VOL. 11, NO. 9, SEPTEMBER 2016 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

ASSESSMENT OF SALINITY STRESS AND THE PROTECTIVE EFFECTS OF GLYCINE BETAINE ON LOCAL WHEAT VARIETIES

Mohammad Sayyar Khan, Syed Jawad Shah and Mazhar Ullah
Faculty of Crop Production Sciences, Institute of Biotechnology and Genetic Engineering (IBGE), the University of Agriculture Peshawar, Pakistan
E-Mail: sayyarkhankazi@aup.edu.pk

ABSTRACT

Salinity is a major abiotic stress that affects plant growth and productivity. Wheat as a major fodder crop is severely damaged by salinity. In the current research, the effect of various salt stress conditions was determined on morphological and biochemical parameters of three locally developed wheat varieties. The ameliorating effect of Glycine betaine (GB), on salt damages in the three varieties under various salt stress conditions was also determined. The three varieties used were Atta-Habib, Siren and variety A (VA). Plants were subjected to three different salt stress conditions i.e. 0 mM, 50 mM, and 100 mM in the presence of 100 mM GB. Mostly non-significant differences were shown for morphological traits by all the three varieties from control to 100 mM salt stress with application of GB. However, in comparison to Atta-Habib and VA, the Siren variety performed better. Siren showed significantly high ($P \leq 0.05$) number of roots (19.0), as compared to Atta-Habib (14.3), and VA (14.3) under 100 mM salt stress with GB application. Similarly, significantly high ($P \leq 0.05$) shoot length was observed in Siren (55.9 mm) as compared to that of Atta-Habib (37.0 mm) and VA (39.3 mm) under 100 mM salt stress with GB application. The Siren variety retained significantly high water content as compared to the other two varieties. At high salt stress (100 mMNaCl), Siren showed high chlorophyll content (13.0 µg/g fresh weight) than that of Atta-Habib (11.7 µg/g), and VA (6.8 µg/g). Atta-Habib and VA accumulated comparatively high proline content at high salt stress. Our results revealed that GB protected plants against the damaging effects of salt stress. The Siren variety showed high tolerance and recovery from salt stress as compared to Atta-Habib and VA with GB application.

Keywords: wheat, salt stress, glycine betaine, chlorophyll, proline.

INTRODUCTION

Wheat is an important grain and fodder crop for the entire world community. It is cultivated on about 220 million hectares around the globe with an average yield of 564.6 million tons, about 2500 kg per hectare. China being the leader cultivating it on 30 million hectare alone followed by Russian federation, US, India, Australia, Turkey, Canada and Pakistan. France is the largest producer with an average of 7200 kg per hectare in the European world because of its longest winter season for wheat cultivation. Pakistan usually grow wheat to full-fill its staple food requirement, thus growing it on about 36% of its area reserved for cultivation, giving about 30% of the value generated by major crops and 76% of the total weightage of food grains (Ashraf and Fooland, 2007). Pakistan occupies 10th place in terms of area (8.5 million hectares) and 59th in terms of yield (21.0 m ton) among the world pioneers for wheat production. The production rate deviates from area to area and time to time depending on a number of factors needed to be considered, while estimating the yield of the crop per hectare e.g. weather and the availability of the irrigation water are but few that contribute to the yield (Mehmood *et al*., 2009).

Soil and water salinization is a major limiting factor for the average global production of different crop varieties (Bhatti *et al*., 204).Approximately more than 800 million hectares (Mha) of land are affected by salinity throughout the world (Munns, 2005). Lack of fresh water resources and the use of brackish or saline water for irrigation are the limiting factors restricting plant growth and productivity. In many countries, agriculture production is hampered by lack of fresh water resources and the use of brackish water for irrigation (Bustan *et al*. 2004). Salt stress affects plant growth and productivity by disturbing biochemical and physiological processes. Salt stress causes osmotic stress, ion toxicity, oxidative stress and nutrient deficiency (Chinnusamy *et al*., 2006).

In response to environmental stresses, plants adopt various defense strategies to counteract the damaging effects on growth and productivity. One such strategy is the accumulation of osmoprotectants or compatible solutes to maintain cellular homeostasis during exposure to abiotic stresses such as salt and drought (Yancey *et al*., 1982). In recent years, much emphasis has been placed on the multi-faceted role of Glycine betaine (GB), a quaternary ammonium compound (Takabe *et al*., 1998; Khan *et al*., 2009). The main role of GB has been proposed to be protection of cellular organelles, membranes, and proteins of important phisological processes such as photosynthesis and respiration (Sakamoto and Murata, 2002). For many years, exogenous GB is applied to plants under abiotic stress conditions to minimize the resulting damages. Several studies conducted on the effects of abiotic stresses revealed that exogenous GB application exerted positive effects on plant growth and recovery from drought stress (Iqbal *et al.* 2005; Ma *et al*. 2007)and salt stress (Hu and Hu, 2012; Cha-Um and Kirdmanee, 2010; Rezaei *et al*., 2012). GB application has also been studied in different wheat varieties, enhancing its capability under stressful conditions (Raza *et al*., 2006; Raza *et al*., 2014).

The current study is aimed to evaluate the effect of salinity on local wheat varieties and the ameliorating effects of exogenous GB application.

MATERIALS AND METHODS

The research was conducted at Recombinant DNA Technology Laboratory, Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar during the year 2013. During the course of this experiment, three local wheat varieties were used. The varieties were tested against different levels of NaCl stress. In order to maintain the osmotic potential, exogenous GB was applied. The data was collected on morphological and biochemical parameters.

Greenhouse experiment

Three locally developed wheat varieties (Siren, Atta-Habib and VA) were used in the greenhouse experiment. The seeds of these varieties were grown in CRD (Completely Randomized Design) at the greenhouse at IBGE. Four pots per variety were used in which one pot was used as control while other three pots contained 0 mM, 50 mM and 100 mM NaCl concentrations along with GB (100 mM). Concentration of NaCl and GB was fixed using the following formula:

$$\text{Concentration} = \frac{\text{Molarity} \times \text{molecular weight} \times \text{Volume}}{1000}$$

This experiment was repeated three times. First time in late growing season (February), and second and third times during October and November, respectively.

PHYSIOLOGICAL PARAMETERS

Data was taken each time at different intervals of each experiment. The parameters were checked and their respective protocols were:

Root and shoot length

The plants grown at control and salt supplemented media were checked for their root and shoot length using ruler (scale marked with centimetres) and the data obtained was compared graphically.

Number of leaves and roots

Number of leaves and roots were calculated for all three genotypes under control and salt stress conditions and data obtained was compared graphically.

BIOCHEMICAL PARAMETERS

Proline content

For proline extraction, Bates *et al*. (1973) protocol was used. A sample of 500 mg of frozen plant material was homogenized with 3% aqueous sulfo-salicyclic acid and then centrifuged at 9000 g. After that supernatant was collected and 1 ml of acid ninhydrin and glacial acetic acid was added to 250 µl of extract. The sample was then boiled at 100 ºC for 1hour in water bath. For extraction, 4 ml of toluene was added and optical density of proline was measured at 520 nm. From standard curve in the range of 0-20 ug/ml of L-proline the amount of proline was determined.

Chlorophyll content

Leaf material (0.1-0.3g) was taken from sample. Liquid nitrogen was used for crushing and then extraction for 15 minutes on ice in 700 ul of 80% acetone, cell debris was layered by centrifugation at 18000 g at 4ºC for 10 minutes. The pellet was re-suspended in same volume of 80% acetone as before, mixed thorough vortexing and after sedimentation of cell debris using centrifuge machine. The supernatant was combined with first extraction. Using photometer, the OD of extract (against 80% acetone) was determined at 645 nm and 663 nm. Total chlorophyll in µg/mg was determined by using equation,

$$C = 20.2*OD645+8.02*OD663$$

The experiment was repeated thrice.

Relative water content

In order to find the fresh weight, the plant was taken from pots, weighed using balance and then dried in oven. After that the dry weight was found using balance.

Relative water quantity = fresh weight- dry weight

Statistical analysis

Analysis of variance (ANOVA) was used to determine the effect of salt stress and GB application on morphological and biochemical parameters in three wheat varieties. In the greenhouse experiment, soil pots were arranged in a CRD design with 5 replications/each line. Tukey's test was used for multiple comparisons at p-level ($P \leq 0.05$).

RESULTS

In order to find the effect of salinity on local wheat varieties in the presence of GB, three wheat varieties (Atta-Habib, Siren and VA) were grown in completely randomized design (CRD) and watered regularly with salt concentrations, 0 mM, 50 mM and 100 mM. Foliar GB was applied at a concentration of 100 mM.

MORPHOLOGICAL PARAMETERS

Number of leaves

On comparing the wheat varieties, Siren showed increase in number of leaves at control and control plus GB as compared to Atta-Habib and VA. The lowest value was shown by Atta-Habib at 50 and 100 mM salt and GB, Siren at 50 mM salt and GB, VA at control and GB and at 50 and 100 mM salt and GB, whereas the highest value was shown by Siren at control only (Table-1). In case of number of leaves, the results were non-significant in all the three conditions i.e variety, treatment and variety into treatment interaction.

Number of roots

Increasing salt stress negatively affected average number of roots particularly in Siren and VA. Atta-Habib

remained unaffected by various salt concentrations. Under control condition, Siren showed significantly high (P≤ 0.05) root number (23.7) as compared to Atta-Habib (14.0) and VA (17.7). Salt stress decreased root number in Siren and VA; however, as compared to VA (14.3) and Atta-Habib (14.3), the Siren variety maintained high root number (19.0) under 100 mM salt stress with GB application (Table-1).

Root length

Root length was also negatively affected by increasing salt stress in all the three varieties. Maximum root length (10.5 mm) was observed in Atta-Habib under control condition. While Siren and VA showed root length as 8.4 and 9.8 mm, respectively (Table-1). High salt stress (100 mM) significantly (P≤ 0.05) decreased root length in all the three varieties. Atta-Habib, Siren and VA showed root length as 6.9, 5.9 and 7.7 mm, respectively under 100 mM salt stress with GB application.

Shoot length

Shoot length was slightly affected by salt stress. Siren showed increase in shoot length as compared to Atta-Habib and VA. However, all three varieties showed increase in shoot length especially at 50 mM salt stress with GB application. High salt stress (100 mM) non-significantly decreased shoot length in Atta-Habib and VA. Atta-Habib showed shoot length as 37.0 mm, while VA showed 39.3 mm. Siren variety was less affected by high salt stress with GB application and maintained high shoot length (55.9 mm) (Table-1).

Table-1. Effect of salt stress and exogenous GB on various morphological growth parameters of wheat varieties.

Varieties	Conditions	Growth parameters			
		No of leaves	No of roots	Root length	Shoot length
Atta-Habib	Control	22.0±3	14.0±3.1	10.5±2.2	45.7±4.8
	Control + GB	23.0±2.5	14.3±2.8	9.5±2.3	37.8±5.5
	50 mM + GB	22.0±3.5	13.3±2.2	8.2±1.8	40.7±6.4
	100 mM + GB	22.0±2.3	14.3±3.4	6.9±1.3	37.0±7.4
Siren	Control	26.0±3.8	23.7±2.2	8.4±2.3	57.6±8.3
	Control + GB	25.0±2.5	16.7±2.5	7.9±1.2	49.1±5.4
	50 mM + GB	22.0±3.1	15.3±3.1	7.9±1.8	59.6±5.3
	100 mM + GB	24.0±3.5	19.0 3.4	5.9±1.1	55.9±6.7
VA	Control	23.0±2.4	17.7± 3.2	9.8±2.2	45.2±7.1
	Control + GB	22.0±2.3	18.7±4.1	8.5±2.3	38.7±4.3
	50 mM + GB	22.0±2.1	14.7±4.2	8.4±2.3	48.7±5.2
	100 mM + GB	22.0±3.8	14.3±2.6	7.7±1.8	39.3±4.7

GB: Glycine betaine; ±: Standard error.

Relative water content

Siren variety showed significantly high (P≤ 0.05) water content (42.5 gm), than that of Atta-Habib (24.3 gm) and VA (20 gm) (Figure-1). Salt stress significantly (P≤ 0.05) decreased relative water content in all the three varieties. However, slight stability was observed in all three varieties under 50 mM salt with GB compared to that under control condition with GB. Under high salt stress (100 mM) and GB application, Siren maintained significantly high (P≤ 0.05) water content (25.0 gm) as compared to that of Atta-Habib (9.1 gm) and VA (10.1 gm).

ARPN Journal of Agricultural and Biological Science

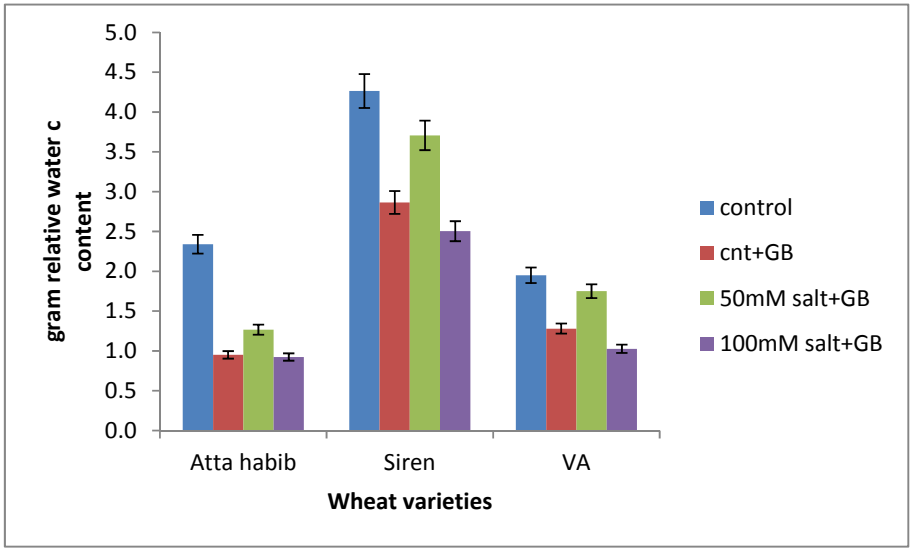

Figure-1. Effect of salt stress on relative water content of wheat seedlings in the presence of GB.Values are averages ± SE.

Chlorophyll content

Under control condition, Siren showed significantly high chlorophyll content as compared to that of Atta-Habib and VA (Figure-2). The chlorophyll level usually increases with slight increase in salt concentration; however as the salt concentration increases, chlorophyll content tends to decrease. Atta-Habib remained unaffected by increasing salt concentration from 0 mM to 100 mM. However, Siren and VA showed decrease in chlorophyll content under 50 and 100 mM salt stress with GB application. Under 100 mM salt and GB, the Siren variety showed significantly high chlorophyll content (12.5 µg/g fresh weight) as compared to that of Atta-Habib (11.5 µg/g fresh weight) and VA (7.5 µg/g fresh weight).

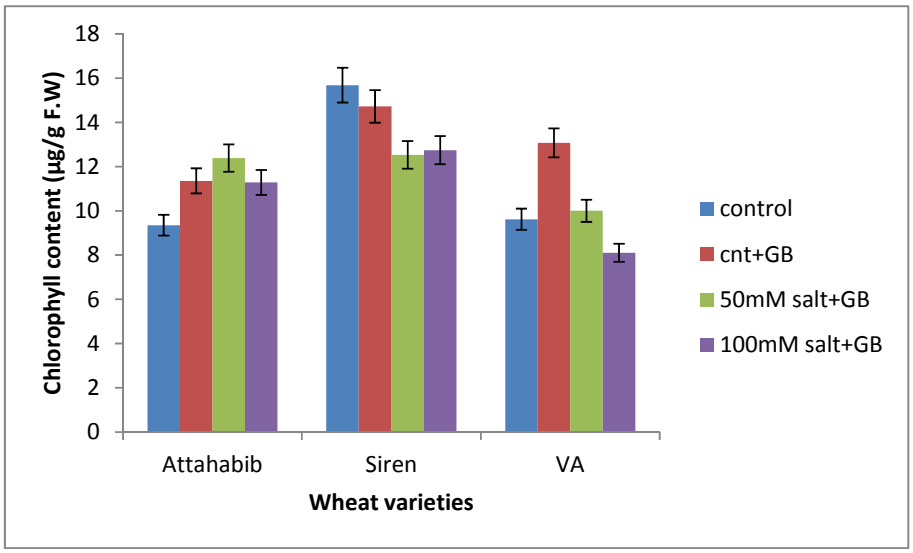

Figure-2. Effect of salt stress on chlorophyll content of wheat seedlings in the presence of GB. Values are averages ± SE.

Proline content

The proline content fluctuated significantly in Atta-Habib and VA from control to salt stress conditions with GB application (Figure-3). In comparison to control condition, Siren showed slight decrease in proline content under salt stress with GB. Atta-Habib and VA showed proline content as 5.0 µg/g fresh weight each as compared to that of Siren which accumulated above 7.0 µg/g. Upon high salt stress (100 mM with GB) Atta-Habib and VA tended to proline content significantly; however, Siren showed non-significant reduction in proline content.

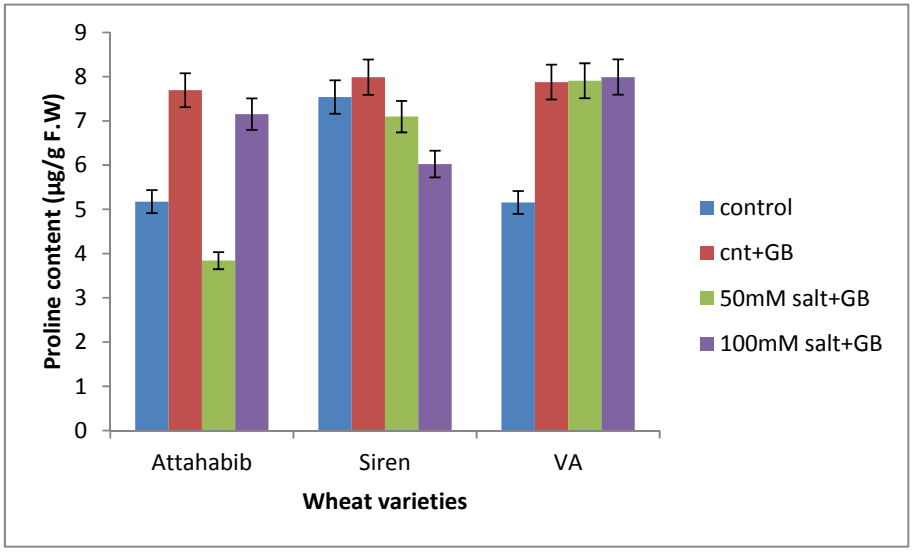

Figure-3. Effect of salt stress on proline content of wheat seedlings in the presence of GB. Values are averages ± SE.

DISCUSSIONS

Salinity is one of the most limiting factors affecting wheat productivity. Several strategies are available to counteract the negative effects of salt stress on plants. The use of exogenous GB is one of such strategies. GB is an osmoprotectant widely used to maintain the osmotic homeostasis in plants under environmental stresses including salt stress. A number of studies reported positive effects of exogenously applied GB on various physiological and biochemical parameters of growth under stress conditions (Iqbal *et al*. 2005; Ma *et al*. 2007; Hu and Hu, 2012; Rezaei *et al*., 2012).

Crop varieties may show different responses to environmental stresses due to their genetic adaptation. In one study, Raza *et al*. (2006) investigated the influence of exogenous GB on the photosynthetic potential of two differentially adapted wheat cultivars under salt stress. Salt stress reduced photosynthesis in both salt tolerant and salt sensitive cultivars. Exogenous GB application ameliorated the negative effects due to salt stress; however, it was more prominent in the salt tolerant cultivar than the sensitive cultivar. Hu and Hu (2012) reported similar results in perennial ryegrass when subjected to salt stress and the exogenous GB application ameliorated the adverse effects of salt stress. Similar results were produced in our study. Salt stress reduced growth parameters more prominently in Atta-Habib and VA than that in Siren. GB application ameliorated the damaging effects; however, Siren showed comparatively strong response to GB than Atta-Habib and VA.

Siren comparatively produced better results for all morphological traits. However, it is clear that the GB application protected all the three varieties from the adverse effects of salinity. Similarly, in case of number of roots, Siren performed well as compared to other two varieties. Same results were obtained for Siren in case of shoot and root length, after the salinity stress being neutralized by GB. Tammam *et al*. (2008) reported same results while investigating wheat cultivar Banysoif 1. They found no effect on fresh and dry weight of the cultivar up to 120 mM NaCl. Maqsood *et al*. (2006) reported similar results in maize while studying effect of salinity on morphological parameters. They concluded that no wide differences existed between treatments at the time of harvest. They associated it with crop type, timing and rate of application of GB, salt and other environmental conditions. Yang *et al*. (2008) reported similar results in case of transgenic tobacco plants.

The chlorophyll level was improved through application of GB even in the presence of salt stress up to 100 mM at which plant usually dies. Improvement in photosynthesis by GB in salt stressed plants was also reported by Hayashi *et al*. (1997). Exogenous application of GB improved growth and carbon dioxide assimilation in maize plants (Yang and Lu, 2005). Similar GB effects against salt in bacterial species i.e. synechococcus were detected by Ohnishi and Murata. (2006). The same type of protection of photosynthetic machinery under various abiotic stresses was reported for a number of other accumulating and non-accumulating GB crops (Sakamoto *et al*., 1998;Takabe *et al*., 1998; Gao *et al*., 2000; Holmstrom *et al*., 2000; Prasad *et al*., 2000; Ashraf and Fooland, 2007).Based on the recent and previous findings, we may conclude that the exogenous application of GB improves photosynthetic efficiency of plants.

Proline, an important osmoprotecatnt is accumulated under stress conditions (Larher *et al*., 1993). Our results indicated that Atta-Habib and VA increased their proline content upon salt stress. As these varieties showed more sensitivity to salt stress than that of Siren; therefore more proline was accumulated by salt stress and GB application to ameliorate the adverse effects. On the contrary, Siren showed more tolerance to salt stress in terms of improved morphological traits and chlorophyll

content and therefore accumulated less proline content. This is based on the speculation that sensitive genotypes may produce more proline as compared to tolerant genotypes. From these findings, it is clear that application of GB against salinity may be useful to develop the resistance capability of wheat crop.

CONCLUSIONS

Based on our results, we conclude that among all the three tested wheat varieties, Siren showed better physiological and biochemical indicators of growth under salt stress and responded more efficiently to exogenous GB application as compared to the other two varieties. These results should be further expanded to investigate the molecular basis of comparative performance of Siren under salt stress with or without GB application.

REFERENCES

Ashraf M. and M.R. Fooland. 2007. Roles of glycine betaine and proline in improving abiotic stress resistance. J. Env. Exp. Bot. 59(2): 206-216.

Bates L.S., R.P Waldran and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant.Soil. 39: 205-208.

Bhatti M.A., Z. Ali, A. Baksh, A. Razaq and A.R. Jamal. 2004. Screening of wheat lines for salinity tolerance. Int. J. Agri. Biol. 6(4): 627-628.

Bustan A., M. Sagi, Y.D. Malach and D. Pasternak. 2004. Effects of saline irrigation water and heat waves on potato production in an arid environment.Field.Crop. Res. 90: 275-285.

Cha-Um S and C. Kirdmanee. 2010. Effects of Glycine betaine on proline, water use, and photosynthetic efficiencies, and growth of rice seedlings under salt stress. Turk. J. Agric. For. 34: 517-527.

Chinnusamy V., J. Zhu and J.K. Zhu. 2006. Salt stress signaling and mechanisms of plant salt tolerance. Genet. Eng. 27: 141-177.

Gao M., A. Sakamoto, K. Miura, N. Murata, A. Tao and R. Suguira. 2000.Tranformation of Japanese persimmon (*Diospyros kaki*Thunb) with a bacterial gene for choline oxidase. J. Mol. Breed. 6(5): 501-510.

Ghogdi E.A., A.I. Darbandi and A. Borzouei. 2012. Effects of salinity on some physiological traits in wheat (*Triticumaestivum*L.) cultivars. Indian J. Sci. Technol. 5(1): 1901-1906.

Hayashi H., Alia, L. Mustardy, P. Deshnium, M. Ida and N. Murata. 1997. Transformation of *Arabidopsis thaliana* with the *codA*gene for choline oxidase: accumulation of glycinebetaine and enhanced tolerance to salt and cold stress. Plant. J. 12: 133-142.

Holmstrom K.O., S. Somersalo, A. Mandal, E.T. Palvaand and B. Welin. 2000. Improved tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. J. Exp. Bot. 51(343): 177-185.

Hu L. and T. Hu. 2012. Exogenous Glycine betaine ameliorates the adverse effect of salt stress on perennial ryegrass. J. Amer. Soc. Hort. Sci. 137(1): 38-46.

Iqbal N., M.Y. Ashraf and M. Ashraf. 2005. Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage. Pak. J. Environ. Sci. Technol. 2(2): 155-160.

Khan M.A., E. Islam, M.U. Shirazi, S. Mumtaz, S.M. Mujtaba, M.A. Khan and G.M. Kaleri. 2010. Physiological responses of various wheat genotypes to salinity. Pak. J. Bot. 42(5): 3497-3505.

Khan M.S., X. Yu, A. Kikuchi, M. Asahina and K.N. Watanabe. 2009. Genetic engineering of glycine betaine biosynthesis to enhance abiotic stress tolerance in plants. Plant Biotechnol. 26: 125-134.

Larher F., L. Leport, M. Petrivalsky and M. Chappart. 1993. Effectors for the osmoinducedproline response in higher plants. Plant. Physiol. Biochem. 31: 911-922.

Ma X.L., Y.J. Wang, S.I. Xie, C. Wang and W. Wang. 2007. Glycinebetaine application ameliorates negative effects of drought stress in tobacco. Russ. J. Plant. Physiol.54: 472-479.

Maqsood A., M. Shahbaz and N.A. Akram. 2006. Influence of exogenously applied glycinebetaine on growth and gas exchange characteristics of maize (*Zea Mays* L.). Pak. J. Agri. Sci. 43(1-2): 1-2.

Mehmood E., R. Kausar, M. Akram and S.M. Shahzad. 2009. Is boron required to improve rice growth and yield in saline environment.Pak. J. Bot. 41(3): 1339-1350.

MunnsR. 2005. Genes and salt tolerance: bringing them together. New.Phytol. 167: 645-663.

Munns R. and M. Tester. 2008. Mechanisms of salinity tolerance. Ann. Rev. Plant. Bio. 59: 651-681.

Ohnishi N. and N. Murata. 2006. Glycinebetaine counteracts the inhibitory effects of salt stress on the degradation and synthesis of the D1 protein during photo inhibition in *Synechococcus*sp. PCC 7942. Plant. Physiol.141: 758-765.

Prasad K.V.S.K., P. Sharmila, P.A. Kumar and P.S. Pardha. 2000. Transformation of brassica juncea with a

bacterial *cod A* gene enhances its tolerance to salt stress. J. Mol. Breed. 6(4): 489-499.

Raza S.H., H.U.R. Athar and M. Ashraf. 2006. Influence of exogenously applied Glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. Pak. J. Bot. 38(2): 241-251.

Raza M.A.S., M.F. Saleem, G.M. Shah, I.H. Khan and A. Raza. 2014. Exogenous application of glycinebetaine and potassium for improving water relations and grain yield of wheat under drought. J. Soil Sci. Plant Nutr. 14(2).

Rezaei M.A., B. Kaviani and A.K. Masouleh. 2012. The effect of exogenous glycine betaine on yield of soybean [*Glycine max* (L.) Merr.]in two contrasting cultivars Pershing and DPX under soil salinity stress.Plant.Omics. J. 5(2): 87-93.

Sakamoto A. and N. Murata. 2002. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant.Cell. Environ.25: 163-171.

Sakamoto A., Alia and N. Murata. 1998. Metabolic engineering of rice leading to biosynthesis of glycinebetaine and tolerance to salt and cold. Plant. Mol. Biol.38: 1011-1019.

Takabe T., Y. Hayashi, A. Tanaka, T. Takabe and S. Kishitani. 1998. Evaluation of glycinebetaine accumulation for stress tolerance in transgenic rice plants. In: Proceedings of International Workshop on Breeding and Biotechnology for Environmental Stress in Rice. Hokkaido Agricultural Experiment Station, Sapporo. pp. 63-68.

Tammam A.A., M.F. AbouAlhamd and M.M. Hemeda. 2008. Study of salt tolerance in wheat (*Triticumaestium*L.) cultivar Banysoif 1. Aust. J. Crop. Sci. 1:115-125.

Yancey P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero. 1982. Living with water stress: evolution of osmolyte systems. Science.217: 1214-1222.

Yang, X. and C. Lu. 2005. Photosynthesis is improved by exogenous glycinebetaine in salt stressed maize plants. Physiol. Plant. 1-10.doi:10.111/j.1399-3054.2005.00518.x.