthetica 20: 50-55.
Rizhsky L, Liang H, Mittler R.2002. The combined effect of drought stress and heat Shock on gene expression in tobacco. Plant Physio. 130:1143–51.
Schutzendubell A, Polle A.2002. Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53:1351-1365.
Shahbaz M, Ashraf M, Athar H.2008. Dose exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (Triticum aestivum L.)? Plant Growth Regul. 55:51-64.
Somogy M.1952. Notes on sugar determination. J. Biol. Chem. 195: 19–23.
Tattini M, Gucci R, Coradeschi MA, Ponzio C, Everard JD.1995. Growth, gas exchange and ion content in Olea europaea plants during salinity stress and subsequent relief. Physiol. Plant 98: 117-124.
Wright STC.1977. The relationship between leaf water potential and the levels of abscisic acid and ethylene in excited leaves. Planta 134: 183-189.
Yu JQ, Huang LF, Hu WH, Zhou YH, Mao WH, Ye SF, Nogues S.2004. A role of brassinosteroids in the regulation of photosynthesis in Cucumis sativus. J. Exp.Bot. 55: 1135-1143.
Zhang M, Zhai Z, Tian X, Duan L, Li Z.2008. Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (Glycine max L.). Plant Growth Regul. 56(3): 257-264.
Zhu JK.2001. Cell signaling under salt, water and cold stresses. Cur. Opin. *Plant Biol* 4:401–406.