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Influence of exogenous glycinebetaine on hot pepper under water stress

R.M. Bhatt, N.K. Srinivasa Rao and A.D.D.V.S. Nageswara Rao
Division of Plant Physiology and Biochemistry
ICAR-Indian Institute of Horticultural Research
Hesaraghatta Lake Post, Bengaluru - 560 089, India
E-mail: rmbt@iihr.ernet.in

ABSTRACT

A study was conducted to evaluate the effect of exogenous application of glycinebetaine (GB) on physiological response in hot-pepper (*Capsicum annuum* L. vs. Arka Lohit and Pusa Jwala) under water stress. Glycinebetaine was applied to seeds as well as plants through foliar applications. Water stress affected considerably the morpho-physiological parameters in both the cultivars. However, in glycinebetaine (GB) treated plants, plant height, leaf area (LA), flower and fruit number and total dry matter (TDM) were greater compared to the untreated stress plants (T4) under water stress. Glycinebetaine application enhanced the photosynthesis (P_N) in water deficit experiencing plants, mostly due to a greater stomatal conductance (g_s) and carboxylation efficiency of CO_2 assimilation. In both the cultivars after 12 day of stress, the P_N decreased from 10.1 to 1.0-1.3 µ mol m^{-2} s^{-1} in untreated stressed plants (T4), while in the treated stressed plants P_N had reduced to 2.0 – 3.0 µ mol m^{-2} s^{-1} (T1 – T3). The application of GB increased the WUE in both the cultivars. The better WUE in treated plants of hot-pepper under stress was attributed to the improved P_N. The higher per plant yield in the GB applied plants under stress in both the cultivars associated with higher P_N rate, gs and WUE in treated plants. Though there was an increase in P_N rate, WUE and plant yield in the treated plants (T1 – T3), the better results were found in the plants (T2) where seeds were treated and foliar application was given at the time of imposing stress.

Key words: Glycinebetaine, hot pepper, photosynthesis, stomatal conductance

INTRODUCTION

Among various problems faced by crop plants, water stress is considered to be the most critical one (Boyer, 1982). Since plants being immobile, cannot evade water stress in the same way as other mobile organisms. Therefore, plants adopt many morphological and physiological alterations to acclimatize to stressful environment (Sakamoto and Murata 2002). One such mechanism that is ubiquitous in plants is the accumulation of organic metabolites collectively called as compatible solutes (Bohnert *et al*, 1995). These compatible solutes act as an osmoprotectant (Burnett *et al*, 1995). Among the osmoprotectant, glycinebetaine considered to be one of the most predominant and most effective osmoprotectant (Burnett *et al*, 1995). However, not all plants can produce osmolytes in sufficient quantities to combat drought. In many crops genotypic engineering was adopted to increase or initiate the synthesis of glycinebetaine. However, biosynthesis of glycinebetaine through genetic engineering is costly (Hanson and Wyse, 1982) and most of the plants do not normally accumulate sufficient amount of osmolytes. The exogenous application of glycinebetaine has been suggested as an alternative approach to improve tolerance under water stress (Makela *et al*, 1996). Application of glycinebetaine has been shown to protect functional protein, vital enzymes and photosynthetic machinery (Xing and Rajashekar, 1999) and has been found to improve the crop water productivity under limited and well watered conditions (Hussain *et al*, 2008).

However, such studies are lacking in the horticultural crops like hot-pepper, though it is being grown under tropical and sub-tropical conditions. Therefore, the present study was conducted to evaluate the effect of exogenous application of glycinebetaine on physiological response in hot-pepper under water stress.

MATERIAL AND METHODS

Plant material and glycinebetaine treatment: The seedlings of hot-pepper genotypes, Arka Lohit and Pusa Jwala were raised in seedling trays containing coco peat. One month old seedlings were transplanted in the plastic pots (12''dia.) containing sandy soil and farmyard manure (3:1 v/v) under natural environmental conditions. The day temperature varied from 34 to 36°C and photosynthetic photon flux density (PPFD) from 1000 to 1900 μ mol $m^{-2}s^{-1}$ during the study. The plants were irrigated regularly and the recommended package of practices was followed to grow the plants. Water stress was imposed at flowering stage (35 days after transplanting) by withholding irrigation for a period of 12 days. The GB treatment was given to seed (6.0%) before sowing and plants through foliar spray (1.0%) at the time of imposing water deficit stress. The treatments were defined as follows: T1 = water deficit stress + seeds treatment with 6% GB, T2 = water deficit stress + seeds treatment with 6% GB + foliar spray (1.0%), T3 = water deficit stress + foliar spray only, T4 = water deficit stress, T5 = irrigated plants. Soil moisture content as measured gravimetrically was 20-22% in the control and 11-13% under the stress.

Morpho-physiological observations: Gas exchange parameters such as net photosynthesis rate (P_N), stomatal conductance (g_s) and internal CO_2 (Ci) were measured using portable Photosynthesis Analysis System (Model LCA 3, Analytical Development Corporation, Huddesdon, U.K). Fully expanded leaf (5th leaf) from top was used clamped to the leaf chamber and the observations were recorded when P_N, g_s and Ci were reached to stable value under natural conditions. All the gas exchange parameters were recorded between 10.00 and 11.30h. The osmotic potential (ψ_s) was measured using Wescor osmometer (model 5520). The observations were also recorded on plant height, leaf area, number of flowers and fruits. Leaf area was measured by leaf area meter (LI-3000). Plant samples were collected at the time of releasing the stress and dried in oven at 80°C for 48h to determine the total dry matter (TDM). The plant fruit yield was measured on fresh weight basis.

RESULTS AND DISCUSSION

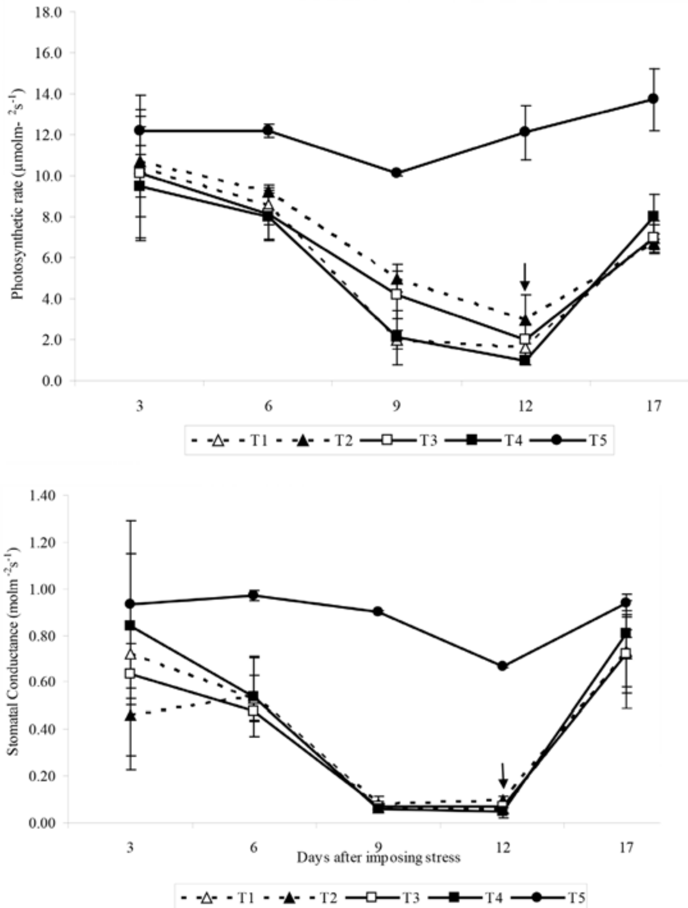
The effect of GB on plant height, leaf area, flower and fruit number and total dry matter (TDM) were shown in Table 1. There was considerable effect of GB treatments on these parameters under water stress. The TDM accumulation was 8.7 to 11.5% in Arka Lohit and 3.0 to 36.7% higher in Pusa Jwala in treated plant (T1–T3) as compared to untreated plants (T4) under stress (Table 1). In both the cultivars, the response was better to GB treatments applied to both seeds + foliar treatment (T2). A significant decrease in P_N and g_s was found by 12 days after stress in both the cultivars (Fig. 1a and 1b). Reduction in P_N was sharp in Pusa Jwala as compared to Arka Lohit. At the end of 12 days stress, P_N decreased from 10.1 to 1.0-1.3 μ mol m^{-2} s^{-1} in untreated stressed plants (T4), while in GB treated stressed plants P_N reduced to 2.0 – 3.0 μ mol m^{-2} s^{-1} (T1 – T3). Similarly, gs reduced from 0.84 mol m^{-2} s^{-1} to 0.04 mol m^{-2} s^{-1} in untreated stress plants, while in treated stress plants, it was 0.89 to 0.06 mol m^{-2} s^{-1}. Among the treated plants, the higher g_s (0.08 to 0.10 mol m^{-2} s^{-1}) was found in T2 in both the cultivars. The recovery in P_N and g_s after releasing 12 days stress was almost the same in both the cultivars (Fig. 1a and 1b). The Ci value was higher in untreated stress plants (T4) as compared to the plants treated with GB (T1 – T3) in both the cultivars (Table 2). Similar trend was observed for A/Ci ratio. The WUE reduced in both the cultivars under stress in untreated plants (0.26 – 0.38 μ mol CO_2/ mol H_2O $m^{-2}s^{-1}$). However, the decrease in WUE was less in the plants treated with GB and ranged from 0.53 to 0.70 μ mol CO_2/ mol H_2O $m^{-2}s^{-1}$ in Arka Lohit and 0.30 to 0.47 μ mol CO_2/ mol H_2O $m^{-2}s^{-1}$ in Pusa Jwala. The ψ_s in the irrigated plants varied from -1.10 to -1.16 MPa. Under stress, it decreased up to -2.0 to -2.32 MPa under stress (T4) in both the cultivars. GB treated plants (T1 – T3) of Arka Lohit had the ψ_s of -2.45 to -3.00 MPa and Pusa Jwala -2.59 to -2.90 MPa (Table 2). The per plant yield was 267 – 294g $plant^{-1}$ in Arka Lohit and 204 – 213 g $plant^{-1}$ in Pusa Jwala in the treated stress plants (T1 – T3), while in untreated stress plants (T4) it was 252 g $plant^{-1}$ in Arka Lohit and 156g $plant^{-1}$ in Pusa Jwala (Fig. 2). The effect of GB on plant response to water stress was better in T2.

Table 1. Morpho-physiological parameters as affected by glycinebetaine application under stress in two cultivars of hot pepper

Variety	Treatment	Plant height (cm)	Leaf area (cm^2)	Flower No.	Fruit No.	Total dry matter (g $plant^{-1}$)
Arka Lohit	T1	76.0	659.2	50	8	22.629
	T2	84.0	1094.0	53	7	23.223
	T3	92.0	1374.0	65	7	22.635
	T4	75.0	720.6	50	6	20.810
	T5	80.0	2615.2	70	20	46.394
	SEM	1.38	158.86	2.40	1.17	2.16
Pusa Jwala	T1	62.0	1017.0	25	8	16.589
	T2	68.0	1328.6	50	8	20.291
	T3	56.0	1145.0	58	5	15.309
	T4	50.0	778.0	41	8	14.84
	T5	70.0	2049.0	53	21	34.236
	SEM	1.66	96.53	2.59	1.26	1.62

T1 = water deficit stress + seeds treatment with 6% GB, T2 = water deficit stress + seeds treatment with 6% GB + foliar spray (1.0%), T3 = water deficit stress + foliar spray only, T4 = water deficit stress and T5 = irrigated plants

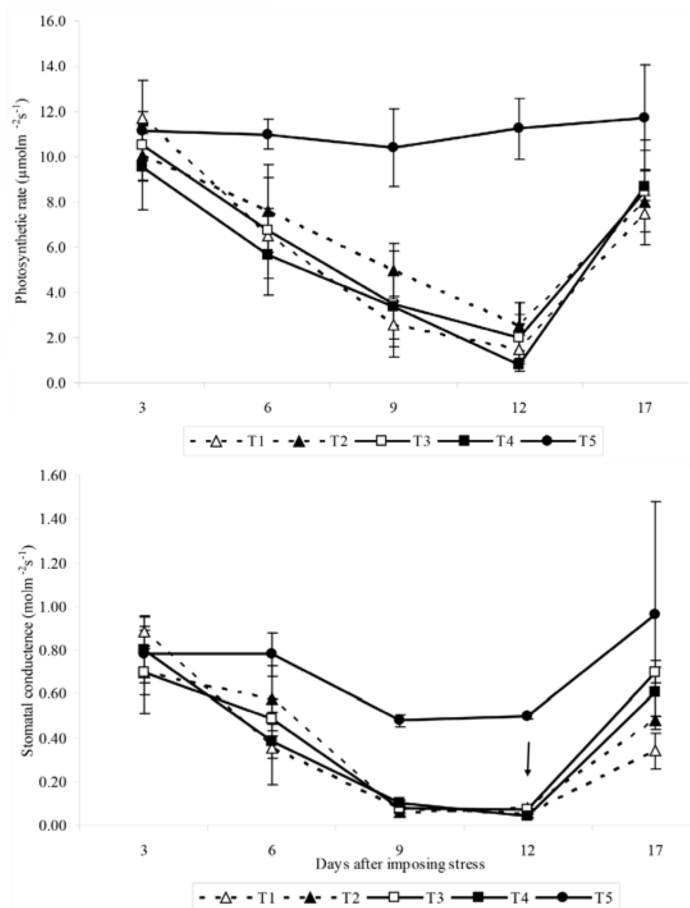
Our studies confirmed that the application of GB improved the LA production, plant height and TDM



T1 = water deficit stress + seeds treatment with 6% GB, T2 = water deficit stress + seeds treatment with 6% GB + foliar spray (1.0%), T3 = water deficit stress + foliar spray only, T4 = water deficit stress and T5 = irrigated plants

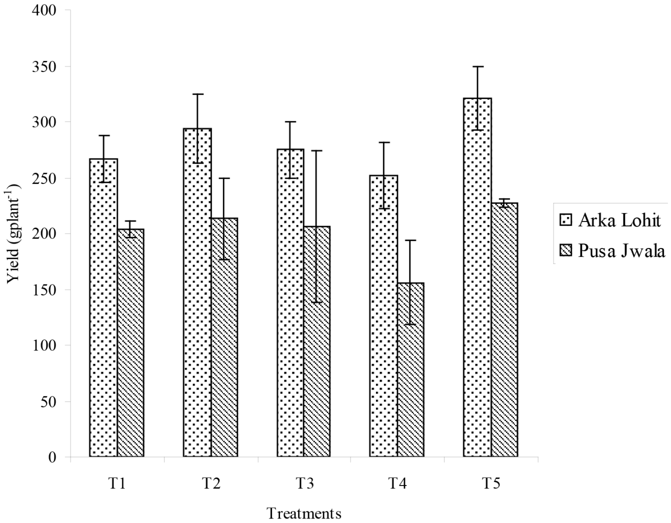
Fig.1a. Pattern of photosynthesis and stomatal conductance as affected by glycinebetaine application during water stress in hot pepper var. Arka Lohit

accumulation and P_N rate under water deficit. Generally, the reduction in P_N under water-deficit is caused by either stomatal closure and/or photosynthetic apparatus damage. Stomatal closure has an effect on CO_2 entering cells, whereas continuous moderate or sever absence of water can damage the photosynthetic apparatus (Makela *et al*, 1999). Our study indicated that water deficit considerably reduced the P_N and g_s in hot pepper. The GB application alleviated these disturbances caused by water deficit in both the cultivars (Fig. 1a and Fig. 1b). There was an increase in Ci under stress condition (T4). An increase in Ci with a decrease in P_N and g_s in untreated plants under stress (T4) suggests a decline in biochemical capacity of the plants. The greater A/Ci ratio in the GB applied plants indicates the improvement in carboxylation efficiency in the GB applied plants under water stress. The application of GB improved g_s and protected the photosynthetic apparatus, which resulted in the higher P_N under water deficit and alleviation of negative effects (Fig. 1a & b and Table 2). Ma *et al*, (2007) also found that in tobacco the foliar application of GB improved the P_N under water stress mostly due to a greater g_s and carboxylation efficiency of CO_2 assimilation. The GB application also resulted in a decrease in ψ_s in both the cultivars (Table 2). Glycinebetaine is thought to act as a compatible solute; therefore, its accumulation decreases ψ_s and improves the leaf water status in these cultivars. The decrease in ψ_s may improve the leaf water status. Earlier studies also shown the application of GB improved water status of plants under stress (Xing and Rajashekar, 1999). Further, the application of GB increases the WUE in both the cultivars. Makhdum and Shabad-ud-Din (2007) found an increase in WUE in GB applied plants of cotton under



T1 = water deficit stress + seeds treatment with 6% GB, T2 = water deficit stress + seeds treatment with 6% GB + foliar spray (1.0%), T3 = water deficit stress + foliar spray only, T4 = water deficit stress and T5 = irrigated plants

Fig. 1b. Pattern of photosynthesis and stomatal conductance as affected by glycinebetaine application during water stress in hot pepper var. Pusa Jwala



T1 = water deficit stress + seeds treatment with 6% GB, T2 = water deficit tress + seeds treatment with 6% GB + foliar spray (1.0%), T3 = water deficit stress + foliar spray only, T4 = water deficit stress and T5 = irrigated plants

Fig. 2. Effect of glycinebetaine on plant yield under water stress in two cultivars of hot pepper

Table 2. Net photosynthetic rate (P_N, µmol m^{-2}s^{-1}), stomatal conductance (gs, mol m^{-2}s^{-1}), intercellular carbon dioxide (Ci, ppm), water use efficiency (WUE, µmol CO_2/ mol H_2O m^{-2} s^{-1}) and leaf osmotic potential (ψ_s,-MPa) as influenced by glycinebetaine application under stress in hot pepper

Variety	Treatment	A	Gs	Ci	A/Ci	WUE	ψ_s
Arka Lohit	T1	1.6	0.06	337	0.005	0.59	2.45
	T2	3.0	0.10	306	0.010	0.70	3.00
	T3	2.0	0.07	303	0.007	0.53	2.50
	T4	1.0	0.05	351	0.003	0.26	2.00
	T5	12.1	0.67	289	0.042	1.11	1.16
	SEM	0.92	0.05	5.15	0.003	0.06	0.14
Pusa Jwala	T1	1.5	0.06	314	0.004	0.30	2.59
	T2	2.5	0.08	300	0.008	0.47	2.90
	T3	2.0	0.07	333	0.006	0.40	2.60
	T4	0.8	0.04	331	0.002	0.38	2.32
	T5	11.2	0.50	304	0.037	0.98	1.10
	SEM	0.86	0.04	3.03	0.003	0.05	0.14

T1 = water deficit stress + seeds treatment with 6% GB, T2 = water deficit stress + seeds treatment with 6% GB + foliar spray (1.0%), T3 = water deficit stress + foliar spray only, T4 = water deficit stress and T5 = irrigated plants

water stress. The better WUE in treated plants of hot-pepper under stress was attributed to the improved P_N. The higher per plant yield in the GB applied plants under stress in both the cultivars of hot-pepper attributed to higher P_N rate, g_s and WUE in treated plants. Among the treatments, best results were found in T2, where GB treatment was given to seeds and foliar application.

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