Int. J. Adv. Res. Biol.Sci. 2(7): (2015): 81–93

International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Effects of paclobutrazol on growth and physiological attributes of Soybean (*Glycine max*) plants grown under water stress conditions

Sharifa S. Abu-Muriefah*

Faculty of Science, Princess Norah Bint Abdulrahman University, KSA
*Corresponding author

Abstract

This study investigated ameliorative effect of paclobutrazol on growth, hormones, antioxidant system and protein pattern of soybean (*Glycine max* L.) plants grown under water deficit conditions. Pot-grown plants were foliar treated with paclobutrazol at 40 mg L^{-1} at vegetative growth and the beginning of bloom and then exposed to water deficit stress at pod initiation for 7 d. Paclobutrazol promoted biomass accumulation and seed yield under both water conditions. Plants treated with paclobutrazol showed higher leaf water potential only in water-stressed condition. Water stress decreased the chlorophyll content, but those of paclobutrazol -treated plants were higher than the stressed control. Paclobutrazol-treated plants contained less GA_3 and ABA under well-watered condition than untreated plants, while the IAA and zeatin levels were increased substantially under both water conditions, and ABA concentration was also increased under water stressed condition. Under water-stressed conditions, paclobutrazol increased the content of proline and soluble sugars, and the activities of superoxide dismutase and peroxidase in soybean leaves but not the malondialdehyde content or electrical conductivity. These results suggest that paclobutrazol-induced tolerance to water deficit stress in soybean was related to the changes of hormones and antioxidant system of leaves.

Keywords: Paclobutrazol; Antioxidant enzymes; *Glycine max*; Endogenous hormone;

Introduction

Soybean (*Glycine max* L.) is an important dicot crop due to the high content of oil and protein in its seeds. Because of its potential for large-scale production, soybean has excelled in the world agricultural economy as a major oilseed crop. At present, soybeans are grown primarily for oil extraction and for use as a high protein meal for animal feed (Singh and Shivakumar, 2010). According to Li-Juan and Ru-Zhen (2010), soybean has a protein content of approximately 40% and an oil content of approximately 20%. Soybean production has been increased from about 26 million tons in 1960 to 223 million tons in 2010 due to increases in harvest area and yield (FAO, 2012). One major factor influencing growth and yield of soybean is water deficit and drought stress.

Drought is a regular and common feature in Saudi Arabia. Severe water stress reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Abdalla, 2011; Azadeh *et al*., 2014). Water stress has been found to activate the production of the reactive oxygen species (ROS) which is toxic for plant cells (Miyake, 2010). The common adverse effect of water stress on crops is the reduction in fresh and dry biomass production as well as crop yield (Lisar *et al*., 2012). Drought stress causes closing stoma and reducing leaf area (Kumudini, 2010); consequently decreasing photosynthetic pigments and activity. Exogenously applied plant growth regulators are being used increasingly to enhance tolerance of crops to environmental stresses. Paclobutrazol, an active member of the triazole family, was developed for use as a plant growth retardant (Brian, 2015). Paclobutrazol applied foliarly to oilseed rape has enhanced tolerance to stresses, such as, waterlogging (Qiu et al., 2005), heat (Wang et al., 2014), and salt stress (Hajihashemi et al., 2014). It also has enhanced drought tolerance in strawberry (Parvin et al., 2014). Recent researches showed that paclobutrazol applied as a foliar spray at the pollination time increased wheat yield significantly (Hajihashemi et al., 2014) and soybean yield (Zhang et al., 2007).

The purpose of this study is to determine whether paclobutrazol increases drought tolerance of soybean (*Glycine max* L.) plants, and if such tolerance is associated with changes in growth, phytohormones and antioxidant enzymes.

Materials and Methods

Experiment

The experiment was conducted during the soybean growing season of May to September 2014. Seeds of soybean (*Glycine max* L., cv. Ransom) were sown in pots (25 cm diameter×30 cm tall), containing 10 kg of a sandy soil mixed with peat moss (2:1 ration) and 2.5% organic matter content, nutrients of 200 mg N kg−1, 100 mg P kg−1, and 200 mg K kg−1. After the first trifoliolate leaf emerged, seedlings were thinned to four per pot. Water content of the pots was kept at 80% of soil field capacity by manual watering until the water treatments were initiated.

At the vegetative growth stage (eight weeks after planting), 40 pots with uniform seedlings were selected. Paclobutrazol at 40 mg L^{-1} plus Tween 20 surfactant at 0.1% (v/v) in water was applied to the plants until incipient runoff, using a hand-held aerosol-propelled sprayer. This treatment was repeated just before flowering stage. Plants of the other 20 pots were the non-stressed controls and received water and Tween 20 surfactant at 0.1% (v/v).

Two water treatments were imposed at 7 d after the first application of paclobutrazol treatment following the methods of Desclaux and Roument (1996). Soil water content of well-watered controls (10 pots of paclobutrazol treated and 10 untreated) was maintained at 80% of soil field capacity, while the water content of water-stressed pots (10 pots of paclobutrazol treated and 10 untreated) was set to 60% of the soil field capacity by withholding watering for certain time.

Plant height and biomass measurement

Four pots from each treatment were kept growing until harvest. The water content of water-stressed pots after 7 d of water stress was recovered to 80% of soil field capacity for the remainder of the experiment. Plant height, biomass (dry weight), and seed weight were measured at full maturity.

Leaf water potential measurement

Leaf water potential (Ψleaf) of the uppermost fully expanded leaves was measured at midday (11:30 am) on Day 7 after initiating water stress, when the sky was clear. A pressure chamber (Model 3000, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) was used to measure the Ψleaf, with one leaf per plant from plants of three pots for each treatment.

Chlorophyll content, and Photosynthetic rate

Chlorophyll content, and Photosynthetic rate (Pn) of the uppermost fully expanded leaves were measured on Day 7 after initiating water stress. Chlorophyll content was measured with a portable chlorophyll meter (Minolta SPAD-502, Japan), following the method of Turner and Jund (1991), and Pn was measured with a photosynthesis system (LI-6400, LI-COR., USA). The measurements were made between 09.00 and 11.00 am when photosynthetically active radiation above the canopy was 1000–1200 μmol m−2 s−1. Plant leaves of three pots were used for each treatment as described above.

Biochemical measurements

Leaves were sampled at 7 d after initiation of water stress for each paclobutrazol and water treatment between 9:30 and 10:30 am. The uppermost fully expanded leaf from plants, of 4pots in each treatment, was detached and immediately immersed in liquid N2. Leaf samples were pooled by pot, ground to fine powder in liquid N2 with a mortar and pestle, and stored in liquid N2 until used.

The activities of SOD and POD and malondialdehyde (MDA) content were analyzed by the method of Leul and Zhou (1999). Soluble sugars were evaluated using the anthrone method described by Fales (1951). Proline was extracted and determined spectrophotometrically following the method of Bates et al. (1973). Extraction, purification, and determination of endogenous levels of IAA, GA3, ABA, and cytokinine by HPLC technique were performed as described by Guo et al. (2011).

Statistical analysis

The collected data were analyzed statistically using completely randomized block design and analysis of variance (ANOVA) according to Gomez and Gomez (1984) with the aid of COSTAT computer program. Treatment means were compared using the least significant difference test (LSD) at 5% level.

Results and Discussion

Growth and yield

Plant height, dry weight, and seed yield were decreased by water stress treatment (Table 1). Conversely, paclobutrazol treatment, under the well-watered treatment, caused an increase in the total dry weight by about 14.5% and in seed yield by 23.2%. While, the corresponding increases under water-stressed conditions were about 14% and 20%, respectively, as compared with control. It was clear that paclobutrazol caused reductions in plant heights either in well-watered or in water-stressed plants. These reductions were 10.9% and 30.2%, respectively, as compared with control, while, water stress treatment caused a reduction in plant height by about 18.6% as compared with control, in the absence of paclobutrazol treatment.

Table 1. Effect of water stress (WS) and paclobutrazol (PBZ) on plant height, dry weighy and seed yield. Values are means of three replicates ±SE.

Treatments	Plant height (cm)	Fresh weight (g/plant)	Dry weight (g/plant)	Seed yield (g/plant)	Water content (%)
Control	62.5±8.4	116.6±12.4	25.6±3.6	12.5±2.6	82%
WS	50.9±9.5	073.7±11.5	20.8±4.2	08.6±1.1	78%
PBZ	55.7±9.9	148.5±13.5	28.3±4.9	15.4±2.5	84%
WS + PBZ.	43.6±8.5	094.4±10.6	23.6±2.8	10.4±2.4	80%

It was found that triazole compounds protect plants from various environmental stresses, including chilling, drought, heat, waterlogging, air pollutants, and heavy metals (Zhang et al., 2007; Fletcher et al., 2010). Drought is a major abiotic factor that limits agricultural crop production. To improve agricultural productivity within limited land and water resources, it is imperative to ensure high crop yields against unfavorable environmental stresses. Our results confirmed that paclobutrazol could enhance water deficit tolerance in soybean, which led to higher biomass accumulation and seed yield under water stress conditions than without paclobutrazol (Table 1).

One of the most widely used agricultural indices to define stress tolerance is data for plant biomass and yield (Juan et al., 2005). The results obtained from these experiments herein show that water stress caused a significant reduction in plant growth parameters and yield. However, supplementary paclobutrazol enhanced these parameters compared to water-stressed plants. Triazole growth regulators have been reported to protect plants from various environmental stresses, including drought, salinity, and heavy metals (Zhang et al., 2007; Aly and Latif, 2011). Our results demonstrate that exogenous application of PBZ compound improved the plant height, plant fresh and dry weights as well as water content of soybean plants under water stress. These findings are in agreement with other published studies. It was found that Soybean is sensitive to drought stress (van Heerden and Krüger, 2002), and a drought-induced decrease in photosynthesis reduces soybean yield (Liu et al., 2004). In the present study, water stress affected leaf RWC, and water-stressed plants showed significantly lower values compared to respective control plants in response to water stress. It is hypothesized that protection of salinity in triazole compound-treated plants was associated with longer roots and smaller leaves for absorbing more water and losing less water, which improve stress tolerance in water-stressed plants (Fletcher et al., 2010).

Leaf water potential

Data recorded in Figure (1) showed that water stress caused a decreased in the Ψleaf, while pacrlobutrazol caused an increase of about 29% in the Ψleaf under water-stressed condition as compared to WS treatment in the absence of paclobutrazol, indicating that the paclobutrazol-treated leaves might lose less water. However, under the well-watered conditions, there was no significant difference in Ψleaf between paclobutrazol-treated (-0.65 MPa) and control plants (-0.58 MPa).

Considerable variation in leaf Ψw was observed among the treatments of both water stress and PBZ (Fig. 1). Water-stressed plants without PBZ showed a significant reduction in leaf Ψw than non-water-stressed plants without PBZ. The application of PBZ significantly increased the leaf Ψw in water-stressed plants. The maximum leaf Ψw was observed in PBZ (40 mg l^{-1}) as compared to the control (PBZ 0.0 mg l^{-1}). The data pertaining to interaction effect of water stress and paclobutrazol clearly indicated an effective role of PBZ for improving leaf Ψw and paclobutrazol (40 mg l-1) was found to be efficient in mitigating the stress by increasing the leaf Ψw. Water-stressed plants with PBZ had significant reductions in the decrease in leaf Ψw than water-stressed plants without PBZ treatments (Fig. 1). It is worth mentioning here that as compare to non-water-stressed and non-PBZ-treated plants (control), the water-stressed plants without PBZ treatment showed a significant reduction in leaf Ψw whereas application of PBZ in water-stressed plants significantly reduced this reduction in leaf Ψw. Thus, it appears that PBZ has a role for improving leaf Ψw in soybean. In this study, the plants treated with PBZ appear to have been more resistant to water stress than those without PBZ treatment, as shown by the alleviation of the reduction in leaf Ψw. The reduction in the decrease in leaf Ψw for the PBZ-treated plants was particularly significant. Similar results have been reported for barley (Trebichalský et al. 2014), pea (Wang et al., 1992), oak (Thomas and Gausling, 2000) and apple (Atkinson et al., 2000). The PBZ influence was, however, reduced the decline in leaf water potential. Moreover, upregulation of stress protective bio-molecules in PBZ-treated plants have also enhanced the capacity to limit the damage

caused by species of reactive (Rady and Gaballah, 2012). Traiazoles-treated plants characteristically use less water and have increased tolerance to drought and a higher water potential than control plants (Fletcher et al., 2000). Reduced inhibition of growth under drought stress appears to be associated with maintenance of relatively high water potential in leaves (Ohashi et al., 2006). Higher leaf water potential is vital for soybean yield formation (Liu et al., 2004).

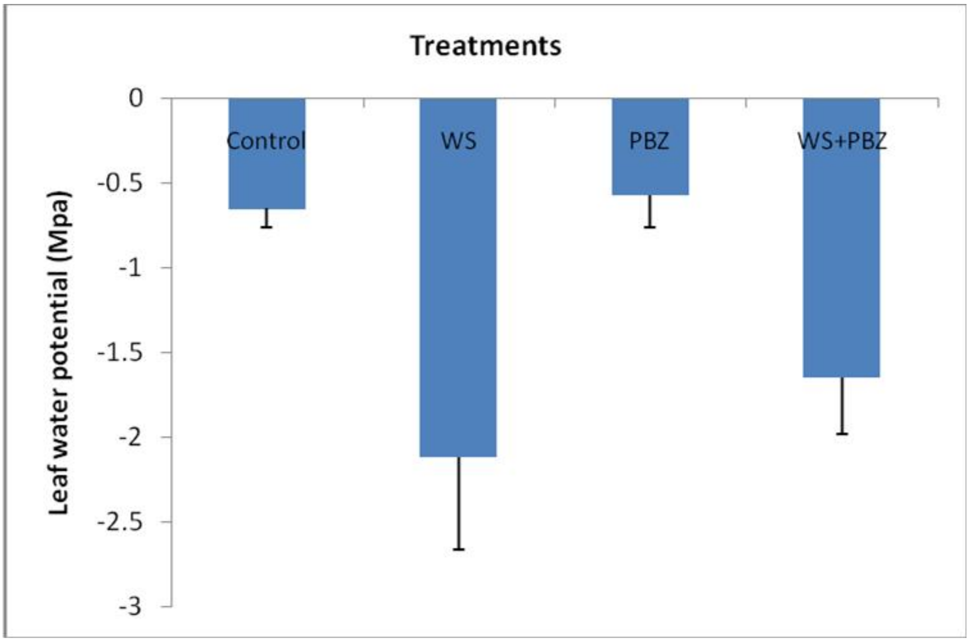

Fig. 1: Effect of water stress (WS) and paclobutrazol (PBZ) on leaf water potential of soybean leaves. Values are means of three replicates (Vertical lines indicate±SE).

Chlorophyll content

Data obtained in Figure (2) indicated that water deficit decreased chlorophyll content of soybean leaves by about 20% as compared with well-watered plants. Paclobutrazol, on the other side caused an improvement in chlorophyll content of water-stressed plants compared to that recorded at water stress treatment without paclobutrazol. In well-watered plants, chlorophyll content increased by about 10% as compared with control treatment.

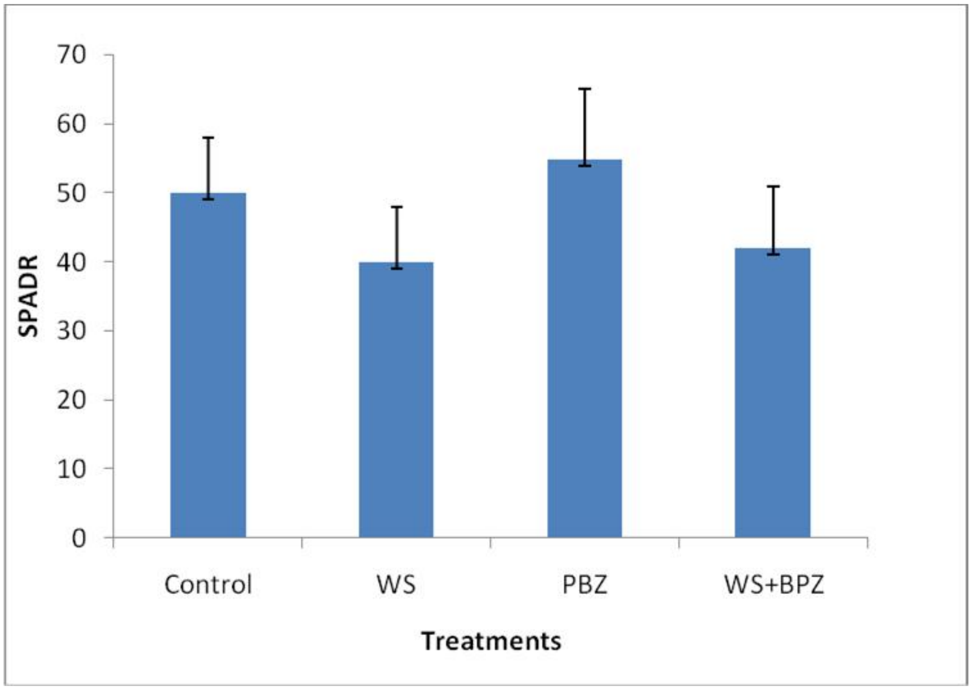

Fig. 2: Effect of water stress (WS) and paclobutrazol (PBZ) on chlorophyll content of soybean leaves. Values are means of three replicates (Vertical lines indicate±SE).

The chlorophyll content of leaves generally decrease underwater stress conditions (Karimi et al., 2005). The reduction in leaf chlorophyll under stress is attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex (Yokas et al., 2008). Moreover, Tuna (2014) reported that light-scattering spectroscopy and microscopy have established that the cross-sectional areas of triazole-treated chloroplasts are significantly larger compared to those observed in untreated leaves. An increase in cytokinins by triazoles could lead to the observed enhanced chloroplast size and chlorophyll levels. For example, in maize, triazole treatment did not change the number of chloroplasts, although the findings indicated more chlorophyll per chloroplast (Basra, 2000). In the present study, water stress treatment caused a major decline in the chlorophyll content while PBZ treatment of the stressed plants significantly increased chlorophyll content compared to water-stressed plants without PBZ. Nouriyani et al. (2012) reported that an increase in paclobutrazol concentrations increased chlorophyll content significantly in two wheat cultivars. Pinhero and Fletcher (1994) observed an increase in chlorophyll and carotenoid pigments after treatments with the triazole compound paclobutrazol in maize seedlings. Our results also confirmed these findings.

Photosynthetic rate (Pn)

Figure (3) showed that Pn of soybean plants drastically decreased under water stress condition. The reduction in Pn caused by WS treatment was about 33% as compared to well-watered plants. On the other hand, Pn of either well-watered plants or water-stressed plants increased by paclobutrazol treatment. In this regard the increase in Pn of unstressed plants reached about 8% of control, while the increase in Pn of water-stressed plants was about 12% as compared to water-stressed plants in the absence of paclobutrazol.

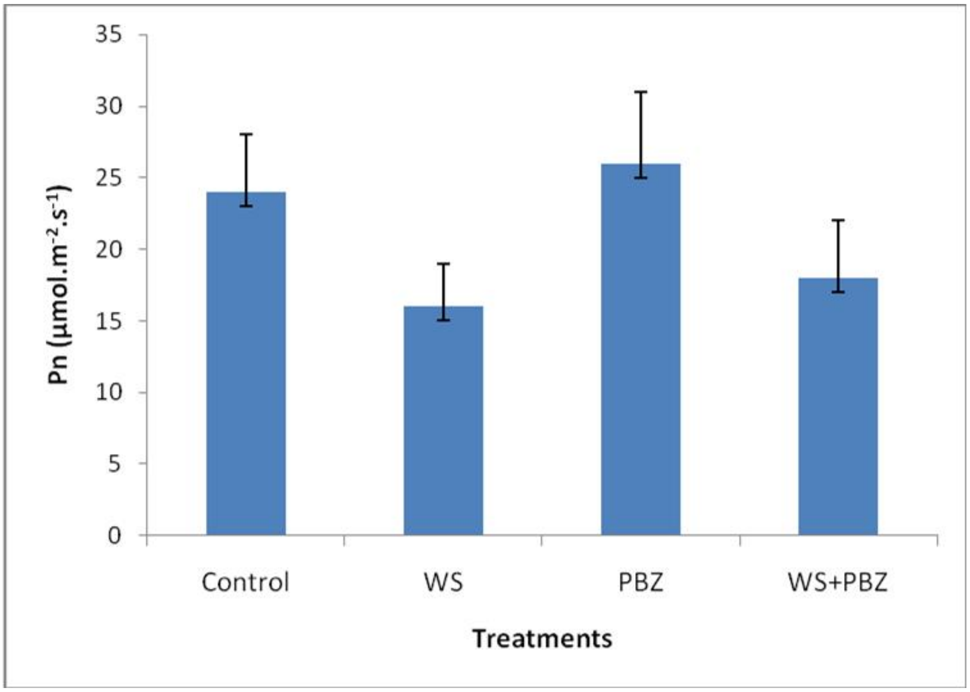

Fig. 3: Effect of water stress (WS) and paclobutrazol (PBZ) on photosynthetic rate (Pn) of soybean leaves. Values are means of three replicates (Vertical lines indicate±SE).

It was reported that water stress adversely impacts many aspects of the physiology of plants, especially photosynthetic capacity (Osakabe et al., 2014). Water stress directly affects rates of photosynthesis due to the decreased CO_2 availability resulted from stomatal closure (Chaves et al., 2009), and/or from changes in photosynthetic metabolism (Lawlor, 2002). Drought has a negative effect on photosynthesis when the rates of photosynthesis are reduced by water stress (Osakabe and Osakabe, 2012). A strong interconnection between the responses to drought stresses has been suggested, and around 70% genes are induced by drought (Chan et al., 2010; Estavillo et al., 2011). Water stress also stimulates the production of ROS, such as H_2O_2, superoxide (O_2^-) and singlet oxygen ($^1O_2$), by specific photochemical and biochemical processes, which also exerts deleterious effects on photosynthesis (Li et al., 2008). In another studies, it was found that water stress substantially alters plant metabolism, decreasing plant growth and photosynthesis (Aldesuquy et al, 2012). The relative water content (RWC), water potential and turgor of cells are decreased and the concentrations of ions and other solutes in the cells are increased, thereby decreasing the osmotic potential (Ribas-Carbo et al., 2005). Stomatal pores in the leaf surface progressively close, decreasing the conductance to water vapour and thus slowing transpiration and the rate at which water deficits develop. Also, photosynthetic assimilation of CO2 decreases, often concomitant with, and frequently ascribed to, decreasing conductance to CO2 (Tezara et al., 1999). In the present study, it was found that soybean seedlings treated with paclobutrazol had increased Pn regardless of soil water status, but leaf water potential and chlorophyll content were increased only under water-stressed conditions with PBZ (Figs 1 and 3) . Improvement of photosynthesis by paclobutrazol is the main contribution for better yield regardless of water conditions, as was indicated by other studies (Qiu et al., 2005; Tuna, 2014). Thus, water-stressed plants treated with PBZ maintained a greater Pn than those without PBZ (Fig. 2).

Proline and soluble sugars

Data recorded in Fig (4) showed clearly that proline and soluble sugar contents substantially increased water-stressed plants. In this regard, WS treatment caused an increase of about 64% in proline and about 73% in soluble sugars as compared with well-watered plants. Moreover, PBZ treatment caused further increases in both compounds. Thus, Proline concentration and soluble sugar content increased with WS+PBZ treatment by about 10% and 6%, respectively, compared with those values obtained under WS treatment in the absence of PBZ.

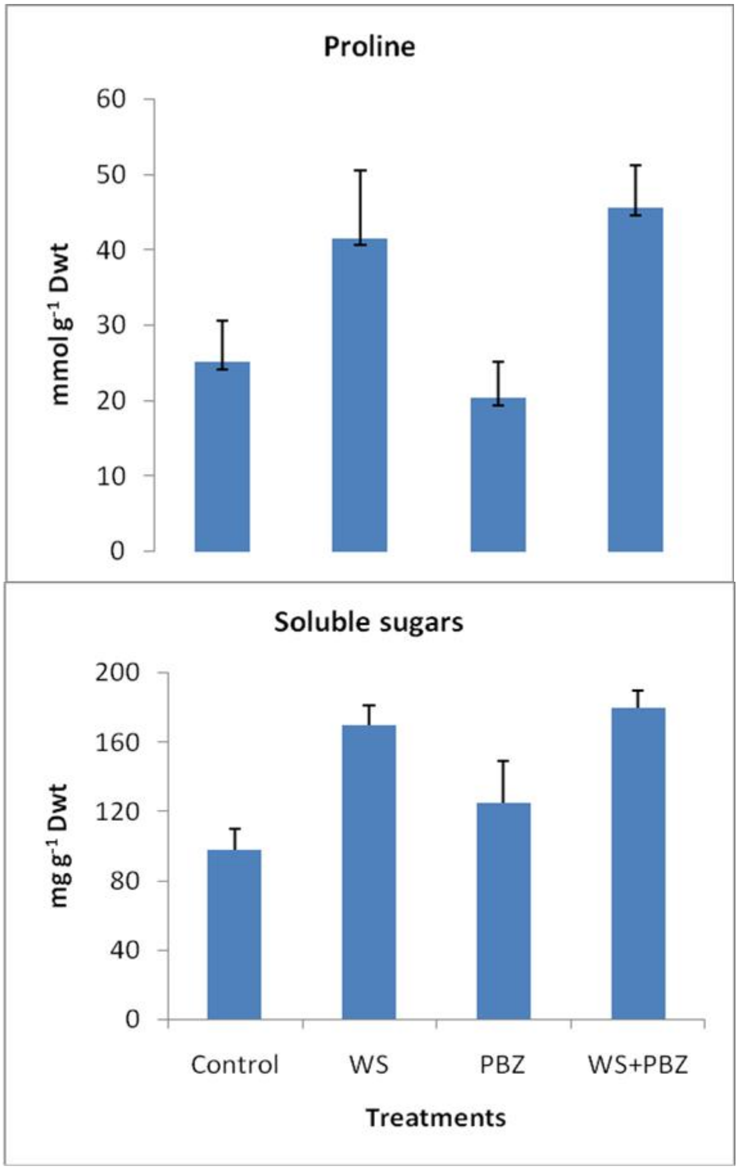

Fig. 4: Effect of water stress (WS) and paclobutrazol (PBZ) on proline and soluble sugar contents of soybean leaves. Values are means of three replicates (Vertical lines indicate±SE).

It is well known that proline and sugars are considered compatible and osmoregulator solutes and both compounds accumulate in many plant species under a broad range of stress conditions, such as water shortage, salinity, extreme temperatures, and high light intensity (Abbaspour, 2012). In our study, proline and soluble sugar contents in the leaves of soybean plants grown under water stress increased compared to the unstressed control plants. Moreover, externally supplied PBZ promoted this effect and increased proline and soluble sugar content (Fig 4). The literature survey showed that biochemical effects of the triazoles include increased levels of proline. For example, the triadimefon treatment also increased the proline content in the leaves, stem, and root of *C. roseus* plants compared to the control (Jaleel et al., 2007). Similarly, paclobutrazol increased the proline content in *Eruca sativa* seedlings (Mathur and Bohra, 1992). Consistent with these results, Baninasab and Ghobadi (2011) observed that paclobutrazol ameliorated the injuries caused by heat stress by increasing the leaf proline content and preventing an increase in leaf electrolyte leakage. Moreover, it was reported that soybean plants cope with drought stress by accumulating some osmolytes, including proline and soluble sugars (van Heerden and Krüger, 2002). Drought increased proline and soluble sugar accumulation in soybean leaves, and paclobutrazol increased osmolyte contents compared with the water-stressed control (Fig. 4). Proline accumulation is positively correlated with drought tolerance (Reddy et al., 2004). Osmolyte accumulation induced by PBZ may be an important factor to prevent water loss and to maintain high photosynthesis activity in soybean.

Phytohormones

Data reported in Tables (5 and 6) showed that all phytohormone concentrations in soybean leaves were changed with both water stress and paclobutrazol treatments. In the absence of paclobutrazol, water stress alone caused a reduction in GA3, IAA and CK by about 76%, 50% and 42%, respectively, and caused an increase of about 98% in ABA concentration, as compared with control treatment. Paclobutrazol-treated plants had much lower GA3 and higher IAA and CK than untreated soybean within each water condition but ABA content was increased by paclobutrazol only under water-stressed conditions. In this regard, patrobutrazol treatment alone caused a decrease of about 41% in GA3 and 11% in IAA levels and an increase of about 86% and 68% in CK and ABA of well-watered plants, with respect to control treatment. Moreover, WS + PBZ treatment caused a decrease in GA and IAA concentrations and an increase in CK and IAA concentrations as compared with WS treatment alone

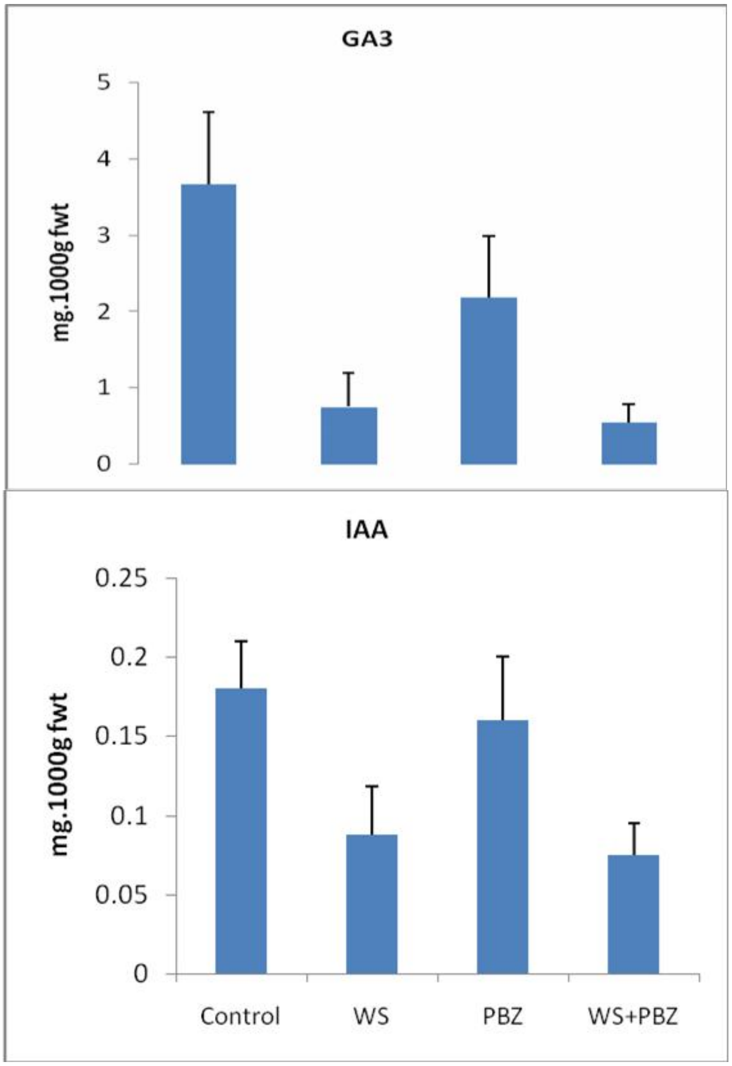

Fig. 5: Effect of water stress (WS) and paclobutrazol (PBZ) on endogenous GA3 and IAA levels of soybean leaves. Values are means of three replicates (Vertical lines indicate±SE).

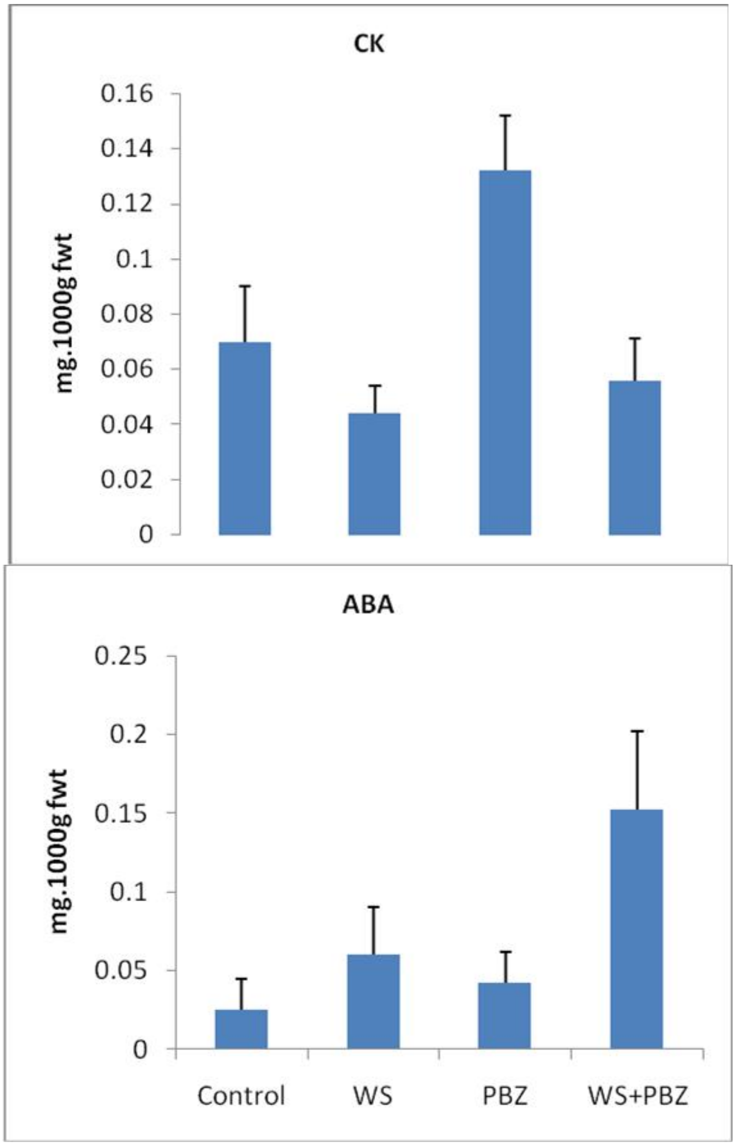

Fig. 6: Effect of water stress (WS) and paclobutrazol (PBZ) on endogenous CK and ABA levels of soybean leaves. Values are means of three replicates (Vertical lines indicate±SE).

The endogenous hormonal balance in soybean leaves was affected after plants were treated with uniconazole and exposed to water deficit stress (Figs 5 and 6). Leul and Zhou (1998) reported that changes in morphological characteristics and physiological activities by triazole compounds were closely related with alterations in hormone concentrations in leaves following treatment. Paclobutrazol-treated plants had decreased GA3 and IAA, but increased CK, and ABA contents under water stress condition (WS+PBZ treatment). These changes resulted in a new balance of endogenous hormones, which would be favorable to drought tolerance (Davis et al., 1988). The effects of triazoles and gibberellic acid (GA) are mutually antagonistic, as seen in examples of inhibition of triazole-induced physiological and biochemical processes. Inhibited gibberellin biosynthesis and increased cytokinin and abscisic acid content induced by these triazoles might be the cause of increased root growth and corresponding dry or fresh weight in plants (Basra, 2000; Rajalekshmi et al., 2009). It was interesting to find that PBZ caused an increase in ABA accumulation by 98% when soybean experienced water deficit (Fig. 6). An increase in ABA after triazole treatment under water stress conditions was also observed by Grossmann et al. (1994). ABA accumulation in triazole-treated rape leaves decreased under water logging conditions (Leul and Zhou 1998). Conversely, triazoles, including PBZ, inhibited GA biosynthesis and stimulated. ABA accumulation

(Fletcher et al., 2000). Asare-Boamah et al. (1986) reported that the transient rise in ABA levels partly contributed to increased drought tolerance by reducing leaf transpiration. High leaf ABA content induced stomatal closure in water-deficit stressed plants, which resulted in a high leaf water potential, and the net effect was that water-stressed plants maintained a greater Pn. Liu et al. (2004) showed that high leaf water potential is vital for soybean pod set. Also, triazoles -induced tolerance to heat or low-temperature stresses has been associated with increased levels of endogenous ABA (Fletcher et al., 2000). In the present experiment, when plants are exposed to water-deficit stress, drought-induced ABA plays a major role in response and tolerance to water deficit (Reddy et al., 2004). Accumulation of ABA by PBZ-treated soybean might have helped to prevent water loss.

Antioxidant enzymes

Data in Table (2) indicated that activities of SOD, PPO and POX increased under water stress condition. Soluble protein showed slight increase also under WS treatment as compared with control. Paclobutrazol was found to increase POX activity and protein concentration in well-watered plants, but SOD and PPO activities were not changed significantly at PBZ treatment. Conversely, in water-stressed plants, PBZ caused significant increases in the activity of SOD, PPO and POX as well as the concentration of soluble proteins in plant leaves. These increases of enzyme activities and proteins in water-stressed plants under PBZ treatment were about 166%, 14%, 94% and 42%, respectively, as compared with well-watered control plants. But at water-stressed plants, PBZ treatment caused an increase in SOD activity by about 7% and soluble protein by about 29%, as compared to WS-plants without PBZ treatment. Triazoles have increased the activity of antioxidants (Kraus and Fletcher, 1994), which help plants to tolerate stress conditions. Foliar application of triazoles caused an increase in SOD and POX activities of soybean plants during water stress. Fletcher et al. (2000) indicated that triazole-induced stress tolerance in plants may be caused, at least in part, by increased antioxidant activity, which in turn reduces oxidative injury to membrane and/or enzyme activity.

Table 2. Effect of water stress (WS) and paclobutrazol (PBZ) on activities of superoxide dismutase (SOD), polyphenol oxidase (PPO) and peroxidase (POX) of soybean leaves. Values are means of three replicates ±SE.

Treatments	SOD (U mg^{-1} protein)	PPO (U x 100 mg^{-1} protein)	POX (Δ A470 $min^{-1} mg^{-1}$ protein)	Soluble protein (mg g^{-1} Fwt)
Control	065.8±9.4	72.6±5.3	08.5±1.8	2.23±0.45
WS	164.4±11.5	85.8±6.6	20.7±3.2	2.46±0.28
PBZ	068.5±7.5	70.4±4.8	10.9±2.6	2.88±0.33
WS+PBZ	175.8±10.3	86.6±7.5	23.4±3.2	3.17±0.64

In the present study, we found that paclobutrazol is involved in the protection and activation of the antioxidative system under conditions of water stress because PBZ treated plants exhibited higher antioxidative activities after being exposed to water stress compared to WS treatment without PBZ (Table 2). These data are in agreement with those of earlier studies, which showed that various triazoles-treated plants are more tolerant to environmental stresses (Kishorekumar et al., 2008) compared to untreated plants. Reactive oxygen species (ROS) cause damage to lipids, proteins, and DNA. The triazole compounds enhance different H2O2 scavenging enzymes, like superoxise dismutase and ascorbate peroxidase, and also various other antioxidants in many plant species. This enhancement would help in scavenging of ROS, like H2O2 (Tuna, 2014). The stress protection offered by triazoles depends heavily on the modulation of the activity and levels of antioxidants. A literature survey indicates that triazoles have increased the activity of antioxidant potential (Sivakumar and Panneerselvam, 2011), which helps plants tolerate stress conditions. These results are in agreement with those of Nair et al. (2012) who reported that triazols caused an increase in the activities of antioxidant enzymes, like superoxide dismutase, peroxidase and polyphenol oxidase. Therefore, paclobutrazol seem to induce increased stress resistance by increasing photosynthetic efficiency, thereby reducing the generation of free radicals and increasing the levels of antioxidants involved in the prevention of free radical generation (Singh, 2005; Tuna, 2014).

Conclusion

It can be concluded that the application of paclobutrazol reduced the deleterious effects of drought stress on soybean growth. Paclobutrazol caused an increase in the leaf water potential (Ψ_{leaf}) and in the activities of antioxidant enzymes. It also promoted accumulation of proline, soluble sugars and the rate of photosynthesis under water-stress condition. Moreover, paclobutrazol induced significant accumulation of leaf ABA by plants exposed to water deficit stress, which played a major role in soybean tolerance to drought stress.

References

Abbaspour, H. 2012. Effect of salt stress on lipid peroxidation, antioxidative enzymes, and proline accumulation in pistachio plants. J Med Plant Res. 6: 526-529.

Abdalla M.M. 2011. Beneficial effects of diatomite on the growth, the biochemical contents and polymorphic DNA in *Lupinus albus* plants grown under water stress. Agriculture and Biology Journal of North America 2: 207-220.

Aldesuquy, H.S., Baka, Z.A., El-Shehaby, O.A. and Ghanem, H.E. 2012. Varietal differences in growth vigor, water relations, protein and nucleic acids content of two wheat varieties grown under seawater stress. Journal of Stress Physiology and Biochemistry, 8: 24-47.

Aly, A.A and Latif, H.H. 2011. Differential effects of paclobutrazol on water stress alleviation through electrolyte leakage, phytohormones, reduced glutathione and lipid peroxidation in some wheat genotypes (*Triticum aestivum* L.) grown *in-vitro*. Romanian Biotechnological Letters, 16(6): 6710.

Asare-Boamah, N..K., Hofstra, G., Flecher, R.A. and Dumbroff, E.B. 1986. Triadimefon protects bean plants from water stress through its effects on abscisic acid. Plant Cell Physiol, 27: 383–390.

Azadeh, R., Maryam, F. and Saeed, S. 2014. The effects of seed priming by ascorbic acid on some morphological and biochemical aspects of rapeseed (*Brassica napus* L.) under drought stress condition. Int. J. Biosci. 4 (1): 432-442.

Baninasab, B. and Ghobadi, C. 2011. Influence of paclobutrazol and application methods on high-temperature stress injury in Cucumber seedlings. J Plant Growth Regul. 30: 213–219.

Basra, A.S. 2000. Plant growth regulators in agriculture and horticulture: Their role and commercial use. In: Fletcher RA, Sopher CR, Vettakkorumakankav NN (eds) Regulation of gibberellins is crucial for plant stress protection Published by The Haworth Pres, Binghamton, NY.

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

Brian, W.E. 2015. Plant Growth Regulators for Annuals. Fine American Inc., NC, USA. Pp. 48.

Chan, K.X., Crisp, P.A., Estavillo, G.M. and Pogson, B.J. 2010. Chloroplast-to-nucleus communication: current knowledge, experimental strategies and relationship to drought stress signaling. *Plant Signal. Behav.* 5 1575–1582.

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

Davis, T.D., Steffens, G.L. and Sankhla, N. 1988. Triazole plant growth regulators Hort Rev, 10: 63–105.

Estavillo, G.M., Crisp, P.A., Pornsiriwong, W., Wirtz, M., Collinge, D., Carrie, C., et al. 2011. Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in *Arabidopsis*. Plant Cell, 23: 3992–4012.

Fales, F.W. 1951. The assimilation and degradation of carbohydrates by yeast cells. J Biol Chem, 193: 113–124.

FAO – Food And Agriculture Organization Of The United Nations 2012. *Production crops.* Roma, Food And Agriculture Organization Of The United Nations Disponível em: < http://faostat. fao. org/site/567/default.

Fletcher, R., Gilley, A., Sankhla, N. and Davis, T. 2010. Triazoles as plant growth regulators and stress protectants. Horticultural Reviews, 27: 55-138.

Fletcher, R.A., Angella, G., Sankala, N. and Tim, D. 2000. Triazoles as plant growth regulators and stress protectors. Hort. Rev., 24: 55-105.

Fletcher, R.A., Gilley, A., Davis, T.D. and Sankhla, N. 2000. Triazole as plant growth regulators and stress protectants. Hort Rev, 24: 55–138.

Gomez K.A. and Gomez A.A. 1984. Statistical Procedures for Agricultural Research, 2nd ed. John Wiley and Sons, New York, USA, 8–29**.**

Grossmann, K. Kwiatkowski, C., Häuser, C. and Siefert, F. 1994. Influence of the triazole growth retardant BAS 111W on phytohormone levels in senescing intact pods of oilseed rape. Plant Growth Regul, 14: 115–118.

Guo, X.F., Zhou, Y., Tu, F.Q., Xiong, X.J., Wang, H. and Zhang, H.S. 2011. Determination of phytohormones in plant samples based on the precolumn fluorescent derivatization with 1,3,5,7-tetramethyl-8-aminozide-difluoroboradiaza-s-indacene by HPLC for routine use. Journal Separation Science. 34: 789–795.

Hajihashemi, Sh., Kiarostami, Kh., Enteshari, Sh., and Saboora, A. 2014. Effect of paclobutrazol on wheat salt tolerance at pollination stage. Russian Journal of Plant Physiology, 56(2): 251-257.

Jaleel, C.A., Gopi, R., Manivannan, P., Kishorekumar, A., Gomathinayagam, M. and Panneerselvam, R. 2007. Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage. J Zhejiang Univ Sci B. 8: 283-288.

Juan, M., Rivero, R.M., Romero, L. and Ruiz, J.M. 2005. Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. Environ Exp Bot. 54: 193-201.

Karimi. G., Ghorbanli, M., Heidari, H., Khavari Nejad, R.A. and Assareh, M.H. 2005. The effect of NaCl on growth water relations, osmolytes and ion content in *Kochia prostrate*. Biol Plant. 49: 301-304.

Kishorekumar, A., Jaleel, C.A., Manivannan, P., Sankar, B., Sridharan, R., Murali, P.V. 2008. Comparative effects of different triazole compounds on antioxidant metabolism of *S. Rotundifolius*. Colloids Surfact B: Biointerfaces 62: 307-311.

Kumudini, S. 2010. Soybean Growth & Development. In: B Singh, (Ed.). *The Soybean: Botany, Production and Uses.* CAB International, Oxfordshire, UK, p. 48–73.

Lawlor, D.W. and Cornic, G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell and Environment 25: 275–294.

Leul, M. and Zhou, W.J. 1998. Alleviation of waterlogging damage in winter rape by application of uniconazole: Effects on morphological characteristics, hormones and photosynthesis. Field Crops Res, 59: 121–127.

Li, W.-X., Oono, Y., Zhu, J., He, X.-J., Wu, J.-M., Iida, K., et al. 2008. The *Arabidopsis* NFYA5 transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance. Plant Cell 20 2238–2251.

Li-Juan, Q. and Ru-Zhen, C. 2010. The Origin and History of Soybean. In: B Singh, (Ed.). *The Soybean: Botany, Production and Uses.* CAB International, Oxfordshire, UK, p. 01–23.

Lisar, S.Y.S., Motafakkerazad, R., Hossain, M.M. and Rahman, I.M.M. 2012. Water Stress in Plants: Causes, Effects and Responses. In:Rahman, I. M. M. & Hasegawa, H. *Water Stress,* InTech, Croatia, p. 01-14.

Liu, F.L., Jensen, C.R. and Andersen, M.N.2004. Pod set related to photosynthetic rate and endogenous ABA in soybean subject to different water regimes and exogenous ABA and BA at early reproductive stages. Ann Bot, 94: 405–411.

Mathur, R., Bohra, S.P. 1992. Effect of paclobutrazol on aminotransferases; Protein and proline content in *Eruca sativa* var. T-23 seedlings. J Phytol Res. 5: 93-95.

Miyake, C. 2010. Alternative electron flow (water-water cycle and cyclic electron flow around PSI) in photosynthesis: molecular mechanisms and physiological function. Plant and Cell Physiol. 51: 1951-1963.

Nair, V.D., Gopi, R., Mohankumar, M., Kavina, J. and Panneerselvam, R. 2012. Effect of triadimefon: a triazole fungicide on oxidative stress defense system and eugenol content in *Ocimum tenuiflorum* L. Acta Physiol Plant. 34: 599-605.

Nouriyani, H., Majidi, E., Seyyednejad, S.M., Siadat, S.A., and Naderi, A. 2012. Effect of paclobutrazol under different levels of nitrogen on some physiological traits of two wheat cultivars (*Triticum aestivum* L.). World Appl Sci J. 16: 01-06.

Ohashi, Y., Nakayama, N., Saneoka, H. and Fujita, K. 2006. Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. Biol Plant, 50: 138–141.

Osakabe, K., Osakabe, Y. 2012. Plant light stress, in *Encyclopaedia of Life Sciences* ed. Robinson S. A., editor. (London: Nature Publishing Group).

Osakabe, Y., Osakabe, K., Shinozaki, K. and Tran, L.S.P. 2014. Response of plants to water stress. Front Plant Sci., 5: 86-93.

Parvin, S., Javad, T. and Ghader, N. 2015. Effects of different water stress levels and paclobutrazol on strawberry. Cercetări Agronomice în Moldova, 1 (161) : 107-114.

Pinhero, R. and Fletcher, R.A. 1994. Paclobutrazol and ancymidol protect corn seedlings from high and low temperature stresses. Plant Growth Reg. 15: 47-53.

Qiu, J., Wang, R.M., Yan, J.Z. and Hu J. 2005. Seed film coating with uniconazole improves rape seedling growth in relation to physiological changes under waterlogging stress. Plant Growth Regul, 47: 75–81.

Rady, M.M. and Gaballah, M.S. 2012. International Science Congress Association: 1 Improving Barley Yield Grown Under Water Stress Conditions. Res.J.Recent Sci. 1(6): 1-6.

Rajalekshmi, K.M., Jaleel, C.A., Azooz, M.M. and Panneerselvam, R. 2009. Effect of triazole growth regulators on growth and pigment contents in *Plectranthus aromaticus* and *Plectranthus vettiveroids.* Adv Biol Res. 3: 117-122.

Reddy, A.R., Chaitanya, K.V. and Vivekanandan, M. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol, 161: 1189–1202.

Ribas-Carbo, M., Taylor, N.L., Giles, L., Busquets, S., Finnegan, P.M.m Day, D.A., Lambers, H., Medrano, H., Berry, J.A. and Flexas, J. 2005. Effects of Water Stress on Respiration in Soybean Leaves. Plant Physiol., 139(1): 466–473.

Singh, G. and Shivakumar, B.G. 2010. The role of soybean in agriculture. In: B Singh, (Ed.). *The Soybean: Botany, Production and Uses.* CAB International, Oxfordshire, UK, pp. 24–47.

Singh, V.P. 2005 Metal toxicity and tolerance in plants and animals. Published by Sarup & Sons, Delhi, India 79.

Sivakumar, T. and Panneerselvam, R. 2011. Triadimefon mediated changes in antioxidant and indole alkaloid content in two species of *Datura*. Amer J Plant Physiol. 6: 252-260.

Tezara, W., Mitchell, V.G., Driscoll, S.D. and Lawlor, D.W. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401: 914-917.

Thomas, F.M. and Gausling, T. 2000. Morphological and physiological responses of oak seedlings (*Quercus petraea* and *Q. robur*) to moderate drought. Ann. For. Sci., **57**, 325–333**.**

Trebichalský, P., Harangozo, L., Tóth, T., Bystrická, J. and Musilová J. 2014. Reduction of herbicide and water stress in spring barley by regulators of polyamine. Biosynthesis. J Microbiol Biotech Food Sci, 3 (2): 171-173.

Tuna, A.L. 2014. Influence of foliarly applied different triazole compounds on growth, nutrition, and antioxidant enzyme activities in tomato (*Solanum lycopersicum* L.) under salt stress. Australian Journal of Crop Science 8(1):71-79.

Turner, F.T. and Jund, M.F. 1991.Chlorophyll meter to predict nitrogen topdress requirement for semidwarf rice. Agron J, 83: 926–928.

van Heerden, P.D.R. and Krüger, G.H.J. 2002. Separately and simultaneously induced dark chilling and drought stress effects on photosynthesis, proline accumulation and antioxidant metabolism in soybean. J Plant Physiol, 159: 1077–1086.

Wang, L.H. and Lin, C.H. 1992. The effect of paclobutrazol on physiological and biochemical changes in the primary roots of pea, J. Exp. Bot., 43, 1367–1372**.**

Wang, L.L., Chen, A.P., Zhong, N.Q., Liu, N., Wu, X.M., Wang, F., Yang, C.L., Romero, M.F. and Xia, G.X. 2014. The *Thellungiella salsuginea* tonoplast aquaporin tstip1;2 functions in protection against multiple abiotic stresses. Plant Cell Physiol.55 (1): 148-161.

Yokas, I., Tuna, A.L., Burun, B., Altunlu, H., Altan, F. and Kaya, C. 2008. Responses of the tomato (*Lycopersicon esculentum* Mill.) plant to exposure to different salt forms and rates. Turk J Agric Forest. 32: 319-329.

Zhang, M., Duan, L., Tian, X., He, Z., Li, J., Wang, B. and Li, Z. 2007. Uniconazole-induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. J Plant Physiol. 164: 709-717