▼ Journal of Research (Science), Bahauddin Zakariya University, Multan, Pakistan.
Vol. 18, No. 1, January 2007, pp. 13-17 ISSN 1021-1012

INFLUENCE OF FOLIAR APPLICATION OF GLYCINE BETAINE ON GAS EXCHANGE CHARACTERISTICS OF COTTON (*GOSSYPIUM HIRSUTUM* L.)

Muhammad Iqbal Makhdum and Shabab-ud-Din
Central Cotton Research Institute, Multan

Abstract

Water is the most limiting factor in cotton production and numerous efforts are being made to improve crop drought tolerance. A field study was conducted with the objectives to determine the effects of different application rates of glycine betaine in field grown cotton at Central Cotton Research Institute, Multan. Four levels of glycine betaine (0.0, 1.0, 3.0 and 6.0 kg ha^{-1}) were applied at three physiological growth stages i.e. at squaring, first flower and peak flowering. Cotton cultivar CIM-448 was used as test crop. Results showed that crop sprayed with glycine betaine at the rate of 6.0 kg ha^{-1} maintained 120.0, 62.1, 69.7 and 35.5 percent higher net CO_2 assimilation rate (P_N), transpiration rate (E), stomatal resistance (gs) and water use efficiency (P_N/E), respectively over that of untreated crop. Crop spayed with glycine betaine at peak flowering stage maintained higher P_N, E, gs and P_N/E compared to at other stages of growth.

Keywords: Cotton, foliar application, gas exchange, glycine betaine.

INTRODUCTION

The plants lack the capability of locomotion as a means of responding to changes in their environment. They are exposed to various environmental stresses and must adapt to them in other ways. The most typical kind of stress plants receive from their surroundings is water stress and temperature stress. The severity of stresses experienced by plants varies both spatially and temporally at several different scales.

Water availability often exerts more pressure on the survival of arable crops than any other single environmental factor. Higher plants are usually faced with some degree of water stress during development [Morgan 1984]. Plants attempt to tolerate or resist stress due to decreased water availability by making osmotic adjustments to cells through the increase in inorganic ions or organic solutes [Hendrix and Pierce 1983]. Recently, the quaternary ammonium compound, i.e., glycine betaine has received attention as a compatible osmolyte [Makela *et al*. 1996, Agboma *et al*. 1997]. Glycine betaine has been exogenously applied to many non-accumulating and accumulating crops in an effort to improve stress tolerance and yield. The various researchers reported a positive response in offsetting the water stress on maize and sorghum [Agboma *et al*. 1997] and cotton [Gorham and Jokinen 1998]. Meek *et al*. [1999] reported that glycine betaine treated cotton plants had significantly higher mid-season boll numbers, stomatal resistance rates, number of effective sympodia and boll retention in the second fruiting positions. However, no significant differences were observed between treatments in yield measurements at time of harvest. Results are varied and appear to depend on numerous factors such as type of crop, timing and rate of application, and environmental conditions. More research needs to be conducted to quantify the effects of exogenous application of glycine betaine on yield of crop plants. Therefore, the objective of the present study was to determine the effects of different application rates of glycine betaine and time of foliar application on gas exchange characteristics of cotton crop under an arid environment.

MATERIALS AND METHODS

Field studies on cotton cultivar CIM-448 (*Gossypium hirsutum* L.) were conducted during the cotton season 2002-2003 at the experimental farm of Central Cotton Research Institute, Multan. Cotton planting was carried out in mid-May on silt loam soil. The plot size was 90 m^2 and planting intensity was approximately 42000 ha^{-1} in rows 75 cm apart. The crop was irrigated and received 150 kg N, 50 kg P_2O_5 and 50 kg K_2O ha^{-1}. All phosphorus, potassium and 1/3 nitrogen were applied at the time of planting and remaining 2/3 of nitrogen applied at squaring and flowering stages in two equal split doses. Treatments were replicated four times in a split plot design and consisted of an untreated control and glycine betaine sprayed at the rates of 1.0, 3.0, 6.0 kg ha^{-1}. The foliar application of glycine betaine was done at three stages of growth i.e. squaring, first flower and peak flowering. The doses of glycine betaine were kept in main plots. The crop was sprayed with a knapsack sprayer using two nozzles per row and operated at 4-km ha^{-1} using 275 k Pa pressure to deliver 200 l ha^{-1}. Standard production practices were followed throughout the growing season.

The instantaneous measurements of net photosynthetic rate and transpiration rate were made on fully expanded youngest leaves of 10 plants (4^{th} leaf from top) using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Measurements were performed when plants were 60-d old at 11:00 with the following conditions: molar flow of air per unit leaf area 408.5 m mol m^{-2} s^{-1}, atmospheric pressure 97.8 kPa, water vapor pressure inside chamber 1120-1220 Pa, photosynthetic active radiation (PAR) at leaf surface was maximum up to 1280 µmol m^{-2} s^{-1}, temperature of leaf was maximum up to 34.4°C, ambient temperature (32.3-33.9°C), ambient CO_2 concentration (351.3 µmol mol^{-1}). Stomatal resistance measurements were made with automatic porometer MK-3 (Delta-T Devices, Burnwell, Cambridge, England). Water-use efficiency (WUE) was computed [WUE = net photosynthesis (µmol (CO_2) m^{-2} s^{-1}) / transpiration rate (mmol (H_2O) m^{-2} s^{-1})]. Data were analyzed statistically according to the methods described by [Gomez and Gomez 1984].

RESULTS AND DISCUSSION

Data for net assimilation rate (P_N) differed significantly due to different levels of glycine betaine and time of application (Table 1). The P_N increased with increasing levels of glycine betaine. Averaged across the time of application, the maximum P_N [24.2 μ mol (CO_2) m^{-2} s^{-1}] was recorded in crop treated at the rate of 6.0 kg ha^{-1} glycine betaine. The P_N values ranged from 11.0 to 24.2 μ mol (CO_2) m^{-2} s^{-1}. Averaged across the levels of glycine betaine, P_N increased with ontogeny. The maximum P_N [18.8 μ mol (CO_2) m^{-2} s^{-1}] was observed at peak flowering stage and minimum [16.5 μ ml (CO_2) m^{-2} s^{-1}] at squaring stage. There was significant interaction between doses of glycine betaine and time of application. This show that glycine betaine at the rate of 6.0 kg ha^{-1} sprayed at peak flowering stage increased P_N substantially. Meek *et al*. [1999] reported that foliar sprays of glycine betaine on cotton caused increase in P_N. They explained that accumulation of glycine betaine by plant through biochemical mechanism made the plant to withstand environmental stresses and thus this situation resulted in increased P_N.

Table 1: Effect of Different doses of Glycine Betaine and Time of Application on Net Assimilation Rate (P_N) [μ mol (CO_2) m^{-2} s^{-1}].

Glycine Betaine (kg ha^{-1})	Time of Foliar Application			Mean
	Squaring	First flower	Peak flowering	
0	9.3	11.2	12.6	11.0
1.0	14.5	15.0	16.1	15.2
3.0	18.9	20.3	21.1	20.1
6.0	23.3	24.1	25.3	24.2
Mean	16.5	17.7	18.8	
LSD (p<0.05)	Doses	Time of application	Interaction	
	0.26**	0.34**	0.67*	

*, ** = significant at the 0.05 and 0.01 probability levels, respectively.

The significant differences in transpiration rate (*E*) were observed in levels of glycine betaine and time of application (Table 2). The *E* increased with increasing doses of glycine betaine. Averaged across time of application, the maximum *E* [5.51 m mol (H_2O) m^{-2} s^{-1}] was recorded in the crop, which was foliated with 6.0 kg ha^{-1} glycine betaine. The values ranged from 3.40 to 5.51 m mol (H_2O) m^{-2} s^{-1}. Averaged across the levels of glycine betaine, maximum *E* (4.79 m mol H_2O m^{-2} s^{-1}) was observed at peak flowering stage compared to minimum [4.35 m mol (H_2O) m^{-2} s^{-1}] at squaring stage. There was significant interaction between doses of glycine betaine and time of application. These data show that glycine betaine at the rate of 6.0 kg ha^{-1} sprayed at peak flowering stage resulted in increased *E*. These results agree with those of [Meek *et al*. 1999] and Makhdum *et al*. [2004] who reported that cotton plant made osmotic adjustments by accumulating glycine betaine that resulted in increased *E*.

Table 2: Effect of Different Levels of Glycine Betaine and Time of Application on Transpiration Rate (*E*) [mmol (H_2O) m^{-2} s^{-1}].

Glycine Betaine (kg ha^{-1})	Time of Foliar Application			Mean
	Squaring	First flower	Peak flowering	
0	3.07	3.41	3.71	3.40
1.0	4.00	4.30	4.55	4.28
3.0	4.93	5.08	5.24	5.08
6.0	5.39	5.51	5.64	5.51
Mean	4.35	4.58	4.79	
LSD (p<0.05)	Doses	Time of spray	Interaction	
	0.07**	0.05**	0.05**	

**= significant at the 0.01 probability level.

Table 3: Effect of different Doses of Glycine Betaine and Time of Application on the Stomatal Resistance (s cm^{-1})

Glycine Betaine (kg ha^{-1})	Time of Foliar Application			Mean
	Squaring	First flower	Peak flowering	
0	7.06	7.31	7.67	7.35
1.0	6.11	6.47	6.85	6.48
3.0	5.09	5.49	5.75	5.44
6.0	4.10	4.30	4.59	4.33
Mean	5.59	5.89	6.22	
LSD ($p<0.05$)	Doses 0.04**	Time of application 0.03**	Interaction 0.06**	

**= significant at the 0.01 probability level.

Data for stomatal resistance differed significantly due to levels of glycine betaine and time of application (Table 3). Stomatal resistance decreased with increasing levels of glycine betaine. Averaged across levels of glycine betaine, the minimum stomatal resistance of 4.33 s cm^{-1} was recorded in the crop treated with glycine betaine at the rate of 6.0 kg ha^{-1}. The values ranged from 4.33 to 7.35 s cm^{-1}. However, stomatal resistance increased with the advancement of age. Averaged across the levels of glycine betaine, the crop maintained stomatal resistance at 5.59 s cm^{-1} at squaring phase compared that of 6.22 s cm^{-1} at peak flowering stage. The similar results have been reported by [Meek *et al*. 1999] that cotton plant treated with glycine betaine had significantly higher stomatal resistance compared to untreated crop. Gorham and Jokinen [1998] also reported that foliar application of glycine betaine on cotton increased stomatal resistance, which made crop to economize water.

Table 4: Effect of Different Doses of Glycine Betaine and Time of Application on Water use Efficiency (P_N/E) [µ mol (CO_2) m^{-2} s^{-1}/ mmol (H_2O) m^{-2} s^{-1}].

Glycine Betaine (kg ha^{-1})	Time of Foliar Application			Mean
	Squaring	First flower	Peak flowering	
0	3.03	3.28	3.40	3.24
1.0	3.63	3.49	3.54	3.55
3.0	3.83	4.00	4.03	3.95
6.0	4.32	4.37	4.49	4.39
Mean	3.70	3.78	3.87	
LSD($p<0.05$)	Doses 0.14**	Time of application 0.08**	Interaction 0.09 ^{ns}	

ns= significant at the 0.05 probability level.
**= significant at the 0.01 probability level.

The water use efficiency (WUE) [P_N/E] differed significantly due to different doses of glycine betaine and time of application (Table 4). Averaged across time of foliar application, exogenous glycine betaine improved WUE significantly. Maximum P_N/E of 4.39 was recorded in crop sprayed at the rate of 6.0 kg ha^{-1} glycine betaine. There was 35.5 percent higher P_N/E in crop sprayed with 6.0 kg ha^{-1} glycine betaine compared to untreated crop. Averaged across does of glycine betaine, maximum WUE was achieved by foliar application of glycine betaine at peak flowering stage. There was no significant interaction between different doses of glycine betaine and time of foliar application. The significant increase in WUE resulted due to enhanced accumulation of glycine betaine by the plant, which resulted in combating the environmental stress during the growth period. Some earlier studies [Hendrix and Pierce 1983, Naidu *et al*. 1998, Meek *et al*., 1999, Makhdum *et al*. 2004] also support these data. Naidu *et al*. [1998]

reported that plants accumulate betaines through a biochemical mechanism which improves the ability of plants to withstand stresses.

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