J. Appl. Environ. Biol. Sci., 5(6)352-357, 2015

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

Effect of Ascorbate and Methylamine Treatment on Glycinebetaine, Sugar, and Proline Contents of Soybean (*Glycine max* L.) Seeds 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: March 11, 2015
Accepted: May 23, 2015

ABSTRACT

Drought stress is one of abiotic stresses and has always been faced by farmers and crop growers as an important challenge. Soybean is regarded as one of the first farming plants. Water is very vital of growth and development of soybean. The present study aimed at determination of the effect of ascorbate and methylamine treatments on glycinebetaine, sugar, and proline contents of soybean (*Glycine max* L.) seeds 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. 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). It is concluded that treating plants under PEG-induced drought stress by using ascorbate and methylamine has varying effects on proline, glycinebetaine, and sugar contents of seeds, seedlings, leaves, and roots of soybean.

KEYWORDS: Drought stress, ascorbate, methylamine, glycine betaine, proline, sugar.

1- INTRODUCTION

It is evidently important to determine environmental stressors and their roles in prediction and evaluation of growth and efficiency of crops. Lack of water is one of limiting factors in production of agricultural products in many parts of the world. In arid and semiarid areas, the cultivars with the highest ability of resistance toward drought seems very crucial. Drought stress is one of abiotic stresses and has always been faced by farmers and crop growers as an important challenge. One of the most important ways of prevention from unsuitable use of water is adoption of drought-resistant plants. This is very important as drought is the main cause of losses in crops and one of the most important abiotic stresses which affects growth and development of plants (Arji, 2003).

Glycinebetaine (N, N, N-trimethyl glycine) is one of the most widely evaluated quaternary ammonium compounds and compatible solutes in plants, animals and bacteria (Wahid et al., 2007; cited in Farooq et al., 2009). Several investigation establish that glycinebetaine has a key part in increasing plant tolerance under various abiotic stresses including drought (Quan et al., 2004; cited in Farooq et al., 2009). The introduction of genes synthesizing glycinebetaine into non-accumulators of glycinebetaine proved to be influential in enhancing tolerance to many abiotic stresses (Sakamoto and Murata, 2002; cited in Farooq et al., 2009). Cotton cultivars adapted to water stress conditions accumulated higher glycinebetaine than the non-adapted ones under drought (Naidu et al., 1998; cited in Farooq et al., 2009). Beside direct protective roles of glycinebetaine either via positive effects on enzyme and membrane integrity or as an osmoprotectant, glycinebetaine might also safeguard cells from environmental stresses indirectly by joining signal transduction pathways (Subbarao et al., 2000; cited in Farooq et al., 2009).

Osmotic adjustment is a mechanism to preserve water relations under osmotic stress. It includes the accumulation of various osmotically active molecules/ions like soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions, etc. Under water shortage and because of solute accumulation, the osmotic potential of the cell decrease, which brings water into the cell and helps with the conservation of turgor. Through osmotic adjustment, the organelles and cytoplasmic activities occur at about a usual rate and aid plants to have a better performance in terms of growth, photosynthesis and assimilate partitioning to grain filling (Farooq et al., 2009).

Osmotic adjustment is performed with the accumulation of compatible solutes. One of these compatible solutes is proline which is one of the most vital cytosolutes and its free accumulation is a prevalent response of higher plants, algae, animals and bacteria to low water potential (Zhu, 2002; Wahid and Close, 2007). Its synthesis in

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

leaves at low water potential is triggered by a blend of augmented biosynthesis and slow oxidation in mitochondria. In spite of some debate, several physiological roles have been allocated to free proline including stabilization of macromolecules, a sink for excess reductant and a store of carbon and nitrogen for use after relief of water deficit (Zhu, 2002; cited in Farooq et al., 2009). Proline contents were increased under drought stress in pea cultivars (Alexieva et al., 2001; cited in Farooq et al., 2009). Drought-tolerant petunia (*Petunia hybrida*) varieties accumulated free proline under drought that acted as an osmoprotectant and induced drought tolerance (Yamada et al., 2005; cited in Farooq et al., 2009).

Soybean is regarded as one of the first farming plants. It contains plentiful amounts of protein, carbohydrate, oil, phosphorus, calcium, iron, magnesium, zinc, fiber, and vitamins (thiamin, riboflavin, and niacin) (Akparobi, 2009). Water is very vital of growth and development of soybean. The present study aimed at determination of the effect of ascorbate and methylamine treatments on glycinebetaine, sugar, and proline contents of soybean (*Glycine max* L.) seeds 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:

$S = - (1.18\times10^{-2}) C - (1.18\times10^{-4}) C^2 + (2.67\times10^{-4}) CT + (8.39\times10^{-7}) C^2T$

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.

In order to estimate glycinebetaine content, 4 g iodine was mixed with 6 g potassium iodide and then mixed with distilled water to achieve 100 ml solution. Then, 0.5 g dry sample was solved in 5 ml distilled water and agitated at 25°C. The solution was filtered and diluted by sulfuric acid 2 N at a ratio of 1:1 and kept in ice for 1 h. next, 0.2 ml Lugol's iodine solution with potassium iodide was added to 5 ml of the sample and slowly agitated by a vortex mixer. The solutions were kept at 0-4°C for 16 h. Then, they were centrifuged at 10000g for 15 min. 0.5 of supernatant was mixed with 4.5 ml 1,2-dichloroethane and vortexed. The absorbance of the lower phase was read at 365 nm (Sairam et al., 2002).

To estimate sugar content, the samples were weighed and kept in 10 ml ethanol 70% for a week. Then, 1 ml diluted alcoholic solution, 1 ml distilled water, 1 ml phenol 5%, and 5 ml concentrated sulfuric acid were poured in a lab tube. The tubes were kept at ambient temperature and absorbance was read at 485 nm (Kochert, 1978).

Proline content was estimated by the method of Bates (1973). 2 g of wet sample were mixed with 5 ml sulfosalicylic acid and then filtered through Whatman ® qualitative filter paper, Grade 2. 1 ml of the resulted extract was mixed with 1 ml ninhydrin acid and 1 ml pure acetic acid. The tubes were put in hot bath at 100°C for 1 h. To block the reaction, the tubes were immediately put in ice. Next, 2 ml toluene was added and agitated for 20 s to have two phases. The colored supernatant was separated and cooled. The absorption of supernatant was read at 520 nm.

Statistical analyses were performed by one-way ANOVA through Duncan Test at $p\leq0.05$ in SPSS (Version 21) in three iterations. Graphs were drawn in Excel Software (Microsoft Office, 2010).

3- RESULTS

Figs. 1 shows the glycinebetaine content in experimental and control samples in leaves, roots, 24-h seeds, and 7-d seedlings. Fig. w represents sugar content in experimental and control samples in 24-h seeds and 7-d seedlings and Fig. 3 shows proline content in experimental and control samples in leaves, roots, 24-h seeds, and 7-d seedlings.

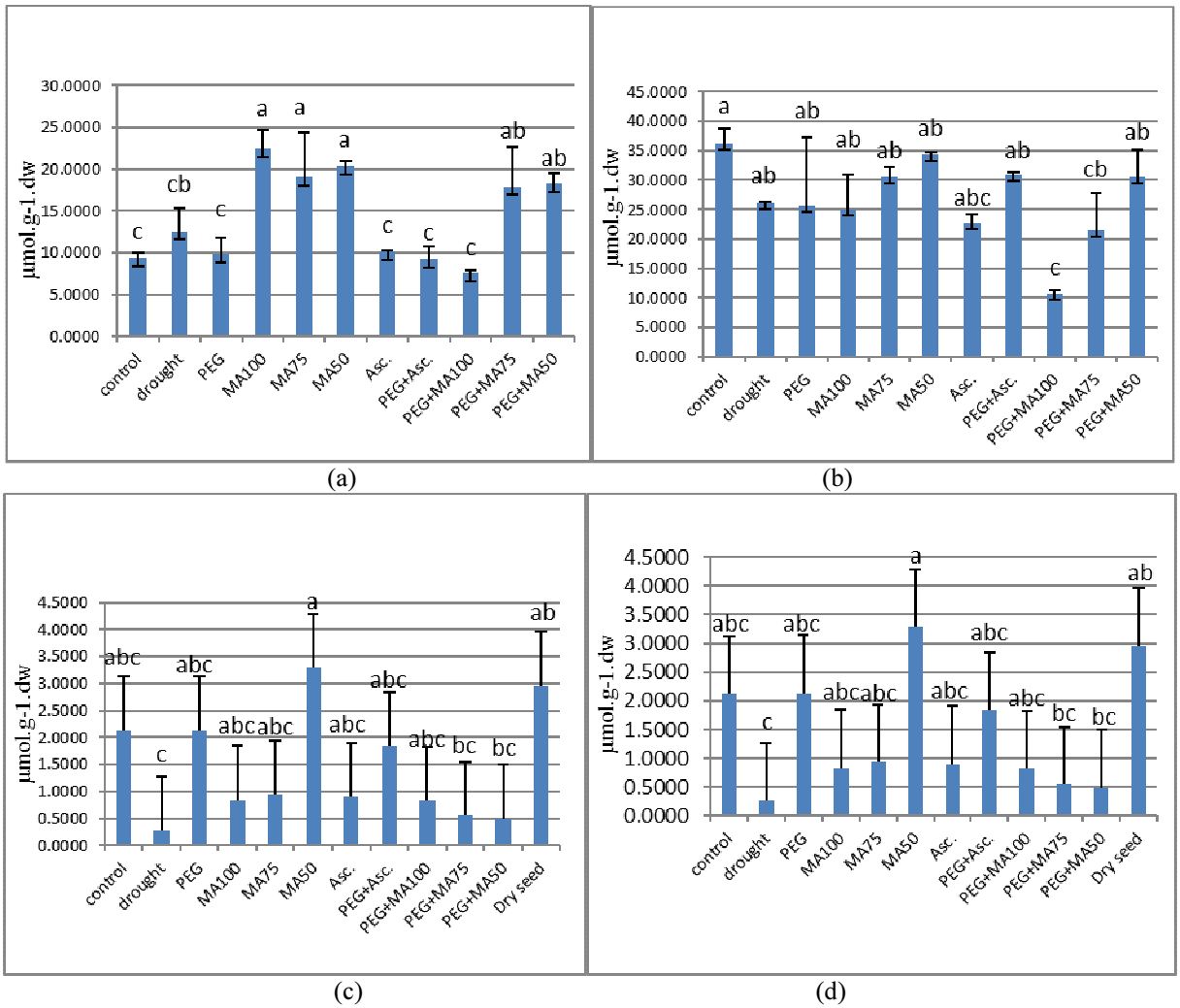
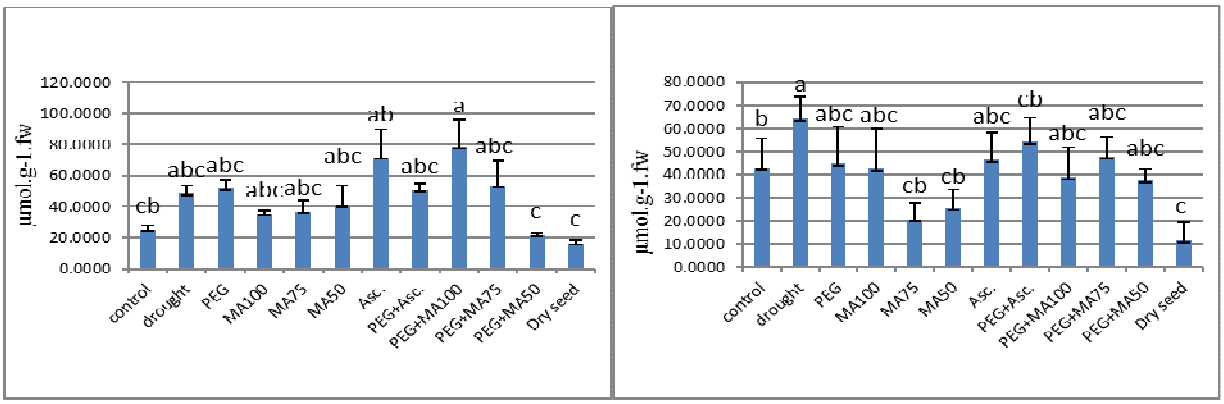


Figure 1: Glycinebetaine content in experimental and control samples; (a) leaves, (b) roots (c) 24-h seeds, and (d) 7-d seedlings.

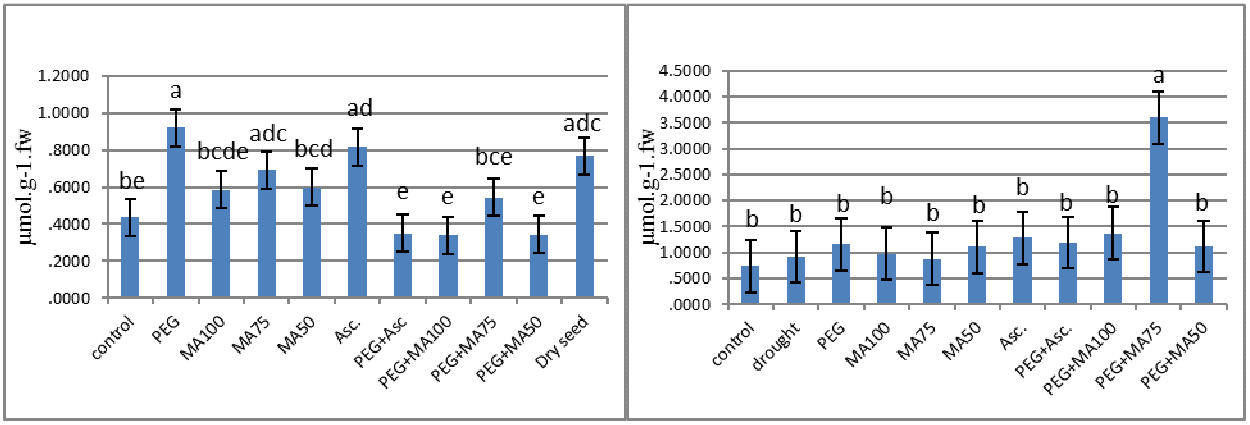
According to Fig. 1, the highest amount of glycinebetaine of leaves were found in MA100, MA75, and MA 50. The lowest amount of glycinebetaine of leaves were in control, PEG, Asc, PEG+Asc and PEG+MA100. Significant differences were detected between these two groups of samples ($p<0.05$). The highest and lowest root glycinebetaine contents were found in control and PEG+MA100, respectively. Significant difference was detected between these two samples ($p<0.05$). The highest glycinebetaine content of 7-d seedlings were seen in MA50 which had a significant difference with other samples ($p<0.05$). The lowest glycinebetaine content of 7-d seedling was detected in Asc which had significant difference with MA50, PEG+MA100, and PEG+MA50 ($p<0.05$). The highest and lowest glycinebetaine contents of 24-h seeds were detected in MA50 and control samples. A significant difference was detected between these two samples ($p<0.05$).



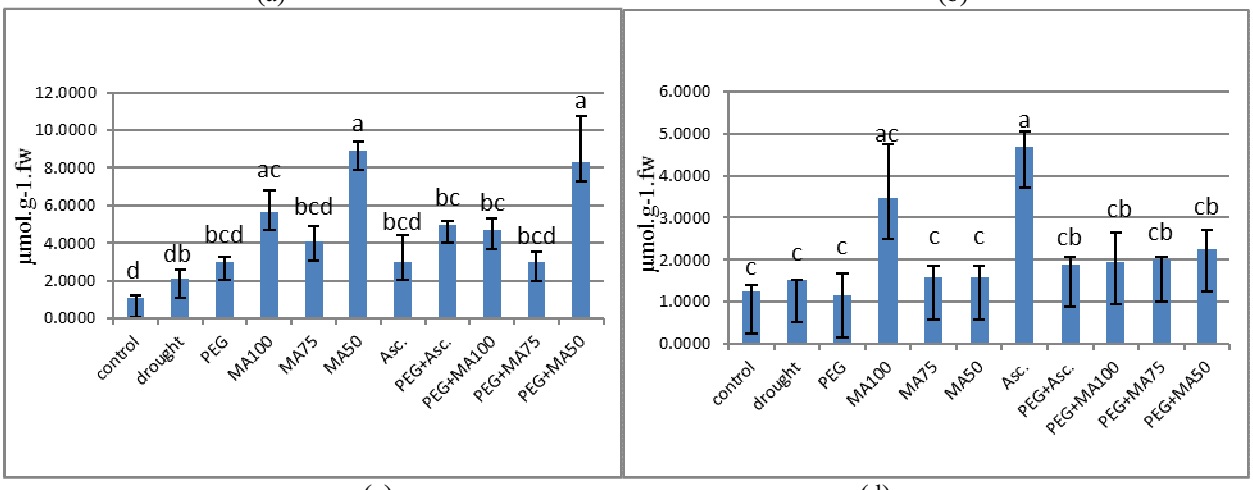
(a) (b)

Figure 2: Sugar content in experimental and control samples; (a) 7-d seedlings and (b) 24-h seeds.

The highest sugar content in 7-d seedling was in PEG+MA100. The lowest sugar content in 7-d seedling was in dry seed and PEG+MA50. Significant differences were seen between PEG+MA100 and Asc and dry seed and PEG+MA50 ($p<0.05$). Also, the highest and lowest sugar content in 24-h seeds were in control and dry seed, respectively. There was a significant difference between these two samples ($p<0.05$). Moreover, there was a significant difference between dry seed and PEG+Asc ($p<0.05$).



(a) (b)



(c) (d)

Figure 3: Proline content in experimental and control samples; (a) 24-h seeds, (b) 7-d seedlings, (c) leaves, and (d) roots.

The highest proline content in 24-h seeds was found in PEG; however, it had no significant difference with MA75, Asc, and dry seed ($p>0.05$). In addition, the lowest proline content in 24-h seeds was in PEG+Asc, PEG+MA100, and PEG+MA50; they had significant difference with control sample ($p>0.05$). The highest proline content in 7-d seedling was detected in PEG+MA75 which had significant difference with other samples ($p<0.05$). No significant difference was found between other samples ($p<0.05$). Also, the highest proline content in leaves were found in MA50 and PEG+MA50 which had significant difference with all the samples ($p<0.05$) except MA100 ($p>0.05$). The lowest proline content in leaves was seen in control sample. The highest content of proline in roots was in Asc which had significant differences with other samples ($p<0.05$) except MA100 ($p>0.05$). The lowest proline content of root was in PEG which had significant difference with MA100 and Asc ($p<0.05$).

4- DISCUSSION AND CONCLUSION

When exposed to drought stress, plants produce more dry matter due to stomata closure during stress and varied water use efficiency. Plants response to drought stress depends on stress severity and duration, genotype, and plant age and development. Therefore, different species of plants present a wide range of drought-resistance mechanisms resulting in morphological, physiological, and biochemical adaptations. One of important responses of plants to drought stress is reduction of leaf area. Reduction of leaf area can be caused by decreased cell division and leaf senescence. Osmotic adjustment is known as a part of drought-tolerance mechanisms in plants. In varying environmental situations, plants synthetize low molecular weight solutes known as compatible solutes including amino acids (protein and glycine), sugar (sucrose and glucose), sugar alcohols (mannitol and sorbitol), ions, organic acids, amides, amines, and betaine groups which are synthetized in response to drought stress and do not interfere with normal biochemical reactions in cells. Accumulation of these solutes reduces water potential in plant organs and therefore, plants can absorb water (Pagter et al., 2005).

The present study was formulated in order to evaluate the effect of ascorbate and methylamine treatments on glycinebetaine, sugar, and proline contents of soybean (*Glycine max* L.) seeds under polyethylene glycol-induced drought. According to the results, the highest amount of glycinebetaine of leaves were found in MA100, MA75, and MA 50. The lowest amount of glycinebetaine of leaves were in control, PEG, Asc, PEG+Asc and PEG+MA100. Therefore, it can be claimed that ascorbate and methylamine treatments could not improve glycinebetaine content in leaves in PEG-induced drought stress. The same situation can be seen about the content of glycinebetaine in roots as the highest and lowest glycinebetaine content were detected in control and PEG+MA100 samples, respectively. Additionally, the highest glycinebetaine content of 7-d seedlings were seen in MA50 which had a significant difference with other samples ($p<0.05$). The lowest glycinebetaine content of 7-d seedling was detected in Asc which had significant difference with MA50, PEG+MA100, and PEG+MA50 ($p<0.05$). This is indicative of the fact that methylamine cannot improve glycinebetaine content in PEG-induced drought stress. The same trend was seen for the 24-h seeds. This is not consistent with the results of Rontein et al. (2002). They reported that methylamine increased glycinebetaine in different parts of plant.

Also, in the present study, the highest sugar content in 7-d seedling was found in PEG+MA100 and the lowest sugar content was in dry seed and PEG+MA50. This indicates that methylamine 100 mM alleviates adverse effects of PEG-induced drought stress in 7-d seedlings. Drought stress result in accumulation of dissolved sugars and osmotic adjustment (Pattanagul and Madore, 1999). This keeps turgor and membrane stability (Mundree et al., 2002) and facilitates water conservation in cytoplasm (Bohnert et al., 1995). Furthermore, Yordanov et al. (2003) stated that during drought stress, dissolved sugars interact with polar groups and replace water molecules and therefore, block structural changes and prevent from enzyme retardation.

In addition, the highest proline content in 24-h seeds was found in PEG; however, it had no significant difference with MA75, Asc, and dry seed ($p>0.05$). In addition, the lowest proline content in 24-h seeds was in PEG+Asc, PEG+MA100, and PEG+MA50; they had significant difference with control sample ($p>0.05$). This indicates that ascorbate and methylamine treatments cannot improve proline content of 24-h seed in drought stress. Also, the highest content of proline in 7-d seedling was found in PEG+MA75 which had significant difference with other samples ($p<0.05$). This shows that methylamine 75 $mM.l^{-1}$ can greatly improve proline content in 7-d seedling in PEG-induced drought. This is in agreement with the results of Rontein et al. (2002). They claimed that methylamine can increase proline content of plant. It is noteworthy that this was not the case for ascorbate. This is consistent with the result of Behairy et al. (2012). They reported that proline content in the stressed seeds treated with ascorbate decreased compared to the ones untreated with ascorbate. Also, Tabatabaei and Naghibalghora (2013) reported that ascorbate increased seed proline in normal situation without any stress while it decreased seed proline in stress situation.

Drought stress is an abiotic stress and it has always been regarded as chief challenge for farmers and growers of crop plants. It can be concluded that treating plants under drought stress by using ascorbate and methylamine can sometimes improve proline, glycinebetaine, and sugar contents of seeds, seedlings, leaves, and roots.

REFERENCES

Akparobi S.O. (2009). Effect of farmyard manures on the growth and yield of *Amaranthus cruentus*. Agricultura Tropica et Subtropica, 42(1): 1-4.

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.

Arji, A. (2003). Effects of drought stress on physiological, morphological, and biochemical characteristics of some cultivars of olive. A dissertation for PhD in Horticulture, Tarbiat-Modarres University.

Bates L.S, Waldern R. P., Teare I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39: 205- 207.

Behairy R.T., El-Danasoury M., Craker L. (2012). Impact of Ascorbic Acid on Seed Germination, Seedling Growth, and Enzyme Activity of Salt-Stressed Fenugreek. 1 (3): 106-113.

Bohnert H.J., Nelson D.E., Jensen R.G. (1995). Adaptation to environmental stresses. Plant Cell, 7: 1099-1111.

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.

Kochert G. (1978). Carbohydrate determination by the phenol sulfuric acid method. In Helebust, J. A., Craig, J.S., Handbook of physiological methods, Cambridge University Press, Cambridge, 9697.

Mundree S.G, Baker B., Mowla S., Peters S., Marais S., Vander Willigen C., Govender K., Maredza A., Muyanga S., Farrant J.M., Thomson J.A. (2002). Physiological and molecular insight into drought tolerance. African J Biotechnol, 1: 28–38.

Naidu B.P., Cameron D.F., Konduri S.V. (1998). Improving drought tolerance of cotton by glycinebetaine application and selection. In: Proceedings of the 9th Australian agronomy conference, Wagga Wagga.

Pagter M, Bragato C, Brix H (2005). Tolerance and physiological responses of *Phragmites australis* to water stress. Aquatic Bot. 81:285-299.

Pattanagul W., Madore M.A. (1999). Water Deficit Effects on *Raffinose* Family Oligosaccharide Metabolism in Coleus. Plant Physiol. 1999 Nov, 121(3): 987-993.

Quan R.D., Shang M., Zhang H., Zhang J. (2004). Improved chilling tolerance by transformation with betA gene for the enhancement of glycinebetaine synthesis in maize. Plant Sci., (166): 141–149.

Rontein D., Basset G., Hanson A.D. (2002) Metabolic engineering of osmoprotectant accumulation in plants. Metabolic Engineering, 4: 49-56.

Sairam, R.K., Rao K.V., Srivastava G.C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolite concentration. Plant Sci., 163: 1037-1046.

Sakamoto A., Murata N. (2002). The role of glycinebetaine in the protection of plants from stress: clues from transgenic plants. Plant Cell Environ. 25: 163–171.

Subbarao G.V., Nam N.H., Chauhan Y.S., Johansen C. (2000). Osmotic adjustment, water relations and carbohydrate remobilization in pigeon pea under water deficits. J. Plant Physiol. 157: 651–659.

Tabatabaei S.A, Naghibalghora S.M. (2013). The effect of ascorbic acid on germination characteristics and proline of sesame seeds under drought stress. International Journal of Agriculture and Crop Sciences, 6 (4): 208-212.

Wahid A., Close T.J. (2007). Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. Biol. Plantarum 51: 104–109.

Wahid A., Gelani S., Ashraf M., Foolad M.R. (2007). Heat tolerance in plants: an overview. Environ. Exp. Bot., 61: 199–223.

Yamada M., Morishita H., Urano K., Shiozaki N., Yamaguchi-Shinozaki K., Shinozaki K., Yoshiba Y. (2005). Effects of free proline accumulation in petunias under drought stress. J. Exp. Bot. 56: 1975–1981.

Yordanov, I., Velikova V., Tsonev T. (2003). Plant responses to drought and stress tolerance. Bulg. J. Plant Physiol., (Special Issue): 187-206.

Zhu J.K. (2002). Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53: 247–273.