J. Appl. Environ. Biol. Sci., 5(8)161-165, 2015
© 2015, TextRoad Publication

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Effect of Ascorbate and Methylamine Treatments on DPPH-Scavenging Activity of Soybean Plants (*Glycine max* L.) Under Polyethylene Glycol-Induced Drought

Fatemeh Bagheri[1], Arian Satei[2]

[1]M.Sc. in Plant Physiology – Islamic Azad University of Iran – Gorgan Branch
[2]Assistant professor in Plant Physiology - Islamic Azad University of Iran – Gorgan Branch

Received: February 27, 2015
Accepted: April 21, 2015

ABSTRACT

In environmental stress tolerance, such as drought, high activities of antioxidant enzymes and high contents of non-enzymatic constituents are vital because oxidative stress caused by abiotic stresses can cause irreversible damages in plants and crop yield. The present study aimed at evaluation of the effect of ascorbate and methylamine treatments on DPPH-scavenging activity of soybean plants (*Glycine max* L.) under polyethylene glycol-induced drought. The seeds were planted and irrigated with Asc, PEG, PEG+Asc, MA50, PEG+MA50, MA75, PEG+MA75, MA100, PEG+MA100, and control solutions in 8-hour intervals. The antioxidant activity of the samples were evaluated through DPPH radical scavenging activity assay. The results derived from the present study revealed that the highest and lowest DPPH-scavenging activity of 24-h seeds were detected in PEG+Asc and PEG+MA50, respectively; however, no significant difference was detected between the samples ($p>0.05$). Also, the highest and lowest DPPH-scavenging activity of 7-d seedlings were detected in PEG and MA75, respectively; however, no significant difference was detected between the samples ($p>0.05$).

KEYWORDS: Ascorbate, methylamine, drought stress, antioxidant activity, polyethylene plycol, DPPH.

1- INTRODUCTION

Environmental stresses result in many plant responses, changing from change in gene expression to metabolic processes. Preserving higher plant efficiency under environmental stresses is reasonably the chief concern in recent agricultural practice (Gill and Tuteja, 2010; cited in Anjum et al., 2012). Drought stress is chief limitation which decreases the yield of crop plants all around the world (Khamssi et al. 2011; cited in Anjum et al., 2012). Thorough knowledge about physio-biochemical responses of plants to drought is important for increasing plant tolerance mechanisms to drought stress (Jaleel et al., 2006; cited in Anjum et al., 2012). In general, plants perceive drought stress either when the water source to roots is limited or when the transpiration rate increases very much (Manivannan et al., 2007; cited in Anjum et al., 2012). Plants can evade from drought stress by enhancing water absorption or declining transpiration (Ruiz-Sánchez et al., 2007; cited in Anjum et al., 2012). The responses of plants to water shortage are detected as phenological responses, morphological variations, physiological changes, and biochemical adaptations, like alterations in plant structure, growth rate, tissue osmotic potential and antioxidant defenses (Duan et al., 2007; cited in Anjum et al., 2012).

The antioxidant defense system in the plant cell includes both enzymatic and non-enzymatic mechanisms. Enzymatic mechanisms contain superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase. Non-enzymatic mechanisms have cystein, reduced glutathione and ascorbic acid (Gong et al., 2005; cited in Farooq et al., 2009).

In environmental stress tolerance, such as drought, high activities of antioxidant enzymes and high contents of non-enzymatic constituents are vital. The reactive oxygen species in plants are removed by various antioxidant enzymes and/or lipid-soluble and water-soluble scavenging molecules (Hasegawa et al., 2000; cited in Farooq et al., 2009). Aside from catalase, many peroxidases and peroxiredoxins, four enzymes play roles in the ascorbate-glutathione cycle, a pathway that permits the scavenging of superoxide radicals and H_2O_2. These are ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase (Fazeli et al., 2007; cited in Farooq et al., 2009). Most of the ascorbate-glutathione cycle enzymes are situated in the cytosol, stroma of chloroplasts, mitochondria and peroxisomes (Jiménez et al., 1998; cited in Farooq et al., 2009). Ascorbate peroxidase is a key antioxidant enzyme in plants (Orvar and Ellis, 1997; cited in Farooq et al., 2009).

***Corresponding Author:** Fatemeh Bagheri, M.Sc. in Plant Physiology – Islamic Azad University of Iran – Gorgan Branch. Email: f.bagherii2015@gmail.com

Reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide and hydroxyl radical can bring about lipid peroxidation and subsequently membrane injury which causes leakage of cellular content, protein degrading, enzyme inactivation, pigment bleaching and disruption of DNA strands and accordingly, cell death (Scandalios, 1993; cited in Abbas et al., 2014). Higher production of oxygen free radicals leads to peroxidation of membrane lipids and the degree of peroxides damage of cell was controlled by the potency of peroxidase enzyme system (Sairam and Tyagi, 2004; cited in Abbas et al., 2014).

Polyethylene Glycol (PEG) causes water stress in plants (Ruf et al., 1967; Kaufman and Eckard, 1971; cited in Abbas et al., 2014). PEG is a non-penetrating inert osmoticum that decreases the osmotic potential of nutrient solutions, but it is not taken and is not phytotoxic (Lawlor, 1970; cited in Abbas et al., 2014). PEG induces water stress in cultured plant cells in the similar way it does in the cells of intact plants (Azhar et al, 2012; cited in Abbas et al., 2014).

The present study was formulated in order to determine the effect of ascorbate and methylamine treatments on DPPH-scavenging activity of soybean plants (*Glycine max* L.) under polyethylene glycol-induced drought.

2- MATERIALS AND METHODS

Ascorbate and methylamine were purchased from Pajohan-Sanaat-Homehr. The required amount of PEG with molecular mass of 6000 was derived by the following relation in order to provide osmotic potential of 0.3 MPa:

S = - (1.18×10-2) C – (1.18×10-4) C2 + (2.67×10-4) CT + (8.39×10-7) C2T

where C, T, and S stand for concentration of PEG 6000 (g.l-1), temperature (°C), and osmotic potential (MPa), respectively. PEG concentration was found to be 35.42 g. Solutions were made on Aug 2013 in Research Laboratory of Islamic Azad University of Gorgan – Iran. Methylamine soultions were prepared in three concentrations (50, 75, and 100 mM.l-1).

Soybean (*Glycine max* L.) seeds were purchased from Araghi-Mahalleh Station – Gorgan – Iran and 600 seeds were placed in hypochlorite sodium 10% for 10 min after rinsing with distilled water. Petri dishes were rinsed with boiling water and then disinfected with hypochlorite sodium 10%. Petri dishes and seeds were rinsed immediately after disinfection. Cleansing fabrics were disinfected with distilled water and hypochlorite sodium and finally they were washed with distilled water. The seeds were sorted in 5 petri dishes between two cleansing fabrics. In each petri dish, seeds were sorted in 5 rows each with 20 seeds. Then, the petri dishes were placed at 25°C at darkness and were irrigated with Asc, PEG, PEG+Asc, MA50, PEG+MA50, MA75, PEG+MA75, MA100, PEG+MA100, and control solutions in 8-hour intervals.

The antioxidant activity of the samples were evaluated through DPPH radical scavenging activity assay. In brief, 0.004 g solution of DPPH radical was added to the sample solution in methanol (100 ml). The mixture was centrifuged at 1000g for 10 min. 1.5 ml DPPH solution and 1.5 ml sample extract were poured in lab tube and left to stand for 30 minutes in the dark, and the absorbance was measured at 517 nm.

$$A: 1/5(mL) + 1/5\ (mL)\ DPPH \xrightarrow{\text{dark}} OD517$$

Then, 1.5 ml DPPH solution and 1.5 ml pure methanol were poured in lab tube and left to stand for 30 minutes in the dark, and the absorbance was measured at 517 nm.

$$B: 1/5(mL) + 1/5\ (mL)\ DPPH \xrightarrow{\text{dark}} OD517$$

The scavenging capacity of the DPPH radical was calculated using the following equation

$$\text{DPPH scavenging percentage} = \frac{ODB - ODA}{ODB} \times 100$$

All experiments were carried out in triplicate. Statistical analyses were performed by one-way ANOVA through Duncan Test at $p \leq 0.05$ in SPSS (Version 21) in three iterations. Graphs were drawn in Excel Software (Microsoft Office, 2010).

3- RESULTS AND DISCUSSION

Fig. 1 shows DPPH-scavenging capacity in experimental and control samples in 24-h seeds. As it can be seen, the highest and lowest DPPH-scavenging activity of 24-h seeds were detected in PEG+Asc and PEG+MA50, respectively; however, no significant difference was detected between the samples ($p>0.05$).

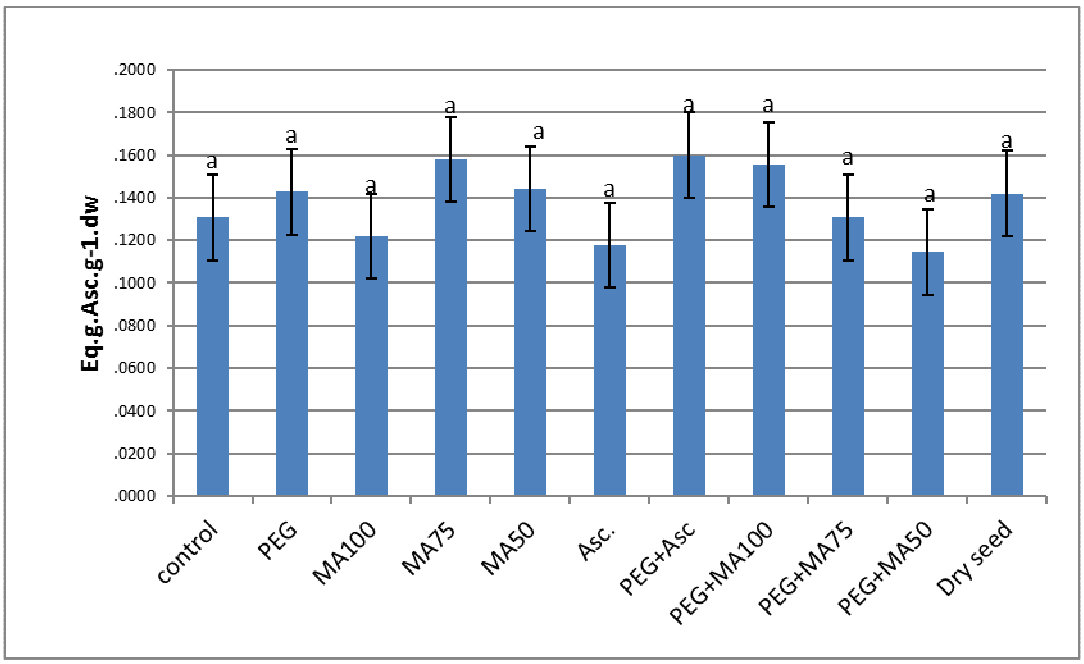

Figure 1: DPPH –scavenging activity of experimental and control samples in 24-h seeds

Fig. 2 represents DPPH-scavenging capacity in experimental and control samples in 7-d seedlings. As it can be seen, the highest and lowest DPPH-scavenging activity of 7-d seedlings were detected in PEG and MA75, respectively; however, no significant difference was detected between the samples ($p>0.05$).

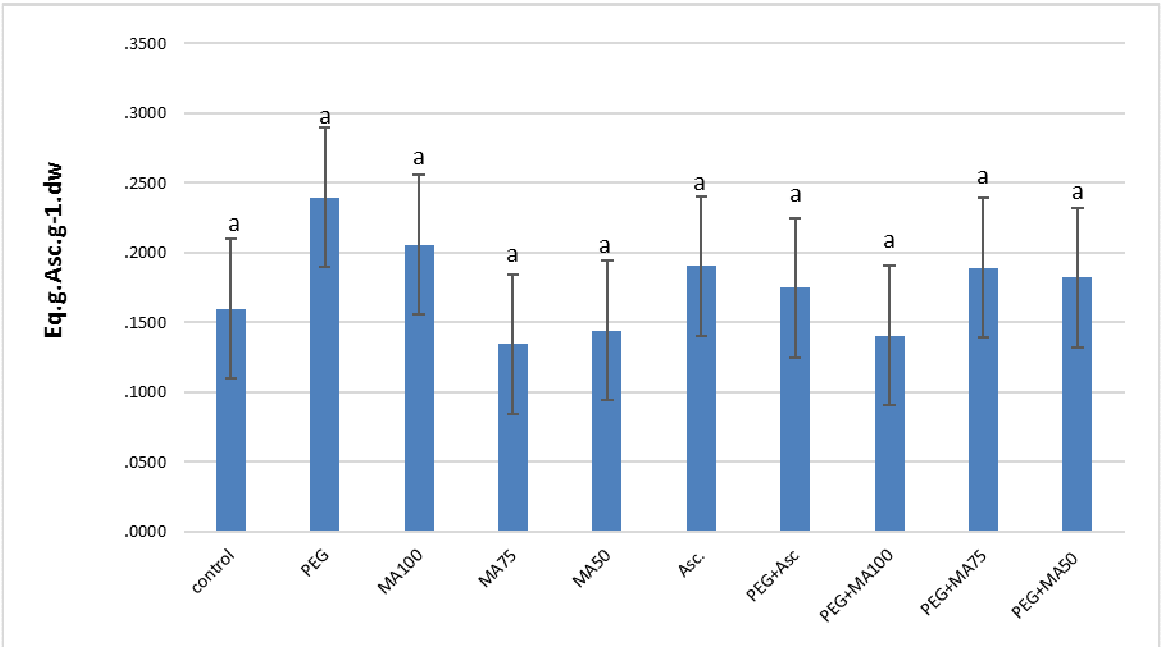

Figure 1: DPPH –scavenging activity of experimental and control samples in 7-d seedlings

Unlike methylamine, ascorbate could have a positive effect on DPPH-scavenging activity in PEG-induced drought. Also, the results indicate that no significant difference exist between the samples in DPPH-scavenging activity under PEG-induced drought ($p>0.05$). Drought stress is considered as one of the dominated factors to determine geographical distribution of vegetative cover and limitation of agricultural products. Environmental stress renders severe influence on plants because it cause imbalance in formation and retardation of reactive oxygen

species. The symptoms of injuries caused by drought stress include chlorophyll degeneration, protein denaturation, reduced membrane permeability, peroxidation, slower development rate of leaves, leaflet epinasty, and stomata closure. Stomata closure results in reduced internal CO_2 concentration. Then, a concurrent reduction in photosynthesis occur as a result of lower availability of CO_2 for carbon fixation. Reduced concentration of CO_2 leads to higher levels of reactive oxygen species in leaves because of the light reaction which brings about plant senescence or even death. Roots are injured by long-term water deprivation leading to distracted respiration in electron transport level. Lack of a proper electron receptor triggers saturated redox chains, accumulation of NAD(P)H, and reduction of ATP formation. In plant cells, oxidative stress reactions are triggered by toxic free radicals caused by molecular oxygen reduction to superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), and peroxyl radicals ($ROO^{\cdot}$). These radicals can inactivate a variety of kelvin cycle enzymes and play role in oxidative systems. Toxic radicals can be scavenged both enzymatically and chemically to protect plant cells against oxygen toxicity and prevent from adverse effects of reactive oxygen species under stress conditions.

One of the widely-used free radicals, especially in laboratory studies, is DPPH. DPPH-scavenging activity by systems has been considered as an important factor in literature. Plants possess an efficient defensive systems against oxidative stress which can scavenge and/or inactivate free radicals. This defensive system include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) as well as non-enzymatic system including ascorbate, tocopherol, carotenoids, and other compounds such as flavonoids, mannitol, and polyphenols). Frequency of defensive systems is because reactive oxygen species are formed in cells and various subcellular parts.

Ascorbate is one of the most important plant antioxidants which takes part a range of cellular processes such as cell growth and division, metabolic reaction in the onset of germination, blocking cell poisoning, cell preservation against oxidative reactions, and prevention from cell death. Soaking seeds in ascorbate has widely been adopted so that it results in higher efficiency and tolerance against different external factors such as high salinity and nematodes. Adverse environmental situations have adverse effects on seeds (Aspinall and Paleg, 1981; Benson, 1990; cited in Ghandian Zanjan et al., 2011).

4- Conclusion

Drought stress is one of abiotic stresses and it has always been a challenge for farmers. Beside a lot of other adverse effects of drought stress, formation of toxic free radicals cause several injuries in plants which reduces the final yield. The present study determined the effect of treating soybean seeds and seedlings under PEG-induced drought by ascorbate and methylamine. The results obtained from the present study showed that ascorbate could have a positive effect on DPPH-scavenging activity in PEG-induced drought. Also, the results indicate that no significant difference exist between the samples in DPPH-scavenging activity under PEG-induced drought ($p>0.05$). Further studies are required to understand the effect of other treatment, such treatment by polyamines, on antioxidant activity of plants under different abiotic stresses.

REFERENCES

Abbas S.R., Ahmad S.D., Sabir S.M., Shah A.H. (2014). Detection of drought tolerant sugarcane genotypes (*Saccharum officinarum*) using lipid peroxidation, antioxidant activity, glycine-betaine and proline contents. Journal of soil science and plant nutrition, 14 (1): http://dx.doi.org/10.4067/S0718-95162014005000019.

Anjum S.A., Saleem M.F., Wang L.C., Bilal M.F., Saeed A. (2012). Protective role of glycinebetaine in maize against drought-induced lipid peroxidation by enhancing capacity of antioxidative system. Australian Journal of crop Science, 6(4): 576-583.

Aspinall, D., Paleg, L.G. (1981). Proline accumulation: Physiological aspects. In Paleg, L.G. and Aspinall, D. (Eds.), The physiology and biochemistry of drought resistance in plants. Sidney, Academic Press, pp. 205-241.

Azhar, H.S., Safdar, H.S., Habib, A., Zahoor, A.S., Fida, M.A., Farhatullah, A., Abrar, H.S. (2012). Adaptation to polyethylene stress mentains totipotency of cell lines of *Oryza sativa* L. Swaft-1 for a longer period. Pakistan Journal of Botany, 44: 313-316.

Benson E.E. (1990). Free radical damage in stored plant germplasm. International Board for Plant Genetic Resources, Italy.

Duan B., Yang Y., Lu Y., Korpelainen H., Berninger F., Li C. (2007). Interactions between drought stress, ABA andgenotypes in *Picea asperata*. Journal of Experimental Botany, 58: 3025-3036.

Farooq M., Wahid A., Kobayashi N., Fujita D., Basra S.M.A. (2009). Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29: 185–212.

Fazeli F., Ghorbanli M., Niknam V. (2007). Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. Biol. Plant. 51: 98–103.

Gill S.S., Tuteja N. (2010). Polyamines and abiotic stress tolerance in plants. Plant Signal Behavior, 5: 26-33.

Ghandian Zanjan M., Eradatmand Asli D., Yousefirad M. (2011). Determination of effect of ascorbic acid on germination of canola under high salinity stress. The 1st National Conference on Modern Issues of Agriculture, Islamic Azad University, Saveh Branch, Saveh, Iran.

Gong H., Zhu X., Chen K., Wang S., Zhang C. (2005). Silicon alleviates oxidative damage of wheat plants in pots under drought, Plant Science, 169: 313-321.

Hasegawa P.M., Bressan R.A., Zhu J.K., Bohnert H.J. (2000). Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology, 51: 463-499.

Jaleel C.A., Gopi R., Manivannan P., Kishorekumar A., Sankar B., Panneerselvam R. (2006). Paclobutrazol influences vegetative growth and floral characteristics of *Catharanthus roseus* (L.) G. Don. Indian Journal of Applied & Pure Biology, 21: 369-372.

Jiménez A., Hernandez J.A., Ros Barcelo A., Sandalio L.M., del Rio L.A., Sevilla F. (1998). Mitochondrial and peroxisomal ascorbate peroxidase of pea leaves. Physiol. Plant. 104: 687-692.

Kaufman, M.R., Eckard, A.N. (1971). Evaluation of water stress control with PEG by analysis of guttation. Plant Physiology, 47: 453-458.

Khamssi N.N., Golezani K.G., Najaphy A., Zehtab S. (2011). Evaluation of grain filling rate, effective grain filling period and resistance indices under acclimation to gradual water deficit stress in chickpea cultivars. Australian Journal of crop Science, 5: 1044-1049.

Lawlor, D.W. (1970). Absorption of PEG by plants and their effects on plant growth. New Phytologist, 69: 501-513.

Orvar B.L., Ellis B.E. (1997). Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase show increased susceptibility to ozone injury. Plant J., 11: 1297-1305.

Manivannan P., Jaleel C.A., Kishorekumar A., Sankar B., Somasundaram R., Sridharan R., Panneerselvam R. (2007). Drought stress induced changes in the biochemical parameters and photosynthetic pigments of cotton (*Gossypium hirsutum* L.). Indian Journal of Applied & Pure Biology, 22: 369-372.

Ruf, R.H., Eckard, E.R., Gifford, R.O. (1967). Compounds of osmotic adjustment of plants to rapid changes in root medium osmotic pressure. Soil Science, 104: 159-62.

Ruiz-Sánchez M.C., Domingo R., Pérez-Pastor A. (2007). Daily variations in water relations of apricot trees under different irrigation regimes. Biol Plant, 51: 735-740.

Sairam, R.K., Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. Current Science, 86: 407-421.

Scandalios, J.G. (1993). Oxygen stress and superoxide dismutases. Plant Physiology, 101: 7-12.