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Drought and Gibberellic Acid-dependent Oxidative Stress: Effect on Antioxidant Defense System in Two Lettuce Cultivars

Dina Z. Abdel-Kader
Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Abstract : The purpose of the present work was to evaluate the oxidative stress and the antioxidant defense system in two-lettuce cultivars *Lactusa sativa* var. longifolia-baladi (baladi cv.) and *Lactusa sativa* var. crispa-mignonette (mignonette cv.). The plants were subjected to two drought levels and treated with two concentrations of gibberellic acid (GA_3). The application of drought stress increased significantly the lipid peroxidation and catalase activity in the two cultivars while it induced significant decreases in protein content, peroxidase and ascorbic acid oxidase activities as compared to control. Treatment with gibberellic acid alleviated the adverse effects of drought. It could be concluded that the lowest concentration of GA_3 was more effective on alleviating the adverse effect than the highest concentration. Also it was found that mignonette cultivar was more tolerant to drought than baladi cultivar.

Keywords: Drought, gibberellic acid, lipid peroxidation, antioxidants.

Introduction

Under natural conditions plants are often exposed to various environmental stresses that decrease the production. At the whole-plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth. At the molecular level, the negative effect of stress on leaves may be in part a consequence of the oxidative damage to important molecules, as a result of the imbalance between production of activated O_2 and antioxidant defenses (Foyer *et al.*, 1994).

A consequence of the drought-induced limitation of photosynthesis is the exposure of plants to excess energy, which, if not safely dissipated, may be harmful to photosystem II (PSII), because of over-reduction of reaction centers (Demmig-Adams and Adams, 1992) and increased production of reactive oxygen species in chloroplast (Smirnoff, 1993).

Lipid peroxidation is commonly taken as an indicator of oxidative stress. Inaki *et al.* (1998) concluded that application of severe water stress to pea led to increase in catalytic Fe, lowering of antioxidant protection and accumulation of malondialdehyde and oxidized proteins. To counteract the toxicity of active oxygen species, different antioxidants like ascorbic acid, glutathione, carotenoids, α-tochopherol, superoxide dismutase, catalase, peroxidase and the enzymes of the ascorbic glutathione cycle are present in plants (Foyer *et al.*, 1994).

The principal H_2O_2 scavenging enzyme in plants is catalase enzyme which is located in peroxisomes/glyoxysomes and ascorbate peroxidase which is primarily found in cytosol and chloroplasts (Asada, 1999). The sub-cellular distribution of these enzymes suggests that chloroplastic ascorbate peroxidase removes H_2O_2, produced during Mehler reaction and other chloroplastic processes, whereas catalase scavenges photorespiratory H_2O_2 (Willekens *et al.*, 1997). They also suggested that the function of catalase in the cell is to remove the bulk of H_2O_2, whereas peroxidase would be involved mainly in scavenging H_2O_2 that is not taken by catalase. The catalase/peroxidase system may thus act cooperatively to remove H_2O_2 at a minimum expense of reducing power. Peroxidase also has been implicated in a number of higher plant processes such as host defense mechanism, cross-linking of hydroxyproline-rich glycoprotein monomers in cell walls, cross-linking pectic polysaccharides with phenolic acids in cell walls, lignification and suberization (Mellon, 1991).

Ascorbic acid oxidase is a plant specific, copper containing oxidase that catalyses the aerobic oxidation of ascorbic acid to dehydro-ascorbic acid (DHA) via a free radical semidehydro-ascorbic acid (Lin and Varner, 1991). This enzyme is of special interest because of the high concentrations of ascorbate in plant cells, the high reactivity of the ascorbate/DHA redox pair, and its cell wall localization. It is conceivable that DHA could cause cell wall loosening (Horner and Tand-Wagner, 1980). A related discovery is that ascorbate free radical, an intermediate of the ascorbic acid oxidase reaction, enhances vacuolization, and hence cell elongation in onion root tip cells (Hidalgo *et al.*, 1989).

Schwanz and Polle (2001) reported that the development of drought stress in *Pinus pinaster* and *Quercus rebur* caused the gradual reduction in antioxidant protection, increased lipid peroxidation, and increased oxidation of ascorbate.

During prolonged periods of drought, the decrease in water availability for transport-associated processes leads to changes in the concentrations of many metabolites followed by disturbances in amino acid and carbohydrate metabolism. (Girousse *et al.*, 1996).

Riccardi *et al.* (1998) described protein changes occurring in leaves of two maize genotypes after progressive dehydration of the plants. They concluded that protein quantity was differently modified by stress, according to genotype. Also, de-Ronde *et al.* (2000) detected, that with decreasing water content there was a progressive increase in free proline in six cotton cultivars, whereas Schwanz and Polle (2001) reported that drought stress caused significant loss in soluble proteins and carotenoids.

It was reported that GA_3 alleviated the drought stress on peanut plants by increasing carbohydrate content, amino-N and total nitrogen (El-Meleigy *et al.*, 1999). Same was the opinion of Aliyev *et al.* (2000) with wheat seedlings. The growth promoting effects of gibberellins (GAs) on plants are well documented (Nagel *et al.*, 2001).

The aim of present study was: a), to evaluate the effect of drought on antioxidative defense system in two cultivars of lettuce b), to study the effect of GA_3 application on alleviating the adverse effect of drought and c), to compare the response of two cultivars to drought and GA_3 effect.

Materials and Methods

Seeds of two lettuce cultivars, *L. sativa* var. longifolia-baladi (Baladi cv.) and *L. sativa* var. crispa-mignonette (mignonette cv.) were planted in 15-cm diameter pots, half filled with

sandy soil. Starting from 2 weeks after sowing, the following treatments were applied:

Water deficit: This was induced by withholding irrigation for 2 weeks (D1) and 3 weeks (D2).

GA_3 application: Another two groups of droughted plants were sprayed with 50 and 100 ppm of GA_3, three times at 2-days intervals.

Control: Plants were kept in optimal conditions over the experimental period. Control plants were sprayed with 0, 50, and 100 ppm GA_3 at the same time with the droughted plants.

Lipid peroxidation: Lipid peroxides were extracted by grinding 0.5 g of leaves with 5% metaphosphoric acid. Homogenate were filtered and centrifuged at 6000 rpm for 20 min. The reaction mixture was formed by mixing 0.5 mL of supernatant, 0.25 mL of 1% (w/v) TBA (in 50 mm NaOH), and 0.25 mL of 25% (v/v) HCL and by incubating the reaction mixture at 95 °C for 30 min (Minotti and Aust, 1987).

Antioxidant enzymes (catalase, peroxidase and ascorbic acid oxidase): Antioxidant enzymes were extracted from 0.5 g frozen leaves with phosphate buffer (pH 7.0). Peroxidase activity was determined following the dehydrogenation of guaicol at 436 nm (Malik and Singh, 1980). Catalase activity was determined using the method of Luck (1974) and ascorbic acid oxidase activity was measured according to method of Oberbacker and Vines (1963).

Protein electrophoresis: Samples were prepared for electrophoresis by solubilization in equal volumes of SDS buffer (0.0625 M Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% mercaptoethanol and bromophenol blue, 0.001%). Six reference proteins differing in their molecular weights were used as markers and run in parallel with the protein under study. The method used was recommended by King and Laemmli (1971).

Statistical analysis: Analysis of variance (ANOVA), student t-test was performed on all data using SPSS program.

Results

Lipid peroxidation: The results in Fig. 1 show that increasing the severity of drought stress (from D1 to D2) induced significant increase in the level of lipid peroxidation in the two plant cultivars under investigation as compared to controls. Application of GA_3 (50 and 100 ppm) significantly reduced lipid peroxidation in the two droughted cultivars during two experimental periods. It was noticed that the application of 50 ppm GA_3 was more effective than 100 ppm GA_3.

Catalase: Two-weeks drought period (D1) resulted in highly significant increases in catalase activity in both cultivars. However, three-weeks drought period (D2) induced a significant decrease in catalase activity at baladi cultivar and a significant increase in mignonette cultivar Fig. 2.
It was found that application of 50 ppm GA_3 on control plants induced a highly significant increase in catalase activity at baladi cultivar whereas it induced a highly significant decrease in mignonette cultivar. On the other hand, 100 ppm GA_3 concentration recorded non-significant effect on catalase activity at both cultivars.
Application of the two GA_3 concentrations on two-weeks drought period (D1) induced a significant decrease in catalase activity in both cultivars. However, the same concentrations were found to induce a highly significant increase in catalase activity at D2 treatment. It was noticed that as GA_3 concentration increases, the catalase activity also increases.

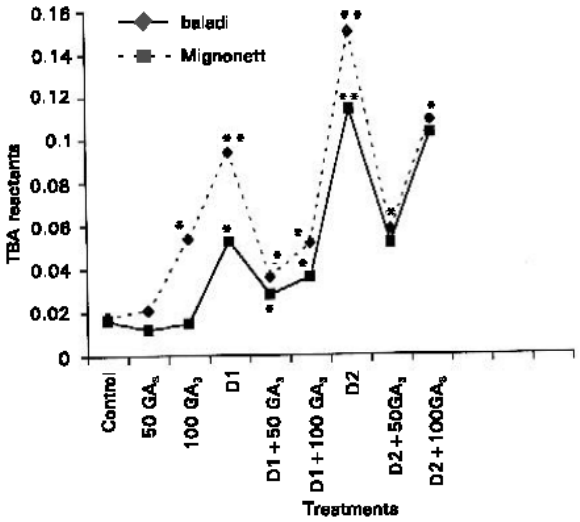

Fig. 1: Effect of two drought treatments (D1 and D2) and two GA_3 concentration (50 and 100 ppm) on lipid peroxidation (as TBA reactants). * Significant at ($P<0.05$) ** significant at ($P<0.01$)

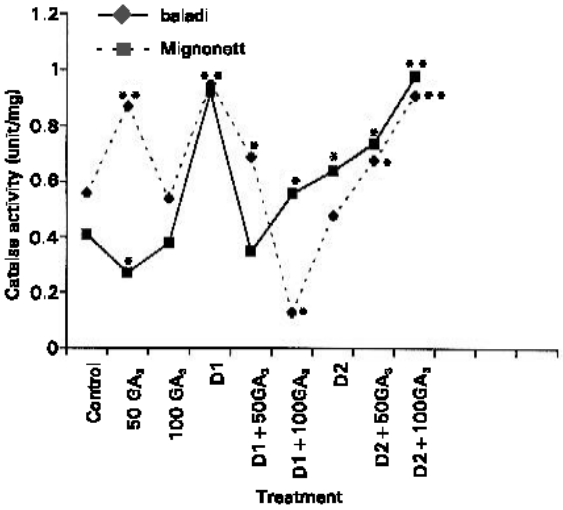

Fig. 2: Effect of two drought treatments (D1 and D2) and two GA_3 concentrations (50 and 100 ppm) on catalase activity (unit/mg). * Significant at ($P<0.05$) ** significant at ($P<0.01$)

Peroxidase: In baladi cultivar, it was found that with increasing drought periods there is a significant increase in peroxidase activity. However, mignonette cultivar showed an increase in peroxidase activity only at D1 drought period. Application of 50 ppm GA_3 at three water regimes (control, D1 and D2) induced highly significant increases in peroxidase activity in both cultivars. These increases were inversely proportional to increasing drought severity. The same trend was noticed during application of 100 ppm GA_3 on mignonette cultivar

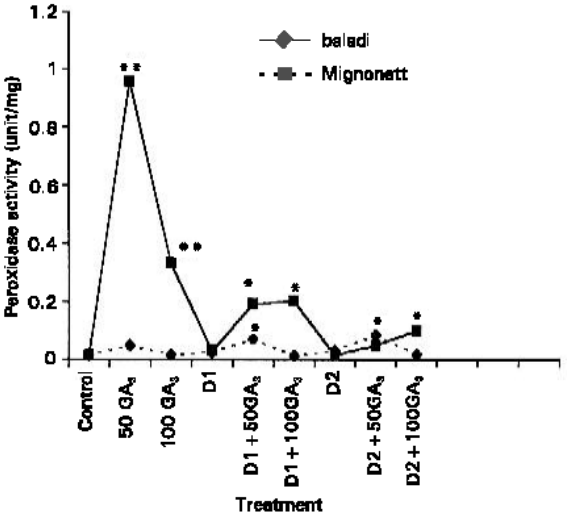

Fig. 3: Effect of two drought treatments (D1 and D2) and two GA_3 concentration (50 and 100 ppm) on peroxidase activity (units/mg). * Significant at ($P<0.05$) ** significant at ($P<0.01$)

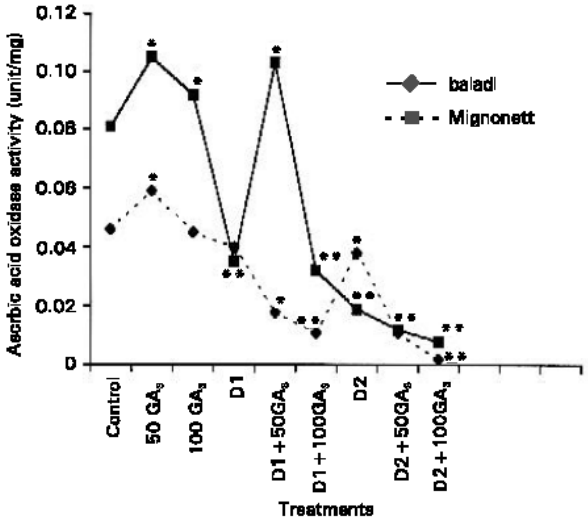

Fig. 4: Effect of two drought treatments (D1 and D2) and two GA_3 concentration (50 and 100 ppm) on ascorbic acid oxidase activity (unit/mg). * Significant at ($P<0.05$) ** significant at ($P<0.01$)

while it showed non-significant effect when applied on baladi cultivar (Fig. 3).

Ascorbic acid oxidase: Two drought stress periods significantly decreased the activity of ascorbic acid oxidase when compared to their corresponding controls. It was found that mignonette cultivar showed more decrease in ascorbic acid oxidase activity than baladi cultivar Fig. 4.

The two GA_3 concentrations induced highly significant decrease in ascorbic acid oxidase activity during the two drought periods in baladi cultivar. The same results were obtained in mignonette cultivar except at D1 drought, where the application of 50 ppm GA_3 induced a highly significant increase in enzyme activity.

Protein: The electrophoretic patterns of protein in baladi & mignonette cultivars are shown in Fig. 5 (A, B and C). It was noticed that the two drought conditions (D1 and D2) caused a degradation of all polypeptide bands as compared with control plants except the band between 67,000 and 43,000 kD in baladi cultivar Fig. 5 A. In mignonette cultivar (Fig. 5 B and C) D1 drought stress induced a decrease in staining intensity which reflect the decrease in polypeptide concentrations, while D2 treatment induced the appearance of 2 new bands between 67,000 and 43,000 kD. Also, a polypeptide 94,000 kD (Phosphorylase-b) appeared in all treatments except in control and 50 ppm GA_3. Increasing concentration of GA_3 increased the concentration of polypeptide in the two cultivars, so did the staining intensity of most bands.

Discussion

Some authors have proposed that water stress should be considered as an oxidative stress (Burke *et al.*, 1985), since it was reported that lipid peroxidation caused alterations in the membrane similar to those noticed under certain conditions of dehydration. Also, it has been proposed that water stress conditions, may trigger an increased formation of the superoxide radical and hydrogen peroxide, which can directly attack membrane lipids and inactivate the SH- containing enzymes (Menconi *et al.*, 1995).

It was found from present study that drought stress induced a high significant increase in lipid peroxidation in the two cultivars under investigation (Fig. 1). These could be ascribed to de-esterification reactions by free radical reaction on phospholipids (Navarri-Izzo *et al.*, 1995). Also, Bartoli *et al.*, (1999) found a significant increase in hydroxyl radical production after drought and even higher after hydration. They suggested that water stress could mainly act at cytosolic level. The results from Fig. 2 showed a significant increase in catalase activity in response to D1 stress in baladi and to D1 & D2 in mignonette cultivars. This increase in catalase activity could be a tolerant response of the plant to increase the net photosynthesis by decreasing H_2O_2 levels, which increased in response to drought stress. The first response to drought stress is the stomata closing, and hence lowering CO_2 influx (Malinowski and Belesky, 2000). Under conditions favoring rapid photorespiration, such as increased O_2, low CO_2 and temperature, about 25% of the glycolate metabolized during photorespiration is released as CO_2 (Hanson and Petrson, 1986). With insufficient catalase activity, excess H_2O_2 may rapidly decarboxylate the ketoacids such as hydroxy pyruvate and glyoxylate to generate additional CO_2 (Zelitch, 1992). This additional loss of assimilated CO_2 might be avoided with higher catalase activity, thereby re-establishing the stoichiometry closer to 25% and increasing net photosynthesis. The relation of catalase activity to net photosynthesis was supported by studies with a tobacco mutant, in which a correlation was obtained between elevated catalase and decreased photorespiration (Zelitch, 1992). The result of the present investigation showed a significant increase in peroxidase activity as a response to drought stress. These results are in agreement with Bacon *et al.* (1997), who reported that prolonged drought caused a 200-300% increase in cell wall associated peroxidase activity. Mader *et al.* (1986) had suggested that some cell wall associated activity is in fact an artifact of the homogenization which takes place to extract activity. Ionic peroxidase within the cytoplasm may associate

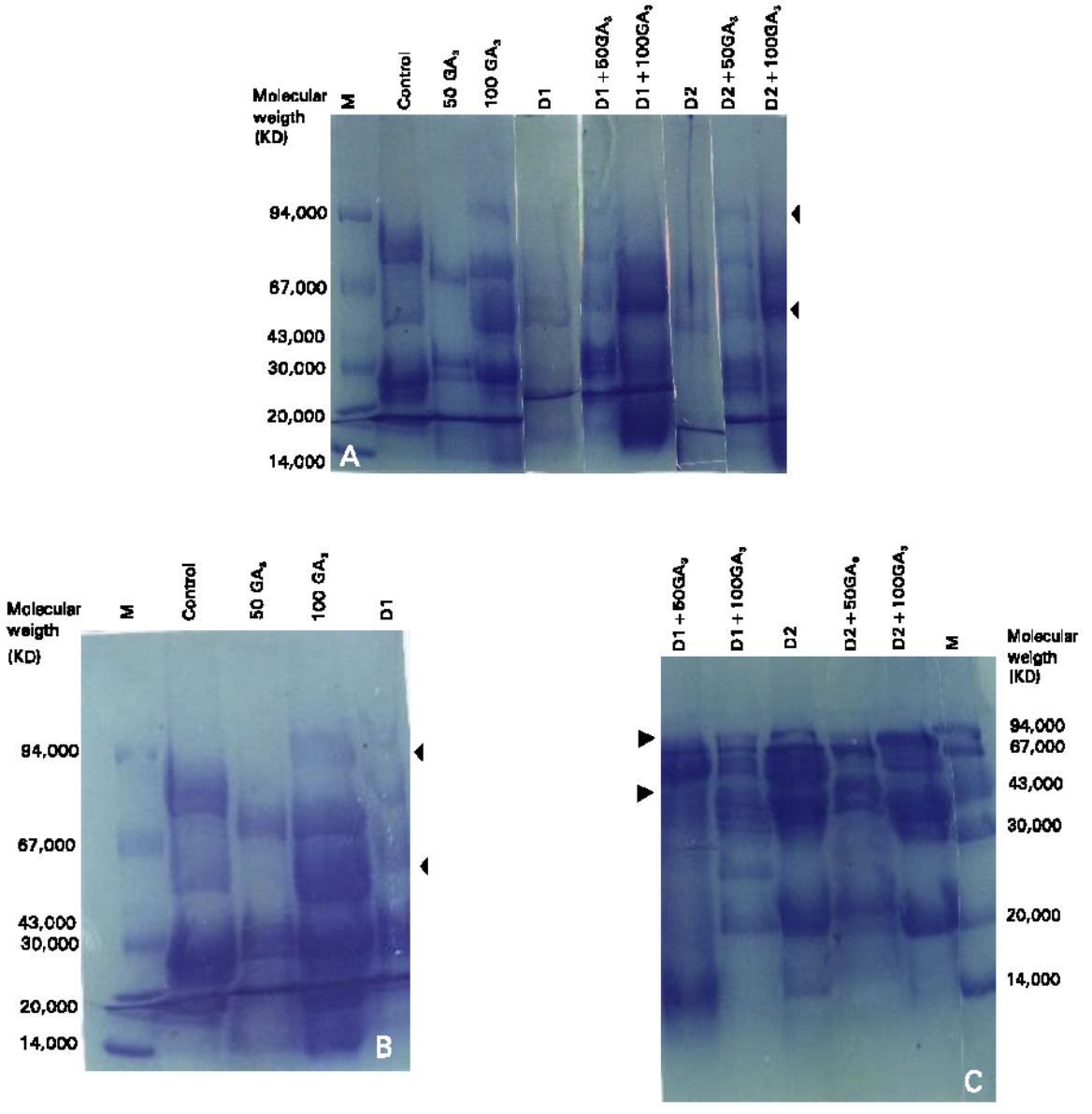

Fig. 5: Electrophoretic profiles of protein in response to 2 drought treatments (D1 and D2) and two gibberellic acid concentration (50 and 100 ppm). A-baladi cultivar. B and C-mignonette. (M = biomarkers with different molecular weights, arrows points to the new polypeptides appeared).

with the cell wall upon homogenization. Changes in cytoplasmic activity are often associated with induction of plant antioxidant systems (Zhang and Kirkham, 1996). While changes in cell-wall peroxidase activity during water stress may play a central role in controlling the expansion rate of cells (Bacon *et al.*, 1997).

The decrease in ascorbic acid oxidase activity could be attributed to the decrease in ascorbate content. It could be due to the consumption of ascorbate for zeaxanthin synthesis and tocopherol regeneration (Smirnoff, 1993). The decrease in ascorbic acid content induced by severe water stress was reported by Òrmaetxe *et al.* (1998) and Bartoli *et al.* (1999). They suggested that the decrease in ascorbic acid may be due to its direct destruction by O_2 and derived species.

It was found, from the present study Fig. 5: A, B and C, that drought stress decreased protein concentration. These results are in agreement with those obtained by Riccardi *et al.* (1998) and Schwanz and Polle (2001). Proteins are susceptible to oxidation by reactive oxygen species, where the type of damage induced is the characteristic of denaturing species (Griffiths, 2000). Foyer *et al.* (1998) reported that during water stress the decrease in maximal nitrate reductase (NR) activity was accompanied by a sharp decline in NR transcript levels. They suggested that this decrease in the quantity of NR

message might have been caused by the decrease in foliar NO_3. A severe or prolonged NO_3 deficit may reduce the stability of both NR transcripts and NR protein (Ferrario *et al.*, 1995).

The effect of GA_3 in alleviating the water stress responses in two cultivars under investigation was shown in all measured parameters. Gibberellic acid could enhance plant tolerance to water stress through the increase in protein synthesis (El-Meleigy *et al.*, 1999). It was found that GA_3 brought about a 3-fold stimulation of adenosyl methionine synthetase activity in wheat aleurones (Mathur *et al.*, 1992). They also reported that the activity of adenosyl methionine synthetase was considerably decreased by the simultaneous presence of abscisic acid. Jia and Zhang (2001) reported that signaling process of water-stress induced abscisic acid (ABA) accumulation in maize leaf and root. They also recorded that potent free radical scavenger and reducing agents, N-acetyl cysteine and cystein significantly inhibited or nearly completely blocked the dehydration-induced ABA accumulation. So, gibberellic acid could alleviate the adverse effects of water stress by its antagonism with abscisic acid. In this respect, White and Rivin (2000) suggested that GA_3 antagonizes ABA signaling in developing maize embryos, and the changing hormone balance provides temporal control over the maturation phase. Also, Gomez *et al.* (2001) reported the antagonism between gibberellins and abscisic acid. On the other hand, Schopfer *et al.* (2001) reported that germination of radish seeds could be inhibited under far-red light or ABA, while the application of GA_3 restores full germination under far-red light. They concluded that far-red light and ABA inhibit reactive oxygen intermediate in both seed coat and embryo and GA_3 reverses this inhibition when initiating germination under far-red light. Gibberellic acid could alleviate water stress effect by reversing the action of ethylene, which is formed as a degradation product of lipid hydroperoxide (Bradley and Minn, 1992). Kelev and Aloni (1999) found that apical application of GA_3 reversed the trachid polarity disorder induced by ether. The effect of GA_3 could also be due to its increase in free radical scavengers. Zhang and Schmidt (2000) reported that the application of hormone containing products enhanced α-tochopherol and ascorbic acid concentrations in two turfgrass species subjected to drought stress and thus promote the growth and drought tolerance.

It could be concluded that the lowest concentration of GA_3 was more effective on alleviating the adverse effect of drought than the highest one. Also it was found that mignonette cultivar was more tolerant to drought than baladi cultivar.

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