

Response of cotton crop to exogenous application of glycinebetaine under sufficient and scarce water conditions

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Abstract Irrigation water is the basic resource to exploit the potential of cotton crop under an arid or a semi-arid environment. Under limited water conditions, various compatible solutes like glycinebetaine are being used to ameliorate the drought stress. Exogenous application of glycinebetaine (GB): GB₁ (100 mg L⁻¹), GB₀ (no application) was done in adequate water (DS₀) and in drought stress condition (DS₁) at arid environment during the growing seasons of years 2009 and 2010. Results demonstrated that application of GB improved the physiological processes of crop leading to better crop performance. Exogenous application of GB increased the drought tolerance with remarkable improvement in photosynthetic process and exhibited higher growth and yield. It was further noted that this technique was economically sustained; predicted higher radiation and water-use-efficiency; and can be applied in limited water condition to avoid yield losses.

Keywords Cotton · Glycinebetaine and drought · Growth · Yield

Introduction

Cotton crop is widely cultivated in more than 50 % countries of the world and is known as silver fiber in Pakistan due to its immense importance. Pakistan ranks fourth in production and third in consumption in the world.

The cotton crop is considered as an economic engine because it provides 60 % of the total foreign exchange earnings and employs more than 50 % labor force (Anonymous 2013).

Agricultural production systems in the world demands utilization of available resources with an efficient and effective approach. Productivity of all crops especially cotton in Pakistan has remained below compared to other cotton growing countries namely, Australia, South Africa, and China. Poor agronomic practices including various inputs like weather, land, genetic resources and water (Deng et al. 2004; Maqsood et al. 2006; Mahavishnan and Rekha 2007), nitrogen fertilizers (Ferrari et al. 2011), choice of the most promising cultivars (Killi et al. 2005; Wilde et al. 2008), and plant density (Kreig 1998) are of prime importance resources.

Water stress leads toward lower crop growth and yield which is a bigger threat under arid or semi-arid regions. Under these conditions, various emerging approaches are under study-like seed priming, exogenous application of growth regulators/compatible solutes and other growth tonics/micronutrients to ameliorate the effect of drought on crops (Azam et al. 2005; Mahmood et al. 2009; Sarwar et al. 2013). Among various compatible solutes, application of glycinebetaine is most likely to be used as it is highly effective to mitigate the effect of drought stress. Glycinebetaine stabilizes the enzymes, protein, and lipids involved in photosynthesis processes under drought stress (Khan et al. 2009; Hossian and Fujita 2010). Application of glycinebetaine improves the drought tolerance in plants and increases growth and yield of crops such as rice, wheat, sunflower, and maize (Farooq et al. 2008; Hussain et al. 2008). Accumulation of glycinebetaine varied among different crop cultivars and species, but it is also affected with environmental factors such as salinity, drought, and

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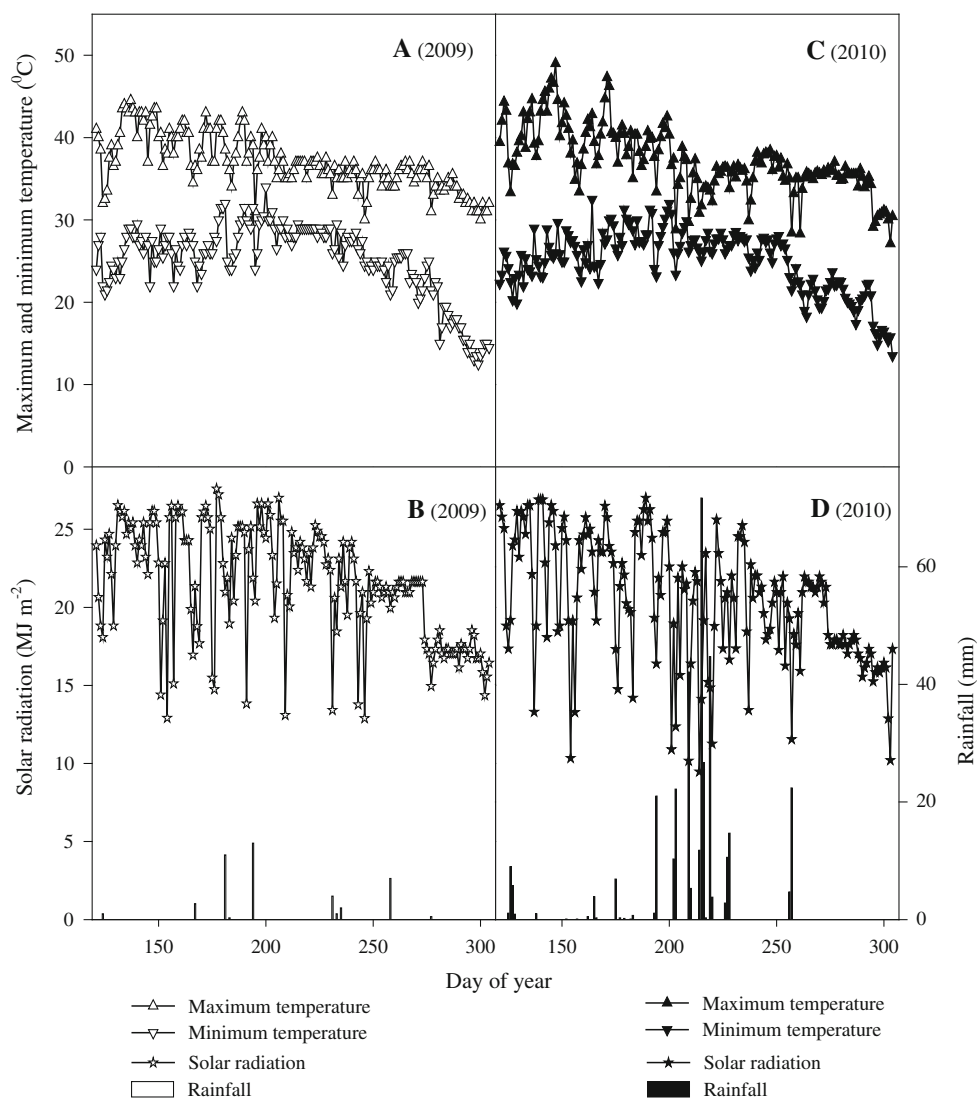
temperature (Moghaieb et al. 2004; Zhang et al. 2008; Cui et al. 2008; Shahbaz et al. 2011). Although it has been reported that glycinebetaine is naturally synthesized in plants under drought stress, the level achieved seems to be lower than the amount required to withstand water deficit condition (Subbarao et al. 2001; Martinez et al. 2005). Application of glycinebetaine has been tried on different crops for creating drought tolerance, but it is still contradictory whether biosynthesis mechanisms of plants is effective or exogenous application is required (Meek et al. 2003). Some researchers commented in favor of application of glycinebetaine for drought tolerance in cotton crop (Patil et al. 2011; Shallah et al. 2012), while in another study no significant crop performance was recorded with exogenous application of glycinebetaine (Meek et al. 2003). In Pakistan, cotton growers are facing the problem of water scarcity which is affecting crop growth, yield, and quality. The present study was therefore conducted with the

objective to verify its exogenous application of glycinebetaine ameliorates the water stress in cotton crop under water deficit condition.

Materials and methods

The study was conducted under field conditions at Central Cotton Research Institute, Multan, Pakistan during crop seasons 2009 and 2010 to quantify the impact of application of glycinebetaine on cotton under varying irrigation regimes. The experimental site is located at latitude $30^{\circ}12'N$, longitude $71^{\circ}28'E$, and altitude 123 m in the Punjab province. The site lies in the heart of cotton belt of the country. The area experiences greater diurnal fluctuations during summer and winter seasons. The climate is very hot, and mercury may rise to $48 \pm 2^{\circ}C$ and minimize from 8.0 to $14.0^{\circ}C$ during winter season. The weather

Fig. 1 Daily maximum and minimum air temperatures (A, C), rainfall, and solar radiation (B, D) during the cotton crop seasons 2009 and 2010 at Multan, Pakistan



remained favorable for production of cotton crop during the cropping seasons (Fig. 1). The soil is alluvial, calcareous, and alkaline in reaction (Table 3.1). The treatments consisted of two variables: (a) two watering regimes No drought stress (DS_0), at 50 % field capacity/drought stress (DS_1) and (b) exogenous spray of glycinebetaine untreated check (GB_0) Foliar application of 0.1 % Tween-80; glycinebetaine, 100 mg L⁻¹ (GB_1). The experimental plots were arranged in a split-plot design with four replications. The cotton genotypes CIM-496 was used as test crop. The crop was sown during second week of May 2010, crop seasons 2009–2010 and 2010–2011. The various standard tillage operations were performed for seed bed preparation to plant cotton crop. The crop was sown on bed-furrow planting technique as vogue in this area. The pre-emergence herbicide Pendimethalin 33 % at the rate of 2.5 L ha⁻¹ was sprayed before planting. The plant density was maintained at 45,000 plants ha⁻¹ at planting geometry of 75 cm between rows and 30 cm from plant to plant. The acid-delinted 25 kg cotton seed ha⁻¹ was drilled mechanically along the edges of the beds. The gap-filling and thinning operations were carried out at days 5 and 20 after sowing for maintaining the required plant density. The good agricultural practices were adopted by controlling infestation of insects-pests at below economic threshold level (ETL) by spraying recommended pesticides during the seasons. The weeds were controlled by inter-culturing manually and/or mechanically to avoid nutrient losses and refuge for insects-pests. The measured quantity of irrigation water as per treatment was applied using “Cut-Throat Flume”. The “cut-out” irrigation was done in mid-September during both the seasons. The crop was fertilized with basal dose of phosphorus (50 kg P₂O₅ ha⁻¹) in the form of triple superphosphate and potassium (50 kg K₂O ha⁻¹) in the form of potassium sulfate and nitrogenous fertilizers according to standards. The osmoprotectant glycinebetaine was exogenously applied at the rate of 100 mg L⁻¹ at days 30, 45, and 60 after sowing of the crop, and endogenous phenolic compounds were prepared in 0.1 % Tween-80.

For data recording, five plants from each respective treatment were selected on random basis and were tagged for calculating various observations on growth, seed cotton yield, and its components. The various technological properties of cotton lint, i.e., fiber length, fiber fineness, fiber strength, fiber uniformity ratio, were determined by employing High Volume Instrument (HVI), a Fiber Testing System, Zellweger Uster Ltd., Switzerland. After 70 days after sowing (DAS), fully expanded upper most leaves were collected from each treatment and were analyzed for glycinebetaine contents ($\mu\text{mol g}^{-1}$ LFW) following procedure of Grieve and Grattan (1983). Growth parameters: leaf area index (LAI) was calculated as the ratio of leaf

area to land area, and then LAD and NAR were calculated as suggested by Watson (1952) and Hunt (1978).

$LAI = \text{Leaf area} / \text{Land area}$

$LAD = (LAI_1 + LAI_2) \times (t_2 - t_1) / 2$

$NAR = TDM / LAD$

Intercepted radiation and radiation-use-efficiency

The fraction of intercepted radiation (F_i) was calculated from LAI using exponential equation suggested by Monteith and Elston (1983): $F_i = 1 - \exp(-k \times LAI)$, where k is an extinction coefficient for total solar radiation. The PAR intercepted was calculated by multiplying 0.5 to the total incident radiation (Szeicz 1974): $S_a = F_i \times S_i$, where S_i is the total amount of incident PAR. The radiation-use-efficiency (RUE) was calculated using the following formula: $RUE_{SC} = \text{Seed cotton} / \sum S_a$

Water use and water-use-efficiency

Actual cumulative crop evapotranspiration was estimated by multiplying potential evapotranspiration (PET) with appropriate value of crop coefficient, which usually corresponds closely to the green crop cover as suggested by Doorenboss and Pruitt (1977). Daily Penman's PET was calculated using standard program of “CROPWAT”. Water-use-efficiency (WUE) was calculated using the following formula: $WUE_{SC} = \text{Seed cotton} / CCET$ (kg ha⁻¹ mm⁻¹), where CCET is the cumulative crop evapotranspiration.

Statistical analysis

Data were analyzed using MSTAT-C statistical package on a computer (MSTAT Development Team 1989). The least significance differences (LSD) at 0.05 and 0.01 probability levels were applied to test the significance of treatments and means (Steel et al. 1997).

Results and discussion

Growth behavior

Chlorophyll content (SPAD)

Among the irrigation regimes, the crop which was water stressed showed a reduction of 4.85 % and 5.34 % in 2009 and 2010 compared to the fully irrigated crop. The application of glycinebetaine showed significant effect on the chlorophyll content as the SPAD values increased by the foliar-applied glycinebetaine. In 2009, maximum chlorophyll content (51.01) was recorded for exogenous

Table 1 Effect of irrigation regimes and glycinebetaine on growth parameters of cotton crop during the crop seasons 2009 and 2010

Treatments	SPAD value		LAI		LAD (days)		NAR ($\text{gm}^{-2}\text{day}^{-1}$)	
	2009	2010	2009	2010	2009	2010	2009	2010
Irrigation regimes								
DS ₀	46.21a	44.55a	3.91a	3.80a	297.51a	292.71a	3.44a	3.42a
DS ₁	43.97b	42.17b	3.38b	3.44b	244.98b	242.71b	3.31b	3.26b
LSD _{0.05}	0.36	0.79	0.12	0.36	7.16	7.13	0.09	0.11
Contrast (Linear)	**	**	*	**	**	**	**	**
Glycinebetaine								
GB ₁	51.01a	49.22a	3.75a	3.86a	279.65a	278.10a	3.53a	3.48a
GB ₀	39.17b	37.51b	3.54b	3.38b	262.84b	257.32b	3.22b	3.20b
LSD _{0.05}	5.82	5.89	0.15	0.25	5.05	5.91	0.19	0.18
Contrast (Linear)	**	**	**	**	**	**	**	**
Interaction	NS	NS	NS	NS	NS	NS	NS	NS

DS₀ no drought stress, DS₁ drought stress, GB₁ with glycinebetaine application, and GB₀ without glycinebetaine application

glycinebetaine-treated crop, whereas minimum (39.17) was noticed for untreated check. Crop on which glycinebetaine was applied exogenously attained greater chlorophyll content during the crop season 2009–2010 compared to 2010–2011 (Table 1).

Leaf area index

The results indicated that the maximum LAI increased steadily up till 105 days after sowing but thereafter showed a declined trend (Fig. 1). The water stress caused a decline of 13.55 and 9.47 % in 2009 and 2010 in maximum LAI compared to fully irrigated crop. With glycinebetaine application, the maximum LAI (3.75) was attained in 2009 by the crop under exogenously applied glycinebetaine treatments compared to untreated check (3.54). Moreover, foliar spray of glycinebetaine helped in simulating the crop toward attaining a higher LAI, and this response was more pronounced in 2009–2010 compared to 2010–2011 (Table 1)

Leaf area duration

The crop under stress reduced leaf area duration 17.65 % in 2009 and 17.08 % in year 2010 compared with fully irrigated crop. Likewise, the spray of glycinebetaine simulated the crop toward lengthening cumulative leaf duration. Greater cumulative leaf duration (279.65 and 278.10 days) was recorded under exogenously applied glycinebetaine treatments in 2009 and 2010, respectively (Table 1)

Net assimilation rate

Water-stressed crop showed a reduction of 3.78 and 4.68 % in net assimilation rate in 2009 and 2010 compared to the fully irrigated crop. A response similar to varying irrigation regimes was also noticed in case of glycinebetaine applied

exogenously. It is evident from the results that due to the better response of foliar-sprayed glycinebetaine regarding other parameters, a maximum net assimilation rate (3.53 and 3.48 $\text{g m}^{-2} \text{day}^{-1}$) was also recorded in the treatment with glycinebetaine sprayed exogenously (Table 1).

Yield and yield parameters

Number of bolls plant⁻¹

Data indicated that the number of bolls plant⁻¹ in 2009–2010 and 2010–2011 produced by the crop exposed to the water stress condition was 26.78 and 23.49, respectively. In contrast, the treatment with no water stress attained 15.7 and 3.6 % greater number of bolls plant⁻¹ in 2009–2010 and 2010–2011, respectively, compared to the water stressed crop. Exogenous application of glycinebetaine led to maximum number of bolls plant⁻¹ (27.29 and 25.04) which was 19.6 and 15.61 % higher than untreated crop (Table 2).

Seed cotton yield

The water stress caused a reduction of 17.88 and 17.67 % in seed cotton yield compared to fully irrigated crop in 2009 and 2010. Crop attained greater seed cotton yield under exogenously applied glycinebetaine treatments compared to untreated check. Moreover, the spray of glycinebetaine simulated the crop toward attaining higher seed cotton yield (2264 and 2211 kg ha^{-1}) compared to untreated crop (1861 and 1658 kg ha^{-1}) (Table 2).

Lint yield

Highest lint yield (953 and 878 kg ha^{-1}) was obtained under full water condition compared to the water deficient crop (769 and 724 kg ha^{-1}) in 2009 and 2010. In case of glycinebetaine application, a reduction of 21.18 % and

Table 2 Effect of irrigation regimes and glycinebetaine on yield and yield parameters of cotton crop during the crop seasons 2009 and 2010

Treatments	No. of Bolls plant ⁻¹		Seed cotton yield (kg ha ⁻¹)		Lint Yield (kg ha ⁻¹)		RUE (g MJ ⁻¹)		WUE (kg ha ⁻¹ mm ⁻¹)	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Irrigation regimes										
DS ₀	26.78a	23.49a	2265a	2122a	953a	878a	0.191a	0.179a	3.35a	3.18a
DS ₁	23.14b	22.68b	1860b	1747b	769b	724b	0.171b	0.164b	2.76b	2.62b
LSD _{0.05}	1.69	0.40	168.45	107.42	102.70	52.82	0.012	0.009	0.25	0.16
Contrast (Linear)	**	*	**	**	**	**	*	*	**	**
Glycinebetaine										
GB ₁	27.29a	25.04a	2264a	2211a	963a	934a	0.196a	0.192a	3.35a	3.32a
GB ₀	22.62b	21.13b	1861b	1658b	759b	669b	0.166b	0.150b	2.76b	2.49b
LSD _{0.05}	1.81	2.25	169.50	126.31	78.28	44.64	0.016	0.006	0.25	0.19
Contrast (Linear)	**	**	**	**	**	**	**	**	**	**
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

DS₀ no drought stress, DS₁ drought stress, GB₁ with glycinebetain application, and GB₀ without glycinebetaine application

Table 3 Effect of irrigation regimes and glycinebetaine on biophysical characteristics of cotton crop during the crop seasons 2009 and 2010

Treatments	Net photosynthetic rate (μmol m ⁻² s ⁻¹)		Transpiration rate (m mol m ⁻² s ⁻¹)		Intercellular CO ₂ concentration (mmol m ⁻² s ⁻¹)		Stomatal conductance (mmol (CO ₂) m ⁻² s ⁻¹)	
	2009	2010	2009	2010	2009	2010	2009	2010
Irrigation regimes								
DS ₀	31.55a	29.71a	6.09a	6.18a	561.38a	556.63a	318.75a	311.25a
DS ₁	20.12b	19.51b	5.15b	4.92b	502.63b	498.00 b	296.25b	290.88b
LSD _{0.05}	1.21	1.73	0.41	0.45	52.28	52.15	6.90	7.61
Contrast (Linear)	**	**	**	**	*	*	**	**
Glycinebetaine								
GB ₁	29.50a	27.53a	5.97a	5.84a	591.63a	585.75a	321.63a	314.38a
GB ₀	22.18b	21.70b	5.28b	5.27b	472.38b	468.88b	293.38b	287.75b
LSD _{0.05}	1.61	1.66	0.43	0.26	45.95	42.44	9.39	9.61
Contrast (Linear)	**	**	**	**	**	**	**	**
Interaction	NS	NS	NS	NS	NS	NS	NS	NS

DS₀ no drought stress, DS₁ drought stress, GB₁ with glycinebetain application, and GB₀ without glycinebetaine application

28.37 % was recorded in the untreated plants in 2009 and 2010 (Table 2).

Biophysical characteristics

Net photosynthetic rate

It is evident from the results that maximum net photosynthetic rates 31.55 and 29.71 μmol m⁻²s⁻¹ were recorded in plants grown under optimum water condition, while minimum net photosynthetic rates (20.12 and 19.81 μmol m⁻²s⁻¹) were found in water deficient crop during 2009 and 2010, respectively. The effect of exogenously applied glycinebetaine corresponded to a net

photosynthetic rate approximately 25 % higher over the untreated plants in 2009 and 2010 (Table 3).

Transpiration rate

Crop plants which were grown under well water condition recorded higher transpiration rate (6.09 and 6.18 m mol (H₂O) m⁻²s⁻¹) compared with plants grown under drought stress in 2009 and 2010. Furthermore, exogenously applied glycinebetaine at the rate of 3 kg ha⁻¹ imparts significant effect on transpiration rate. During 2009, the highest transpiration rate (5.97 m mol (H₂O) m⁻²s⁻¹) was noticed in case of crop on which glycinebetaine was sprayed compared to untreated check which

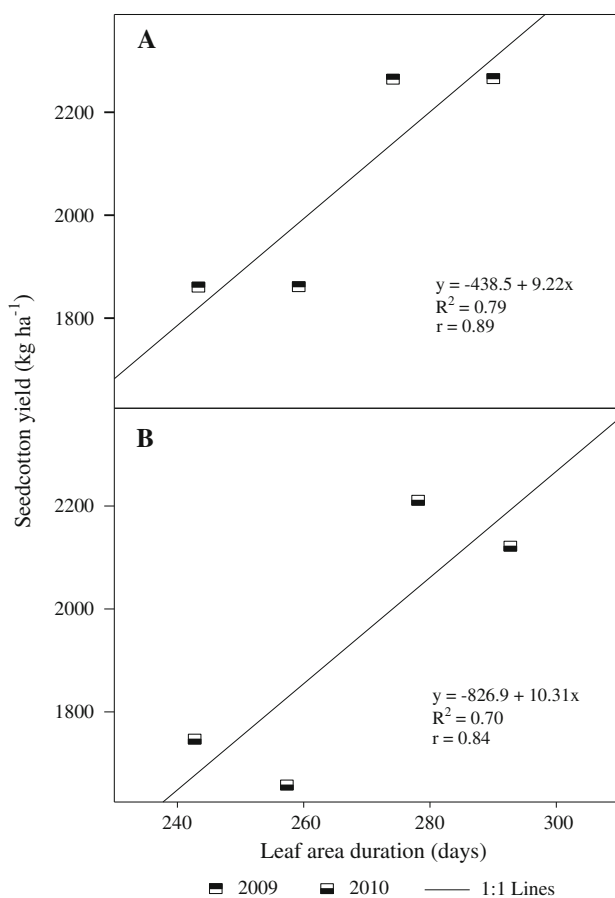


Fig. 2 Relationship between leaf area duration (days) and seed cotton yield (kg ha^{-1}) during 2009 (A) and 2010 (B)

had the lowest transpiration rate ($5.28 \text{ m mol (H}_2\text{O) m}^{-2}\text{s}^{-1}$) (Table 3).

Intercellular CO_2 concentration

In 2009, intercellular CO_2 was 10.46 % higher in crop grown under well water condition than water-stressed plants. It is also evident from the results that during 2009, foliar application of glycinebetaine at the rate of 3 kg ha^{-1} showed 20.1 % increase in intercellular CO_2 concentration compared to the untreated check. Similar type of observation was seen in next year regarding both treatments (Table 3).

Stomatal conductance

The crop receiving an adequate amount of irrigation water and/or not facing any water shortage attained 7.06 and 6.54 % greater stomatal conductance compared to water-stressed crop. The water stress caused a remarkable reduction in stomatal conductance compared to fully irrigated crop. Likewise, during 2009, remarkable increase of 8.78 % in stomatal conductance was noticed due to the

exogenously applied glycinebetaine compared to the untreated check (Table 3).

Glycinebetaine ($\mu\text{mol g}^{-1} \text{ LFW}$)

Data showed that different treatments significantly varied the internal plant status of glycinebetaine contents. Under drought stress, plants significantly increased the level of glycinebetaine contents compared to sufficiently irrigated plants. Similarly, the exogenous application of glycinebetaine improved the plant glycinebetaine contents (Figs. 3, 4).

Physiological efficiency

Radiation-use-efficiency

Comparing the two irrigation regimes, it was noticed in 2009 that water-stressed crop showed 10.47 % reduction in RUE for seed cotton yield compared to the fully irrigated crop. Foliar application of glycinebetaine also significantly enhanced RUE and recorded maximum value (0.196 g MJ^{-1}) compared to the untreated check (0.166 g MJ^{-1}) (Table 2).

Water-use-efficiency

The crop matured under optimum water condition used 2945 m^3 water, while 1917 m^3 irrigation water was given to crop grown under water stress. Results showed that in 2009, adequate irrigation helped in producing maximum WUE for seed cotton yield ($3.35 \text{ kg ha}^{-1} \text{ mm}^{-1}$) compared to the water-stressed crop which had minimum WUE for seed cotton yield ($2.76 \text{ kg ha}^{-1} \text{ mm}^{-1}$). Comparing the effect of exogenous applied glycinebetaine, it was noticed that foliar-applied glycinebetaine simulated the crop in attaining 22.38 % higher WUE for seed cotton yield compared to the untreated check (Table 2).

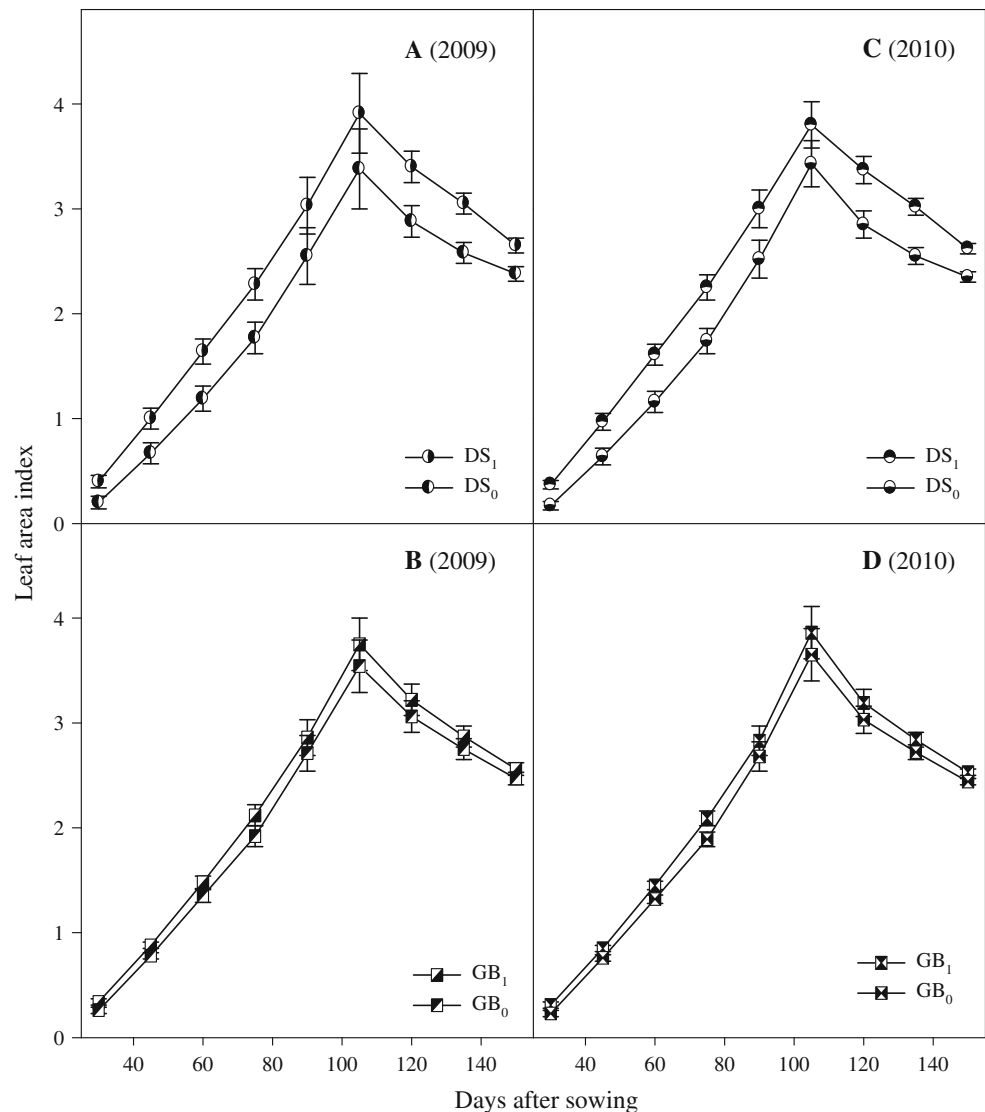
Economic analysis

Data for economic analysis showed that crop grown under well water condition was found to be economically superior compared with water deficit crop. Exogenous application of glycinebetaine also enhanced the productivity of crop therefore resulting in greater gross and net returns during both the crop seasons (Table 4).

Discussion

Experimental results showed that crop matured under sufficient water condition and with glycinebetaine application performed well compared with deficient crop. As water is a

Fig. 3 Seasonal leaf area index as affected by irrigation regimes (A, C) and glycinebetaine (B, D) during 2009 and 2010. Bars represent LSD values at 5 %



limited source in arid or semi-arid regions, various techniques such as exogenous application of growth hormones, soluble salts, and micronutrients are being used to ameliorate the water stress in plants. Our experiment showed that application of glycinebetaine was found to be an efficient means for enhancing drought tolerance under water deficit condition. Glycinebetaine is generally absorbed by the leaves and enables its stability, representing protective capability on long-term basis. Plants attained full water condition for their maturity, or drought tolerant showed higher photosynthetic ability, transpiration rate, and improved intercellular CO₂ concentration. Overall crop yield is also related with physiological performance or how plants are assimilating CO₂, water, or nutrients. Higher transpiration rate also increases the uptake of nutrients through transpiration pull which in turn improves the crop growth and yield/quality. Under drought stress, cotton plant

biosynthesizes glycinebetaine which increases the plant tolerance that is a good mechanism and have potential for further improvement by exogenous application. Our experimental results revealed that plant under drought stress and with exogenous application of glycinebetaine (GB) increased the biosynthesis of GB which in turn enhanced the ability of crop to withstand drought condition (Fig. 4).

Exogenous application of glycinebetaine increased its concentration in plant and plant tolerated water deficiency, as all biophysical characteristics were improved. Reduction in photosynthesis (Sikuku et al. 2010) and intercellular CO₂ concentration (Esha 2011) will ultimately reduce the crop performance under water stress condition. Improvement in crop growth performance with glycinebetaine might be due to the effective exchange of gasses and higher photosynthetic rate. Positive correlation

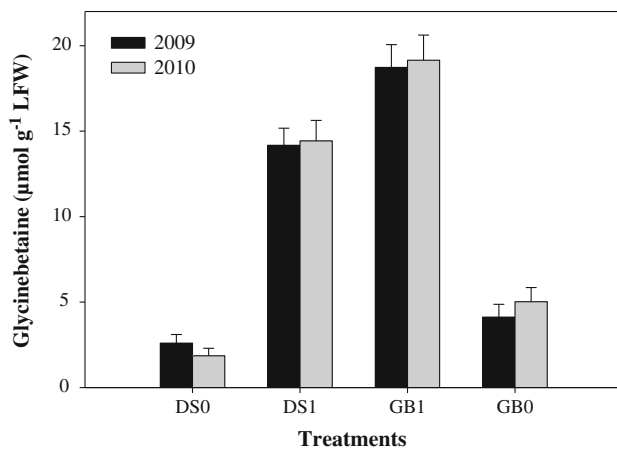


Fig. 4 Leaf Glycinebetaine (GB) contents (70DAS) as affected by exogenous application of GB under different water regimes

was observed among LAI and seed cotton yield (Fig. 2). Previous research work also elaborated that application of glycinebetaine fasten the CO_2 assimilation and gas exchange system which leads toward better crop growth/ yield (Makela et al. 1998; Shallah et al. 2012)

In addition, adequate water supply and exogenous application of glycinebetaine resulted in intercepting and absorbing photosynthetically active radiation to the greater extent and therefore resulting in recording higher RUE. On the other hand, water stress showed remarkable decrease in these characteristics similar to that reported in case of no foliar-applied glycinebetaine. Exogenously applied glycinebetaine

resulted in improved plant growth and yield of cotton (Gorham et al. 2000). Similarly, an increase in seed cotton yield and its components such as number of flower buds plant^{-1} , bolls plant^{-1} , and boll weight due to the glycinebetaine accumulation under water stress conditions was reported by Sarwar et al. (2006), and these findings corroborate the results of the present study. The reason for higher seed cotton yield might be due to the exogenous application of glycinebetaine helped in maintaining osmotic potential, increased turgor pressure, and thus resulted in the reduction of water loss which ultimately leads to better crop production.

Water-use-efficiency is an important factor regarding efficient crop of the applied water. In developing countries like Pakistan, water input is a precious source and is becoming more precious due to its increasing shortage (Sarwar et al. 2013). Reduced water inputs can also be eliminated with foliar application of growth tonics, soluble salts etc. Our experiment highlighted that application of glycinebetaine increases the WUE which might be due to the improved physiological mechanism of plants grown under drought stress. Adoption of any application/technique will ultimately depend on money involved which is a crucial factor in developing countries. This experiment showed that exogenous application of glycinebetaine is economically sustained as it recorded higher benefits under application in both years. Farmers can increase the crop yield with little extra cost by glycinebetaine application. Moreover, it is an efficient technique to increase crop tolerance under arid or semi-arid zone.

Table 4 Economic analysis of cotton as affected by various irrigation regimes and exogenous application of glycinebetaine

Treatments	Total variable cost (Rs ha^{-1})	Gross return (Rs ha^{-1})	Total cost of cultivation (Rs ha^{-1})	Net income (Rs ha^{-1})	Benefit cost ratio
2009–2010					
Irrigation Regime					
DS ₀	6919	120045	83792	36253	1:43
DS ₁	6064	98580	82937	15643	1:19
Glycinebetaine					
GB ₁	8500	119992	82123	37869	1:46
GB ₀	–	98633	73623	25010	1:34
2010–2011					
Irrigation Regime					
DS ₀	7907	225738	106208	119530	2:12
DS ₁	6919	185846	105220	80626	1:77
Glycinebetaine					
GB ₁	9000	235206	104301	130905	2:26
GB ₀	–	176378	95301	81077	1:85

DS₀ no drought stress, DS₁ drought stress, GB₁ with glycinebetaine application, and GB₀ without glycinebetaine application

References

- Anonymous (2013). Economic Survey of Pakistan. Government of Pakistan, Finance Division, Economic Advisor's Wing, Islamabad, pp 15.
- Azam MM, Waris A, Nahar NM (2005) Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. *Biomass Bioenergy* 29:293–302
- Cui XH, Fu-Shun H, Chen H, Chen J, Wang XC (2008) Expression of the *Vicia faba* VFPIP gene in *Arabidopsis thaliana* plant improves their drought resistance. *J Plant Res* 121:207–214
- Deng XP, Shan L, Zhang H, Turner NC (2004). Improving agriculture water-use efficiency in arid and semi arid areas of China. In: Proceedings of the 4th International Crop Science Congress Brisbane: 1–14.
- Doorenboss J, Pruitt WO (1977). Guidelines for predicting Crop Water Requirements. Irrigation and Drainage paper 24, Food and Agriculture Organization of the United Nations, Rome.
- Esha BS (2011). Mechanism of drought tolerance in cotton-Response of cotton cultivars to irrigation in the Texas high plains. MSc. Agricultural Thesis in Texas Technical University, Texas.
- Farooq M, Basra SMA, Wahid A, Cheema ZA, Cheema MA, Khaliq A (2008) Physiological role of exogenously applied glycinebetaine to improve drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *J Agron Crop Sci* 194:325–333
- Ferrari S, Junior EF, Ferrari JV, Pereira GA (2011) Cotton development and yield according to nitrogen application and cover crops. *Ciênc Agrár Londrina* 32:1405–1416
- Gorham J, Jokinen K, Malik MNA, Khan IA (2000). Glycinebetaine treatment improves cotton yields in field trials in Pakistan. In: Proceedings of the World Cotton Research Conference II, Athens, pp 624–627
- Grieve CM, Grattan SR (1983) Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil* 70:303–307
- Hossain MA, Fujita M (2010) Evidence for a role of exogenous glycinebetaine and proline in antioxidant defence and methylgl-oxal detoxification system in mungbean seedlings under salt stress. *Physiol Mol Biol Plants* 16:19–29
- Hunt R 1978. *Plant Growth Analysis*. The Inst. of Bio. Studies in Bio. Edward Arnold (Pub.) Ltd., 96: 8–38.
- Hussain M, Malik MA, Farooq M, Ashraf MY, Cheema MA (2008) Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J Agron Crop Sci* 194:193–199
- Khan MA, Shizari MU, Khan MA, Mujtaba SM, Islam E, Mumtaz S, Shereen A, Ansari RU, Ashraf MY (2009) Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pak J Bot* 41:633–638
- Killi F, Efe L, Mustafayev S (2005) Genetic and environmental variability in yield, yield components and lint quality traits of cotton. *Int J Agri Biol* 7:1007–1010
- Kreig DR 1998. Interaction between plant density and water supply affects cotton yield. *Fluid J* 1–4.
- Mahavishnan K, Rekha KB (2007) Response of cotton (*Gossypium hirsutum* L.) to inorganic nitrogen, legume incorporation and FYM- A Review. *Agri Revolut* 28:289–294
- Mahmood T, Ashraf M, Shahbaz M (2009) Does exogenous application of glycinebetaine as a pre-sowing seed treatment improve growth and regulate some key physiological attributes in wheat plants grown under water deficit conditions? *Pak J Bot* 41:1291–1302
- Makela P, Munns R, Colmer TD, Cundon AG, Peltonen-Sainio P (1998) Effect of foliar applications of glycinebetaine on stomatal conductance, abscisic acid and solute concentrations of salt and drought stressed tomato. *Aust J Plant Physiol* 25:655–663
- Maqsood M, Hussain T, Tayyab M, Ibrahim M (2006) Effect of different irrigation levels on the yield and radiation-use-efficiency of cotton (*Gossypium hirsutum* L.) under two sowing methods. *Pak J Agri Sci* 43:21–24
- Martinez AT, Speranza M, Ruiz-Duenas FJ, Ferreira P, Camarero S, Ghillen F, Martinez MJ, Del Gutierrez A, Rio JC (2005) Biodegradation of lignocelluloses: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *Int J Microbiol* 8:195–204
- Meek C, Oosterhuis D, Gorham J (2003) Does foliarapplied glycinebetaine affect endogenous betaine levels and yield in cotton? *Crop Manag.* doi:10.1094/CM-2003-0804-02-RS
- Moghaieb REA, Saneoka H, Fujita K (2004) Effect of salinity and osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *saueda maritima*. *Plant Sci* 166:1345–1349
- Monteith JL, Elston JF (1983). Performance and productivity of foliage in field. In the growth and functioning of leaves. Dale JE, Milthorpe FL (eds) Cambridge University Press, Butterworths, Lord. pp 499–518.
- MSTAT Development Team (1989). MSTAT user's guide. A micro computer program for the design and analysis of agronomic research experiments. Michigan State University, East Lansing
- Patil MP, Biradar DP, Patil VC, Janagoudar BS (2011) Response of cotton genotypes to drought mitigation practices. *Am Eurasian J Agri Environ* 11:360–364
- Sarwar MKS, Ullah I, Rehman MU, Ashraf MY, Zafar Y (2006) Glycinebetaine accumulation and its relation to yield and yield components in cotton genotypes grown under water deficit condition. *Pak J Bot* 38:1449–1456
- Sarwar N, Ali H, Maqsood M, Ullah E, Shahzad M, Mubeen K, Shahzad AN, Shahid MA, Ahmad S (2013) Phenological response of rice plants to different micronutrients application under water saving paddy fields on calcareous soil. *Turkish J Field Crops* 18:52–57
- Shahbaz M, Masood Y, Perveen S, Ashraf M (2011) Is foliar applied glycinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.). *J Appl Biol Food Quality* 84:192–199
- Shallan MA, Hassan HMM, Namich AAM, Ibrahim AA (2012) Effect of sodium, nitroprusside, putrescine and glycinebetaine on alleviation of drought stress in cotton plant. *Am Eurasian J Agri Environ* 12:1252–1265
- Sikuku PA, Netond GW, Onyango JC, Musyimi DM (2010) Effects of water deficit on physiology and morphology of three varieties of nerica rain-fed rice (*Oryza sativa* L.). *ARPJ Agri Biol Sci* 5:23–29
- Steel RGD, Torrie JH, Dickey DA 1997. *Principles and Practices of Statistics, A Biometric Approach*. 3rd Edition. McGraw Hill, International Book Co, Inc, Singapore.
- Subbarao GV, Wheeler RM, Levine LV, Stutte JW (2001) Glycinebetaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *J Plant Physiol* 158:767–776
- Szeicz G (1974) Solar radiation for plant growth. *J Appl Ecol* 11:617–636
- Watson D (1952) Comparative physiological studies on the growth of field crops. I. variation in net assimilation rate and leaf area between species and varieties and within and between years. *Ann Bot* 11:41–76
- Wilde C, Johnson J, Keeling W, Johnson P (2008) Effect of cotton variety selection and irrigation levels on economic returns: Texas Southern High Plains. Beltwide Cotton Conf. Nashville, Tennessee, Jan, pp 8–11
- Zhang Y, Hu W, Gao Y, Yao Y, Tang M, Hu G (2008) Fertilizing irrigated cotton for high yield and high nitrogen use efficiency. *Better Crops* 92:6–7