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# The effects of brassinosteroid on the induction of biochemical changes in *Lycopersicon esculentum* under drought stress

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**Abstract:** Drought stress is considered a restricting factor for plant products; therefore many compounds were applied to minimise the harmful effects of stress. One type of these compounds that have antioxidative characteristics is brassinosteroids. In this experiment, when 4 fully expanded leaves of tomato plants appeared, 24-epibrassinolide (24-EBL) was sprayed onto the leaves at 0.01 and 1 µM concentrations for 3 days with a 1-day interval. Three levels of drought stress (0, 3, and 5 days withholding water) were applied. Thereafter, the effects of brassinosteroids and water stress were investigated on some biochemical and antioxidative parameters of tomato plants. Lipid peroxidation, peroxide hydrogen, and proline content increased in plants subjected to drought stress. Reduction in protein content in drought conditions showed that drought stress affected protein synthesis and degradation. Decline in the activity of antioxidant enzymes (POD and APX) and increases in the SOD, GR, and CAT activities were observed in drought stressed plants. Reduction in peroxidation of lipids and $H_2O_2$ content in the plants treated with both 24-EBL and drought stress was observed. Based on our results it seems that brassinosteroids considerably alleviated oxidative damage that occurred under drought stress. Increase in activity of antioxidant enzymes (POD, CAT, APX, GR, and SOD) and change in isoenzymes pattern with higher intensity as well as increases in proline and protein content in drought stressed plants treated with BR will probably show the role of brassinosteroids in increased tolerance of plants to water stress. Therefore, 24-epibrassinolide may have a role in the mitigation of damage caused by water stress.

**Key words:** Drought stress, brassinosteroid, antioxidative, tomato, proline, lipid peroxidation

## Introduction

Drought stress is defined as a condition in which water available to plants is so low that it is unfavourable for the growth of a plant species (Zhu, 2001; Egert & Tevini, 2002). Plants will respond to conditions of drought stress through a number of physiological and developmental changes (Inze & Montagu, 2000). Under stressful conditions, the stress factors, or the toxic molecules derived from the stress, attack the most sensitive molecules (primary targets) in cells to impair their function (Ingram & Bartels, 1996; Inze & Montagu, 2000). The damaged targets recover either by repair or replacement via de novo biosynthesis. When the stress is too intense and

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severely damages target molecules, catastrophic cascades of events set in, leading to cell death (Inze & Montagu, 2000). Cells could be protected either by the endogenous molecular systems or exogenous applied compounds that mitigate the stress (Ingram & Bartels, 1996). Capacity of plants to detoxify reactive oxygen species has been related to the stress tolerance of plants (Egert & Tevini, 2002; Apel & Hirt, 2004).

Oxidative stress is a key underlying component of most abiotic stresses (Mittler, 2002; Apel & Hirt, 2004; Yin et al., 2008) and a major limiting factor to plant growth in the field (Scandalios, 2002; Blokhina et al., 2003; Mittler, 2006). Production of reactive oxygen species (ROS) and other radicals increases dramatically during abiotic stress conditions and oxidative stress occurs when the rate of ROS production outstrips the capacity of antioxidant systems to detoxify them (McCord, 2000; Mittler, 2002; Mittler et al., 2004). The result is oxidative damage to key biomolecules such as proteins, lipids, and DNA, leading to cellular dysfunction and ultimately cell death (Wagner et al., 2004; Halliwell, 2006; Baxter et al., 2007). To avoid oxidative damage, plants invoke a molecular response that allows them to cope with and adapt to the oxidative stress situation. Many stress-related genes have been identified and the list of antioxidant enzymes, hormones, and metabolites present in plants continues to be extended (Nakashita et al., 2003; Cao et al., 2005). One type of these compounds that have antioxidative characteristics is brassinosteroids (BRs) (Bishop & Koncz, 2002; Haubrick & Assmann, 2006).

BRs are common plant-produced compounds that can function as growth regulators (Khripach et al., 2000; Davis, 2005; Bishopp et al., 2006). In addition, it has been suggested that BRs could be included in the category of phytohormones (Clouse & Feldmann, 1999; Haubrick & Assmann, 2006). Exogenous application of BRs may influence a range of diverse processes of growth and development in plants (Cao et al., 2005; Clouse & Sasse, 1998; Yu et al., 2004; Montoya et al., 2005). In addition, it is becoming clear that BRs interact both negatively and positively with other major signalling pathways including those regulated by auxin and ethylene (Amzallage & Vaiseman, 2006; Haubrick et al., 2006). It is clear that BRs provide protection against a number of abiotic stresses (Sasse, 1999; Rao & Vardhini, 2002; Krishna, 2003; Nemhauser, 2004). Treatment with BRs enhanced the growth of wheat (Sairam, 1994), French bean (Upreti & Murti, 2004), and sugar beet plants (Schilling, 1991) under drought stress. Application of BRs improved tolerance against salt in rice (Anuradha & Rao, 2001, 2003; Özdemir et al., 2004), groundnut (Vardhini & Rao, 1997), wheat (Sairam et al., 2005), and chickpea (Ali et al., 2007). It was reported that BL induced thermotolerance in tomato (Dhaubhadel et al., 1999; Mazorra et al., 2002; Ogweno et al., 2008), rice (Wang & Zang, 1993), and brome grass (Wilen et al., 1995). Moreover, BRs increased tolerance against high temperatures in *Brassica napus* (Singh & Shono, 2005) and brome grass (Wilen et al., 1995). One mechanism that may be involved in the resistance to many types of stress is the increased activity of the antioxidant pathway. Several studies have shown that BRs alter the antioxidant capacity of plants under stress conditions (Kamuro & Takatsuto, 1999; Yardanova, 2004; Yin et al., 2008). The present study was an attempt to carry out investigations of the effect of brassinolide on the water stress responses of tomato plants, with the aim to elucidate possible mechanisms that might be involved in the BR-promoted antioxidant responses to drought.

***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; SOD-Superoxide dismutase; $H_2O_2$- peroxide hydrogen; NBT- ρ-nitro blue tetrazolium chloride; EDTA-Ethylendiamine tetraacetic acid; GR- Glutathione reductase.

## Materials and methods

24-Epibrassinolide (EBL, C28H48O6, MW = 480) was purchased from Sigma (USA). Tomato (*Lycopersicon esculentum* Mill. var. *Tomba* (BB204)) seeds were used in this study. Seeds were sown in plastic pots containing sand, loam, and peat (2:1:1). When 4 fully expanded leaves appeared (the tomato plants were 1 month old), the tomato was left to grow in 26/18 °C and 16/8 h (light/dark) conditions in a growth chamber. Hoagland's nutrient solutions were

used every 7 days and the pH was maintained at about 6.7 (Meidner, 1984). 24-EBL was dissolved in distilled water and ethanol. Tween-20 (0.01%) was used as surfactant. Preliminary screening was performed for various concentration of 24-epibrassinolide to obtain the optimum responses and the concentrations of 0.01 and 1 μM 24-EBL were selected. 24-EBL was sprayed onto the leaves at 0.01 and 1 μM concentration for 3 days on alternate days. Three levels of water stress (0,3, and 5 days withholding water) were applied. Light intensity was 6000 Lux at the plant level. Four replicates were assigned for each treatment. After treatment, the third leaves of plants were harvested, and samples were either rapidly dried in an oven at 80 °C to constant weight, which were used for determination of dry weight and further analyses, or were frozen in liquid nitrogen and stored at –80 °C for biochemical analysis.

**Biochemical assays**

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

Proline was extracted and its concentration determined as described by Bates et al. (1973). Leaf tissues were homogenised with 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 ×g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Proline (Sigma) was used for the standard curve.

Soluble proteins were determined as described by Bradford (1976) using BSA (Merck) as standard.

The content of $H_2O_2$ was determined according to Alexieva et al. (2001). Leaf tissue (500 mg) was homogenised in an 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.

**Enzyme Assays**

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 a pre-chilled mortar and pestle. 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 activity of ascorbate peroxidase (APX; EC 1.11.1.11) enzyme was measured using the method of Nakano and Asada (1981). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0) containing 0.5 mM ascorbic acid, 0.15 mM $H_2O_2$, 0.1 mM EDTA, and 50 μL of enzyme extract (supernatant). Oxidation of ascorbic acid was considered a decrease in absorbance at 290 nm that was followed 2 min after starting the reaction. One unit of APX oxidises 1 mM ascorbic acid in 1 min at 25 °C.

Catalase (CAT; EC 1.11.1.6) activity was assayed with spectrophotometry by monitoring the decrease in 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 of enzyme extract to the reaction mixture and the change in absorbance was followed 1 min after starting of the reaction. Unit of activity was taken as the amount of enzyme that decomposes 1 mM of $H_2O_2$ in 1 min.

Peroxidase (POD; 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 photometrically. The enzyme was assayed in a solution that contained 50 mM

phosphate buffer (pH 7.0), 0.3% $H_2O_2$, and 1% guaiacol. The reaction was started by the addition of 20 µL of the enzyme extract at 25 °C and was followed 3 min after starting the reaction. The enzyme unit was calculated for the formation of 1 mM tetraguaiacol for 1 min.

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 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.

Glutathione reductase (GR; EC 1.6.4.2) activity was measured according to Foyer and Halliwell (1976), which depends on the rate of decrease in the absorbance of oxidised glutathione (GSSG) at 340 nm. The reaction mixture contained 25 mM Na-phosphate buffer (pH 7.8), 5 mM GSSG, and 1.2 mM $NADPHNa_4$. The reaction was carried out for 3 min and activity of GR was calculated from the reduced GSSG concentration by using the extinction coefficient 6.2 $mM^{-1}$ $cm^{-1}$. One enzyme unit was defined as µmol $mL^{-1}$ oxidised GSSG per min.

**Native PAGE and activity stain**

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 with 12% polyacrylamide gel, for POD with 10% and for CAT with 7%. SDS was omitted from the PAGE. Activity stain for each enzyme was carried out as follows. APX activity was detected by the procedure described by Mittler and Zilinskas (1993). Gel equilibrated with 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM ascorbate for 30 min was incubated in a solution composed of 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 solution of the following buffer containing 28 mM TEMED and 2.45 mM NBT for 10-20 min with gentle agitation in the presence of light. For POD, gel was incubated in 25 mM potassium phosphate buffer (pH 7.0) and then the gel was submerged again in the same buffer (fresh amount) containing 18 mM guaiacol and 25 mM $H_2O_2$, until the POD activity-containing band was visualised carefully (Fielding and Hall, 1978). For CAT, gel was incubated in 3.27 mM $H_2O_2$ solution for 25 min and then the gel was washed in distilled water and submerged in a solution composed of potassium ferricyanide 1% and ferric chloride 1% for 4 min (Woodbury et al., 1971).

**Statistical analysis**

All experiments were performed with 4 replicates, using a completely randomised design. Data were statistically analysed by one-way analysis of variance using SPSS (version 11) and the means were separated by Duncan's multiple range test at 0.05 probability level.

## Results and discussion

The content of proline progressively increased in plants as the drought levels increased (Figure 1). In those plants that were either under drought stress or 24-EBL treatments increases in the content of proline were observed. It has been reported that BR treatments induced expressing of biosynthetic genes

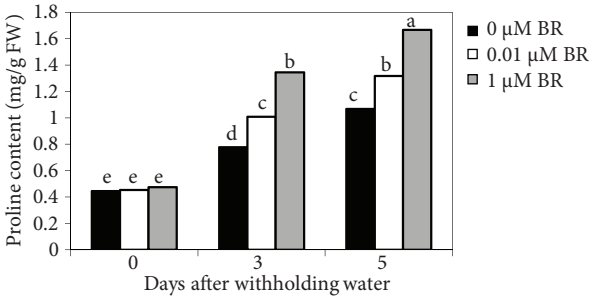
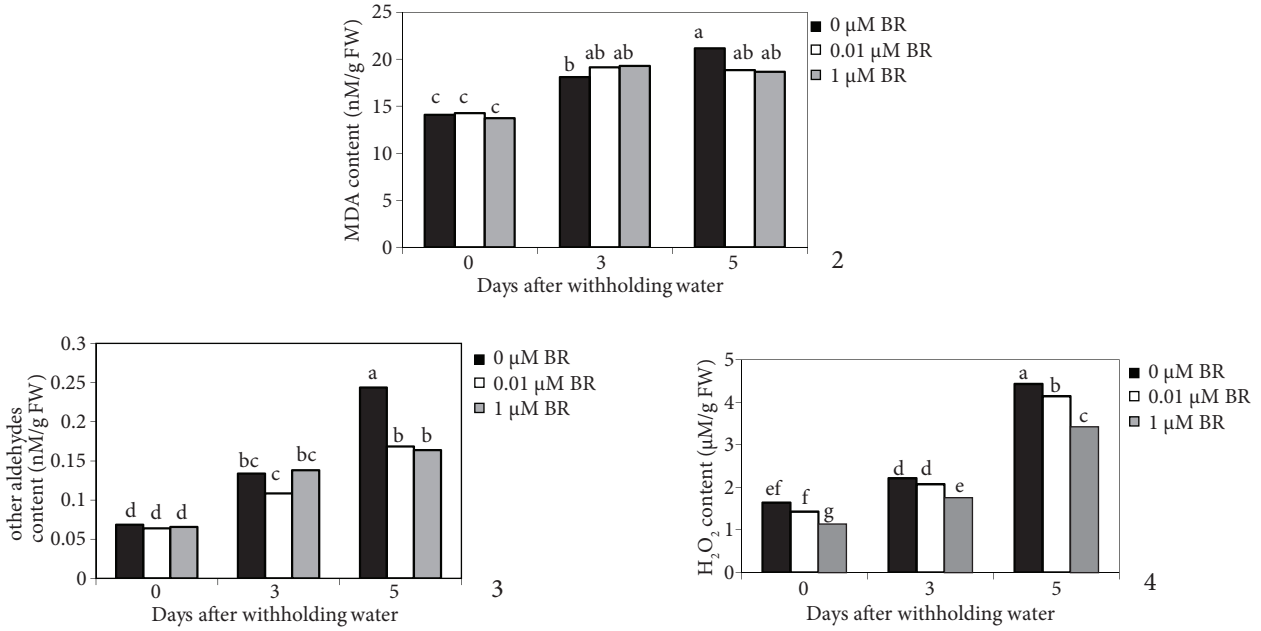



Figure 1. Effects of BR and drought stress on proline content of *Lycopersicon esculentum*. Plants were grown for 1 month under controlled conditions and were divided into 3 groups. Two groups were pretreated with BR and the other group was just sprayed with distilled water. All 3 groups were exposed to water stress for 3 and 5 days. The control group was irrigated daily. Values are the means of 4 replicates, and letters show significant differences among the means at $P < 0.05$ according to Duncan's test.

of proline (Özdemir et al., 2004). There are also reports that show that application of BRs (28-HBL and 24-EBL) increased proline content in sorghum plants exposed to osmotic stress (Vardhini & Rao, 2003). Proline as a cytosolic osmoticum and as a scavenger of $OH^{\cdot}$ radical can interact with cellular macromolecules such as enzymes, DNA, and membranes and stabilise the structure and function of such macromolecules (Anjum et al., 2000; Matysik et al., 2002; Kavir Kishor et al., 2005). In addition, among various compatible solutes, proline is the only molecule that has been shown to protect plants against singlet oxygen and free radical induced damage resulting from stress (Alia et al., 1997). Our data showed that proline was accumulated in BR-treated plants under stress conditions. Therefore, a role of BR in the accumulation of proline as an important component of protective reactions in tomato plants in response to drought stress is possible.

Formation of MDA and others aldehydes in plants exposed to water stress is a reliable indicator of free radical formation in the tissue, and is currently used as an indicator of lipid peroxidation (Meirs et al., 1992; Borsanio et al., 2001). Considering that drought stress results in increases in reactive oxygen species (Apel & Hirt, 2004; Gong et al., 2006), peroxidation of lipid membranes is both a reflection and measure of stress-induced damage at the cellular level (Meirs et al., 1992; Borsanio et al., 2001). In the present study the content of MDA, other aldehydes, and $H_2O_2$ increased significantly as drought progressed in plants (Figures 2-4). It was previously found that the MDA content increased in 3 genotypes of wheat under drought stress (Sgherri et al., 2000). It is affirmed that under water stress the content of MDA and $H_2O_2$ is different among different cultivars of bean, banana, and rice. In drought-tolerant cultivars, since there is high antioxidative potential, they are able to scavenge $H_2O_2$ radicals and with decreasing $H_2O_2$ content lower MDA content is produced compared to sensitive-tolerance cultivars (Li & Van Staden, 1998; Sharma & Shanker, 2005; Türkan et al., 2005). Furthermore, in *Lilium longiflorum* plants under short-term heat stress, $H_2O_2$ and MDA contents slightly varied at 37 and 42 °C, while increasing significantly at 47 °C (Yin et al., 2008). In our study, BR-pretreated plants that




Figures 2-4. Effects of BR and drought stress on MDA, other aldehydes, and $H_2O_2$ content of *L. esculentum*. Plants were grown for 1 month under controlled conditions and were divided into 3 groups. Two groups were pretreated with BR and the other group was just sprayed with distilled water. All 3 groups were exposed to water stress for 3 and 5 days. The control group was irrigated daily. Values are the means of 4 replicates, and the letters show significant differences among the means at $P < 0.05$ according to Duncan's test.

were thereafter put under drought stress had much less $H_2O_2$, MDA, and others aldehydes' content than drought stressed plants (Figures 2 and 3). It is assumed that BRs act as secondary messengers for the induction of antioxidant defences in stressed plants (Khripach et al., 2000); thus, based on our results in MDA measurement, it is very possible that 24-EBL effectively scavenged ROS by increasing the activity of antioxidant enzyme systems.

A decrease in protein content is a common phenomenon in drought stress (Zhu, 2001). The reason for this is that the amino acid of proteins will react with active radical and will be degraded (Wagner et al., 2004). In this study, decreases in protein content in water stressed plants were observed. 24-EBL application increased the protein content both in normal and stressed plants (Figure 5). BRs participate in the processes of gene expression, transcription, and translation in normal and stressed plants (Sasse, 1999; Mazorra et al., 2002). It has been observed that the tolerance to high temperature in tomato leaves (Dhaubhadel et al., 1999; Ogweno et al., 2008) and brome grass (Kulaeva et al., 1991) is due to application of BRs, which is associated with induction of de novo polypeptide synthesis. The promotion growth in seedlings by BRs under saline conditions was related to enhanced levels of nucleic acids and soluble proteins (Anuradha & Rao, 2001, 2003; Shahbaz et al., 2008). In addition, Sairam (1994) reported the

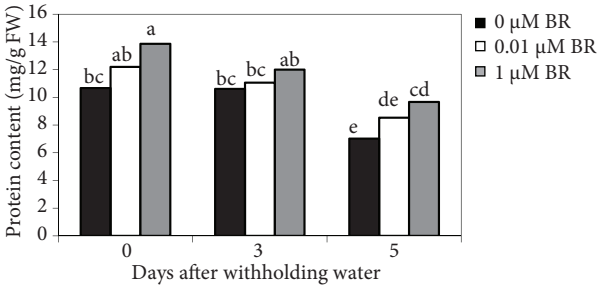
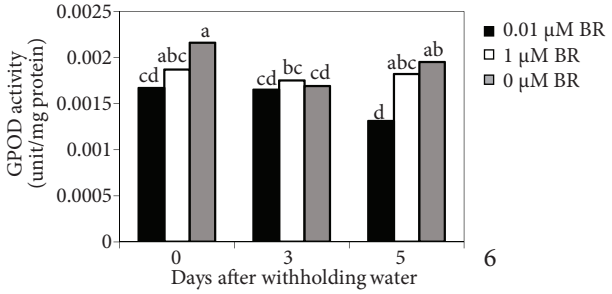
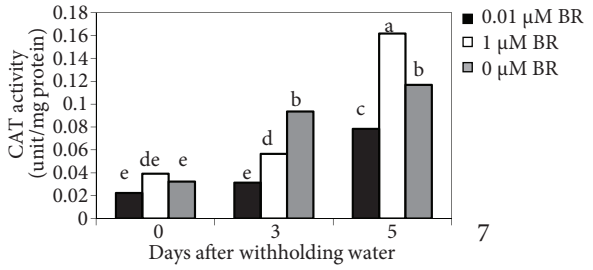



Figure 5. Effects of BR and drought stress on protein content of *L. esculentum*. Plants were grown for 1 month under controlled conditions and were divided into 3 groups. Two groups were pretreated with BR and the other group was just sprayed with distilled water. All 3 groups were exposed to water stress for 3 and 5 days. The control group was irrigated daily. Values are the means of 4 replicates, and the letters show significant differences among the means at $P < 0.05$ according to Duncan's test.

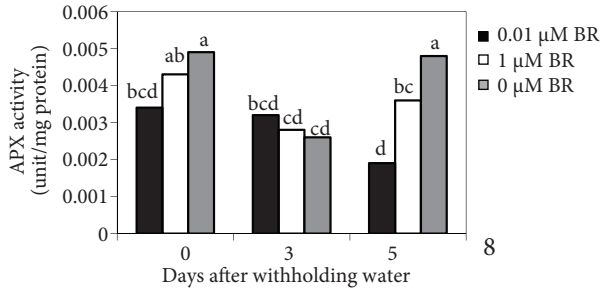
enhancement of proteins in wheat plants by homobrassinolide under moisture stress conditions. It has been reported that osmotic stress resulted in considerable reductions in the protein content in the seedlings of both susceptible and tolerant varieties of sorghum plants but treatment with brassinosteroids resulted in not only restoring the protein levels but also further improvement (Vardhini & Rao, 2003). In this study, with pretreatment with BR when plants were under severe drought, protein content was much lower than that in mild stress. Possibly induction oxidative stress is responsible, because protein degradation may occur under severe drought stress. A number of reports have shown that cells exhibit increased rates of proteolysis following exposure to oxidative-inducing agents (Inze & Montagu, 2000; Jung, 2003).

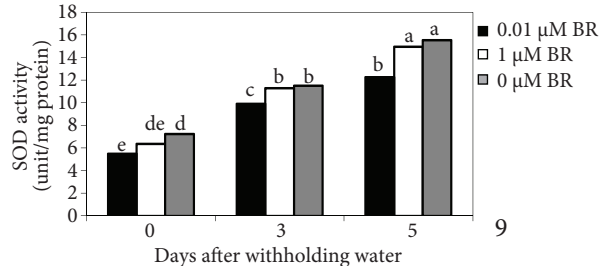
In the present study, drought stress decreased the peroxidase activity in 5-day drought stressed plants (Figure 6). 24-EBL application positively influenced POD activity in control and stressed plants. The highest activity was observed in control and 5-day drought stressed plants. The activity of enzyme was 2-fold higher in those plants treated with 1 μM BR when compared with BR-free plants (Figure 6). In 5-day drought stress conditions, the activity of catalase significantly increased (Figure 7). 24-EBL pretreatment increased CAT activity in drought stressed plants. In the severe drought stressed plants, the activity was much higher than that in the control unstressed plants (Figure 7). Drought stress resulted in a decrease in ascorbate peroxidase (APX) activity. In those plants under 5-day drought, APX activity was 33% less than that in unstressed plants (Figure 8). 24-EBL application caused an increase in APX activity in the control and 5-day drought stressed plants. The highest activity was observed under condition of 1 μM 24-EBL and 5-day drought, and that was about 4 times more than that in BR-free, stressed plants (Figure 8). Drought stress significantly increased the activity of SOD. Exogenous application of EBL in drought stressed plants enhanced the activity of this enzyme at both levels of 24-EBL concentration (Figure 9). Drought treatment induced GR activity on both days 3 (30%) and 5 (65%) in tomato plants (Figure 10). Moreover, the induced level of GR activity in 24-EBL treated groups was higher on both days 3 and 5 days after withholding water.

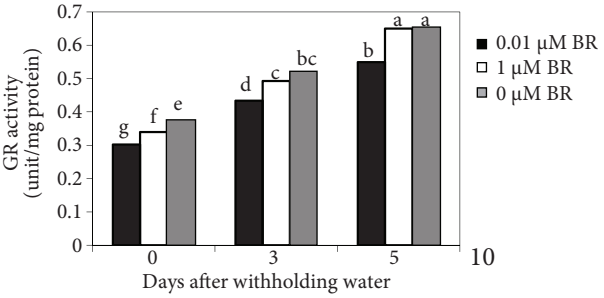



Figures 6, 7. Effects of BR and drought stress on POD and CAT activity of *L.esculentum*. Plants were grown for 1 month under controlled conditions and were divided into 3 groups. Two groups were pretreated with BR and the other group was just sprayed with distilled water. All 3 groups were exposed to water stress for 3 and 5 days. The control group was irrigated daily. Values are the means of 4 replicates, and the letters show significant differences among the means at $P < 0.05$ according to Duncan's test.



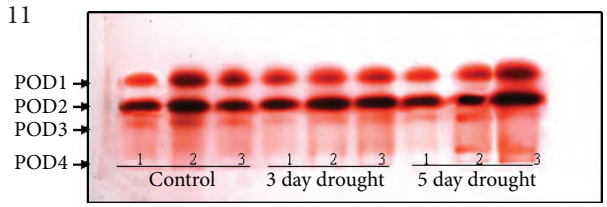



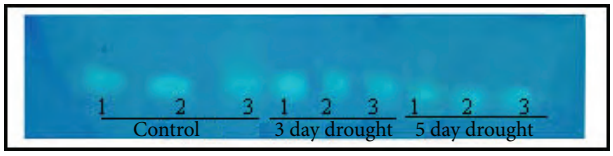



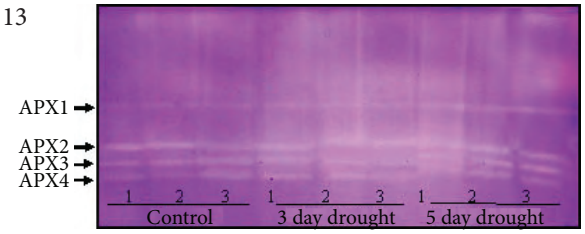

Figures 8-10. Effects of BR and drought stress on APX, SOD, and GR activity of *L. esculentum*. Plants were grown for 1 month under controlled conditions and were divided into 3 groups. Two groups were pretreated with BR and the other group was just sprayed with distilled water. All 3 groups were exposed to water stress for 3 and 5 days. The control group was irrigated daily. Values are the means of 4 replicates, and the letters show significant differences among the means at $P < 0.05$ according to Duncan's test.

In part of the native polyacrylamide gel electrophoresis (PAGE) analysis, 4 isoenzymes were detected for peroxidase enzyme, especially in BR-treatment and drought stress conditions (Figure 11). In addition, one isoenzyme was detected for CAT enzyme. Especially in BR-treated drought stressed plants, CAT isoenzyme showed higher intensity (Figure 12). Native polyacrylamide gel electrophoresis (PAGE) analysis on ascorbate peroxidase enzyme showed 4 isoenzymes for this enzyme. In BR-treated, drought stressed plants, these isoenzymes were markedly detected (Figure 13).

The increase in peroxidase activity that was observed in our study could reflect a similar process of oxidative stress with the implication of peroxidase activity as part of the antioxidant response against




Figures 11-13. Effects of BR and drought stress on POD, CAT, and APX isoforms by native PAGE. Lanes 1-3 represent different levels of BR concentration (0, 0.01, and 1 µM).

$H_2O_2$. Ascorbate peroxidases (APXs) are the most important $H_2O_2$ scavengers operating in both the cytosol and chloroplasts (Yardanova et al., 2004). In the present work, drought stress decreased the activity of POD and APX enzymes under 5-day drought stress but CAT, GR, and SOD activities increased (Figures 7, 9, 10). The reason for this decrease could be the decrease in the amount instead of enzyme activity, because in this condition protein content decline also can be attributed to an increase in CAT activity. These results were accompanied by increases in MDA and proline contents, indicating that serious damage has occurred. It was found that osmotic stress increased the peroxidase activity and decreased the CAT activity in susceptible varieties of sorghum seedlings but the activity of these enzymes increased in resistant variety under osmotic stress (Vardhini & Rao, 2003). Under heat stress in *Lilium longiflorum* plants, at 37 and 42 °C, antioxidant enzyme activities such as POD, SOD, APX, and CAT were stimulated. However, at 47 °C POD and CAT activity was significantly decreased (Yin et al., 2008). It has been reported that POD activity in wheat leaves decreased when subjected to water stress (Baisak et al., 1994). SOD and CAT activity increased in drought stressed mature leaves of *Arabidopsis thaliana* (Jung, 2003). In this research, the activity of enzymes studied (POD, SOD, CAT, APX) increased when pre-treated with BR. The reason for the increase in the activity of these enzymes may be the effects of BR on expression of biosynthetic genes of these enzymes that resulted in increased oxidation of harmful substrates, as reported by Ogweno et al. (2008), Cao et al. (2005), Nemhauser and Chory (2004), and Shahbaz et al. (2008). These results, accompanied with the decrease in lipid peroxidation and high proline contents, probably represent a decline in ROS and an indicator of removal of stressful conditions by antioxidant enzymes activated by BR. Similarly, it has been observed that BR application caused an increase in CAT activity in both susceptible and resistant varieties of sorghum seedlings under osmotic stress (Vardhini & Rao, 2003). Furthermore, the activities of antioxidant enzymes such as SOD, APX, CAT, and guaiacol POD were significant in EBL-treated tomato plants under heat stress (Dhaubhadel et al., 1999; Ogweno et al., 2008) and Mazorra et al. (2002) reported that EBL and MH5 (BR analogue) stimulated CAT activity in tomato leaf discs under heat stress. Application of 28-HBL or 24-EBL as a foliar spray with varying levels, i.e. 0.05, 1, and 3 µM, increased the catalase activity in groundnut plants with increasing levels of BL (Vardhini & Rao, 2000). The GR activity was investigated to address the question of whether 24-EBL in drought stress causes changes in the other antioxidative enzymes. It was observed at both concentrations of 24-EBL, especially at 1 µM 24-EBL concentration, that GR activity was 120%,

indicating that 24-EBL may be effective in protection from drought stress. GR activity maintains the pool of glutathione in the reduced state, which in turn reduces dehydroascorbate to ascorbate. Increased expression of GR enhances tolerance to oxidative stress (Noctor & Foyer, 1998).

Exogenous applied 24-EBL enhanced SOD, POD, and CAT activities in salt stressed plants of wheat (Shahbaz et al., 2008). Although it has been shown that BRs can induce the expression of some antioxidant genes and enhance the activities of antioxidant enzymes such as SOD, CAT, APX, and POD (Ogweno et al., 2008), it is still unclear whether BRs directly or indirectly modulate the responses of plants to oxidative stress (Cao et al., 2005). Nevertheless, although BRs and ROS are thought to act as secondary messengers for the induction of antioxidant defences in stressed plants (Mazorra et al. 2002), the relationship between BRs and ROS in stress-signal transduction remains unclear (Ogweno et al., 2008).

In conclusion, our results may show that the leaves of tomato plants subjected to drought stress can endure moderate drought stress because of small changes in enzyme activities. However, enzyme activities declined under severe drought conditions accompanied by a significant increase in MDA concentration, indicating serious oxidative damage by the over-accumulation of $H_2O_2$. From this result it could be concluded that the Tomba (BB 204) variety of tomato plant is drought sensitive because a gradual loss in antioxidant protection in the leaves of plants under drought stress was observed. Pre-treatment with BR can ameliorate the adverse effects of drought stress via decreasing the oxidative damage of plant membranes, possibly by the induction of compatible solute for osmotic adjustment and the induction of antioxidant defence system in tomato plants. Finally, the results hint that BR may in future find application for improving plant growth and yield in dry areas.

## References


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

Ali B, Hayat S & Ahmad A (2007). 28-Homobrassinolide ameliorate the saline stress in chickpea (Cicer arietinum L.). *Environ Exp Bot* 59: 217-223.

Alia B, Pardha Saradhi P & Mohanty P (1997). Involvement of proline in protecting thylakoid membranes against free radical-induced photodamage. *J Photochem Photobiol* 38: 253-257.

Amzallag GN & Vaisman J (2006). Influence of brassinosteroids on initiation of the root gravitropic response in Pisum sativum seedlings. *Biologia Plantarum* 50: 283-286.

Anjum F, Rishi V & Ahmed F (2000). Compatibility of osmolytes with Gibbs energy of stabilization of proteins. *Biochem Biophys Ac*ta 1947: 75-84.

Anuradha S & Rao SSR (2001). Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling of rice (Oryza sativa L.). *Plant Growth Regul* 33: 151-153.

Anuradha S & Rao SSR (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.

Apel K & Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55: 373-399.

Baisak R, Rana D, Acharya PBB & Kar M (1994). Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. *Plant Cell Physiol* 35: 489-495.

Bates LS, Waldren RP & Teare ID (1973). Rapid determination of free proline for water stress studies. *Plant and Soil* 29: 205-207.

Baxter CJ, Redestig H, Schauer N, Repsilber D, Patil KR, Nielsen J, Selbig J, Liu J, Fernie AR & Sweetlove LJ (2007). The metabolic response of heterotrophic arabidopsis cells to oxidative stress. *Plant Physiol* 143: 312-325.

Bishop GJ & Koncz C (2002). Brassinosteroids and plant steroid hormone signaling. *Plant Cell* 14: 97-110.

Bishopp A, Mähönem AP & Helariutta Y (2006). Signs of changes: hormone receptors that regulate plant development. *Development* 133: 1857-1869.

Blokhina O, Virolainen E & Fagerstedt KV (2003). Antioxidants, oxidative damage and oxygen deprivation stress; a review. *Ann Bot* 91: 179-194.

Borsanio O, Valpuesta V & Botella MA (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. *Plant Physiol* 126: 1024-1030.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Bioch* 72: 248-254.

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

Clouse SD & Feldmann KA (1999). Molecular genetics of brassinosteroid action. In: Sakurai A, Yokota T and Clouse SD (eds), Brassinosteroids: Steroidal Plant Hormones. Springer–Verlag, Tokyo, Japan, pp. 163-189.

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

Davis PJ (2005). Plant hormones biosynthesis, signal transduction, action. *Publisher Springer* Chapter F2.

Dhaubhadel S, Chaudhary S, Dobinson KF & Krishna P (1999). Treatment with 24-epibrassinolide, a brassinosteroid, increases the basic thermo-tolerance of Brassica napus and tomato seedlings. *Plant Mol Biol* 40: 333-342.

Dhindsa RS, Plumb-Dhindsa P & Thorpe TA (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.

Egert M & Tevini M (2002). Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (Allium schoenoprasum). *Environ Exp Bot* 48: 43-49.

Fielding JL & Hall JL (1978). A biochemical and cytological study of peroxidase activity in roots of Pisum sativum. *J Exp Bot* 29: 969-981.

Foyer CH & Halliwell B (1976). Presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133: 21-25.

Giannopolitis CN & Ries SK (1977). Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol* 59: 309-314.

Gong JR, Zhao AF, Huang YM, Zhang XS & Zhang CL (2006). Water relations, gas exchange, photochemical efficiency, and peroxidative stress of four plant species in the Heihe drainage basin of northern China. *Photosynthetica* 44: 355-364.

Halliwell B (2006). Reactive species and antioxidants: redox biology is a fundamental theme of aerobic life. *Plant Physiol* 141: 312-322.

Haubrick LL & Assmann SM (2006). Brassinosteroids and plant function: some clues, more puzzles. *Plant Cell and Environ* 29: 446-457.

Haubrick LL, Torsethaugen G & Assmann SM (2006). Effect of brassinolide, alone and in contact with abscisic acid on control of stomatal aperture and potassium currents of Vicia faba guard cell protoplasts. *Physiol Plant* 128: 134-143.

Heath RL & Packer L (1969). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125: 189-198.

Ingram J & Bartels D (1996). The molecular basis of dehydration tolerance in plants. *Plant Physiol* 47: 377-403.

Inze J & Montagu MV (2000). Oxidative stress in plants. TJ International Ltd, Padstow, Carnawell. Great Britain 321 pages.

Jung S (2003). Variation in antioxidant metabolism of young and mature leaves of Arabidopsis thaliana subjected to drought. *Plant Sci* 166: 459-466.

Kamuro Y & Takatsuto S (1999). Practical applications of brassinosteroids in agricultural fields. In: Salurai A., Yokota T. and Clouse S.D. (eds), *Brassinosteroids–Steroidal Plant Hornones* Springer, Tokyo, pp. 223-241.

Kavir Kishor PB, Sangam S, Amrutha RN, Sri Laxmi P, Naidu KR, Rao KRSS, Rao S, Reddy KJ & Sreenivasulu N (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Cur Sci* 88: 424-438

Khripach VA, Zhabinskii VN & De Groot AE (2000). Twenty years of brassinosteroids: Steroidal hormones warrant better crops for the XXI *Century Ann Bot* 86: 441-447.

Krishna P (2003). Brassinosteroid-mediated stress response. *J Plant Growth Regul* 22: 289-297.

Kulaeva ON, Burkhanova EA, Fedina AB, Khokhalova VA, Bokebayeva GA, Vorbrodt HM & Adam G (1991). Effect of brassinosteroids on protein synthesis and plant cell ultrastructure under stress condition. In: Cutler HG, Yokota T, Adam G (eds) Brassinosteroids chemistry, bioactivity and applications ACS, *Symposium Series American Chemical Society*, Washington DC, pp 141-155.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Li L & Van Staden J (1998). Effects of plant growth regulators on the antioxidant system in seedlings of two maize cultivars subjected to water stress. *Plant Growth Regul* 25: 81-87.

Matysik J, Alia Bhalu B & Mohanty P (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr Sci* 82: 525-532.

Mazorra LM, Nunez M, Hechavarria M, Coll F & Sanchez-Blanco MJ (2002). Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. *Biol Plant* 45: 593-596.

McCord JM (2000). The evolution of free radicals and antioxidative stress. *Am J Med* 108: 652-654.

Meidner H (1984). Class Experiments in Plant Physiology. *George Allen & Unwin* pp: 156.

Meirs S, Philosophhadas S & Aharoni N (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 & Zilinskas BA (1993). Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate dependent reduction of nitroblue tetrazolium. *Anal Biochem* 212: 540-546.

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

Mittler R (2006). Abiotic stress, the field environment and stress combination. *Trends Plant Sci* 11: 15-19.

Mittler R, Vanderauwera S, Gollery M & Van Breusegem F (2004). Reactive oxygen gene network of plants. *Trends Plant Sci* 9: 490-498.

Montoya T, Nomura T, Yokota T, Farrar K, Harrison K, Jones JGD, Kaneta T, Kamiya W, Szekeres M & Bishop GR (2005). Patterns of dwarf expression and brassinosteroid accumulation in tomato reveal the importance of brassinosteroid synthesis during fruit development. *Plant J* 42: 262-269.

Nakano Y & Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22: 867-880.

Nakashita H, Yasuda M, Nitta T, Asami T, Fujioka S, Aria Y, Sekimata K, Takatsuto S, Yamaguchi I & Yoshida S (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant J* 33: 887-898.

Nemhauser JL & Chory J (2004). Bring it on new insights into the mechanism of brassinosteroid action. *J Exp Bot* 55(395): 265-270.

Noctor G & Foyer CH (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Physiol Plant Mol* 49: 249-279.

Ogweno JO, Song XS, Shi K, Hu WH, Mao WH, Zhou YH, Yu JQ & Nogués S (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.

Özdemir F, Bor M, Demiral T & Turkan I (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.

Plewa MI, Smith SR & Wagner ED (1991). Diethyldithiocarbamate suppress the plant activation of aromatic amines into mutagenes by inhibiting tobacco cell peroxidase. *Mutat Res* 274: 57-64.

Rao SSR, Vardhini BV, Sujatha E & Anuradha S (2002). Brassinosteroids – a new class of phytohormones. *Curr Sci* 82: 1239-1245.

Sairam RK (1994). Effect of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. *Plant Growth Regul* 14: 173-181.

Sairam RK, Stivastava GC, Agarwal S & Meena RC (2005). Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biol Plant* 49: 85-91.

Sasse JM (1999). Physiological action of brassinsteroids. In: Sakurai A. Yokota T. Clouse S.D. (eds), Brassinosteroids: *Steroidal Plant Hormones* Springer–Verlag, Tokyo, Japan, pp. 306-311.

Scandalios JG (2002). Oxidative stress responses – What have genome scale studies taught us? *Genome Biol* 3: 10191-10196.

Schilling G, Schiller C & Otto S (1991). Influence of brassinosteroids on organ relation and enzyme activities of sugar beet plants In: Cutler HG, Yokota and Adam G (eds), Brassinosteroids Chemistry, Bioactivity and Applications ACS symp. Ser. 474 *American Chemical Society*, Washington DC, pp 208-219.

Sgherri CLM, Maffei M & Navari-Izzo F (2000). Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. *Plant Physiol* 157: 273-279.

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

Sharma P & Shanker R (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedling. *J. Plant and Growth Regul* 46: 209-221.

Singh I & Shono M (2005). Physiological and molecular effects of 24-epi brassinosteroid, a brassinosteroid on thermotolerance of tomato. *Plant Growth Regul* 47: 111-119.

Türkan I, Bor M, Özdemir F & Koca H (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant P. acutifolius Gray and drought-sensitive P. vulgaris L. subjected to polyethylene glycol mediated water stress. *Plant Sci* 223-231.

Upreti KK & Murti GS (2004). Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in French bean under water stress. *Biologia Plantarum* 48: 407-411.

Vardhini BV & Rao SSR (1997). Effects of BRs on salinity induced growth inhibition of groundnut seedlings. *Indian J Plant Physiol* 2: 156-157.

Vardhini BV & Rao SSR (2000). Effect of brassinosteroids on the activities of certain oxidizing and hydrolyzing enzymes of groundnut. *Indian J Plant Physiol* 51: 89-92.

Vardhini BV & Rao SSR (2003). Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. *Plant Growth Regul* 41: 25-31.

Wagner D, Przybyla D, Op den Camp R, Kim C, Landgraf F, Lee KP, Wursch M, Laloi C, Nater M, Hideg E & Apel K (2004). The genetic basis of singlet oxygen-induced stress responses of Arabidopsis thaliana. *Science* 306: 1183-1185.

Wang BK & Zang GW (1993). Effect of epibrassinolide on the resistance of rice seedlings to chilling injury. *Acta Phytophysiol Sin* 19: 38-42.

Wilen RW, Sacco M, Gusta LV & Krishna P (1995). Effects of 24-epibrassinolide on freezing and thermal tolerance of brome grass (Bromus inermis) cell culture. *Physiol Plant* 95: 195-202.

Woodbury W, Spencer AK & Stahmann MA (1971). An improved procedure using ferricyanide for detecting catalase isoenzymes. *Anal Biochem* 44: 301-305.

Yardanova RY, Christov KN & Popova LP (2004). Antioxidative enzymes in barley plants subjected to soil flooding. *Environ Exp Bot* 51: 93-101.

Yin H, Chen Q & Yi M (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 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.

Zhu JK (2001). Cell signaling under salt, water and cold stresses. *Current Opinion in Plant Biol* 4: 401-406.