Glycine Betaine Boosts Growth, Chlorophyll a Fluorescence, and Alleviated Drought-induced Damage in Eggplant (*Solanum melongena* L.)

Saad M. Howladar and Fatima Omari Al-Zahrani*

Biology Department, Faculty of Science, Albaha University, Albaha, Saudi Arabia
E-mail address: showladar@bu.edu.sa, fsalomari@bu.edu.sa
** Corresponding author*

ABSTRACT

Osmoprotectants such as glycine betaine (GB) widely accumulate in plants, and mediate various physiological and biochemical processes. In the current study, we investigated the effect on foliar application of GB on enhancing drought stress tolerance in eggplant (*Solanum melongena* L.) during early vegetative growth stages. GB was exogenously applied at three different concentrations (0, 25, and 50 mM) under three levels of irrigation (FI, Ir-80, and Ir-60, i.e., 100%, 80%, and 60% of the soil water-holding capacity [SWHC], respectively).

The results revealed that the deficit irrigation (DI) at Ir-80 and Ir-60) significantly decreased all growth characteristics (i.e., number of leaves per plant, shoot length, stem diameter, leaf area per plant, and shoot fresh weight and dry weight). The physiological attributes were also affected (i.e., chlorophyll *a* fluorescence; Fv/Fm, Fv/F0, and performance index (PI), SPAD chlorophyll, relative water content [RWC], and membrane stability index [MSI]). However, foliar application of GB alleviated DI stress effects in eggplant plants by improving photosynthetic efficiency (evaluated by chlorophyll *a* fluorescence) and enhanced RWC and MSI in the tested eggplants. This in turn reflected in higher plant growth characteristics and improved plant water status under DI stress. Therefore, foliar application of GB may have future commercial applications as a potential osmolyte for improving plant growth under DI of up to 40% of the SWHC.

Keywords: Glycine betaine, drought stress, chlorophyll *a* fluorescence, dehydration tolerance

1. INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the most important crops grown worldwide and in Saudi Arabia, which has a dry climate as it lies in an arid region (Zabin and Howladar, 2015) In such regions, lack of fresh water resources is the major factor restricting agricultural production. Better awareness of water efficiency is considered a successful management strategy and a sustainable agricultural practice in water-limited environments (Howell, 2001; Bacon, 2004). Irrigation using water that is less than the optimum water requirement of the crop is called deficit irrigation (DI). It is a common sustainable practice worldwide (Pereira ***et al.***, 2002), especially in regions characterized by low water resources. There are two main potential benefits of DI: increased water use efficiency and decreased irrigation costs. DI may be performed either by decreased frequency of irrigation or decreased of irrigation water amount

(Patane ***et al***., 2011; Igbadun ***et al***., 2012). DI affects growth and yield of many vegetables and field crops (Abd El-Mageed ***et al***., 2016a, 2016b; Merwad ***et al***., 2018). DI increases water productivity without severe reduction in crop yield (Geerts and Raes, 2009; Semida ***et al***., 2017).

On the other hand, eggplant is a plant with a high water requirement during its growth and development stages; therefore, it is considered drought-sensitive (Fu ***et al.***, 2013). In addition, marketable fruits and yield components of hydroponically cultivated zucchini were considerably affected by irrigation system (Rouphael and Colla, 2005). DI affected the water productivity and water use efficiency of squash (Abd El-Mageed and Semida, 2015a). Different effects of DI are crop-specific; therefore, to accommodate a given crop to a specific location, it is crucial to evaluate the effect of different DI strategies through multi-year open-field experiments before concluding the most suitable irrigation scheduling method (Abd El-Mageed and Semida, 2015b).

Glycine betaine (GB) is an amino acid derivative that accumulates in a wide number of plant species in response to abiotic stresses, including drought (Gorham, 1995). Several reports have elucidated that exogenous GB application improved the growth and yield of wheat and rice plants by regulating some key physiological and molecular attributes, thereby increasing drought tolerance in these plants (Weibing and Rajashekar, 1999; Tahir ***et al***., 2009; Hadiarto and Tran, 2011; Gao ***et al***., 2012; Cha-um ***et al***., 2013).

Therefore, the main objective of this study was to assess the effect of foliar application of GB on the growth and physiological attributes of eggplant grown at 20 or 40% DI in comparison with eggplant grown with full irrigation. The results could be useful in developing additional strategies for the sustainable management of eggplant production under limited irrigation.

2. MATERIALS AND METHODS

From March 2017 to May 2017, a pot experiment with three replicates was conducted during the period of March–May, 2017 in a greenhouse at Albaha University, Alaqiq (latitude 20° 17′ 41″N, longitude 41° 38′ 35″E, 1651.88 m above sea level), Albaha, Saudi Arabia. The climate of the study region is semiarid (Zabin and Howladar, 2015) and is described as having a mean annual temperature ranging from 17.8–29.9°C. The average annual rainfall is reported to be about 62.45 mm, and the relative humidity ranges from 15–87%. The mean wind speed is around 6 kts/deg (PMEP, 2017).

At the fourth leaf stage, eggplant (*Solanum melongena* L.) seedlings were transplanted into plastic pots (25 cm diameter, 25 cm depth) containing 8 kg sandy loam soil. Starting at 10 days after transplanting (DAT), all plants per treatment (n = 30) were sprayed with tap water (0 mM GB) or 25 and 50 mM of GB two times to run-off, at 10-day intervals. The sprays received drops of the surfactant Tween-20 drops to ensure complete penetration of the GB solution into the leaves. Each GB treatment was carried out under three levels of irrigation (full irrigation [FI], Ir-80, and Ir-60, with 100%, 80%, and 60% of soil water-holding capacity [SWHC], respectively). Plants (n = 30) from all the treatment groups received FI until the start of the treatment applications (10 DAT), then all DI treatments were applied with the first GB spray. The electrical conductivity (EC) and pH values and the contents of cations and anions in the soil used for the experiments are shown in Table 1.

Table 1. Physico-chemical characteristics of a sandy loam soil prior to the experiments in two seasons

Bulk density ($g \cdot cm^{-3}$)	CEC ($cmol^{+}/kg$)	pH	EC ($dS \cdot m^{-1}$)	OC*	N	P	K	Ca	Fe	Mn	Zn
				($g \cdot kg^{-1}$)		($mg \cdot kg^{-1}$)					
1.29	7.9	7.7	2.1	8.9	0.8	15	70	85	6.2	4.0	2.1

The SWHC in each pot was 36% (w/v) soil:water, which was measured by saturating the soil in each pot with water and weighing it after it had drained for 48 h. The soil water contents were maintained at approximately 100% (w/v) of the SWHC for the control treatment. The soil moisture level was controlled by weighing each pot, and water loss was supplemented daily according to the irrigation treatments.

According to the recommended doses of agricultural practices, nitrogen (N) as ammonium sulfate (20.5% N) at 2.0 g per pot, phosphorous (P) as calcium superphosphate (15.5% P_2O_5) at 1.2 g per pot, and potassium (K) as potassium sulfate (48% K_2O) at 1.0 g per pot were added to each pot before planting. An additional N dose of 1.2 g ammonium sulfate per pot (20.5% N) was added at 26 DAT. The experiment was arranged in a factorial layout based on RCB design with 15 replicates (pots) per experimental treatment. The experiments were terminated at 42 DAT.

After six weeks of transplanting, five destructive plant samples were randomly chosen from each experimental treatment to determine plant growth, dehydration tolerance, and photosynthetic efficiency.

2.1 Measurements

To measure the growth characteristics of the eggplant plants, five destructive plant samples were randomly chosen from each experimental treatment at the end of the experimental period (42 DAT) to determine plant growth, dehydration tolerance, and photosynthetic efficiency.

The number of leaves per plant was counted and the leaf area per plant (m^2) was assessed using a digital planimeter (Delta-T DIAS image analysis system, Cambridge, UK). Shoot length and stem diameter were measured using the Vernier Caliper (Mahr UK PLC). Shoot fresh weight was assessed, and shoot dry weight (g) was measured after oven-drying the shoots at 70 °C for 48 h.

On a successive period in 2 different (sunny) days, the photosynthetic efficiency (chlorophyll fluorescence) was evaluated with a Handy PEA fluorometer (Hansatech Portable Instruments Ltd., Kings Lynn, UK). A corresponding fourth leaf was evaluated on each randomly chosen plant. The maximum PS II Fv/Fm quantum yield was calculated using the Maxwell and Johnson (2000) formulae; $Fv/Fm = (Fm - F0)/Fm$. The photosynthesis performance index was calculated based on equal absorption (PIABS) (Clark ***et al.***, 2000). The SPAD chlorophyll was measured in the apical third and fourth leaves using a chlorophyll meter (SPAD-502, Minolta, Japan).

Tissue relative water content (RWC) was measured according to the method described by Hayat ***et al.*** (2007) using fully-expanded leaf discs. First, the weights of the discs were recorded (fresh mass; FM) and immediately floated on double-distilled water in petri dishes for 24 h, in the dark, to saturate the discs with water. Any adhering water was gently removed by drying and the turgid mass (TM) was recorded. Dry mass (DM) was taken after drying the discs at 70°C until constant weight was achieved. The RWC was then calculated using the following formula:

$$\mathrm{RWC}(\%) = \left[\frac{(\mathrm{FM}-\mathrm{DM})}{(\mathrm{TM}-\mathrm{DM})}\right] \times 100$$

The membrane stability index of plant tissues (MSI) was determined based on the method of Rady (2011) using two comparable samples of fully-expanded leaf tissues. One sample was transferred into a test tube containing double-distilled water. This test tube was then heated at 40°C in a water bath for 30 min, and the electrical conductivity (EC1) of the solution was registered utilizing a conductivity bridge. The other sample was boiled at 100°C for 10 min, the conductivity was recorded (EC2), and the MSI was calculated using the following formula:

$$\mathrm{MSI}(\%) = \left[1-\left(\frac{\mathrm{EC1}}{\mathrm{EC2}}\right)\right] \times 100$$

2.2 Statistical analysis

The collected data were subjected to analysis of variance using Genstat statistical package (VSN International Ltd, Oxford, UK). Mean multiple comparisons were conducted by the least significant difference test at a probability value of 0.05.

3. RESULTS

Eggplant transplants displayed a significant reduction in growth characteristic and physiological attributes in response to the increased levels of DI treatments (Ir-80 and Ir-60). The DI significantly decreased growth pertaining parameters (i.e., number of leaves, shoot length, stem diameter, shoot fresh weight (FW), shoot dry weight (DW), and leaf area; Tables 2a, b and 3a, b) and physiological attributes (i.e., photosynthetic efficiency evaluated by chlorophyll *a* fluorescence; *Fv/Fm*, *Fv/F0* ratios and PI [Figure 1 and Table 4], SPAD chlorophyll, RWC, and MSI [Tables 5a, b]).

Table 2a. Effect of glycine betaine (GB) foliar application on some growth characteristics i.e., leaves number, shoot length, and stem diameter of eggplant (*Solanum melongena* L.) transplants grown under deficit irrigation regimes

Items	Mean ± SE		
	Leaf number	Shoot length (cm)	Stem diameter (mm)
GB			
GB (0 mM)	18.50 ± 0.96 $^{c\#}$	18.42 ± 0.79 ^{c}	5.74 ± 0.10 ^{b}
GB (25 mM)	23.39 ± 1.26 ^{b}	24.80 ± 0.65 ^{b}	6.06 ± 0.16 ^{b}
GB (50 mM)	27.83 ± 1.61 ^{a}	27.43 ± 0.67 ^{a}	6.46 ± 0.19 ^{a}
Crop evapotranspiration (ETc)			
FI (control)	27.17 ± 1.49 ^{a}	24.37 ± 0.71 ^{a}	6.37 ± 0.18 ^{a}
Ir-80	23.94 ± 1.39 ^{b}	23.85 ± 1.33 ^{ab}	6.32 ± 0.14 ^{a}
Ir-60	18.61 ± 1.14 ^{c}	22.44 ± 1.27 ^{b}	5.57 ± 0.11 ^{b}

[#]Values in each column are the means ± standard error of (n = 6). Mean values followed by a different lowercase letter are significantly different by Duncan's multiple range test at P ≤ 0.05.
FI = irrigation with 100% of soil water-holding capacity (SWHC), Ir-80 = irrigation with 80% of SWHC, and Ir-60 = irrigation with 60% of SWHC.

Table 2b. Mean square, *F* value, and probability for leaf number (LN), shoot length (SL), and stem diameter (SD).

Items	d.f.	Mean square			*F* value and probability		
		LN	SL	SD	LN	SL	SD
GB	2	392.30	386.33	2.32	21.60^{*}	52.53^{*}	8.58^{*}
ETc	2	336.07	17.90	3.62	18.50^{*}	2.43^{ns}	13.38^{*}
GB X ETc	4	14.66	31.89	0.60	0.81^{ns}	4.34^{*}	2.22^{ns}
Exp. Error	40	18.17	7.35	0.27			

^{ns} = non-significant, * Significant at the $P \leq 0.05$ level.

Table 3a. Effect of GB foliar application on some growth characteristics i.e., shoot fresh weight (FW), shoot dry weight (DW), and leaf area of eggplant (*Solanum melongena* L.) transplants grown under deficit irrigation regimes

Items	Mean ± SE		
	Shoot FW/plant (g)	Shoot DW/plant (g)	Leaf area/plant (dm^2)
GB			
GB (0 mM)	$26.34 \pm 1.37^{b\#}$	2.60 ± 0.15^{b}	1.17 ± 0.06^{c}
GB (25 mM)	30.44 ± 1.63^{ab}	2.94 ± 0.15^{ab}	1.41 ± 0.07^{b}
GB (50 mM)	31.11 ± 1.84^{a}	3.27 ± 0.19^{a}	1.61 ± 0.09^{a}
Crop evapotranspiration (ETc)			
FI (control)	32.24 ± 1.57^{a}	3.49 ± 0.18^{a}	1.60 ± 0.08^{a}
Ir-80	31.60 ± 1.53^{a}	2.88 ± 0.11^{b}	1.47 ± 0.07^{a}
Ir-60	24.06 ± 1.21^{b}	2.44 ± 0.14^{c}	1.13 ± 0.05^{b}

#Values in each column are the means ± standard error of (n = 6). Mean values followed by a different lowercase letter are significantly different by Duncan's multiple range test at P ≤ 0.05.
FI = irrigation with 100% of SWHC, Ir-80 = irrigation with 80% of SWHC, and Ir-60 = irrigation with 60% of SWHC.

Table 3b. Mean square, *F* value, and probability for shoot FW, shoot DW, and leaf area.

Items	d.f.	Mean square			*F* value and probability		
		Shoot FW	Shoot DW	Leaf area	Shoot FW	Shoot DW	Leaf area
GB	2	119.86	2.04	0.84	3.00^{*}	6.44^{*}	12.00^{*}
ETc	2	372.39	5.01	1.06	9.32^{ns}	15.78^{*}	15.10^{*}
GB X ETc	4	14.27	0.33	0.08	0.36^{ns}	1.06^{ns}	1.14^{ns}
Exp. error	40	39.98	0.31	0.07			

^{ns} = non-significant, * Significant at the $P \leq 0.05$ level.

Table 4. Mean square, *F* value, and probability for chlorophyll *a* fluorescence i.e., Fv/Fm, Fv / F_0, and PI.

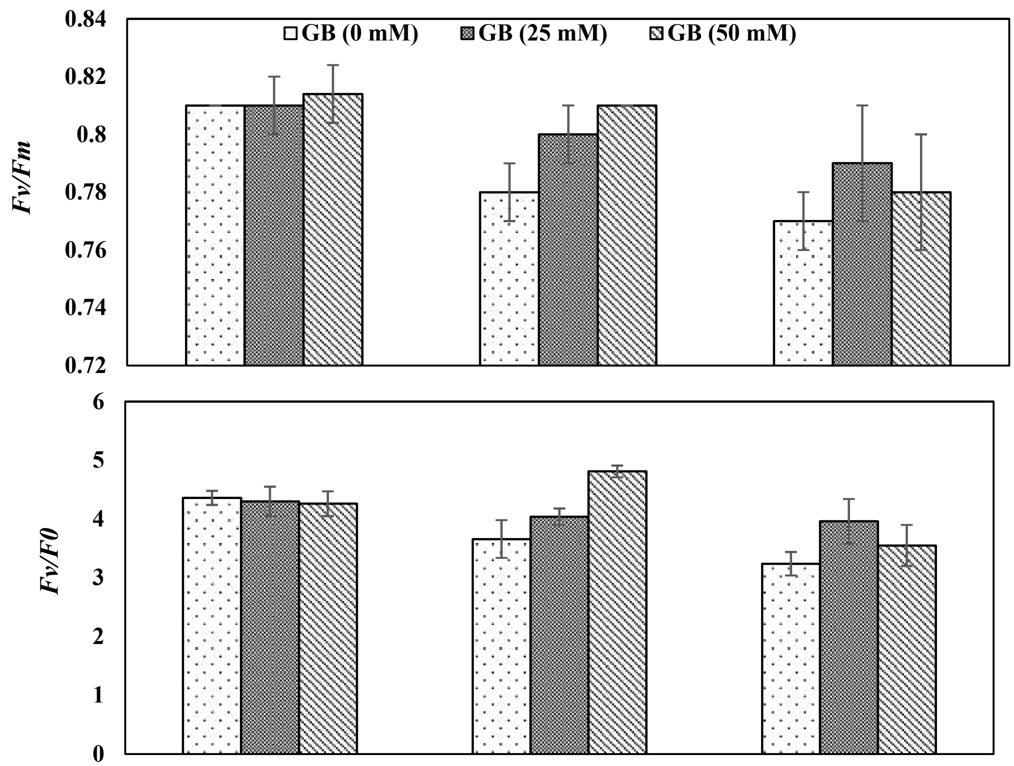
Items	d.f.	Mean square			*F* value and probability		
		Fv/Fm	Fv / F_0	PI	Fv/Fm	Fv / F_0	PI
GB	2	0.002	1.00	22.52	2.21^{ns}	2.77^{ns}	3.09^{ns}
ETc	2	0.005	2.63	10.50	7.66^{*}	7.27^{*}	1.44^{ns}
GB X ETc	4	0.001	0.92	13.72	1.91^{ns}	2.54^{ns}	1.88^{ns}
Exp. error	40	0.001	0.36	7.28			

^{ns} = non-significant, * Significant at the $P \leq 0.05$ level.

Table 5a. Effect of GB foliar application on relative chlorophyll content (SPAD value), relative water content (RWC%), and membrane stability index (MSI%) of eggplant (*Solanum melongena* L.) transplants grown under deficit irrigation regimes

Items	Mean ± SE		
	SPAD	RWC%	MSI%
GB			
GB (0 mM)	42.02 ± 1.67[b #]	70.09 ± 1.57[b]	30.87 ± 1.31[b]
GB (25 mM)	52.28 ± 1.61[a]	74.14 ± 1.47[a]	36.09 ± 1.60[a]
GB (50 mM)	55.01 ± 0.90[a]	75.29 ± 1.57[a]	36.40 ± 0.54[a]
Crop evapotranspiration (ETc)			
FI (control)	49.14 ± 1.83[ab]	79.32 ± 0.70[a]	36.01 ± 1.43[a]
Ir-80	52.29 ± 1.75[a]	72.94 ± 1.08[b]	34.22 ± 1.19[ab]
Ir-60	47.87 ± 2.10[b]	71.06 ± 0.94[b]	32.13 ± 1.12[b]

[#]Values in each column are the means ± standard error of (n = 6). Mean values followed by a different lowercase letter are significantly different by Duncan's multiple range test at P ≤ 0.05.
FI = irrigation with 100% of SWHC, Ir-80 = irrigation with 80% of SWHC, and Ir-60 = irrigation with 60% of SWHC.



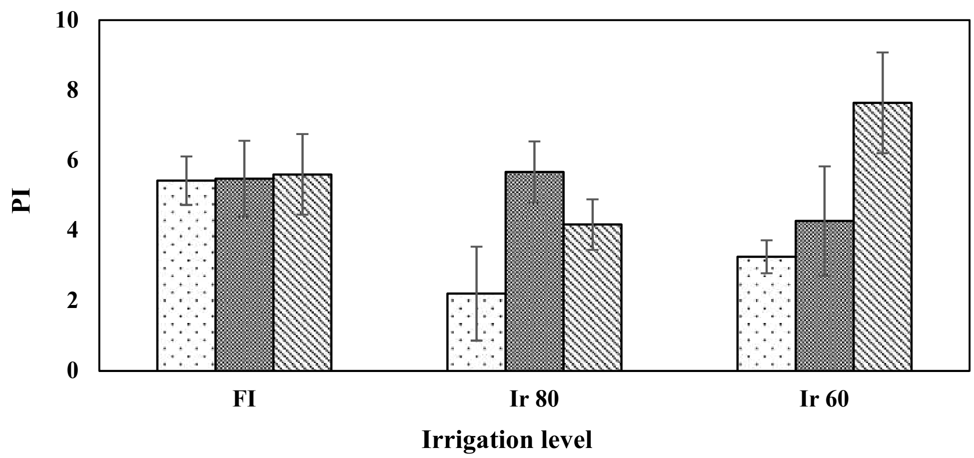


Fig. 1: Effect of glycine betaine (GB) foliar application on chlorophyll *a* fluorescence i.e., *Fv/Fm, Fv / F_0,* and PI of eggplant (*Solanum melongena* L.) transplants grown under deficit irrigation regimes.

Table 5b. Mean square, *F* value, and probability for relative chlorophyll content (SPAD value), RWC%, and MSI%.

Items	d.f.	Mean square			*F* value and probability		
		SPAD	RWC%	MSI%	SPAD	RWC%	MSI%
GB	2	844.08	5.01	40.66	22.64^{*}	0.50^{ns}	3.52^{*}
ETc	2	93.28	168.39	34.03	2.50^{ns}	16.66^{*}	2.94^{ns}
GB X ETc	4	36.68	1.56	17.85	0.98^{ns}	0.15^{ns}	1.54^{ns}
Exp. error	40	37.28	10.11	11.57			

^{ns} = non-significant, * Significant at the $P \leq 0.05$ level.

However, GB application limited the negative effects of DI on the above tested parameters and significantly improved them compared to the controls. Foliar application of 50 mM GB was better than that of 25 mM GB. For example, following foliar application of 50 mM GB, the shoot FW, shoot DW, and leaf area of eggplant were 31.11 ± 1.84, 3.27 ± 0.19, and 1.61 ± 0.09, respectively, while the corresponding values following 25 mM GB treatment were 30.44 ± 1.63, 2.94 ± 0.15, and 1.41 ± 0.07 respectively.

This better GB treatment increased the number of leaves per plant by 50.4%, shoot length by 48.9%, stem diameter by 12.5%, leaf area per plant by 37.6%, shoot FW by 18.1%, shoot DW by 25.8%, Fv/Fm by 6.3%, Fv/F0 by 12.0%, PI by 60.2%, SPAD chlorophyll by 30.9%, RWC by 7.4%, and MSI by 17.9% compared to the untreated plant.

4. DISCUSSION

One of the key results of this study is the positive response of eggplant (*Solanum melongena* L.) transplants to foliar application of GB under drought stress by enhancing dehydration tolerance expressed by RWC, membrane stability index (MSI), and chlorophyll content (SPAD value).

The results of this study indicate that increasing the DI significantly reduced ($P \leq 0.05$) all growth parameters of eggplant. This growth reduction under DI could be attributed to the role of water in increasing the uptake of mineral elements from soil and translocation of photosynthetic assimilates, reflecting increases in the number of leaves and leaf area per plant, as well as shoot FWs and DWs per plant (Ragab ***et al***., 2015). On the other hand, drought stress caused by DI leads to various physio-biochemical adverse effects in plants (Farooq ***et al***., 2009; Bhardwaj and Yadav, 2012; Zhang and Huang, 2013). However, decrease in shoot fresh and dry biomass, shoot length, and plant leaf area have been shown to accompanied with DI stress (Tahir ***et al***., 2009; Mutava ***et al***., 2015), especially in plants grown in sandy soil (Ragab ***et al***., 2015). However, foliar application of GB was found to mitigate all eggplant growth parameters and treatment with 50 mM GB yielded higher significant values. These results are in line with the results reported in other works (Abbas ***et al***., 2010; Ragab ***et al***., 2015), in which foliar application of GB improved all growth characteristics of two eggplant cultivars. Such enhancements could be attributed to the fact that GB has been shown to improve the photosynthetic rate and stomatal conductance. GB penetrates the leaves of the plant directly after it is applied and readily translocates to the roots, meristems, and expanding leaves (Makela ***et al***., 1996). Therefore, developing and expanding plant organs are primarily protected from stress, improving plant growth. In addition, once GB is translocated to plant organs, it acts as an osmoprotectant in plant cells (McCue and Hanson, 1990).

With regard to photosynthetic efficiency, chlorophyll fluorescence is widely used as a stress screening indicator in a wide number of plants to determine injury or tolerance to various environmental stresses (Walker ***et al***., 1990; Weibing and Rajashekar, 1999). Commonly, the rate and yield of variable chlorophyll fluorescence decreased due to stress conditions (Weibing and Rajashekar, 1999). Further, DI stress decreases the photosynthetic rate in plants mainly due to decreased stomatal conductance as a result of increased abscisic acid content in plant leaves (Mutava ***et al***., 2015). The deleterious effect of DI on chlorophyll fluorescence was reduced by GB application. Also, it has been mentioned that the adverse effects of DI on CO_2 uptake and chlorophyll fluorescence in plants were fully or partially prevented by GB application (Weibing and Rajashekar, 1999; Ragab ***et al***., 2015). GB can improve the thermal stability and electron transport in photosystem II (Wiliams ***et al***., 1992). Similarly, GB protects the oxygen evolution in photosystem II against stress in spinach chloroplasts (Papageorgiou ***et al***., 1991).

DI stress treatments, especially Ir-60, significantly decreased the leaf RWC and MSI. These results are in line with results reported in other studies, which refer to the use of DI strategy as a very important approach to increase crop water use efficiency (WUE) (Kirda, 2002). Moreover, the adoption of DI strategies at 60% of the SWHC could be suggested in open-field eggplant for increasing the WUE and maximizing plant growth. Plants treated with GB under DI stress conditions showed a slower decrease in leaf water potential, thus developing wilting symptoms much later than untreated plants. However, GB-treated plants showed better ability to recover from wilting than untreated plants (Weibing and Rajashekar, 1999; Ragab ***et al***., 2015). This is because GB is an osmoprotectant, and it enables plants to maintain adequate amounts of water in tissue for healthy physiological-biochemical processes that can be positively reflected in the healthy growth of eggplant plants under DI stress. This phenomenon leads to saving up to 40% of irrigation water, which may be used in other important activities for human life.

REFERENCES

BACON, M.A. (2004) Water use efficiency in plant biology. Wiley-Blackwell publishing Ltd, Oxford, UK.

HOWELL, T.A. (2001) Enhancing water use efficiency in irrigated agriculture. Agron. J., 93, 281.

PEREIRA, L.S., OWEIS, T., ZAIRI, A. & SANTOS, L. (2002) Irrigation management under water scarcity. Agric. Water Manage. 57, 175-206.

PATANE, C., TRINGALI, S., SORTINO, O. (2011) Effects of deficit irrigation on biomass, yield, water productivity and fruit quality of processing tomato under semi-arid Mediterranean climate conditions. Sci. Hortic. (Amsterdam), 129, 590-596.

IGBADUN, H.E., RAMALAN, A.A. & OIGANJI, E. (2012) Effects of regulated deficit irrigation and mulch on yield, water use and crop water productivity of onion in Samaru, Nigeria. Agric. Water Manage., 109, 162-169.

ABD EL-MAGEED, T.A., SEMIDA, W.M. & ABD EL-WAHED, M.H. (2016a) Effect of mulching on plant water status, soil salinity and yield of squash under summer-fall deficit irrigation in salt affected soil. Agric. Water Manage. 173, 1-12.

ABD EL-MAGEED, T.A., SEMIDA, W.M., MOHAMED, G.F. & RADY, M.M. (2016b) Combined effect of foliar-applied salicylic acid and deficit irrigation on physiological-anatomical responses, and yield of squash plants under saline soil. South African J. Bot. 106, 8-16.

MERWAD, A.M.A., DESOKY, E.M. & RADY, M.M. (2018) Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application. Sci. Hortic. 228, 132-144.

GEERTS, S., RAES, D., 2009. Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. Agric. Water Manage. 96, 1275-1284.

SEMIDA, W.M., EL-MAGEED, T.A.A., MOHAMED, S.E. & EL-SAWAH, N.A. (2017) Combined effect of deficit irrigation and foliar-applied salicylic acid on physiological responses, yield, and water-use efficiency of onion plants in saline calcareous soil. Arch. Agron. Soil Sci. 63, 1227-1239.

FU, Q.S., YANG, R.C., WANG, H.S., ZHAO, B., ZHOU, C.L., REN, S.X. & GUO, Y.D. (2013) Leaf morphological and ultrastructural performance of eggplant (*Solanum melongena* L.) in response to water stress. Int. J. Photosynthesis Res. 51(1), 109-114.

ROUPHAEL, Y. & COLLA, G. (2005) Growth, yield, fruit quality and nutrient uptake of hydroponically cultivated zucchini squash as affected by irrigation systems and growing seasons. Sci. Hortic. 105, 177-195.

ABD EL-MAGEED, T.A. & SEMIDA, W.M. (2015a) Effect of deficit irrigation and growing seasons on plant water status, fruit yield and water use efficiency of squash under saline soil. Sci. Hortic. 186, 89-100.

ABD EL-MAGEED, T.A. & SEMIDA, W.M. (2015b) Organo mineral fertilizer can mitigate water stress for cucumber production (*Cucumis sativus* L.). Agric. Water Manag. 159, 1-10.

GORHAM, J., 1995. Betaines in higher plants–biosynthesis and role in stress metabolism. In: Amino Acids and Their Derivatives in Higher Plants (Wallsgrove, R.M., Ed). pp.171-203. Cambridge University Press, Cambridge.

TAHIR, M., ASRAF, M. & SHAHBAZ, M. (2009) Dose exogenous application of glycine betaine as a pre-sowing seed treatment improve growth and regulate some key physiological attribute in wheat plants grown under water deficit conditions? Pak. J. Bot. 41(3), 1291-1302.

CHA-UM, S., SAMPHUMPHUANG, TH. & KIRDMANEE, C. (2013) Glycinebetaine alleviates water deficit stress in indica rice using proline accumulation, photosynthetic efficiencies, growth performances and yield attributes. Aust. J. Crop Sci. 7(2), 213-218.

HADIARTO, T. & TRAN, L.S.P. (2011) Progress studies of drought responsive genes in rice. Plant Cell Rep. 30, 297-310.

GAO, Y., LI, C. & LOU, K. (2012) Effect of spraying glycine betaine on physiological responses of processing tomato under drought stress. Plant Nutr. Fertil. Sci. 18(2), 426-432.

WEIBING, X. & RAJASHEKAR, C.B. (1999) Alleviation of water stress in beans by exogenous glycine betaine. Plant Sci. 148, 185–192.

P.M.E.P. (2017) Presidency of Meteorology & Environment Protection, National Meteorology & Environment

Center (Albaha Station), Ministry of Defence & Aviation. Saudi Arabia.
ZABIN, S.A. & HOWLADAR, S.M. (2015) Accumulation of Cu, Ni and Pb in Selected Native Plants Growing Naturally in Sediments of Water Reservoir Dams, Albaha Region, KSA. Nature and Science 13(3), 11-17.
MAXWELL, K. & JOHNSON, G.N. (2000) Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51(345), 659–668.
CLARK, A.J., LANDOLT, W., BUCHER, J.B. & STRASSER, R.J. (2000) Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. Environ. Pollut. 109(3), 501-507.
HAYAT, S., ALI, B., HASAN, S.A. & AHMAD, A. (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ. Exp. Bot. 60, 33-41.
RADY, M.M. (2011) Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. Scientia Horticulturae 129, 232-237.
RAGAB, M.E., HELAL, N.A.S., SAWAN, O.M., FAWZY, Z.F. & EL-SAWY, S.M. (2015) Foliar application of glycine betaine for alleviating water stress of tomato plants grown under sandy soil conditions. Int. J. ChemTech Res. 8(12), 52-67.
BHARDWAJ, J. & YADAV, S.K. (2012) Genetic Mechanisms of Drought Stress Tolerance, Implications of Transgenic Crops for Agriculture. Agroecol. Strategies Climate Change (Sust. Agric. Rev.) 8, 213-235.
FAROOQ, M., WAHID, A., KOBAYASHI, N., FUJITA, D. & BASRA, S.M.A. (2009) Plant drought stress: effects, mechanisms and management. In: Lichtfouse, E., Navarrete, M., Debaeke, P., Véronique, S., Alberola, C. (Eds.), Sustainable Agriculture. Springer, Netherlands, pp. 153-188.
ZHANG, CH. & HUANG, Z. (2013) Effects of endogenous abscisic acid, jasmonic acid, polyamines, and polyamine oxidase activity in tomato seedlings under drought stress. Sci. Hortic. 159, 172-177.
MUTAVA, R.N., PRINCE, S.J.K., SYED, N.H., SONG, L., VALLIYODAN, B., CHEN, W. & NGUYEN, H.T. (2015) Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. Plant Physiol. Biochem. 86, 109-120.
ABBAS, W., ASHRAF, M. & AKRAM, N.A. (2010) Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugar beet extracts. Sci. Hortic. 125. 188-195.
MAKELA, P., PELTONEN-SAINIO, P., JOKINEN, K., PEHU, E., SETAIA, H., HINKKANEN, R. & SOMERSALO, S. (1996) Uptake and translocation of foliar-applied glycine betaine in crop plants. Plant Sci. 121, 221-230.
MCCUE, K.F. & HANSON, A.D. (1990) Drought and salt tolerance: Towards understanding and application. TIBTECH 8, 358-362.
WALKER, M.A., SMITH, D.M., PAULS, L.P. & MCKERSIE, B.D. (1990) A chlorophyll fluorescence screening test to evaluate chilling tolerance in tomato. Hort. Sci. 25, 334-339.
WEIBING, X. & RAJASHEKAR, C.B. (1999) Alleviation of water stress in beans by exogenous glycine betaine. Plant Sci. 148, 185-192.
WILIAMS, W.P., BRAIN, A.P.R. & DOMINY, P.J. (1992) Induction of non-bilayer lipid phase separation in chloroplast thylakoid membranes by compatible cosolutes and its relation to the thermal stability of photosystem II. Biochim. Biophys. Acta. 1099, 137-144.
PAPAGEORGIOU, G.C., FUJIMARA, Y. & MURATA, Y.N. (1991) Protection of the oxygen-evolving photosystem II complex by glycine betaine. Biochem. Biophys. Acta. 1057, 361-366.
KIRDA, C. (2002) Deficit irrigation scheduling based on plant growth stages showing water stress tolerance. Deficit irrigation practices. In: FAO Corp, Doc. Rep. 22, Rome, pp. 3-10.