IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 4 Issue 7, July 2017
ISSN (Online) 2348 – 7968 | Impact Factor (2016) – 5.264
www.ijiset.com

Exogenous glycinebetaine mitigate drought stress in wheat (*Triticum aestivum* L.) cultivars

Hossam M Fouda1*, Abd El-Monem M Sharaf1, Magda A.F. Shalaby2 and El sayed KH. A. kasem1

Hossam M. Fouda *Corresponding Author :
Botany and Microbiology Department, Faculty of Science, Al-Azhar University
11884 Nasr City, Cairo, Egypt

[1]**Botany and Microbiology Department, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt**
[2]**Botany Dept., National Research Centre, Dokki, Giza, Egypt.**

ABSTRACT: Water stress, one of the environmental stresses, is the most significant factor restricting plant production on majority of agricultural fields of the world. Wheat is grown on arid agricultural fields and drought often causes serious problems in wheat production in these fields. So, the present study was conducted to investigate whether the application of glycine betaine (GB) could regulate the soluble carbohydrate content, soluble protein content, phenol content and proline content as well as ameliorate the adverse effects of drought stress on wheat. *Triticum sativum* plants which planted , exposed to water stress showed significant decreases in contents carbohydrates, soluble proteins, while phenols, cartenoids and free proline contents were increased throughout the experimental period.Treatment with different concentarion of glycine betaine significantly increased contents of carbohydrate as well as proteins, phenol and proline of *Triticum sativum* plants.
Key words: irrigation interval, glycine betaine, wheat.

1. Introduction:

Today, in a world of 7 billion people, agriculture is facing great challenges to ensure a sufficient food supply. Unfavorable environmental factors such as drought, salinity, chilling, freezing, high temperature etc. significantly limit productivity and quality of crop species worldwide and in extreme cases cause the plant to die **(Sardans et al., 2011).** Almost all of abiotic stresses are often interconnected and associated with plant-water relations. Economic losses associated to water availability reached about one billion dollars, in 2009, only in the United States **(Anderson et al., 2009).** With increasing aridity in conjunction with a fast increase in human population, water will become a scarce commodity in the near future, particularly in the third world countries. Water stress caused by lack of water or by other environmental stresses like extreme temperatures or salinity **(Bartels and Souer, 2004).**

Drought is one of the most significant manifestations of abiotic stress in plants mainly in arid and semi-arid areas and is usually associated with other stresses such as high temperature and irradiance. The predicted increase of dry days per year in many areas of the globe will further exacerbate this problem, especially in arid and semi-arid zones of the Mediterranean **(Luterbacher, 2006).** Drought reduces plant growth by affecting several physiological and biochemical processes, such as photosynthesis, respiration, nutrient transport and hormone balance, leading to the reduction of fresh and dry biomass **(Lei et al., 2006; Farooq et al., 2012).** Plants can escape, avoid or tolerate drought stress according to the type of strategy adopted **(Harb et al., 2010; Farooq et al., 2012).** Therefore, different mechanisms have been evolved to allow plants to adapt and survive for long periods of water deficit **(Cruz de Carvalho, 2008).** Desert plant species have developed different mechanisms to cope with extreme dry conditions, either by regulating their phenology choosing extremely short life cycles, or by osmotic adjustment **(Chaves et al., 2003; Bartels and Sunkar, 2005),** allowing cell enlargement and plant growth during severe drought stress by keeping the stomata partially open to allow CO2 assimilation **(Hare et al., 1998).**

The increasing yield potential has indisputable importance in solving world hunger issue. Since yield has a complex trait and is strongly influenced by the environment, severe losses can be caused by drought, a stress common in most arid and semi-arid areas. Accordingly, drought tolerance is one of the main components of yield stability and its improvement is a major challenge to geneticists and breeders. Drought stress not only affects plant growth and development but ultimately productivity in almost all the cereals, thus it is one of the most serious threats to world agriculture **(Subhani *et al.*, 2011).**

Glycine betaine (N,N,N-trimethyl glycine, GB), which is found in plants, animals, and bacteria **(Prasad and Pardha-Saradhi, 2004)** and many studies indicate that GB might play an important role in enhancing plant tolerance to some abiotic stresses such as salt, drought, and extreme temperatures **(Quan et al., 2004).** The accumulation of endogenous GB is induced under stress conditions, and the levels are correlated with the extent of increased tolerance **(Park et al., 2004).** Significant advances have been made in alleviating the effects of environmental stresses by exogenously applied glycine btaine in different crops, such as wheat **(Sayed et al., 2007), rice (Rahman et al., 2002), sorghum (Ibrahim and Aldesuquy 2003; Ibrahim, 2004) as well as sunflower (Iqbal et al., 2008).**

The aim of the present work was to investigate the effectiveness of glycine betaine in alleviating the negative effects of drought stress. Because of this we hypothesized that GB can mitigate the adverse effects of drought on yield components and physiological characteristics.

2. MATERIALS AND METHODS:

These experiments were carried out in el-gharbia , Egypt . the grains of wheat plant were obtained from the agricultural research centre, ministry of agriculture, giza, Egypt . Soil samples were taken at the depth of 30 cm before planting for physical and chemical analysis as shown in (table 1& 2) according the methods of **nelson and sommers (1996).**

A pot experiments was designed as follows: A homogenous wheat grains were sown in pots (30cm in diameter) containing 8.0 kg of clay soil and subjected to different level of irrigation intervals namely. The pots were divided into six group's representing the following treatments,

A) Irrigation treatments

- Irrigation interval (every 7 days tap water).
- Irrigation interval (every 14 days tap water).
- Irrigation interval (every 28 days tap water).

B) Combined treatments

- Irrigation interval every 7 days + GB (50 ppm).
- Irrigation interval every 14 days + GB (50 ppm).
- Irrigation interval every 28 days + GB (50 ppm).

The plants of wheat were treated twice with the above mentioned treatments (as foliage spraying).the first treatment was made when the age of plants was 30 days, while the second treatment was made when the age of plants was 75 days . The plant samples were collected for analysis when the plants were 58 days old. At the end of the growth seasons 140 days, analysis of the grains yielded from the different treatments as well as the control was done and the irrigation of water level occurs at 15 days throughout the ages of plant.

Table1: Physical properties of the used soil (as percentage %)

Gravels	Fine Gravels	Coarse Sand	Medium Sand	Fine sand	silt	Clay	Texture class
2	4.5	5	45	21	7	15.5	**Sandy-clay soil**

Table 2: Chemical properties of the used soil.

TSS ppm	pH	E.C. mmhos/cm	Cations meq/L				Anion meq/L			
			Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	$SO4^{--}$	$HCO3^-$	$CO3^{--}$
760	7.3	1.85	1.75	0.58	2.55	2	3	1.02	1.5	zero

Chemical analysis:

Contents of soluble carbohydrates were measured according to the method of **Umbriet et al. (1969).** Contents of soluble proteins were estimated according to the methods of **Lowery et al. (1951).** Phenolic compounds were estimated according to the methods of **Daniel and George (1972).** Contents of proline were estimated according to the method of **Bates et al., (1973).**

Statistical methods

Statistical methods all statistical calculations were done using computer programs. Microsoft excels version 10 and spss (statistica package for the social science version 20.00) statistical program. at 0.05 level of probability **(Snedecor and Cochran, 1989).**

RESULTS AND DISCUSSION

1. Soluble Carbohydrates:

1.1 Shoots and yield

Soluble Carbohydrates Shoots and yield Results in the present work Fig (1) recorded decreases in the contents of total soluble carbohydrates in the two stages in shoots and as well as in the yielded seeds in wheat plant growth under the second and third level of irrigation.

Contents of total soluble carbohydrates in grains of wheat plants, mostly, highly significantly increased in response to the treatment with of GB. This was the case in plants grown under the all applied irrigation interval levels Fig (2). The accumulation of soluble sugar in stressed plants has been widely reported as a response to salinity **(Gill et al., 2001; Murakeozy et al., 2003; Juan et al., 2005 and Lacerda et al., 2005). Dawood and Sadak (2014)** stated that GB treatments at different levels (10mM,15mM and 20mM) caused significant increases in IAA, proline, total soluble sugars and significant decreases in MDA, H2O2, in canola plants irrigated with different levels of water (75% FC and 50% FC). All GB treatments caused significant increases in seed yield, oil, carbohydrate, protein, total phenolic content, tannins, and antioxidant activity of the yielded seeds and non-significant increases in flavonoids in the yielded canola seeds either in plants irrigated with 75% FC or 50% FC. The increases in seed yield/plant due to 20 mM GB were 30.80% and 60.28% at 75% FC and 50% FC respectively relative to corresponding controls. Generally, 20 mM GB was the most pronounced and effective treatment in alleviating the deleterious effect of moderate or severe drought stress on canola plants.

Some studies indicated that the application of amino acids as a foliar spray caused an increase in the contents of total soluble sugars. These results are in agreement with the finding of other studies on a variety of plants (**Jianfeng *et al.*, 2005; Abou Dahab and Abdel- Aziz, 2006; Abdel Aziz *et al.*, 2009; Abdel Aziz *et al.*, 2010; Ibrahim *et al.*, 2010).** The promoting effect of the amino acids on the total soluble sugars may be due to their role in biosynthesis of chlorophyll molecules **(Abdel Aziz *et al.*, 2010; Ibrahim *et al.*, 2010).**

2. Soluble Proteins

2.1 Shoots and yield

Results of the present work Figs (3 and 4) revealed that, mostly, highly significant increases in the contents of soluble proteins in shoots and yield of wheat plants were resulted in plants grown under the second and third level of irrigation. This was the case throughout the two stages of growth. Highly significant decrease in soluble protein contents in yielded seeds was observed in response to the aforementioned treatment. On the other hand, the obtained results Figs (3 and 4). revealed that, treating wheat plant with GB at the first level of irrigation, resulted in, mostly, highly significant increases in the contents of soluble proteins in shoots, grains as well as in the yielded grains of the treated plants. GB application allowed Maize plants in the mildly-stressed treatment to overcome water limitation and continue growing which resulted in increased biomass relative to the untreated mildly stressed plants (**Reddy et al; 2013**). GB could counteract the adverse effects of drought on wheat by improvement of growth vigor of root and shoot, leaf area, retention of pigments content, increasing the concentration of organic solutes (soluble sugars and soluble nitrogen) as osmoprotectants, keeping out the polysaccharides concentration and/or stabilization of essential proteins in both wheat cultivars, GB could improve the drought tolerance of both two wheat cultivars (sensitive, Sakha 94) and (resistant, Sakha 93) particularly the sensitive ones (**Heshmat et al; 2012**).

The accumulation of osmolyte compounds such as sugars and amino acids, in the cells as a result of water stress is often associated with a possible mechanism for tolerating the harmful effects of water shortage **(Pirzad et al. 2011).** Also found that yield increased similar results have been reported by **Thalooth et al. (2006), Cox and Cherney (2005).**

3. <u>Total phenol</u>

Results of the present work Fig (5) revealed that, contents of phenol in shoots as well as in the yielded grains were gradually significantly increased in the plants irrigated every 28 day when belong compared with those grown under the first level of irrigation. The similar results have been reported by **Dawood and Sadak (2014)**. The increase in phenol levels have been reported in a number of plants grown under stress condition (**Muthukumarasamy *et al.*, 2000, Navarro *et al.*, 2006 and Farag Abeer 2009).**

4. Total proline

Results of the present work Fig (6) revealed that, contents of proline in shoots as well as in the yielded grains were gradually significantly increased in the plants irrigated every 28 day when belong compared with those grown under the first level of irrigation. The similar results have been reported by **Abd El-Monem (2007)**.

In organisms ranging from bacteria to higher plants, there is strong correlation between increased cellular proline levels and the capacity to survive under stress. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub cellular structure (membrane and proteins) scavenging free radicals and buffering cellular redox potential under stress conditions **(Ashraf and Foolad, 2007).**

Conclusions

In conclusion, water stress negatively affected the biochemical assay of the wheat plant. However, application with GB has beneficial effect on growth and chemical constituents of wheat plants under different levels of irrigation interval.

References

Abd El-Monem, A.A. (2007): Polyamines as modulators of wheat growth, metabolism and reproductive development under high temperature stress. Ph.D. Thesis, Ain Shamas Univ., Cairo, Egypt.

Abdel Aziz, N.G.; Mahgoub, M.H. and Mazher, A.A.M. (2009): Physiological effect of phenylalanine and tryptophan on the growth and chemical constituents of *Antirrhinum majus* plants. Ozean J Appl Sci. 2(4): 399-407.

Abdel Aziz, N.G.; Mazher, A.A.M. and Farahat, M.M. (2010): Response of vegetative growth and chemical constituents of *Thuja orientalis* L. plant to foliar application of different amino acids at Nubaria. J Am Sci. 6(3): 295-301.

Abou Dahab, T.A.M. and Abdel- Aziz, G.N. (2006): Physiological effect of diphyenylamin and tryptophan on growth and chemical constituents of *philodendron erubescens* plants. World J Agric Sci. 2(1): 75- 81.

Anderson DP, Welch JM, Robinson J. 2009. Drought Impact on Agriculture Approaches $1 Billion Early in 2009. 05.05.2011, Available from:http://agecoext.tamu.edu/fileadmin/user_upload/Documents/Resources/Public(C.F. Vasanthaiah, Hemanth (2011). Plants and Environment ISBN 978-953-307-779-6, 272 pages Publisher InTech Published online 17, October, 2011).

Ashraf, M. and Foolad, M.R. (2007): Roles of glycine betaine and proline in improving plant abiotic resistance. Environmental and Experimental Botany, 59:206-216.

Bartels D, Souer E.2004. Molecular Responses of Higher Plants to Dehydration. In: Plant Responses to Abiotic Stress, Hirt, H. & Shinozaki, K. (Eds), Springer-Verlag, Berlin, Heidelberg.

Bartels, D. and R. Sunkar, (2005). Drought and salt tolerance in plants. Crit. Rev. Plant Sci., 24: 23-58.

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

Chaves, M.M., Maroco, J.P. and Pereira, J.S. (2003). Understanding plant responses to drought from genes to the whole plant, Functional Plant Biology; 30:239-264.

Cox, W.J. and D.J.R. Cherney (2005). Timing corn forage harvest for bunker silos. Agron. J., 97: 142-146.

Cruz de Carvalho, M. H. (2008). Drought stress and reactive oxygen species. Plant Signaling & Behavior, 3(3): 156-165.

Daniel, H.D. and George, C.M. (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. J. Amer. Soc. Hort. Sci. 97:651-654.

Dawood MG, Sadak MSh.2014. Physiological role of glycinebetaine in alleviating the deleterious effects of drought stress on canola plants (Brassica napus L.). Middle East Journal of Agriculture Research, 3: 943-954.

Farag, Abeer, A.A. (2009): Increasing tolerance of *Vigna sinensis* L. to salt stress using an organic acid and polyamine. Ph. D. Thesis, Ain Shams Univ. Egypt.

Farooq, M., Ali, A.B., Sardar, A. C. and Zahid, A. C. (2012). Application of Allelopathy in Crop Production. International Journal of Agriculture and Biology, 13S–011/2013/15–6– 1367–1378.

Gill, P.K., A.D. Sharma, P. Singh and S.S. Bhullar, 2001. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. Bulg. J. Plant Physiol., 27: 72-84.

Harb, A., Krishnan, A., Ambavaram, M.M.R. and Pereira A. (2010). Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiology, 154, 1254–1271.

Hare, P.D., Cress, W.A. and Staden, J.V., (1998). Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ. 21, 535–553.

Heshmat. S. Aldesuquy, Samy. A. Abo- Hamed, Mohmed. A. Abbas And Abeer. H. Elhakem; 2012. Role of glycine betaine and salicylic acid in improving growth vigour and physiological aspects of droughted wheat cultivars. Journal of Stress Physiology & Biochemistry, Vol. 8 No. 1 2012, pp. 149-171.

Ibrahim, A.H. (2004) Efficacy of exogenous glycine betaine application on sorghum plants grown under salinity stress. Acta Botanica Hungarica, 46, 307-318.

Ibrahim, A.H. and Aldesuquy, H.S. (2003) Glycine betaine and shiki- mic acid induced modification in growth criteria, water relation and productivity of droughted Sorghum bicolor plants. Phyton (Horn, Austria) 43, 351-363.

Ibrahim, S.M.M.; Taha, L.S. and Farahat, M.M. (2010): Influence of foliar application of pepton on growth, flowering and chemical composition of *Helichrysum bracteatum* plants under different irrigation intervals. Ozean J Appl Sci. 3(1): 143-155.

Iqbal, N., Ashraf, M., Ashraf, M.Y. (2008) Glycinebetaine, an osmolyte of interest to improve water stress tolerance in sunflower (Helianthus annuus L.): water relations and yield. South African Journal of Botany 74, 274– 281.

Jianfeng, N.; Qingsong, Z.; Zhaopu, L. and Jing, S. (2005): Supplemental nitrogen effects on growth, nutrient content and quality of *Aloe vera* seedlings under salt stress. Coll Resour Environ Sci. 32(4): 663-668.

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

Lacerda, C.F., J. Cambraia, M.A.O. Cano and H.A. Ruiz, 2001. Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. Rev. Bras. Fisiol. Veg., 13: 270-284.

Lei Y., Yin C. and Li C. (2006). Differences in some morphological physiological, and biochemical responses to drought stress in two contrasting populations of Populus przewalskii. Physiologia Plantarum 127, 182–191.

Lowery, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the folin-phenol reagent. J. Biol.Chem.193:265-275.

Luterbacher, J., Xoplaki, E., Casty, C., Wanner, H., Pauling, A., Küttel, M., et al., (2006). Mediterranean climate variability over the last centuries: a review. In: Lionello, P., Malanotte-Rizzoli, P., Boscolo, R. (Eds.). The Mediterranean Climate: An Overview of the Main Characteristics and Issues. Elsevier, Amsterdam, pp. 27–148.

Murakeozy, E.P., Z. Nagy, C. Duhaze, A. Bouchereau and Z. Tuba, 2003. Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. J. Plant Physiol., 160: 395-401.

Muthukumarasamy, M.; Gupta, S.D. and Pannerselvam, R. (2000) : Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by trdimefon in NaCl- stressed *Raphanus sativus L*. Biol. Plant., 43:317-320.

Navarro, J.M.; Flores, P.; Garrido, C. and Matrinez, V. (2006): Changes in the contes of antioxidant compounds in pepper fruits at ripening stages, as affected by salinity. Food Chem., 96:66-73.

Park, E.J., Jeknic, Z. and Sakamoto, A (2004) Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. The Plant Journal 40, 474–487.

Pirzad, A., Shakiba, M.R., Zehtab-Salmasi, S., Mohammadi, S.A., Darvishzadeh, R. and Samadi, A. 2011. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in Matricaria chamomilla L. Journal of Mediterranean Plant Research 5(12): 2483-2488.

Prasad, K and Pardha-Saradhi, P. (2004) Enhanced tolerance to photoinhibition in transgenic plants through targeting of glycinebetaine biosynthesis into the chloroplasts. Plant Science 166, 1197–1212.

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

Rahman, M.S., Miyake, H. and Takeoka, Y. (2002) Effects of exogenous glycine-betaine on growth and ultra-structure of salt-stressed rice seedlings (Oryza sativa L.). Plant Production Science 5, 33–44.

Reddy KR, Henry WB, Seepaul R, Lokhande S, Gajanayake B, Brand D. 2013. Exogenous application of glycinebetaine facilitates maize (Zea mays L.) growth under water deficit conditions. American Journal of Experimental Agriculture, 3:1-13.

Sardans J, Pen˜uelas J, Rivas UA. 2011. Ecological metabolomics: overview of current developments and future challenges. Chemoecology, 21:191-225

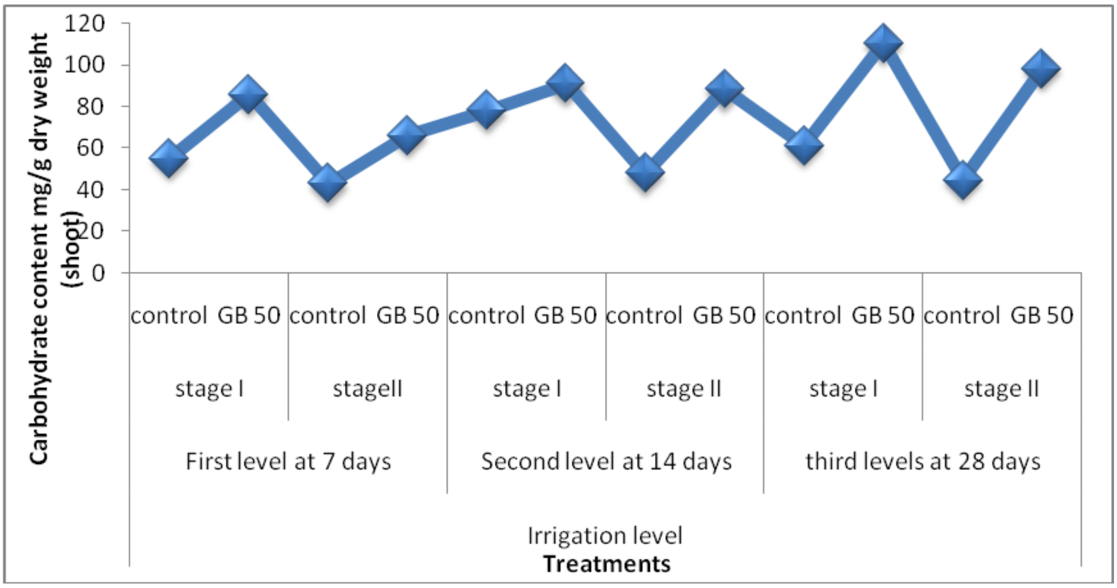
Sayed, H.R.,. Athar, H.A., Ashraf, M. and Hameed, A. (2007) Glycine betaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. Environmental and Experimental Botany 60, 368–376.

Snedecor, G. W. and W. G. Cochran (1989). Statistical Methods. 8th ed. Ames, IA: Iowa State University Press.

Subhani G.M., Hussain M., Ahmad J., Anwar J., 2011 - Response of exotic wheat genotypes to drought stress. Journal of Agricultural Research, 49(3): 293-305.

Thalooth, A.T., Tawfic M.M. and Magda Mohamed H. (2006). A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth, yield and some chemical constituents of mungbean plants growth under water stress condition. World Journal and Agricultural Science, 2: 1. 37-46.

Umbriet, W. W., Burris, R. H., Stauffer, J. F., Cohen, P. P., Johsen, W. J., Lee page, G. A., Patter, V. R. and Schneicter, W. C. (1969). Manometric techniques, manual describing methods applicable to the studs of tissue metabolism. Burgess publishing co., U.S.A .P.P.239.



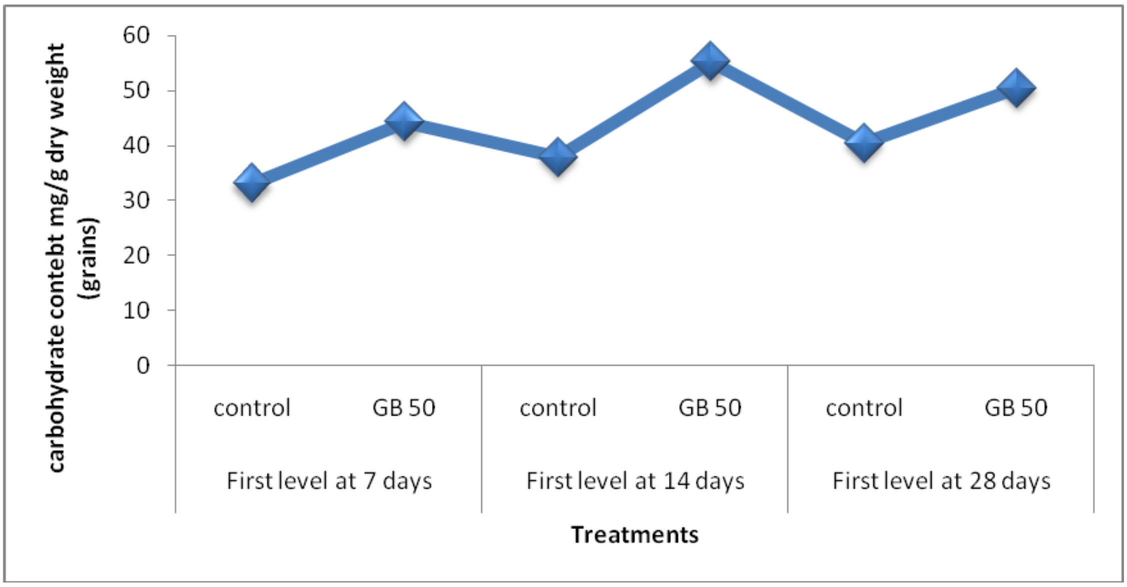
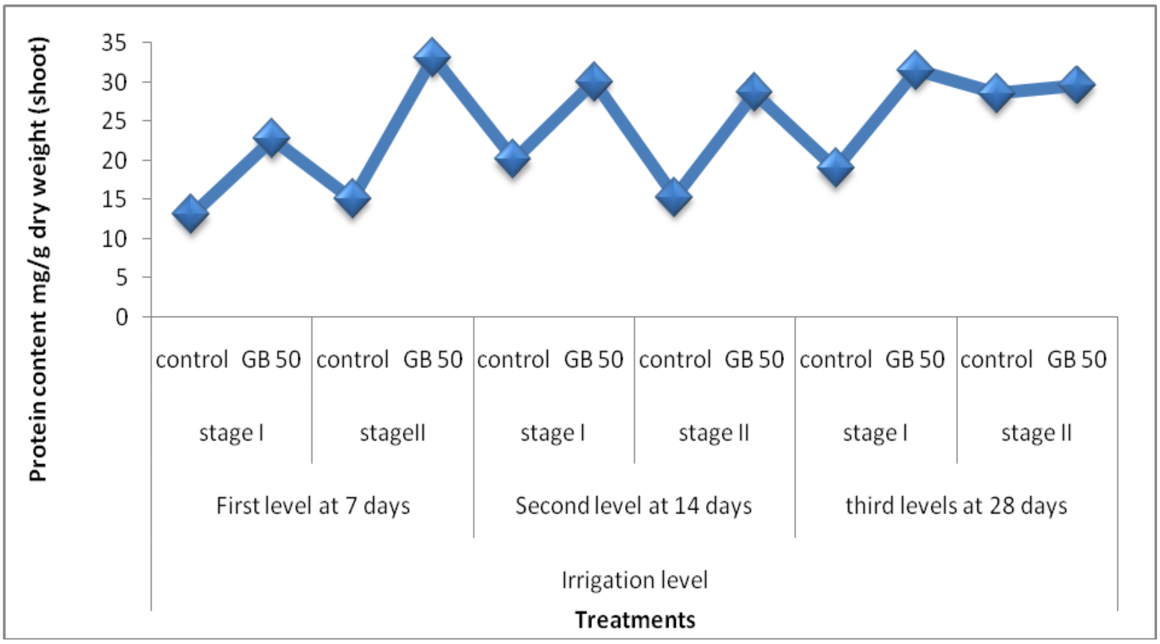


Figure (1, 2). Effect of glycine betaine on the contents of total soluble carbohydrates in shoots and grains of (*Triticum sativum* L.) plants.



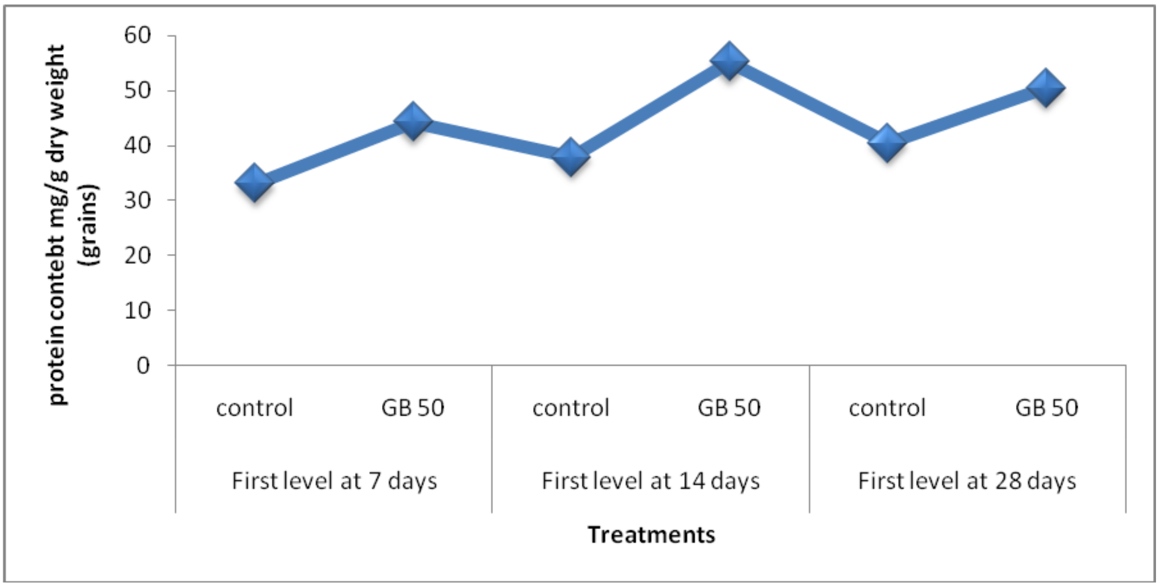


Figure (3, 4). Effect of glycine betaine on the contents of total soluble protein in shoots and grains of (*Triticum sativum* L.) plants.

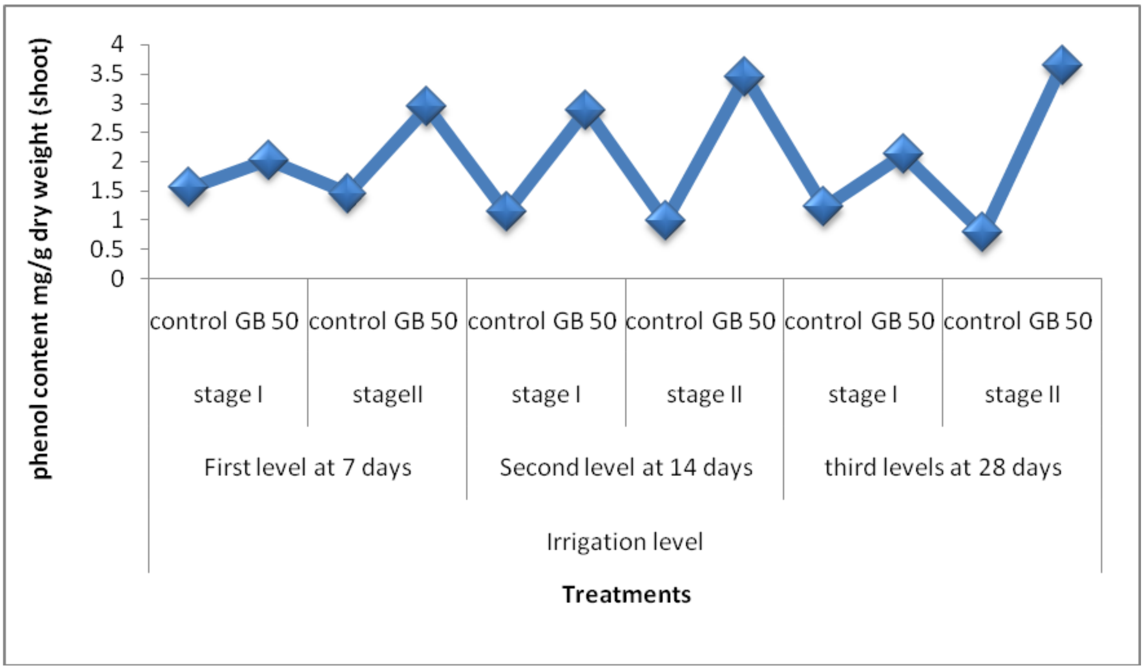


Figure 5. Effect of glycine betaine on the contents of total phenol in the two stages in shoots of (*Triticum sativum* L.) plants.

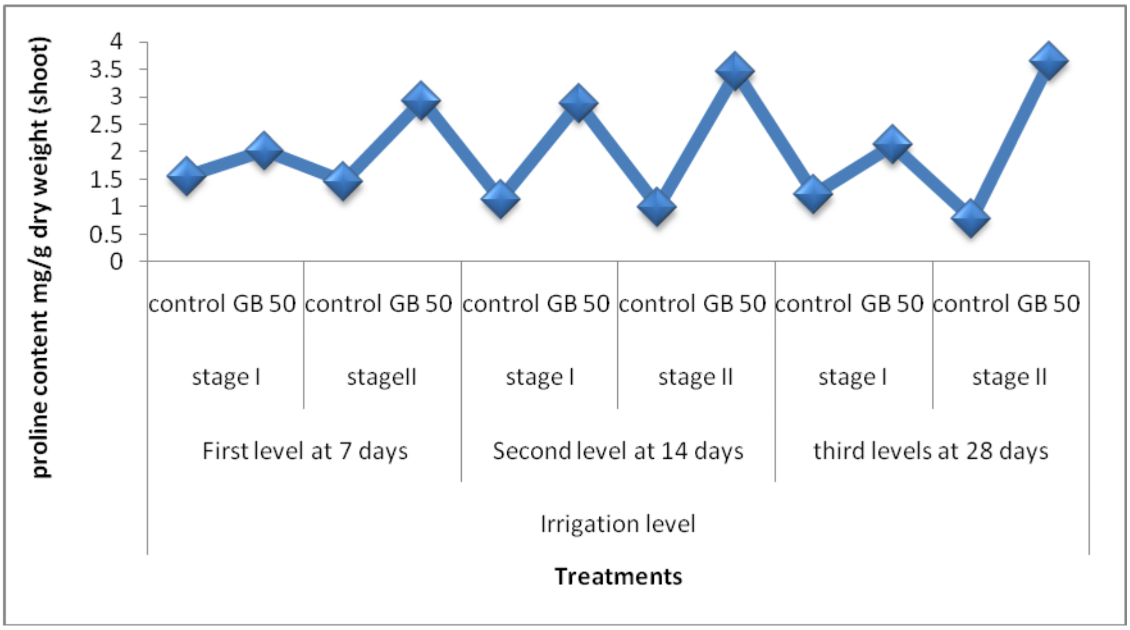


Figure 6. Effect of glycine betaine on the contents of proline in the two stages in shoots of (*Triticum sativum* L.) plants.