Journal of Applied Horticulture, 16(1): 24-28, 2014

Effect of glycinebetaine application on photosynthesis, sugar content, invertase activity and plant yield of hot pepper (*Capsicum annuum* L.) under water stress condition

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Abstract

A study was conducted to evaluate the response of hot pepper (*Capsicum annuum* L.) to foliar applied glycinebetaine (GB) under water stress condition. Three varieties of hot pepper *e.g.* Arka Lohit, Pusa Jwala and Arka Haritha were subjected to water stress at flowering stage. The plants applied with GB had the greater plant height, leaf area, fruit fresh and dry mass under water deficit conditions. GB application increased the P_N under water deficit condition. It was attributed to an improvement in stomatal conductance under water stress. There was a varietal difference in invertase activity and total sugar contents to GB application under water stress. Higher yield and better water use efficiency (WUE) were found in GB applied plants. The plants treated with GB 10 days before and at the time of imposing water stress (T2) responded better. The results suggested that exogenous GB ameliorates the negative effects of water stress in hot pepper.

Key words: Carbon exchange rate, drought, glycinebetaine, hot pepper, plant yield

Introduction

Water availability is considered the major limiting factor in vegetable crop production, and high yields are dependent on adequate water supply. It was observed that under water deficit conditions, plant responds in different ways and follow different strategies for its survival. For example, plants show many morphological and physiological alterations to acclimatize to unfavourable environment (Sakamoto and Murata, 2002). One of the most common stress responses in plants is accumulation of different types of compatible organic solutes (Serraj and Sinclair, 2002). But not all plants can produce osmolytes in sufficient quantities to combat water stress. In many crop plants, the natural accumulation of glycinebetaine (GB) is lower than sufficient to ameliorate the adverse effects of dehydration (Subbarao *et al.*, 2001). Therefore, protective capacity of GB under various stress conditions has prompted numerous investigations in order to increase GB content in plants through genetic engineering (Sakamoto and Murata, 2000; 2002). The exogenous application of GB has been suggested as an alternative approach to improve crop productivity under water stress (Makela *et al.,* 1998; 1999) as it can increases the crop tolerance to water stress (Ma *et al.*, 2007; Hussain *et al.*, 2008). Yang and Lu (2005) found that the exogenous application of GB to low-accumulating or non-accumulating plants may help reduce adverse effects of environmental stresses. Application of GB has been shown to protect functional proteins, 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). There was not much information available on the accumulation of glycinebetaine in relation to water stress in hot pepper (*Capsicum annuum* L.).

The present study was conducted to evaluate the effect of GB on plant-water relation, carbon exchange rate and invertase activity under water stress condition in hot pepper.

Materials and methods

Pot experiment: The seedlings of hot pepper (*C. annuum* L.) varieties. Arka Lohit and Pusa Jwala were raised in seedling trays containing coco peat. One month old seedlings were transplanted in the plastic pots (30 cm dia). The plants were irrigated regularly and the recommended package of practices were followed to grow the plants. Uniformly grown plants were divided into four groups of 25 each. The plants were subjected to water stress by withholding irrigation at flowering stage (35 days from transplanting) and GB treatments (0.1%) through foliar application as: T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress, and T4 = irrigated.

Field experiment: The seedlings of hot pepper varieties *i.e.* Pusa Jwala and Arka Haritha were raised in seedling trays as mentioned in experiment 1. One month old seedlings were transplanted in the field with a spacing of 60 x 50 cm (row to row and plant to plant). Water stress was imposed by withholding irrigation at flowering stage (35 days from transplanting). The GB was applied as defined in experiment 1. The experiments were conducted in completely randomized design. All the data were analyzed statistically using Agris Stat software.

Plant water relation: A portion of the leaf used for the gas exchange parameters was frozen for a week, thawed and sap was used for leaf water potential (ψ_l) determination in leaf water potential system CR7.

Morphological attributes: Plant height, total leaf area, fruit number, fruit fresh weight and total plant dry matter (TDM) were taken in control and stressed plants before releasing water stress. The leaf area (LA) of the plant was measured using leaf area meter (LI-3000). The plant parts were separated and dried in an oven at 80 °C for 72 h to calculate the total dry matter (TDM) of individual plant. The per plant yield was calculated at the end.

Gas exchange parameters and water use efficiency: The observations on P_N and g_s were measured during the water stress period on fully expanded leaves (5th leaf from top) between 1000 and 1130 hr using ADC open portable photosynthesis analysis system (model LCA 3, Analytical Development Corporation, Huddesdon, UK). During the observations, the ambient temperature varied from 34 to 37 °C, irradiance from 1200 to 1600 µmol $m^{-2} s^{-1}$ and the CO_2 between 365 and 375 ppm. Leaf water use efficiency (WUE) was calculated as P_N/E (where, P_N = net photosynthetic rate, E = transpiration rate).

Leaf invertase activity: 500 mg of leaf samples were homogenized in 2 mL ice cold 100 mM acetate buffer pH 5.0 and centrifuged at 2500 g for 20 min at 4 °C. Soluble acid invertase was assayed by incubating 0.2 mL of aliquot (supernatant) with 0.8 mL of 0.1 M sucrose for 30 minutes at 30 °C. Reaction was stopped by adding 1 mL Somogyi's copper reagent and boiled for 10 minutes. Sucrose was added to control sample just before boiling. Blank sample had no sucrose. Reaction mixture was cooled and 1 mL arsenomolybdate was added. The absorbance was read at 630nm. The enzyme activity was estimated as described by Morris and Arthur (1984). The soluble protein was determined by Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as the standard.

Sugar content: Reducing sugars were analysed according to the procedure of Somogyi (1952). 100 mg dry mass of leaves were extracted with 5 mL hot 80% ethanol twice and the supernatant separated out by centrifugation was condensed on a water bath at 80 °C. The residue was dissolved in 100 mL of water. To 2 mL of solution, 1 mL of alkaline copper tartrate reagent was added and the contents were boiled in a water bath for 10 minutes. After cooling to room temperature, 1 mL of arsenomolybdic acid reagent was added to it and volume was later adjusted to 10 mL with water. The absorbance of blue colour formed was recorded at 620 nm. The amount of reducing sugar present in sample was measured using glucose as standard and expressed as mg/g d.wt. Total sugars were estimated by hydrolyzing 10 mL of sugar solution employing 1.0 mL of HCl and keeping solution overnight. After adjusting pH to 6.5 with NaOH, the total sugar was estimated with same way as described for reducing sugars. Non-reducing sugars were calculated by subtracting the reducing sugars from the total sugar content.

Results and discussion

Pot experiment

Plant water relation: The ψ_l varied from -1.2 to -1.4 MPa in Arka Lohit and -1.2 to -1.3 MPa in Pusa Jwala under irrigated condition, and markedly decreased (-1.7 to -1.9 MPa) under stress (Fig 1). The decrease in ψ_l under stress was more in Pusa Jwala as compared to Arka Lohit. But in GB applied plants, ψ_l was relatively higher (less negative) in both the cultivars (-1.4 – 1.7 MPa) under stress.

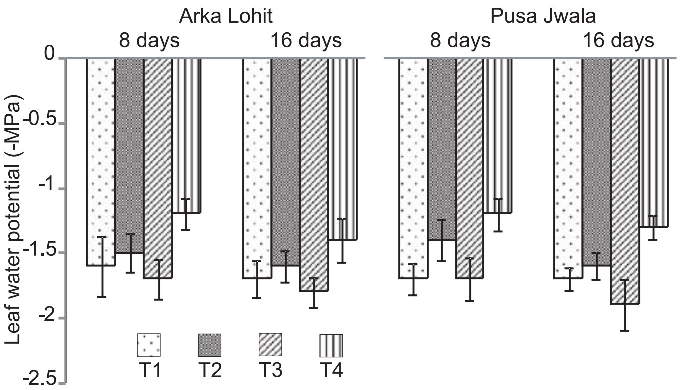

Fig. 1. Leaf water potential (-Mpa) as affected by glycinebetaine under water stress in hot pepper (T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress and T4 = irrigated)

Photosynthesis and its characteristics: Under water-deficit condition, P_N decreased, with a greater effect seen in Pusa Jwala than in Arka Lohit when compared with non stress condition (Fig. 2). However, the P_N was higher in the foliar applied GB plants under water deficit condition, indicating the improvement in P_N under stress. The effect was more pronounced in T2 where plants were treated with GB 10 days before and at the time of imposing stress. Among the varieties, the positive effect was more noteworthy in Arka Lohit. In the GB applied plants, the g_s was also greater than untreated plants under stress. Though the GB increased the g_s in both the varieties, the effect was higher in Arka Lohit than Pusa Jwala.

Invertase activity and sugars: The invertase activity was greater in Pusa Jwala than Arka Lohit irrespective of treatments (Fig. 3). In both the varieties, invertase activity was not significantly affected by water stress. However, the GB application increased the invertase activity by 2 to 4 times in Pusa Jwala and 3 to 8 times in Arka Lohit as compared to untreated plants under stress. The effect of GB was noteworthy in T2. A differential response of sugars to water stress was observed in the hot pepper varieties (Table 1). In Arka Lohit, the sugar contents decreased under stress, while in Pusa Jwala there was an increase in total sugar level under stress (T3).

The GB application increased the sugar level in Arka Lohit under stress and the effect was more pronounced in T2. However, in Pusa Jwala, the sugar level further decreased in GB applied plants under stress. There was no definite trend as far as the accumulation of reducing and non-reducing sugars in GB applied plants under water stress is concerned.

Table 1. Effect of glycinebetaine on reducing, non-reducing and total sugars in hot pepper under water stress (T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress, and T4 = irrigated)

Cultivar	Sugar (mg g^{-1})	T1	T2	T3	T4	S.Em
Arka Lohit	Reducing	1216.2	1101.5	905.5	913.6	37.84
	Non-reducing	119.3	232.5	73.6	185.8	17.58
	Total	1225.5	1333.9	979.1	1099.4	38.44
Pusa Jwala	Reducing	880.2	1343.1	1606.0	795.9	96.09
	Non-reducing	112.7	74.6	187.8	168.0	12.92
	Total	992.9	1417.7	1793.8	963.9	98.39

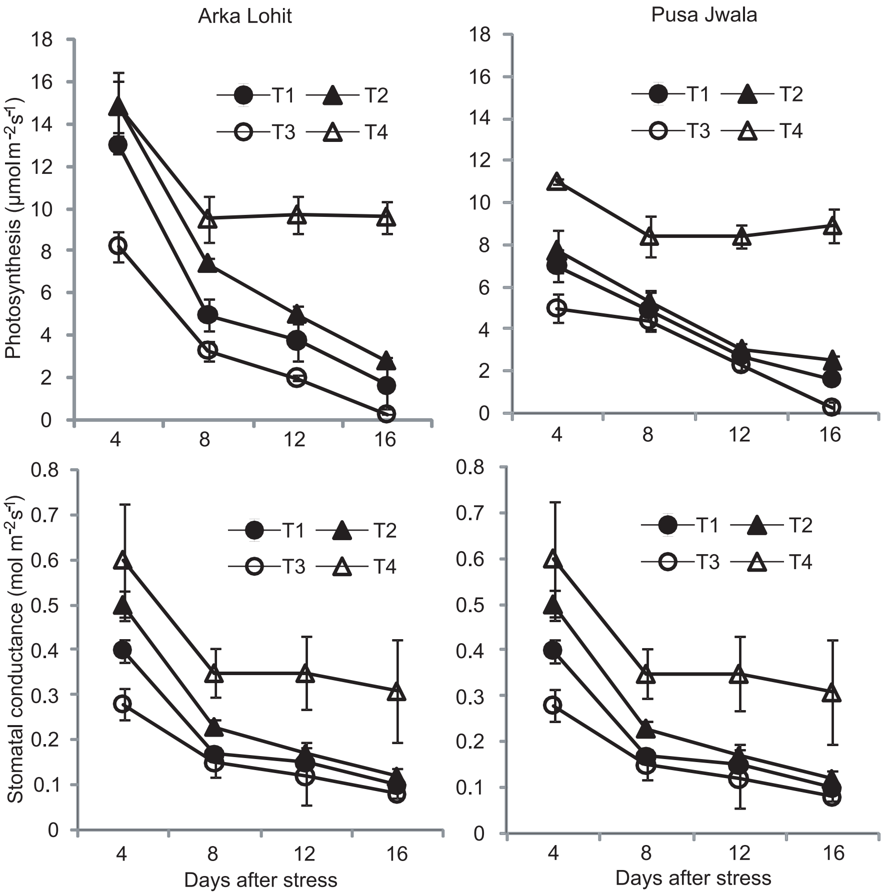

Fig. 2. Photosynthesis and stomatal conductance as affected by glycinebetaine during stress in hot pepper (T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress and T4 = irrigated)

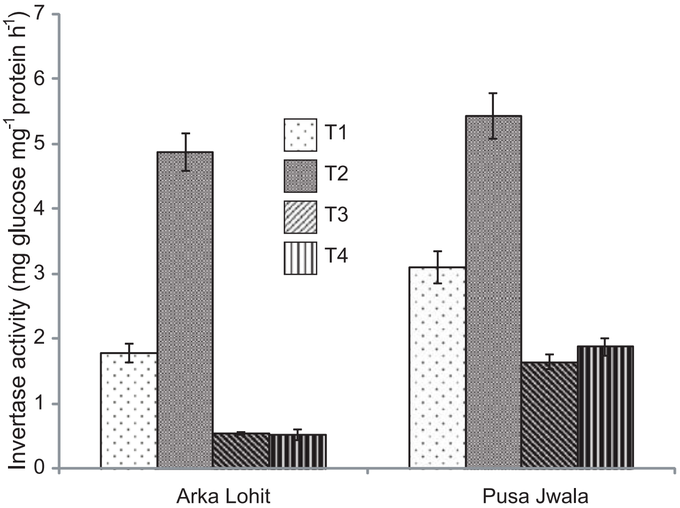

Fig. 3. Invertase activity as influenced by glycinebetaine under water stress in hot pepper (T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress and T4 = irrigated)

Field experiment

Morpho-physiological attributes: The influence of GB application was studied in Pusa Jwala and Arka Haritha under the field conditions. The morpho-physiological attributes such as plant height, leaf area (LA), fruit numbers and fruit fresh weight (FFW) were greater in GB applied plants (T1 and T2) as compared to the untreated (T3) under water stress (Table 2). The positive effects of GB were more noteworthy in Arka Haritha as compared to Pusa Jwala. LA production in Arka Haritha was almost double in the GB applied plants than the untreated plants (T3) under stress. The TDM production was also influenced by GB under water stress (Table 3).

In both the varieties, the TDM was significantly greater in the treated plants under water stress. In Arka Haritha, the TDM was 53.0 to 55.0% and in Pusa Jwala 29.0 to 41.0% higher than the plants without GB application under stress. However, there was no effect of GB on the pattern of dry matter (DM) partitioning to different plant parts. But GB application influenced the DM accumulation in different plant parts under stress as shown by greater values in fruit, stem and leaves in GB applied plants compared to untreated plants under stress in both the varieties.

Water use efficiency: Water use efficiency (WUE) was determined at 10 days and 25 days stress (Fig. 4). In general, WUE irrespective of treatments was higher at 10 days stress and decreased with the progress of stress in both the cultivars. However, in the GB applied plants, the WUE was higher compared to untreated plants (T3) in both cultivars. The plant response was better in T2 in both the cultivars.

Plant yield : A considerable reduction in plant yield was observed in both the varieties (Arka Haritha, 39.6% and Pusa Jwala 66.6%) under water stress (Fig. 5). However, the plant yield was higher in GB treated plants in both cultivars under stress and it was more effective in T2. Arka Haritha was more responsive than Pusa Jwala to GB application under stress.

The present results indicated that the exogenous application of GB improves the morpho-physiological and biochemical performance of hot pepper plants under water stress. There was an increase in TDM production in all the plant parts in GB applied plants of hot pepper under stress indicating the better plant growth in GB treated plants. It was evident from our findings that the

Table 2. Effect of glycinebetaine application on morphological attributes in hot pepper under water stress

Cultivar	Treatment	Plant height (cm)	Leaf area (cm^2)	Fruit number	Fruit fresh weight (g $fruit^{-1}$)
Arka Haritha	T1	56.0	2379.60	136.1	237.60
	T2	57.0	2506.41	85.5	147.85
	T3	45.0	1058.17	80.0	65.70
	T4	61.5	2727.73	124.5	269.71
	S Em	1.7	188.43	7.0	23.03
Pusa Jwala	T1	40.5	1259.98	100.0	88.73
	T2	41.0	1853.68	87.0	87.72
	T3	39.0	1242.54	34.0	34.87
	T4	42.5	2566.77	102.0	97.68
	S Em	0.4	156.39	7.9	7.15

T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress, and T4 = irrigated.

Table 3. Total dry mass (g/plant) and its partitioning (g/plant) to different plant parts in glycinebetaine applied plants under water stress. (T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress, and T4 = irrigated)

Cultivar	Treatment	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Fruit dry weight (g)	Total dry weight (g)
Arka Haritha	T1	18.746	24.694	3.190	25.320	71.950
	T2	16.140	22.924	3.460	30.622	73.145
	T3	9.983	15.522	2.382	18.827	46.714
	T4	21.816	29.190	4.677	42.791	98.474
	SEm	1.260	1.420	0.240	2.540	5.280
Pusa Jwala	T1	12.894	13.879	2.151	15.533	44.455
	T2	10.038	14.141	1.941	21.918	48.038
	T3	9.144	11.816	1.449	11.711	34.119
	T4	17.548	16.202	2.887	24.323	60.960
	SEm	0.950	0.440	0.150	1.450	2.770

GB application increased P_N rate under water stress, and it was more effective when applied 10 days before and at the time of inducing stress (T2). It is well known that generally P_N rate under water stress may be reduced either by stomatal closure and/or photosynthetic apparatus damage. In the present study, there was a considerable increase in g_s in GB treated plants under stress and can be attributed for a higher P_N rate under stress. However, the role of photochemical capacity in increasing P_N of GB-treated water-stressed plants cannot be ruled out. Increased g_s by the GB application was observed in tomato and turnip plants when subjected to drought (Makela *et al.*, 1999). Yang and Lu (2005) also found that GB application in salt stressed plants of maize improved the P_N and such an improvement was associated with an improvement in g_s and the PSII efficiency. The higher ψ_l of hot pepper in GB treated plants under water deficit indicated the maintenance of leaf water balance and leaf turgidity in GB treated plants (Fig 1). Our results support the earlier findings that GB application maintained better leaf water status in the plants (Xing and Rajashekar, 1999; Ma *et al.*, 2007). The GB application improves the WUE in hot pepper and can be attributed to the higher P_N rate under stress. Apart from the P_N and g_s, the biochemical capacity was also affected differentially in hot pepper cultivars by the GB application under stress. GB treatment increased the sugar content under water deficit in cv. Arka Lohit, but it was not the same in cv. Pusa Jwala, indicating the genotypic variability in sugar response to water stress in GB applied plants. The differential trend in sugar accumulation in relation to GB application under water stress indicated the genotypic variability in sugar accumulation by GB application under stress in hot pepper. The increase in the invertase activity in both genotypes of hot pepper by GB application (Fig. 3) may influence the utilization ability of sucrose under stress. Invertase activity has been suggested to be part of mechanism by which sinks maintain sucrose import and sink activity. The results indicated that improvement in the different physiological and biochemical components in GB applied plants resulted in the higher plant yield in hot pepper under water stress (Fig. 5). The plants treated with GB 10 days before and at the time of imposing stress (T2) responded better under water stress. The results suggested that exogenous GB reduced the negative effects of water stress in hot pepper.

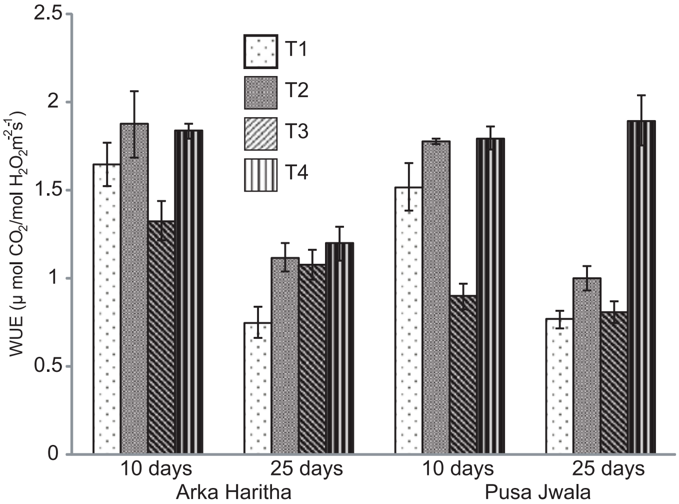

Fig. 4. Effect of glycinebetaine on water use efficiency in hot pepper under water stress (T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress and T4 = irrigated)

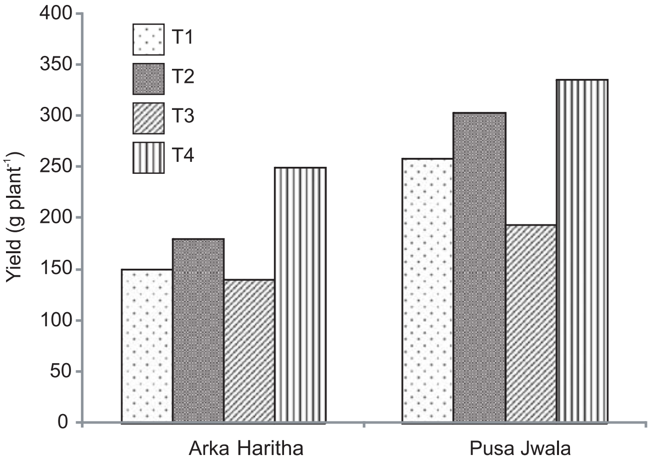

Fig. 5. Effect of glycinebetaine on plant yield (g plant^{-1}) in hot pepper under water stress (T1 = plants treated with GB 10 days before imposing water stress, T2 = plants treated with GB 10 days before and at the time of imposing stress, T3 = no treatment during stress and T4 = irrigated)

In conclusion, exogenous GB application resulted in better plant growth, P_N, WUE and plant yield of hot pepper under water deficit condition. The results suggests that the improved photosynthetic capacity in hot pepper may be associated with an improvement in stomatal conductance, maintenance of better plant water relation and increase in the biochemical capacity in GB treated plants under water stress.

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Received: January, 2014; Revised: March, 2014; Accepted: April, 2014