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EXOGENOUS APPLICATION OF BRASSINOSTEROID ALLEVIATES DROUGHT-INDUCED OXIDATIVE STRESS IN *LYCOPERSICON ESCULENTUM* L.

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Summary. Drought stress is considered as a restricting factor for plant products. Therefore, many compounds have been applied to minimize the harmful effects of stress. Brassinosteroid is one of the compounds with antioxidative characteristics. In this experiment, when the fourth leaf of tomato plants appeared, 24-epibrassinolide was sprayed to the leaves at 0.01 and 1 μM concentrations for 3 days with one day interval. Then three levels of drought stress (control, 3 and 5 days withholding water) were applied. Thereafter, the interaction effects of 24-brassinosteroid (BR_{27}) and water stress on some biochemical and antioxidative parameters of tomato plants were investigated. Peroxidation of lipids and H_2O_2 content were reduced in the plants which were treated with BR_{27}. The intensity of some bands of stress enzymes (GPOD and APX) in the electrophoretic pattern was different in stressed plants treated with 24-epibrassinolide when compared with the plants under drought stress. An increase in the activity of antioxidant enzymes (POD, SOD, CAT, APX) and antioxidative compounds content including ascorbate, carotenoids and proline was observed in drought stress plants after pretreatment with BR. Based on our observations that pretreatment with 24-epibrassinolide caused a decrease in MDA together with an increase in antioxidant enzymes, it is likely that BR mitigated damage caused by water stress.

Key words: drought stress, brassinosteroid, antioxidative system, tomato, proline, lipid peroxidation.

Abbreviations: BR–brassinosteroid; MDA–malondialdehyde; TBA–thiobarbituric acid; TCA–trichloroacetic acid ; EBL-epibrassinolide; HBL–homobrassinolide; CAT–catalase; POD–peroxidase; APX–ascorbate peroxidase; ROS–reactive oxygen species; BR_{27} - 24-epibrassinolide; H_2O_2 - hydrogen peroxide; PMSF-phenylmethylsulfonyl fluoride; PVP-polyvinylpyrrolidone; EDTA-ethylendiamine tetraacetic acid.

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INTRODUCTION

Drought stress is defined as a condition in which water availability to plants is so 1ow that it is unfavorable for the growth of a plant species (Zhu, 2001). Reactive oxygen species (ROS) are enhanced during drought stress through the disruption of electron transport system and oxidizing metabolic activities occurring in chloroplast, mitochondria and microbodies (Sofo et al., 2005). Plants eliminate ROS produced in non-stressful conditions through production of non-enzymatic and enzymatic antioxidants (Inze and Montagu, 2000), whereas during severe drought conditions the production of ROS exceeds the capacity of the antioxidative systems to remove them, causing oxidative stress (Sofo et al., 2005). In these conditions cells could be protected either by the endogenous molecular systems or exogenously applied compounds that mitigate the stress (Ingram and Bartels, 1996).

One of this compounds which has antioxidative characteristics is brassinosteroid (Haubrick and Assmann, 2006). Brassinosteroids (BRs) are common plant-produced compounds that can function as growth regulators (Bishop et al., 2006). In addition, it has been suggested that BRs could be included in the category of phytohormones (Haubrick and Assmann, 2006). Exogenous application of BR may influence a range of diverse processes of growth and development in plants (Cao et al., 2005; Ozdemir et al., 2004). It is now clear that BRs provide protection against a number of abiotic stresses (Vardhini and Rao, 2003). Treatment with BRs enhanced growth of wheat (Shahbaz et al, 2008), French bean (Upreti and Murti, 2004) and tomato plants (Ogweno et al., 2008) under drought stress. Several studies have shown that BRs alter the antioxidant capacity of plants under stress condition (Yin et al., 2008). Zhang et al. (2008) reported that treatment with 24-epibrassinolide increased the activity of some antioxidative enzymes causing mitigation of oxidative stress.

The present study was an attempt to carry out investigations on the effect of brassinolide on tomato plants under drought stress, with the aim 1) to characterize the variation in the antioxidant ability of different concentrations of BR under mild and severe drought stress conditions; 2) to investigate the possible mechanisms responsible for drought tolerance in plants treated with two concentrations of BR, and elucidate the possible mechanisms that might be involved in the BR-promoted antioxidant responses to drought.

MATERIALS AND METHODS

Growth of plants and experimental design

Tomato plants (*Lycopersicon esculentum,* Var. Tomba (BB204) were grown from seeds in trays of compost until the seeds germinated. After germination, 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. Each seedling was placed in one pot. The seedlings were irrigated with water once a day. At the same time, seedlings were also irrigated 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 (about 15 days after growing in the pot), were left to grow in 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 days. After

the adaptation period in the growth chamber, 24-epibrassinolide (Sigma chemicals, USA) dissolved in ethanol was sprayed on the leaves at 0.01 and 1 μM concentrations for 3 days (Tween-20 (0.01%) used as surfactant). Then three levels of water stress (control, 3 days and 5 days withholding water) were applied. Four replicates were assigned for each treatment. 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.

Fresh weight (FW) and dry weight (DW) measurements

The interaction effect of drought and 24-epibrassinolide on FW and DW of the shoot was analyzed. After treatment (24-epiBL and withholding water), the roots were cut off. FW and DW of shoots with the third leaf were also measured. For DW determination, the samples were oven dried at 80°C for 48-72 h.

Biochemical assays

The content of H_2O_2 was determined according to Alexieva et al. (2001). Leaf tissue (500 mg) was homogenized in ice bath with 5 cm^3 of cold 0.1% (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged (12,000×g, 15 min, 4°C) and 0.5 cm^3 of the supernatant was added to 0.5 cm^3 of 100 mM potassium phosphate buffer (pH 7.0) and 1 cm^3 of 1 M KI. The absorbance was read at 390 nm.

The level of lipid peroxidation in plant tissues was measured by determination of MDA (Heath and Packer, 1969) and other aldehydes (Meirs et al., 1992), breakdown products of lipid peroxidation. MDA content was determined with thiobarbituric acid (TBA) reaction. 0.2 g tissue sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10,000×*g* for 5 min. 4 ml of 20% TCA containing 0.5% TBA was added to 1 ml aliquot of the supernatant. The mixture was heated at 95°C for 15 min and cooled immediately. The non-specific absorbance of the supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm for MDA measurement, and at 455 nm for other aldehydes. The level of lipid peroxidation was expressed as μmol of MDA formed using an extinction coefficient of 155 mM^{-1} cm^{-1} and for other aldehydes the extinction coefficient was 0.457×10^5 M^{-1} cm^{-1}.

Proline was extracted and its concentration determined by the method of Bates et al. (1973). Leaf tissues were homogenized in 3 % sulfosalicylic acid and the homogenate was centrifuged at 3,000×*g* for 20 min. The supernatant was treated with acetic acid and ninhydrin, boiled for 1 h, and then the absorbance was determined at 520 nm. Proline (Sigma) was used for a standard curve.

The amount of carotenoids were determined according to the method of Lichtenthaler et al. (1987). 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 chlorophyll assay and 470 nm for carotenoids assay by a UV-Visible spectrophotometer (Cary50, Germany). Pigment content was calculated according to the following formulae:

$$\text{Chl a} = (12.25\ A_{663.2} - 2.79\ A_{646.8})$$

$$\text{Chl b} = (21.21\ A_{646.8} - 5.1\ A_{663.2})$$

$$\text{Car} = (1000\ A_{470} - 1.8\ \text{Chl a} - 85.02\ \text{Chl b})/198$$

Ascorbic acid (ASA) and dehydro-ascorbic acid (DHAS) were determined as described by Mc de Pinto et al. (1999). 0.5 g

of leaf tissue was homogenized in 10 ml meta phosphoric acid (5%) and centrifuged (15 min at 1000×*g*). 300 µl of supernatant was used for the ASA assay and 750 µl potassium phosphate buffer (100 mM, pH 7.2) and 300 µl distilled water were added to the extract. 300 µl of the supernatant was used for the DHAS assay and 750 µl potassium phosphate (100 mM, pH 7.2) and 150 µl of dithiotheritol (10 mM) were added. The samples were incubated at room temperature for 10 min, and then 150 µl of 0.5% N-ethylmaleimide was added. Both samples were vortexed and incubated at room temperature for 10 min. To each sample 600 µl of 10% (w/v) TCA, 600 µl of 44% (v/v) orthophosphoric acid, 600 µl of 4% (w/v) bipyridyl in 70% (v/v) ethanol and 10 µl of 3% $FeCl_3$ were added. After vortex-mixing, samples were incubated at 40°C in a water bath for 20 min, and then samples were vortexed again and incubated at 40°C water bath for another 20 min. The absorbance of samples was recorded at 525 nm. A standard curve of ASA and DHAS was used for the calculation of ASA and DHAS concentration. ASA, DHAS and total ascorbic acid (ASA + DHAS) concentrations were expressed as mg g^{-1} fw.

Assay of enzymes activity

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% PVP-40. Then the extract was centrifuged at 4°C at 17,000×*g* for 10 min. The supernatant was used for measurements of enzyme activity.

A photochemical method published by Giannopolitis and Reis (1977) was used to determine superoxide dismutase (SOD EC 1.15.1.1)) 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) at 78 µmol $m^{-2}s^{-1}$ 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.

The activity of ascorbate peroxidase enzyme (APX, EC 1.11.1.1) was measured using the method of Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.15 mM H_2O_2, 0.1 mM EDTA and 50 µl enzyme extract (supernatant). Oxidation of ascorbic acid was considered as the decrease in the absorbance at 290 nm 2 min after the start of the reaction. One unit of APX oxidizes 1 mM ascorbic acid in one min at 25°C.

Catalase (CAT, EC 1.11.1.6) activity was assayed spectrophotometrically by monitoring the decrease in the absorbance of H_2O_2 at 240 nm using the method of Dhindsa et al. (1981). The assay solution contained 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reaction was started by the addition of 100 µl enzyme extract to the reaction mixture and the change in absorbance was followed 1 min after the start of the reaction. One unit of activity was considered as the amount of enzyme which decomposes 1 mM of H_2O_2 in one minute.

Peroxidase (GPOD, EC 1.11.1.7) activity was determined using the guaiacol test (Plewa et al., 1991). The

tetraguaiacol formed in the reaction has a maximum absorption at 470 nm and thus the reaction can be readily followed spectrophotometrically. The enzyme was assayed in a solution containing 50 mM phosphate buffer (pH 7.0), 0.3% H_2O_2 and 1% guaiacol. The reaction started by the addition of 20 µl enzyme extract at 25°C. One enzyme unit was calculated on the basis of the formation of 1mM tetraguaiacol for 1 min.

Native PAGE and activity staining

Native polyacrylamide gel electrophoresis (PAGE) was performed at 4°C, 180 V, following Laemmli (1970). For APX, the enzyme solutions were subjected to native PAGE on a 12% polyacrylamide gel. For POD and CAT 10% and 7% PAG were used.

APX activity was detected following the procedure described by Mittler and Zilinskas (1993). The gel equilibrated with 50 mM sodium phosphate buffer (pH 7.0) contained 2 mM ascorbate for 30 min. Then gels were incubated in a solution containing the same buffer, 4 mM ascorbate and 2 mM H_2O_2 for 20 min. Then the gel was washed in the buffer for 1 min and submerged in a buffer containing 28mM TEMED and 2.45 mM NBT for 10-20 min with gentle agitation in the presence of light.

For CAT activity detection, the gel was incubated in 3.27 mM H_2O_2 for 25 min and then the gel was washed in distilled water and submerged in a solution composed of 1% potassium ferricyanid and 1% ferric chloride for 4 min (Woodbury et al. 1971).

For POD activity detection, the gel was incubated in 25 mM potassium phosphate buffer (pH 7.0) and then the gel was submerged again in a buffer containing 18 mM guaiacol and 25 mM H_2O_2, till the POD activity-containing band was visualized (Fielding and Hall, 1978).

Statistical analysis

All experiments were performed in 4 replicates, using completely randomized design. 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

Growth

Water deficit decreased the dry and fresh weight of the shoot. However, 24-epibressinolide at the tested concentrations (0.01 and 1 µM) significantly increased shoot fresh and dry matter under normal and stress conditions (Fig. 1A, B). Shoot dry matter increased by 27% and 70% under mild and severe drought stress at 1 µM BR treatment, respectively. 1 µM BR_{27} treatment increased fresh weight by 30% and 50% under normal and severe drought conditions, respectively.

H_2O_2 content and lipid peroxidation

H_2O_2 content increased significantly under drought stress, and nearly tripled in severe drought treatment (Fig. 1E). Both levels of BR treatment decreased significantly H_2O_2 content under both normal and stress conditions. Similarly, the contents of MDA and other aldehydes were reduced in plants treated with 24-epiBL under drought stress, especially in severe stress (Fig. 1C, D). It was obvious that drought stress increased lipid peroxidation in plants.

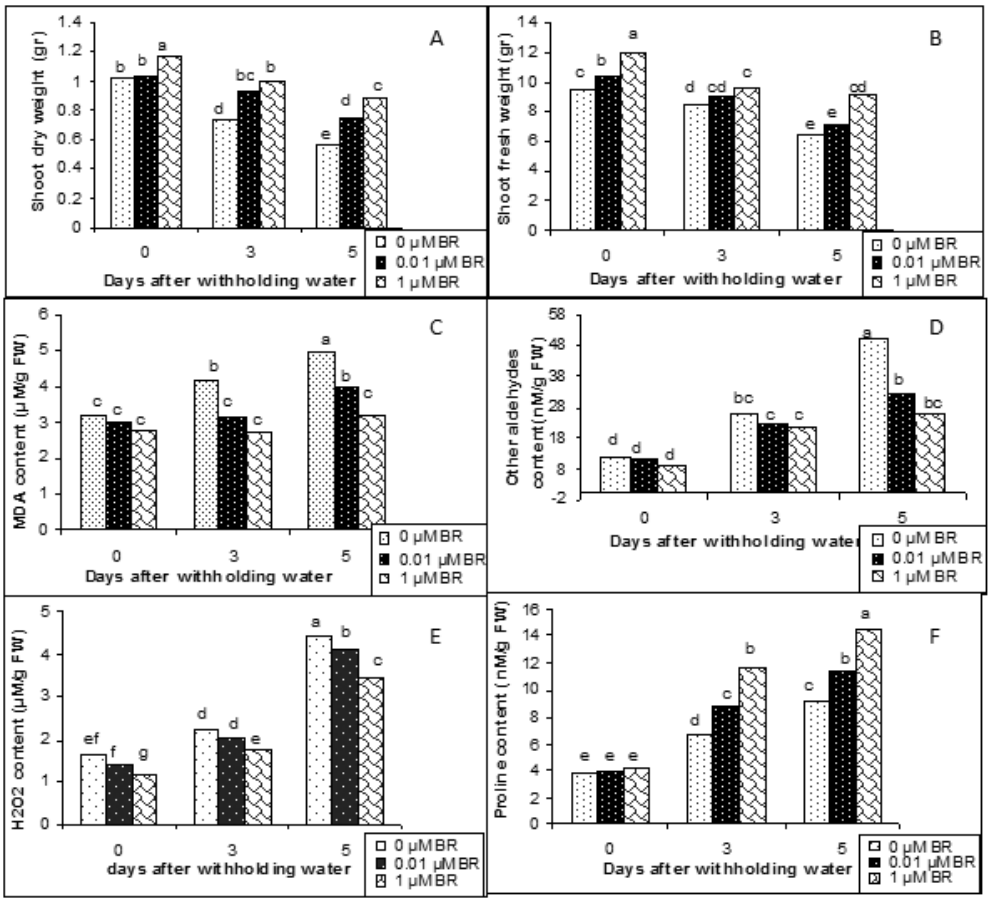

Fig. 1. The effects of BR and drought stress on the shoot weight, lipid peroxidation, H_2O_2 and proline content in *L. esculentum* plants which were grown for one month under controlled conditions. Plants were divided into three groups: two groups were pretreated with BR and another group was sprayed with distilled water. The three groups were subjected to water stress for 3 and 5 days. The control plants were irrigated daily. Values are means of four replicates and SEM significant differences are at $P<0.05$ according to Duncan's test.

Proline content

Drought stress markedly increased proline content. This increase was significantly elevated by pretreatment with BR_{27} at both levels of stress (Fig. 1F). Treatment with 1 µM BR under mild or severe drought stress increased proline content 3- and 4-fold, respectively in comparison with controls.

Ascorbate and carotenoids content

Drought regime caused a significant increase in ascorbate pool. The content of dehydroascorbate was also increased due to drought stress. Mild stress had a more obvious effect on these parameters than severe stress. Drought stress did not significantly affect ascorbic acid content (Fig. 2A, B, C). Exogenous application

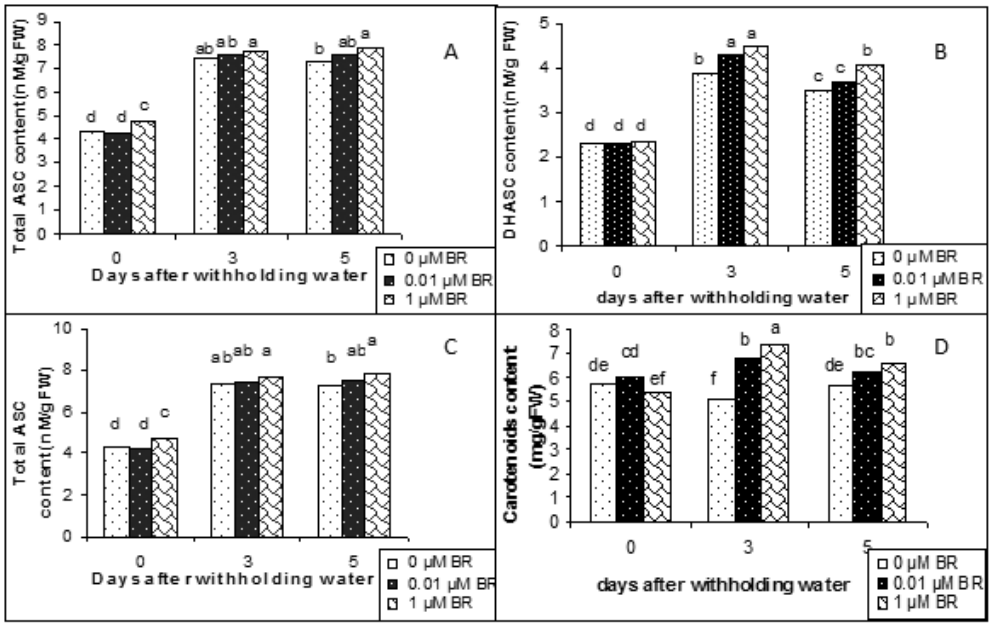

Fig. 2. The effects of BR and drought stress on ascorbate (ASA, DHAS, total AS) and carotenoids content in *L. esculentum* plants which were grown for one month under controlled conditions. Description of variants as in Fig. 1. Values are means of four replicates, and SEM significant differences are at $P<0.05$ according to Duncan's test.

of 0.01 µM BR_{27} did not alter these parameters under normal conditions. Both concentrations of BR elevated these three parameters under drought stress (Fig. 2). Carotenoids content was not influenced by 24-epibrassinolide treatment under normal growth conditions, but it decreased significantly under severe drought stress (Fig. 2D). However, an increase in carotenoids content was found in EBR-treated plants under mild and severe stress.

Enzyme activities

Drought stress increased significantly the activity of SOD and CAT, but GPOD and APX activities declined by severe drought (Fig. 3). Exogenous application of BR in drought stressed plants enhanced the activity of these enzymes at both levels of stress, except APX and SOD activities where there was no significant difference between BR levels applied under mild drought stress (Fig. 3).

Four POD isoforms were visualized on activity staining gels (Fig. 4A). The highest intensity in POD isoforms was observed in POD4 when BR_{27} was applied. Gels stained for CAT revealed only one band (Fig. 4B). The four ASA-specific APX bands were present in tomato plants and they increased not only in drought stressed plants, but also due to BR treatments under stress conditions. Intensity of activity staining of APX increased especially under mild stress and it was higher than that of well-watered controls and severe stressed plants (Fig. 4C).

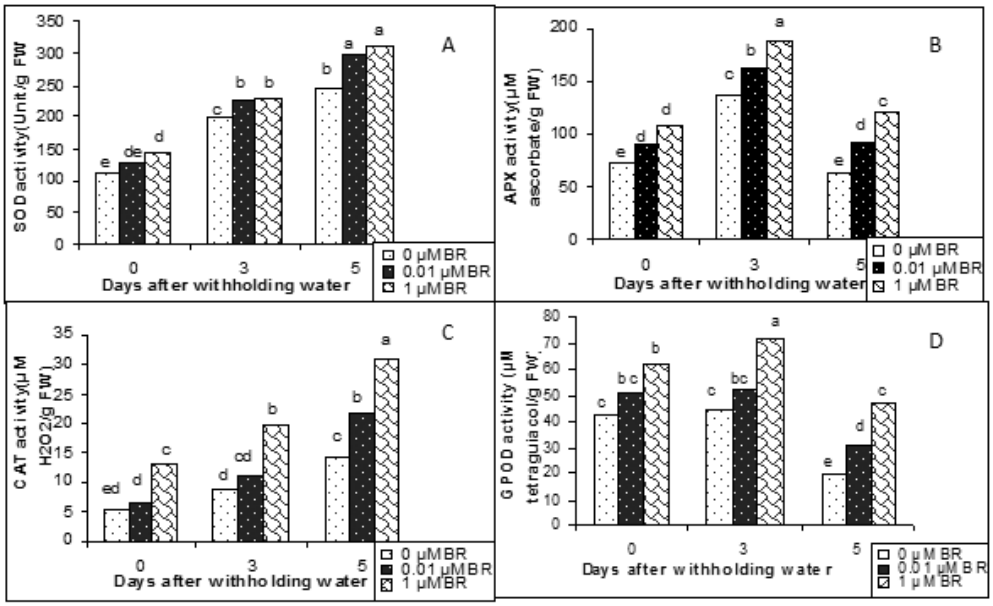

Fig. 3. The effects of BR and drought stress on the antioxidant enzyme activities (SOD, APX, CAT and GPOD) in *L. esculentum* plants which were grown for one month under controlled conditions. Description of variants as in Fig. 1. Values are means of four replicates, and SEM significant differences are at $P<0.05$ according to Duncan's test.

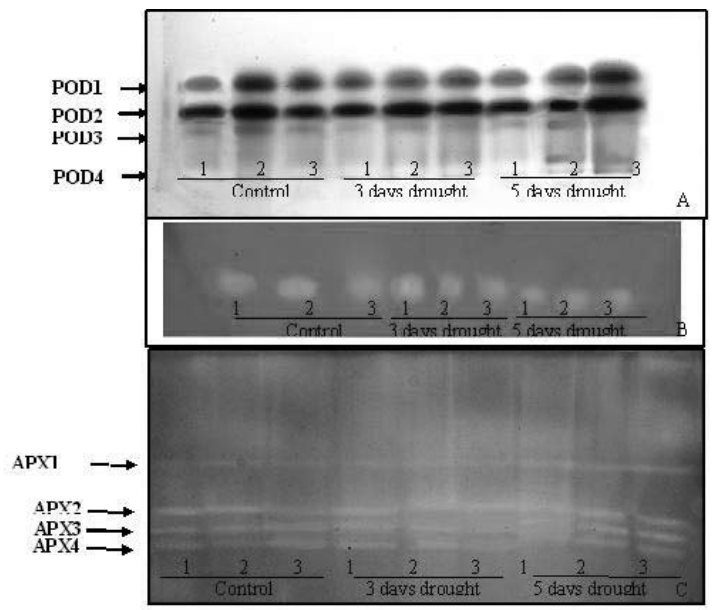

Fig. 4. The effects of BR and drought stress on antioxidant enzyme isoforms (GPOD, CAT and APX) separated by native PAGE. Lanes 1-3 represent different BR concentrations (0, 0.01 and 1 µM).

DISCUSSION

A decrease in plant growth was observed under drought stress (Fig. 1A, B) (Zhu, 2001). Brassinosteroids, especially BR_{27}, caused an increase in plant biomass (Li et al., 2008). Zhang et al. (2008) found that application of BRs could partially alleviate the detrimental effect of water stress on growth of soybean through improving antioxidant system and promoting dry weight accumulation. BRs participate in the processes of gene expression, transcription and translation in normal and stressed plants (Zhang et al., 2008; Mazorra et al., 2002). It was reported that promotion of growth in stressed seedlings of *Robinia Pseudoacacia* (Li et al., 2008), sorghum (Vardhini and Rao, 2003), wheat (Shahbaz et al., 2008) and rice (Anuradha and Rao, 2003) under stress conditions might be related to enhanced levels of nucleic acids, soluble proteins and photosynthesis.

Drought stress caused an increase in the formation of reactive oxygen species (ROS) that are responsible for various damages to macromolecules (Zhu, 2001). In the present study, the H_2O_2 content and lipid peroxidation (MDA and other aldehydes content) increased by increasing the drought stress (Fig. 1C, D, E). Pretreatment with BR_{27} decreased the accumulation of MDA and H_2O_2 contents under drought stress. BRs, especially BR_{27} have anti-stress effects on plants, helping them to overcome low and high temperature stress, drought and pathogen infection (Yin et al., 2008). Zhang et al. (2008) and Li et al. (2008) stated that BR treatment declined MDA content under drought stress in soybean and *Robinia pseudoacacia* plants, respectively. It has also been reported that BR_{27} reduces H_2O_2 and MDA contents in the leaves of tomato plants subjected to high temperature (Ogweno et al., 2008). It has been assumed that BRs act as secondary messengers for the induction of antioxidant defense system in stressed plants and thereby could effectively scavenge ROS in plants under stress (Mazorra et al., 2002).

The content of proline in tomato plants which were under drought stress and 24-epibrassinolide treatments increased progressively with increasing drought level and concentration of BR (Fig. 1F). It has been reported that BR treatments induce the expression of biosynthetic genes of proline (Ozdemir et al., 2004). There are several reports that application of BRs elevated proline content in plants (Vardhini and Rao, 2003). Proline as a cytosolic osmoticum and a scavenger of $OH^{\cdot}$ radical can interact with cellular macromolecules such as DNA, protein and membranes and stabilize the structure and function of such macromolecules (Kavir Kishor et al., 2005).

Carotenoid (Car) pigments protect chlorophylls from photo-oxidative destruction (Presad et al., 2005). The decline in Car content with increasing the stress level was noticeable (Fig. 2B). The content of Car was significantly higher in BR-treated plants under drought stress compared to the controlplants (Fig. 2B). Janeczko et al. (2007) reported that treatments with BR_{27} increased Car content at 2°C in rape leaves.

Drought stress caused accumulation of ascorbate pool (Fig. 2C). There was a significant decrease in ASA concentration under mild to severe stress. Yang and Wang (2007) reported that a decrease in ASC under stress may be due to an increase in oxidation and a reduction in regeneration of ASC. The content of ascorbate in BR-treated plants under drought stress was

significantly higher than controls (Fig. 2A). Ascorbic acid is a key nonenzymatic antioxidant that participates in redox regulation in different cell compartments to protect plant cells from oxidative stress (Chen et al., 2007). Ascorbic acid reduces O_2 and regenerates reduced α-tocopherol (Bartoli et al., 1999). APX oxidizes ASA to dehydro ascorbate (DHAS) through the gluthathione-ascorbate cycle. Thus, the high APX activity likely determined the low level of ASA, which is the main substrate of APX (Fig. 2 A, B). The function of ASC in the detoxification of H_2O_2 is dependent on APX (Chen et al., 2007). As the key redox couple in plant cells, the ratio of ASA/DHAS and its redox cycling can modulate the expression of genes and activities of the redox sensitive transcription factors and enzymes (Pastori and Foyer, 2002).

Antioxidative enzymes aid cells in removing harmful oxygen species. SOD, GPOD, APX and CAT are important antioxidant enzymes that detoxify ROS. Our results showed that treatment of tomato plants with BR_{27} was effective in increasing the activity of these enzymes under drought stress (Fig. 3). It has been found that BRs can induce the expression of some antioxidant genes and enhance the activities of antioxidant enzymes such as SOD, POD, APX and CAT (Mazorra et al., 2002; Cao et al., 2005). Several reports indicated that BRs application resulted in the enhancement of antioxidant enzymes activity under various stresses (Shahbaz et al., 2008; Vardhini and Rao, 2003). Zhang et al. (2008) and Özdemir et al. (2004) showed that BR treatment increased antioxidant enzyme activities in soybean and rice plants under stress conditions. Mazorra et al. (2002) reported that BR-enhanced antioxidant enzyme activities play an important role in drought tolerance. SOD detoxifies superoxide anion free radicals by forming H_2O_2, which is harmful to the chloroplast, nucleic acids and proteins. H_2O_2 can be eliminated by CAT, POD and APX. The induction of SOD activity has been shown to coincide with an increase in POD and CAT activity (Li et al., 2008).

Conclusion

Although BRs and ROS are thought to act as secondary messengers in the induction of antioxidant defenses in stressed plants, the relationship between BRs and ROS in stress-signal transduction remains still unclear (Cao et al., 2005). However, the present study indicated that foliar sprayed brassinolide caused a number of physiological and biochemical changes in the seedlings, including increased shoot mass, content of antioxidants (ascorbate and carotenoids) and free proline content as well as SOD, CAT, POD and APX activities. In contrast, treatment with brassinolide decreased MDA and H_2O_2 content. The response of the antioxidant enzymes to exogenously applied BR_{27} was different. It depended on the level of stress and the BR concentration. The most effective dose of 24-epibressinolide under stress conditions was found to be 1 μM. The highest effect of BR_{27} was found in plants which were under severe drought stress. The mechanism of BR in alleviation of the severe drought stress needs further investigations.

Overall, the results indicated that treatment with brassinolide could reduce the effects of water stress in tomato plants. Thus, it may be an useful management tool in afforestation projects in arid and semiarid areas.

REFERENCES

Alexieva, V., I. Sergiev, S. Mapelli, E. Karanov, 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ., 24, 1337-1344.

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

Bartoli, C. G., M. Simontacci, E. Tambussi, J. Beltrano, E. Montalde, S. Puntarula, 1999. Drought and watering dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. J. Exp. Bot., 50, 375-383.

Bates, L.S., R.P. Waldren, I.D. Teare, 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 29, 205-207.

Bishop, A., A.P. Mähönem, Y. Helariutta, 2006. Signs of changes: hormone receptors that regulate plant development. Development, 133, 1857-1869.

Cao, S., Q. Xu, Y. Cao, K. Qian, K. An, Y. Zhu, H. Binzeng, H. Zhao, B. Kuai, 2005. Loss of function mutation in Det2 gene lead to an enhanced resistance to oxidative stress in Arabidopsis. Physiol. Plant., 123, 57-66.

Chen, K.M., H.J. Gong, S.M. Wang, C.L. Zhang, 2007. Antioxidant defense system in *Phragmites communis* Trin. Ecotypes. Biologia Plantarum, 51(4), 754-758.

Dhindsa, R.S., P. Plumb-Dhindsa, T.A. Thorpe, 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot., 32, 93-101.

Fielding, J.L., J.L. Hall, 1978. A biochemical and cytological study of peroxidase activity in roots of *Pisum sativum*. J. Exp. Bot., 29, 969-981.

Giannopolitis, C.N., S.K. Ries, 1977. Superoxide dismutase. I. Occurrence in higher plants. Plant Physiol, 59, 309-314.

Haubrick, L.L., S.M. Assmann, 2006. Brassinosteroids and plant function: some clues, more puzzles. Plant,Cell and Environ, 29, 446-457.

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

Ingram, J., D. Bartels, 1996. The molecular basis of dehydration tolerance in plants. Plant Physiol., 47, 377-403.

Inze, J., M.V. Montagu, 2000. Oxidative stress in plants. TJ International Ltd, Padstow, Carnawell. Great Britain, p.321.

Janeczko, A., G. Gullner, A. Skoczowski, F. Dubert, B. Barna, 2007. Effects of brassinosteroid infiltration prior to cold treatment on ion leakage and pigment contents in rape leaves. Biologia Plantarum, 51(2), 355-358.

Kavir Kishor, P.B., et al. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current science, 88(3), 424-438.

Laemmli, U. ., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

Li, K.R., H.H. Wang, G. Han, Q. J. Wang, J. Fan, 2008. Effects of brassinolide on the survival, growth and drought resistance of *Robinia pseudoacacia* seedlings under water-stress. New Forests, 35, 255-266.

Lichtenthaler, H. ., A.R. Wellburn, 1983. Determination of total carotenoids and chlorophyll *a* and chlorophyll *b* leaf extracts in different solvents. Biochem. Soc. Trans., 603, 591-592.

Mazorra, L.M., M. Nunez, M. Hechavarria, F. Coll, M.J. Sanchez- Blanco, 2002. Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. Biol. Plant., 45, 593–596.

Meirs, S., S. Philosophhadas, N. Aharoni, 1992. Ethylene-increased accumulation of fluorescent lipid-peroxidation products detected during senescence of Parsley by a newly developed method. J. of the American Society for Hort. Science, 117, 128-132.

Mittler, R., B.A. Zilinskas, 1993. Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate dependent reduction of nitroblue tetrazolium. Anal. Biochem., 212, 540-546.

Nakano, Y., K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol., 22, 867–880.

Özdemir, F., M. Bor, T. Demiral, I. Turkan, 2004. Effect of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidant system of rice (*Oryza sativa* L.) under salinity stress. Plant Growth Regul., 41, 1–9.

Ogweno, J.O., X.S. Song, K. Shi, W.H. Hu, W.H. Mao, Y.H. Zhou, J.Q. Yu, S. Nogués, 2008. Brassinosteroids Alleviate Heat-Induced Inhibition of Photosynthesis by Increasing Carboxylation Efficiency and Enhancing Antioxidant Systems in *Lycopersicon esculentum*. J. Plant Growth Regul., 27, 49-54.

Pastori, G.M., C.H. Foyer, 2002. Common components, networks, and pathways of cross-tolerance to stress. The central role of redox and ascorbic acid-mediated controls. Plant Physiol., 129, 460-468.

Pinto, M. de, D. Francis, L. de Gara, 1999. The redox state of asscorbate-dehydroascorbate pairs a specific sensor of cell division in tobacco BY-Z cells. Protoplasma, 209, 90-97.

Plewa, M.I., S.R. Smith, E.D. Wagner, 1991. Diethyldithiocarbamate suppress the plant activation of aromatic amines into mutagenes by inhibiting tobacco cell peroxidase. Mutat. Res., 274, 57-64.

Prasad, S.M., R. Dwivedi, M. Zeeshan, 2005. Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress. Photosynthetica, 42(2), 177-185.

Shahbaz, M., M. Ashraf, H. Athar, 2008. Dose exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)?, Plant Growth Regul., 55, 51-64.

Sofo, A., A.C. Tuzio, B. Dichio, C. Xiloyannis, 2005. Influence of water deficit and rewatering on the components of the ascorbate-glutathione cycle in four interspecific Prunus hybrids. Plant Sci., 69, 403-412.

Upreti, K.K., G.S. Murti, 2004. Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in french bean under water stress. Biologia Plantarum, 48, 407-411.

Vardhini, B.V., S.S.R. Rao, 2003. Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. Plant Growth Regul., 41, 25-31.

Woodbury, W., A. K. Spencer, M. A. Stahmann, 1971. An improved procedure using ferricyanide for detecting catalase isoenzymes. Anal. Biochem., 44, 301-305.

Yang, S.H., L.J. Wang, S.H. Li, 2007. Ultraviolet-B irradiation-induced freeing tolerance in relation to antioxidant system in winter wheat (*Triticum aestivum* L.) leaves. Environ. Exp. Bot., 60, 300-307.

Yin, H., Q. Chen, M. Yi, 2008. Effects of short-term heat stress on oxidative damage and responses of antioxidant system in *Lilium longiflorum*. Plant Growth Regul., 54, 45-54.

Yu, J.Q., L.F. Huang, W.H. Hu, Y.H. Zhou, W.H. Mao, S.F, Ye, S. Nogues, 2004. A role of brassinosteroids in the regulation pf photosynthesis in *Cucumis sativus*. J. Exp. Bot., 55, 1135-1143.

Zhang, M., Z. Zhai, X. Tian, L. Duan, Z. Li, 2008. Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (*Glycine max* L.). Plant Growth Regul., DOI 10.1007/s10725-008-9305-4.

Zhu, J.K., 2001. Cell signaling under salt, water and cold stresses. Current Opinion in Plant Biol., 4, 401–406.