

## Foliar application of glycine betaine for alleviating water stress of tomato plants grown under sandy soil conditions

<sup>1</sup>Ragab, M.E., <sup>1</sup>Nesreen A.S. Helal, <sup>2</sup>Omaima M. Sawan,  
<sup>2</sup>Z.F. Fawzy and <sup>2</sup>S.M. El-Sawy

<sup>1</sup>Horticulture Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

<sup>2</sup>Vegetable Research Dept., National Research Centre, 33El-Bohouth St.,  
(former El Tahrir St.) Dokki, Giza, Egypt. Postal Code: 12622.

**Abstract:** Two field experiments were carried out during the two growing seasons of 2012/2013 and 2013/2014, in a private farm at Bani Salama region, El-Giza Governorate, Egypt, in order to investigate the effect of foliar application of glycine betaine (GB) on vegetative growth, fruit yield and fruit quality of tomato plants (Marwa hybrid) grown under deficit irrigation (DI) treatments (100% (control), 85%, 70% and 55% of  $ET_0$  (Reference evapotranspiration)) in sandy soil. Four GB concentrations (0, 5, 10 and 20 mM/l), were applied after 2 and 6 weeks from transplanting date. Results indicated that, DI treatments significantly decreased the vegetative growth, photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), leaf relative water content (LRWC), number of flowers per plant and fruit yield of tomato plants. In contrast, there were positive effects on proline content in the leaves, irrigation water use efficiency (IWUE) and some fruit quality characteristics for tomatoes. Foliar application of GB at 10 mM/l ameliorated the negative effects of water stress and produced the highest significant values of plant length, number of leaves/plant, total leaves area/plant, fresh and dry weights of tomato leaves, LRWC and photosynthetic pigments, in both seasons. While, the values of marketable fruit yield of tomatoes were increased significantly also with foliar application of GB at 10 mM/l (by 12.86% and 13.91% in the first and second seasons, respectively), but there were no significant differences were realized among the GB treatments on the fruit quality. Concerning, the effect of interaction between DI treatments and foliar application of GB, the results revealed that application of irrigation water with 100%  $ET_0$  and 10 mM/l of GB was the best treatment for vegetative growth and fruit yield of tomato plants in open field. It was also concluded that the vegetative growth and fruit yield as well as fruit quality of tomato plants which grown under DI, can be enhanced by foliar application of GB with 10 mM/l.

**Key words :** Tomato, Deficit irrigation, Glycine betaine, Yield, Fruit quality, IWUE.

### Introduction

Tomato (*Solanum lycopersicum* L.) is a member of solanaceae family, it's one of the most popular and widely consumed vegetable crops in Egypt and all over the world<sup>1,2</sup>. In Egypt, the tomato's cultivated area amounted by 460.000 feddan<sup>3</sup>. Increasing the productivity per unit area through increasing growth of tomato plants as well as with expanding the cultivated area in newly reclaimed lands, is very important issue especially under climate changes and water shortage conditions<sup>4</sup>. Tomato is a warm season perennial crop<sup>5</sup> and it's sensitive to water stress and there is a high correlation between evapotranspiration (ET) and crop yield<sup>6</sup>.

Drought stress is the major stress affecting crop growth, development and yields<sup>7</sup>. In addition, the agricultural sector consumes more than 85% of the total annual water consumption<sup>8</sup>. As the demand for water is increasing, effective new technologies and agricultural practices are required to save water for expansion of agricultural area and other purposes<sup>9,10</sup>.

Water stress is the major harmful factor affecting the vegetative growth, production and quality of vegetable crops. Several studies have shown that water deficit reduced crop growth, canopy development, morphological characteristics (shoot fresh weight, shoot length and leaf area per plant) and dry matter accumulation of tomato and sweet corn plants<sup>11,12,13</sup>. In addition, maximum leaf area index LAI values for sweet corn plants were found in subjected to full irrigation treatment<sup>14</sup>. Also, several studies have shown that crop yields decreased with decreasing irrigation water for different crops: tomato<sup>15</sup> and squash<sup>16</sup>. As well as, the highest marketable yields of tomatoes were obtained with 100% of crop evapotranspiration ( $ET_c$ ) treatment and the yield reduction has been done when  $ET_c$  is reduced<sup>17,18</sup>. Also, the same results were obtained by<sup>19</sup> and on potatoes<sup>20</sup>. On the other hand, the application of drip irrigation with 75% of  $ET_c$  was found to be the optimum irrigation amount in order to obtain the maximum yield of tomatoes and water productivity<sup>21</sup>. The same results were obtained on sweet corn in Turkey<sup>14</sup> and on processing tomato in Italy<sup>22</sup>.

Conservation of water resources to justify the demand of more crops per drop of water<sup>23,24,9,7</sup>, so using of deficit irrigation (DI) strategy is very important to increase crop water use efficiency (WUE) by reducing the amount of irrigation water<sup>25</sup>. The adoption of DI strategies where a 50% reduction of  $ET_c$  could be suggested for open-field processing tomato in South Italy, for increasing WUE, minimizing fruit losses and maintaining high fruit quality levels<sup>22</sup>. As well as, several studies have shown that DI improved total soluble solids content, titratable acidity, vitamin C content and did not decrease dry mass yield for tomatoes<sup>18,26,27,28,22</sup>. Otherwise, higher irrigation amount increased fruit number, mean fruit weight, fruit diameter and fruit length but decreased TSS and fruit firmness of tomato fruits<sup>29</sup>. In addition, water use efficiency was increased by DI strategy<sup>22</sup>. Also, DI strategy can make a 50% saving of water and an approximately 200% increasing in irrigation water use efficiency compared to full irrigation<sup>26,16,30</sup>.

Glycine betaine (GB) is an amino acid derivative which accumulates in a variety of plant species in response to environmental stresses such as drought, salinity and extreme temperatures. However, there are many important crop species, like potato or tomato are unable to accumulate GB<sup>31</sup>. Thus, as an alternative, exogenous application of GB may be a possible approach to tolerate environmental stress<sup>32,33,34</sup>.

Several studies have shown that, exogenous application of GB increased shoot fresh biomass and leaf area per plant<sup>35,36,37</sup>. Moreover, drought stress decreased the following parameters for tomato plants, shoot height, root length, leaf number, leaf area, total shoot fresh weight, total shoot dry weight, relative water content and stress tolerance index<sup>38,43</sup>. The exogenous application of GB increased all those traits significantly (70%, 73%, 187%, 193%, 168%, 9%, 72% and 122%, respectively). On common bean plants, application of GB at 2 mM/l under depletion of 30% available soil water increased leaf number and plant dry weight, chlorophyll reading, calcium percentage and decreased proline in leaves<sup>39</sup>. In the same trend, under drought stress, the contents of chlorophyll and protein are decline in the leaves of processing tomato, while the contents of proline and soluble sugar are increased and antioxidant enzyme activities are enhanced by spraying GB (20, 40 mM/l)<sup>40</sup>. In addition, GB-treated plants showed better ability to recover from wilting than untreated plants<sup>41</sup>.

The number of flowers, number of fruits and weight of fruit per plant, decreased under drought stress and increased significantly by 86%, 115% and 125%, respectively, under exogenous application of GB<sup>43,42</sup>. In addition, foliar application of GB (2 or 4 mM/l) produced the highest seed yield and quality under the depletion of 30% available soil water<sup>39</sup>.

The present study was conducted to determine the effect of glycine betaine application on ameliorating the deleterious effects of deficit irrigation on the vegetative growth, fruit yield and quality of tomato plants grown under sandy soil conditions.

## Materials and Methods

Field experiment was carried out on tomato plants (*Solanum lycopersicum* L.) during 2012/2013 and 2013/2014 seasons, in a private farm at Bani Salama region, El-Giza Governorate, Egypt, in order to investigate the effect of foliar application of glycine betaine (at concentrations of 0 (control, sprayed with distilled water),

5, 10 and 20 mM/l of GB, which were applied after 2 and 6 weeks from transplanting date) on ameliorating the deleterious effects of deficit irrigation (DI levels were 100% (control), 85%, 70% and 55% of  $ET_o$  (Reference evapotranspiration) and the total amounts of irrigation water were calculated by using Penman–Montieth modified equation<sup>44</sup> and the results are showed in Table (3)) on the vegetative growth, fruit yield and quality of tomato plants grown under sandy soil conditions. The experimental site (Fig.1) is located at latitude: 30°15"N, longitude: 30°47"E, in a newly reclaimed area. Geographical position of the experimental site is shown in Fig.1. Soil and irrigation water samples were taken and analyzed before planting and data are shown in Tables (1 and 2).

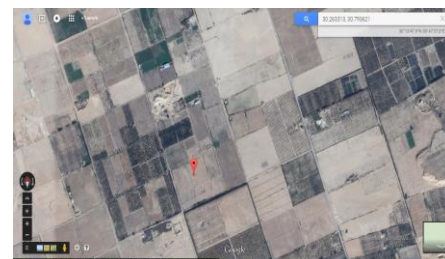
Tomato hybrid "Marwa" was used as test crop. Transplanting dates were on 27<sup>th</sup> of September and 1<sup>st</sup> of October in the first and second seasons, respectively. All agriculture practices were performed as recommended by Egyptian Ministry of Agriculture and Land Reclamation for tomato cultivation under open field conditions. Plants were fertilized with 230 units of N, 45 units of  $P_2O_5$  and 70 units of  $K_2O$ /fed. during the growing season.

### Experimental design:

The experiment was arranged in a split-plot design with three replications. Deficit irrigation treatments were arranged in the main plots and glycine betaine concentrations were assigned in the sub-plots. The area of the experimental plot was 22.5 m<sup>2</sup> consisted of one row with 15 m length and 1.5 m width and the plants were transplanted 75 cm spaced in the rows.

**Table (1): Physical and chemical properties of experimental soil analysis.**

Physical properties		Values
Very coarse sand, % (2-1 mm)		16.60
Coarse sand, % (1-0.5 mm)		54.80
Medium sand, % (0.5-0.25 mm)		1.14
Fine sand, % (0.25-0.1 mm)		16.48
Very fine sand, % (0.1-0.05 mm)		9.64
Silt + Clay, % (0.05> mm)		1.34
Soil texture		Sandy
Field capacity, (%)		12
Wilting point, (%)		3.7
Saturation percent, (%)		29
Chemical properties		
pH		8.12
EC (dS/m)		1.81
Soluble cations (meq./L)	Ca <sup>++</sup>	7
	Mg <sup>++</sup>	3.2
	Na <sup>+</sup>	7.6
	K <sup>+</sup>	0.41
Soluble anions (meq./L)	CO <sub>3</sub> <sup>--</sup>	Nil
	HCO <sub>3</sub> <sup>-</sup>	2.5
	Cl <sup>-</sup>	9
	SO <sub>4</sub> <sup>--</sup>	6.71



**Fig.1: Experimental site (Google map, Satellite)**

**Table (2): Chemical properties of experimental water analysis.**

Items		Values
pH		7.97
EC (dS/m)		1.36
Soluble cations (meq./L)	Ca <sup>++</sup>	6.35
	Mg <sup>++</sup>	4.11
	Na <sup>+</sup>	5.95
	K <sup>+</sup>	0.23
Soluble anions (meq./L)	CO <sub>3</sub> <sup>-</sup>	0
	HCO <sub>3</sub> <sup>-</sup>	2.97
	Cl <sup>-</sup>	4.36
	SO <sub>4</sub> <sup>--</sup>	5.12

**Measured characteristics:**

- *Vegetative growth characteristics:* five plants were randomly chosen from three replications at 65 days from transplanting date to determine the following Characteristics: plant length (cm), number of leaves per plant, total leaves area (m<sup>2</sup>)/plant (total leaves area was estimated with a 20 disc sampling per plant, dried and weighted separately. A relationship between disk dry matter and disk area was applied to total leaf dry matter to find total leaf area<sup>45</sup>, fresh weight and dry weight of leaves (g) per plant.

**- Determination of proline content in the tomato leaves:**

Free proline content was extracted using 3% (w/v) aqueous sulphosalicylic acid and estimated by ninhydrin reagent<sup>46</sup>.

**Table (3): Irrigation requirements (minute/plant per day) for irrigation treatments (100%, 85%, 70% and 55% of ET<sub>0</sub>) for tomato plants in open field cultivation in both seasons of 2012-2013 and 2013-2014.**

Weeks*	First season (2012/2013)				First season (2013/2014)			
	100%	85%	70%	55%	100%	85%	70%	55%
1	13.00	11.05	9.10	7.15	14.00	11.90	9.80	7.70
2	13.50	11.48	9.45	7.43	14.00	11.90	9.80	7.70
3	14.50	12.33	10.15	7.98	15.00	12.75	10.50	8.25
4	15.00	12.75	10.50	8.25	15.50	13.18	10.85	8.53
5	16.00	13.60	11.20	8.80	16.50	14.03	11.55	9.08
6	17.30	14.71	12.11	9.52	17.80	15.13	12.46	9.79
7	18.80	15.98	13.16	10.34	19.00	16.15	13.30	10.45
8	20.50	17.43	14.35	11.28	20.50	17.43	14.35	11.28
9	23.30	19.81	16.31	12.82	23.00	19.55	16.10	12.65
10	26.00	22.10	18.20	14.30	27.00	22.95	18.90	14.85
11	29.00	24.65	20.30	15.95	28.80	24.48	20.16	15.84
12	32.20	27.37	22.54	17.71	32.70	27.80	22.89	17.99
13	33.50	28.48	23.45	18.43	34.00	28.90	23.80	18.70
14	34.60	29.41	24.22	19.03	35.40	30.09	24.78	19.47
15	36.00	30.60	25.20	19.80	37.00	31.45	25.90	20.35
16	38.40	32.64	26.88	21.12	39.50	33.58	27.65	21.73
17	41.00	34.85	28.70	22.55	42.30	35.96	29.61	23.27
18	38.60	32.81	27.02	21.23	40.60	34.51	28.42	22.33

\*Starting from 1<sup>st</sup> of October (2012 and 2013 for the first and second seasons, respectively).

**- Estimation of photosynthetic pigments:**

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids (mg/g fresh weight)) in leaves were assayed<sup>47</sup>. Fifth leaf from the top of the plant (the fully expanded leaf) was chosen to take 0.5g sample fresh weight. The pigments were extracted by grinding the tissue with mortar and pestle using 10 ml N, N dimethyl formamide. The resulting extracts were used to determine the pigments by UV/VIS spectrophotometer (CT 200 spectrophotometer). The absorbance of the solution was measured at 470, 647 and 664 nm for chlorophyll a, b and carotenoids, respectively.

- *Number of flowers and number of fruits per plant* were also measured.

- *Yield:* Tomato fruits were hand harvested when reached to red ripe stage. At harvesting, total marketable yield was measured considering red and disease-free fresh fruits.

- *Fruit quality:* Random samples of fruits were taken from each experimental plot to determine the average fruit weight and total soluble solids (TSS %) using hand refractometer.

**Water measurements:****- Leaf relative water content (LRWC) (%):**

For the estimation of LRWC, 20 leaf discs samples (10 mm in diameter) were taken with a cork borer from three plants per replicate (the fifth leaf from the top) and placed in a reweighed Petri dish to determine

fresh weight (F.Wt.), discs were floated for 24 hours in distilled water inside a closed Petri dish until the discs became fully turgid. Discs samples were weighted after gently wiping the water from the leaf surface with a filter paper to determine turgid weight (T.Wt.). Finally, the leaf discs were placed in a per-heated oven at 70° C to a constant weight (almost 48h) and weighted again to obtain discs dry weight (D. Wt.). So, LRWC % was calculated according to the following equation<sup>48</sup>:  $LRWC \% = [(FW-DW)/(TW-DW)] \times 100$

#### - Irrigation water use efficiency (IWUE) (kg/m<sup>3</sup>):

Irrigation water use efficiency under deficit irrigation treatments was determined using the following equations<sup>49</sup>:  $IWUE = \text{Yield (kg/fed.)} / \text{Applied irrigation water amount (m<sup>3</sup>/fed.)}$ .

#### Statistical analysis:

Analysis of variance of the obtained data from each attribute was computed using the MSTAT Computer Program<sup>50</sup>. The Duncan's New Multiple Range test at 5% level of probability was used to test the significance of differences among mean values<sup>51</sup>.

## Results and discussion

#### Vegetative growth characteristics:

Data in Tables (4 and 5) present the effect of deficit irrigation (DI) treatments and foliar application of glycine betaine (GB) on some plant growth characteristics of tomato plants, i.e., plant length, number of leaves per plant, total leaves area /plant and fresh and dry weights of tomato leaves. The obtained results clearly indicated that increasing the irrigation water deficit led significantly to decrease all plant growth parameters during both seasons of study. The highest values were obtained by the full irrigation (100% ET<sub>o</sub> (control)) treatment, followed by 85% ET<sub>o</sub> treatment with significant differences between them, whereas, the lowest values were obtained by 55% ET<sub>o</sub> treatment. This may be due to the role of water in increasing the uptake of mineral elements from soil and translocation of photosynthetic assimilates, thus reflected increases in the leaf number and leaf area as well as foliage weight/plant<sup>52</sup>. On the other hand, drought stress causes various physiologic and biochemical effects in plants<sup>7,53,54</sup>. However, the reduction in shoot fresh and dry biomass, shoot length, leaf area per plant, net CO<sub>2</sub> assimilation, transpiration rates, and stomatal conductance were accompanied to water stress<sup>55,35,56</sup>, especially, for plants were grown under sandy soil conditions. In addition, the increase of irrigation water from 60% and up to 100% ET<sub>o</sub> significantly increased the vegetative growth parameters, i.e. plant height, number of branches, leaves and pods/plant as well as leaf area and dry weight of stem and whole bean plants<sup>57</sup>.

Concerning the effect of foliar application of GB on the above mentioned characteristics, data showed that the foliar application of GB ameliorated the vegetative growth parameters for tomato plants and the highest significant values for plant length, number of leaves/plant, total leaves area/plant, fresh and dry weights of tomato leaves were obtained with 10 mM/l GB treatment in both seasons and the lowest values were noticed with control treatment (0 mM/l GB). These results are in harmony with other work<sup>58</sup>, found that foliar application of GB caused an improvement in vegetative growth of two eggplant cultivars, such improvement may be due to that GB was found to be related to improve photosynthetic rate and stomatal conductance. In addition, exogenously applied glycine betaine has been shown to penetrate into plant leaves soon after application and is readily translocated to roots, meristems and expanding leaves<sup>32</sup>. Thus, developing and expanding plant organs are primarily protected from stress and enhanced growth and reproduction. Moreover, once glycine betaine is translocated to plant organs, it acts as an osmoprotectant in the cells<sup>59</sup>.

Respecting the interactions between DI treatments and foliar application of GB, tomato plants were irrigated by 100% ET<sub>o</sub> (control) treatment and foliar sprayed by 10 mM/l GB gave the highest values of all mentioned characteristics, followed by 85% ET<sub>o</sub> treatment and foliar sprayed by 10 or 20 mM/l GB with significant differences among the treatments.

**Photosynthetic pigments and proline content in tomato leaves:**

Data in Table (6) present the effect of DI treatments and foliar application of GB on photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) and proline content in tomato leaves. As to the photosynthetic pigments, the highest significant values were obtained with 100% ET<sub>0</sub> (control) treatment, while the lowest values were gained with 55% ET<sub>0</sub> treatment in both seasons. In the second season there were no significant differences were realized between 100% ET<sub>0</sub> and 85% ET<sub>0</sub> treatments. In the same trend, the contents of chlorophyll are declined in the leaves of processing tomato under drought stress<sup>40</sup>. Where, chlorophyll fluorescence has been used widely in a number of plants as a stress screening indicator to determine injury or tolerance to various environmental stresses<sup>41,60</sup>. Typically, the rate and yield of variable chlorophyll fluorescence were reduced by stress conditions<sup>41</sup>. In addition, drought stress reduces photosynthetic rate in soybean which mainly due to the reduction in stomatal conductance caused by increased ABA concentration in the leaves. On contrast, proline content increased in tomato leaves with decreasing the irrigation water<sup>56</sup>.

**Table (4): Effect of deficit irrigation and foliar application of glycine betaine on some vegetative attributes of tomato plants during 2012/2013 and 2013/2014 seasons.**

Treatments		2012/2013				2013/2014							
Deficit irrigation (DI)	Glycine betaine (GB)	Plant length (cm)		Number of leaves/plant		Total leaves area (m <sup>2</sup> /plant)		Plant length (cm)		Number of leaves/plant		Total leaves area (m <sup>2</sup> /plant)	
100% ET <sub>0</sub>	0 mM/l	89.83	b	86.83	b	4.05	bc	90.46	b	86.93	bc	4.24	B
	5 mM/l	90.17	b	88.00	b	4.13	b	90.56	b	89.37	b	4.22	B
	10 mM/l	98.33	a	96.83	a	4.31	a	98.26	a	98.00	a	4.44	A
	20 mM/l	89.00	b	88.83	b	3.98	c	89.33	bc	88.95	b	4.25	B
85% ET <sub>0</sub>	0 mM/l	83.33	C	76.67	def	3.68	d	83.56	d	78.00	e	3.78	C
	5 mM/l	85.50	bc	79.83	cd	3.98	c	85.67	cd	80.74	de	4.07	B
	10 mM/l	88.00	bc	84.50	bc	4.00	c	89.50	bc	84.62	c	4.12	B
	20 mM/l	87.17	bc	80.83	cd	4.02	bc	87.31	bcd	81.19	d	4.16	B
70% ET <sub>0</sub>	0 mM/l	78.00	d	63.33	g	2.37	g	78.83	e	64.41	g	2.48	E
	5 mM/l	79.00	d	71.33	f	2.50	f	79.50	e	72.60	f	2.63	E
	10 mM/l	85.83	bc	77.50	de	3.28	e	86.97	bcd	79.67	de	3.42	D
	20 mM/l	83.50	C	73.50	ef	3.37	e	84.33	d	74.24	f	3.41	D
55% ET <sub>0</sub>	0 mM/l	59.50	G	59.83	g	1.63	j	60.17	h	59.00	h	1.67	H
	5 mM/l	64.83	F	51.92	h	1.77	i	65.58	g	62.38	g	1.81	gh
	10 mM/l	73.67	E	63.73	g	2.16	h	74.67	f	64.92	g	2.14	F
	20 mM/l	72.50	E	63.13	g	1.81	i	73.40	f	63.67	g	1.89	G
100% ET <sub>0</sub>		91.83	A	90.13	A	4.12	A	92.15	A	90.81	A	4.29	A
85% ET <sub>0</sub>		86.00	B	80.46	B	3.92	B	86.51	B	81.14	B	4.03	B
70% ET <sub>0</sub>		81.58	C	71.42	C	2.88	C	82.41	C	72.73	C	2.98	C
55% ET <sub>0</sub>		67.63	D	59.65	D	1.84	D	68.45	D	62.49	D	1.88	D
0 mM/l		77.67	D	71.67	C	2.93	D	78.25	D	72.09	C	3.04	D
5 mM/l		79.88	C	72.77	C	3.10	C	80.33	C	76.27	B	3.18	C
10 mM/l		86.46	A	80.64	A	3.44	A	87.35	A	81.80	A	3.53	A
20 mM/l		83.04	B	76.57	B	3.30	B	83.59	B	77.01	B	3.43	B

Values followed by the same letter (s) within column are not significantly different (P<0.05)

**Table (5): Effect of deficit irrigation and foliar application of glycine betaine on fresh and dry weights of leaves (g) of tomato plants during 2012/2013 and 2013/2014 seasons.**

Treatments		2012/2013				2013/2014			
Deficit irrigation (DI)	Glycine betaine (GB)	Fresh weight of leaves (g)		Dry weight of leaves (g)		Fresh weight of leaves (g)		Dry weight of leaves (g)	
100% ET <sub>0</sub>	0 mM/l	668.80	b	29.09	c	671.60	b	30.97	c
	5 mM/l	662.00	b	29.73	bc	665.60	b	31.53	bc
	10 mM/l	730.50	a	31.83	a	731.10	a	33.53	a
	20 mM/l	670.00	b	30.52	b	673.20	b	32.22	b
85% ET <sub>0</sub>	0 mM/l	591.00	e	22.15	g	596.80	e	23.64	g
	5 mM/l	611.50	d	24.17	f	614.60	d	25.63	f
	10 mM/l	630.00	c	28.09	d	633.30	c	29.68	d
	20 mM/l	612.50	cd	26.38	e	617.50	cd	28.03	e
70% ET <sub>0</sub>	0 mM/l	353.30	g	15.63	j	358.30	g	17.10	j
	5 mM/l	361.80	g	16.65	i	365.50	g	18.20	i
	10 mM/l	390.80	f	19.39	h	394.90	f	20.89	h
	20 mM/l	358.30	g	16.37	ij	362.50	g	17.97	ij
55% ET <sub>0</sub>	0 mM/l	186.70	j	12.23	m	191.10	j	13.77	m
	5 mM/l	208.50	i	13.32	l	213.60	i	14.54	lm
	10 mM/l	229.30	h	14.51	k	233.60	h	16.02	k
	20 mM/l	210.00	i	13.63	l	214.00	i	15.31	kl
100% ET <sub>0</sub>		682.80	A	30.29	A	685.40	A	32.06	A
85% ET <sub>0</sub>		611.30	B	25.20	B	615.50	B	26.75	B
70% ET <sub>0</sub>		366.10	C	17.01	C	370.30	C	18.54	C
55% ET <sub>0</sub>		208.60	D	13.42	D	213.10	D	14.91	D
0 mM/l		450.00	C	19.77	D	454.40	C	21.37	D
5 mM/l		461.00	B	20.97	C	464.80	B	22.48	C
10 mM/l		495.20	A	23.45	A	498.20	A	25.03	A
20 mM/l		462.70	B	21.73	B	466.80	B	23.38	B

Values followed by the same letter (s) within column are not significantly different (P<0.05)

The highest significant values were obtained with 55% ET<sub>0</sub> treatment and the lowest values were gained by 100% ET<sub>0</sub> (control) treatment in both seasons. In the same trend water stress induced an accumulation in proline concentration in wheat grains. Increased proline content in the grain of stressed wheat plants may help to overcome any further stress conditions<sup>61</sup>. Proline accumulates under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress. Similar results are obtained in sorghum plant<sup>62</sup>. In the same trend, under drought stress, the contents of proline and soluble sugar are increased in the leaves of processing tomato<sup>40</sup>.

Respecting the effect of foliar application of GB on photosynthetic pigments and proline content in tomato leaves, results showed that, the highest significant values of chlorophyll a, b and carotenoids were obtained with 10 and 20 mM/l of GB, with no significant differences between them except with chlorophyll a in the first season only. On contrast, the lowest values were obtained with 0 mM/l GB treatment. In this respect, the application of glycine betaine at 1 and 5 mM to salt stressed tomato plants increased the photosynthetic pigments<sup>63</sup>. Moreover, the effect of water stress on fluorescence was reduced by glycine betaine treatment<sup>41</sup>. Also, they mentioned that the adverse effects of water stress on CO<sub>2</sub> uptake and chlorophyll fluorescence in bean were fully or partially prevented by glycine betaine treatment. In support, glycine betaine can improve the thermal stability and electron transport in photosystem II<sup>64</sup>. Similarly in spinach chloroplast, glycine betaine was shown to protect the oxygen evolution in photosystem II against salt stress<sup>65</sup>. On the other hand, the highest significant values of proline content were obtained with 0 mM/l GB (control) treatment and then decreased with increasing GB concentrations in both seasons. In this respect, the reduction of free proline due to the application of glycine betaine was observed, where addition of 1 mM GB to salt affected tomato plants reduced proline accumulation in leaves by 41% while 5 mM GB increased proline by 30%, suggesting its interference in the process of osmotic adjustment by proline production<sup>63</sup>.

**Table (6): Effect of irrigation levels and foliar application of glycine betaine on chlorophyll a, b and carotenoids (mg/g f.wt) and free proline ( $\mu\text{g/g DW}$ ) contents in tomato leaves during 2012/2013 and 2013/2014 seasons.**

Treatments		2012/2013						2013/2014									
Deficit irrigation (DI)	Glycine betaine (GB)	Chlorophyll a (mg/g fresh weight)		Chlorophyll b (mg/g fresh weight)		Carotenoids (mg/g fresh weight)		Proline content ( $\mu\text{g/g DW}$ )		Chlorophyll a (mg/g fresh weight)		Chlorophyll b (mg/g fresh weight)		Carotenoids (mg/g fresh weight)		Proline content ( $\mu\text{g/g DW}$ )	
100% ET <sub>0</sub>	0 mM/l	2.03	e	1.49	bc	1.49	b	36.85	l	2.08	ef	1.54	b	1.56	c	36.52	K
	5 mM/l	2.34	c	1.48	bc	1.61	a	36.60	lm	2.42	c	1.53	b	1.67	b	36.38	K
	10 mM/l	2.66	a	1.76	a	1.69	a	36.00	m	2.74	a	1.79	a	1.75	a	36.08	K
	20 mM/l	2.52	b	1.71	a	1.69	a	36.33	lm	2.61	b	1.74	a	1.76	a	36.10	K
85% ET <sub>0</sub>	0 mM/l	1.89	f	1.31	d	1.12	d	39.75	i	2.01	f	1.39	cd	1.24	e	40.05	H
	5 mM/l	2.03	e	1.48	bc	1.40	c	38.87	j	2.12	e	1.61	b	1.39	d	39.18	I
	10 mM/l	2.22	d	1.64	a	1.48	bc	38.38	j	2.23	d	1.73	a	1.55	c	38.07	J
	20 mM/l	2.12	de	1.69	a	1.44	bc	37.67	k	2.27	d	1.74	a	1.56	c	37.98	J
70% ET <sub>0</sub>	0 mM/l	1.57	hi	1.32	d	0.89	ef	48.53	e	1.63	j	1.34	de	0.94	g	49.35	D
	5 mM/l	1.66	gh	1.28	de	0.97	e	47.22	f	1.75	ghi	1.35	de	1.05	f	47.97	E
	10 mM/l	1.73	g	1.37	cd	1.14	d	46.03	g	1.84	g	1.48	bc	1.24	e	46.25	F
	20 mM/l	1.65	gh	1.51	b	1.16	d	45.05	h	1.77	gh	1.59	b	1.22	e	45.35	G
55% ET <sub>0</sub>	0 mM/l	1.36	j	0.86	g	0.58	i	60.80	a	1.39	k	0.90	f	0.62	j	61.67	A
	5 mM/l	1.52	i	0.92	g	0.67	h	59.97	b	1.61	j	0.97	f	0.75	i	59.00	B
	10 mM/l	1.60	hi	1.13	f	0.81	fg	55.57	c	1.67	hij	1.23	e	0.82	h	54.82	C
	20 mM/l	1.62	ghi	1.17	ef	0.76	g	54.57	d	1.65	ij	1.32	de	0.80	hi	55.38	C
100% ET <sub>0</sub>		2.39	A	1.61	A	1.62	A	36.45	D	2.46	A	1.65	A	1.68	A	36.27	D
85% ET <sub>0</sub>		2.06	B	1.53	B	1.36	B	38.67	C	2.16	B	1.62	A	1.43	B	38.82	C
70% ET <sub>0</sub>		1.65	C	1.37	C	1.04	C	46.71	B	1.75	C	1.44	B	1.11	C	47.23	B
55% ET <sub>0</sub>		1.52	D	1.02	D	0.71	D	57.72	A	1.58	D	1.11	C	0.75	D	57.72	A
0 mM/l		1.71	D	1.25	B	1.02	C	46.48	A	1.78	C	1.29	C	1.09	C	46.90	A
5 mM/l		1.89	C	1.29	B	1.16	B	45.66	B	1.98	B	1.37	B	1.21	B	45.63	B
10 mM/l		2.05	A	1.47	A	1.28	A	44.00	C	2.12	A	1.56	A	1.34	A	43.80	C
20 mM/l		1.98	B	1.52	A	1.26	A	43.40	D	2.07	A	1.60	A	1.33	A	43.70	C

Values followed by the same letter (s) within column are not significantly different ( $P < 0.05$ )

Regarding the interactions between DI treatments and foliar application of GB, the highest significant values of chlorophyll a were obtained with (100% ET<sub>0</sub> (control) and foliar application of 10 mM/l GB) treatment, but the highest significant values for chlorophyll b and carotenoids were gained by (100% or 85% ET<sub>0</sub> and 10 or 20 mM/l GB) treatments. As to proline content in tomato leaves, plants were irrigated with 55% ET<sub>0</sub> and foliar sprayed by GB at 0 mM/l (control treatment) gave the highest significant values in both seasons.

### Flowering and fruit yield:

Data in Table (7) show the effect of DI treatments and foliar application of GB on number of flowers per plant, number of fruits per plant and total marketable yield. Results illustrated that drought stress adversely affected number of flowers per plant and fruit yield of tomato plants. The highest significant values were obtained with 100% ET<sub>0</sub> (control) treatment followed by 85% ET<sub>0</sub> and the lowest values were obtained by 55% ET<sub>0</sub> treatment. DI treatments which negatively affected and reduced plant growth of tomato plants as shown in Tables (5 and 6). So, thus affected the development of flowering and fruiting characteristics. These results are in agreement with other works on bean<sup>66,39</sup> and onion<sup>52</sup>. In addition, maximum marketable tomato yields are obtainable under irrigation with water amounts based on 100% ET<sub>c</sub><sup>67</sup>, or by irrigation water amounts determined using  $K_p \cdot 1.00$ <sup>18</sup>. Besides, a negative trend in response to increasing soil water deficit was observed for fruit yield<sup>28</sup>. In this respect, it was observed that the number of marketable fruits per plant reduced as soil



water tension increased during fruit development<sup>68</sup>. Also, higher yields of tomatoes could be realized with a greater seasonal water application<sup>10</sup>.

Concerning the effect of foliar application of GB on number of flowers per plant and fruit yield. Results showed that the foliar application of 10 mM/l GB gave the highest significant values of the number of flowers, number of fruits per plant and total marketable yield. While, the lowest values were obtained with 0 mM/l GB treatment. Similarly, the application of GB (10 mM) was significant in alleviating the adverse effects of water deficit on yield and yield components of wheat cultivars<sup>69</sup>. These results are in conformity with those obtained on sorghum plant under drought stress<sup>70</sup>. This improvement in yield and yield components due to GB application would result from the beneficial effect of GB on growth and metabolism and its role as osmoprotectant. Moreover, the positive effects of foliar spraying of GB under water limited environment were observed in different crops such as tomato<sup>42</sup> and common bean<sup>44</sup>.

Respecting the interactions between DI treatments and foliar application of GB, tomato plants were irrigated by 100% ET<sub>o</sub> (control) treatment with foliar sprayed with 10 mM/l GB gave the highest significant values of the above mentioned characteristics. In addition, no significant differences were detected with 5 mM/l GB treatment on the number of fruits per plant and 20 mM/l GB treatment on the total marketable yield.

### **Fruit quality:**

Data in Table (8) show the effect of DI treatments and foliar application of GB on fruit quality of tomatoes. Results illustrated that the deficit irrigation treatments significantly decreased the average fruit weight, where the highest significant values were obtained with 100% ET<sub>o</sub> (control) treatment. While, there were no significant differences among 100%, 85% and 70% ET<sub>o</sub> treatments for the fruit diameter and they were superior on 55% ET<sub>o</sub> treatment. The highest significant values for tomato fruit TSS were gained by 55% ET<sub>o</sub> treatment compared to the others studied treatments. Similarly, fruit weight and diameter were the highest at the full irrigated application<sup>18,28</sup>. Moreover, the increase of soil water deficit led to rising in fruit soluble solids content<sup>28</sup>. In the same trend, water deficit improved the quality of fruits, by increasing TSS and acidity in tomato fruits<sup>71</sup>. Also, Moderate water deficits increased the total soluble solids in tomato fruits<sup>72,73</sup>.

Regarding the effect of foliar application of GB on the tomato fruit quality, results showed that no significant differences were noticed among the treatments in both seasons for the mentioned fruit quality characteristics.

Concerning the interactions between DI treatments and foliar application of GB, tomato plants were irrigated by 100% ET<sub>o</sub> (control) treatment with foliar application of 0 mM/l GB, recorded the highest significant values of average fruit weight and followed by 100% ET<sub>o</sub> (control) and 10 mM/l GB treatment. While, there were no significant differences among 100, 85 and 70% ET<sub>o</sub> treatments, and they were superior on 55% ET<sub>o</sub> treatment for the fruit diameter in both seasons. On the contrary, 55% ET<sub>o</sub> treatment with all the GB concentrations recorded the highest significant values for TSS compared to the others studied treatments.

**Table (7): Effect of deficit irrigation and foliar application of glycine betaine on the number of flowers and the number of fruits per tomato plant and the total marketable yield (ton/fed.) of tomatoes during 2012/2013 and 2013/2014 seasons.**

Treatments		2012/2013						2013/2014					
Deficit irrigation (DI)	Glycine betaine (GB)	Number of flowers / plant		Number of fruits / plant		Total marketable yield (ton/fed.)		Number of flowers / plant		Number of fruits / plant		Total marketable yield (ton/fed.)	
100% ET <sub>0</sub>	0 mM/l	112.20	b	81.00	bc	43.82	c	114.80	b	82.82	bc	43.78	c
	5 mM/l	111.70	b	83.33	ab	44.58	b	113.70	b	85.15	ab	45.20	b
	10 mM/l	118.70	a	85.50	a	48.80	a	122.00	a	87.32	a	50.20	a
	20 mM/l	106.80	b	79.50	c	48.82	a	109.50	b	81.32	c	50.07	a
85% ET <sub>0</sub>	0 mM/l	85.67	de	62.67	e	37.52	g	87.88	e	64.49	e	37.55	g
	5 mM/l	88.33	de	65.33	e	39.08	f	90.54	de	67.15	e	39.07	f
	10 mM/l	96.33	c	71.67	d	41.63	d	99.31	c	73.49	d	42.40	d
	20 mM/l	91.83	cd	64.83	e	40.15	e	94.04	cd	66.65	e	40.10	e
70% ET <sub>0</sub>	0 mM/l	64.67	g	44.00	h	32.83	k	67.01	g	45.82	h	32.93	j
	5 mM/l	74.67	f	50.33	g	34.34	j	76.88	f	52.15	g	34.50	i
	10 mM/l	84.83	e	55.33	f	36.15	h	87.04	e	57.15	f	35.98	h
	20 mM/l	73.17	f	50.50	g	35.28	i	75.38	f	52.32	g	35.43	h
55% ET <sub>0</sub>	0 mM/l	44.50	j	35.33	j	22.45	o	46.81	j	37.15	j	23.19	m
	5 mM/l	46.67	ij	37.83	ij	24.57	n	48.31	ij	39.65	ij	25.27	l
	10 mM/l	54.83	h	42.67	h	27.58	l	56.97	h	44.49	h	28.00	k
	20 mM/l	51.83	hi	39.17	i	26.17	m	53.97	hi	40.99	i	25.65	l
100% ET <sub>0</sub>		112.30	A	82.33	A	46.51	A	115.00	A	84.15	A	47.31	A
85% ET <sub>0</sub>		90.54	B	66.13	B	39.60	B	92.94	B	67.94	B	39.78	B
70% ET <sub>0</sub>		74.33	C	50.04	C	34.65	C	76.58	C	51.86	C	34.71	C
55% ET <sub>0</sub>		49.46	D	38.75	D	25.19	D	51.51	D	40.57	D	25.53	D
0 mM/l		76.75	C	55.75	C	34.15	D	79.13	C	57.57	C	34.37	D
5 mM/l		80.33	B	59.21	B	35.64	C	82.35	B	61.03	B	36.01	C
10 mM/l		88.67	A	63.79	A	38.54	A	91.33	A	65.61	A	39.15	A
20 mM/l		80.92	B	58.50	B	37.60	B	83.22	B	60.32	B	37.81	B

Values followed by the same letter (s) within column are not significantly different (P<0.05)

**Table (8): Effect of deficit irrigation and foliar application of glycine betaine on average fruit weight (g), fruit diameter (cm) and TSS (%) of tomato fruits during 2012/2013 and 2013/2014 seasons.**

Treatments		2012/2013					2013/2014						
Deficit irrigation (DI)	Glycine betaine (GB)	Average fruit weight (g)		Fruit diameter (cm)		TSS (%)		Average fruit weight (g)		Fruit diameter (cm)		TSS (%)	
100% ET <sub>0</sub>	0 mM/l	99.75	a	5.31	a	6.14	d	95.60	a	4.97	a	6.19	ef
	5 mM/l	92.05	bc	5.17	a	6.02	d	92.90	ab	5.02	a	6.03	f
	10 mM/l	98.04	ab	5.24	a	6.15	d	94.92	a	5.08	a	6.18	ef
	20 mM/l	89.55	cd	5.18	a	6.13	d	89.63	abc	5.08	a	6.36	de
85% ET <sub>0</sub>	0 mM/l	86.09	cd	5.06	a	6.53	c	86.59	bc	5.17	a	6.66	c
	5 mM/l	85.49	cd	4.98	a	6.39	c	86.65	bc	5.27	a	6.53	cd
	10 mM/l	88.04	cd	5.06	a	6.38	c	85.92	bc	5.12	a	6.23	ef
	20 mM/l	86.05	cd	4.93	a	6.15	d	87.73	bc	5.18	a	6.40	de
70% ET <sub>0</sub>	0 mM/l	82.16	d	4.98	a	6.90	b	83.34	c	5.02	a	6.97	b
	5 mM/l	83.68	d	4.94	a	6.95	b	84.51	c	5.15	a	7.04	b
	10 mM/l	84.98	cd	5.03	a	7.00	b	89.40	abc	5.01	a	7.18	b
	20 mM/l	83.14	d	5.00	a	7.02	b	87.29	bc	5.15	a	7.15	b
55% ET <sub>0</sub>	0 mM/l	70.53	e	3.95	b	7.68	a	67.66	d	4.16	b	7.86	a
	5 mM/l	73.98	e	4.22	b	7.60	a	71.87	d	4.34	b	7.82	a
	10 mM/l	73.28	e	4.30	b	7.63	a	71.43	d	4.32	b	7.78	a
	20 mM/l	71.88	e	4.24	b	7.53	a	70.86	d	4.31	b	7.65	a
100% ET <sub>0</sub>		94.85	A	5.23	A	6.11	D	93.26	A	5.04	A	6.19	D
85% ET <sub>0</sub>		86.42	B	5.01	A	6.36	C	86.72	B	5.18	A	6.46	C
70% ET <sub>0</sub>		83.49	B	4.99	A	6.97	B	86.13	B	5.08	A	7.08	B
55% ET <sub>0</sub>		72.42	C	4.18	B	7.61	A	70.45	C	4.28	B	7.78	A
0 mM/l		84.64	A	4.83	A	6.81	A	83.30	A	4.83	A	6.92	A
5 mM/l		83.80	A	4.83	A	6.74	A	83.98	A	4.94	A	6.85	A
10 mM/l		86.08	A	4.91	A	6.79	A	85.42	A	4.88	A	6.84	A
20 mM/l		82.66	A	4.84	A	6.71	A	83.87	A	4.93	A	6.89	A

Values followed by the same letter (s) within column are not significantly different (P<0.05)

#### Water measurements (LRWC & IWUE):

Data presented in Table (9) show the effect of DI treatments and foliar application of GB on leaf relative water content (LRWC) and irrigation water use efficiency (IWUE). Results revealed that, the DI treatments significantly decreased the LRWC and the highest significant values were obtained with 100% ET<sub>0</sub> (control) treatment. On the other hand, there were positive effects for DI on IWUE. Where 55% ET<sub>0</sub> treatment recoded the highest significant values compared to the others studied treatments. These results are in harmony with other works, which refer to the using of DI strategy is very important to increase crop water use efficiency (WUE)<sup>25</sup>. Moreover, the adoption of DI strategies at 50% reduction of ET<sub>c</sub> could be suggested in open-field processing tomato in South Italy, for increasing WUE, minimizing fruit losses and maintaining high fruit quality levels<sup>28</sup>.

**Table (9): Effect of deficit irrigation and foliar application of glycine betaine on leaf relative water content (LRWC) (%) and the irrigation water use efficiency (IWUE) (kg/m<sup>3</sup>) of tomato plants during 2012/2013 and 2013/2014 seasons.**

Treatments		2012/2013				2013/2014			
Deficit irrigation (DI)	Glycine betaine (GB)	Leaf relative water content (%) (LRWC)		Irrigation water use efficiency (IWUE) (kg/m <sup>3</sup> )		Leaf relative water content (%) (LRWC)		Irrigation water use efficiency (IWUE) (kg/m <sup>3</sup> )	
100% ET <sub>0</sub>	0 mM/l	85.28	d	31.53	j	85.77	c	30.08	i
	5 mM/l	86.02	c	33.20	i	85.97	c	33.62	h
	10 mM/l	88.42	a	38.61	h	89.01	a	40.23	g
	20 mM/l	86.86	b	42.08	g	87.14	b	43.08	f
85% ET <sub>0</sub>	0 mM/l	81.53	f	43.59	f	82.17	e	44.30	f
	5 mM/l	83.07	e	47.56	e	83.27	d	49.06	d
	10 mM/l	84.86	d	54.92	bc	85.14	c	56.13	b
	20 mM/l	84.79	d	54.50	c	85.22	c	55.69	b
70% ET <sub>0</sub>	0 mM/l	65.56	j	46.90	e	66.31	i	47.69	e
	5 mM/l	66.53	i	49.59	d	67.28	h	50.06	d
	10 mM/l	67.49	h	54.33	c	68.65	g	55.56	b
	20 mM/l	68.99	g	56.14	b	69.76	f	56.60	b
55% ET <sub>0</sub>	0 mM/l	60.38	n	53.78	c	61.08	l	53.73	c
	5 mM/l	63.01	m	54.76	bc	63.28	k	55.26	b
	10 mM/l	64.13	l	59.89	a	64.27	j	61.33	a
	20 mM/l	64.87	k	59.86	a	65.47	i	61.01	a
100% ET <sub>0</sub>		86.64	A	36.35	D	86.97	A	36.75	D
85% ET <sub>0</sub>		83.56	B	50.14	C	83.95	B	51.29	C
70% ET <sub>0</sub>		67.14	C	51.74	B	68.00	C	52.48	B
55% ET <sub>0</sub>		63.10	D	57.07	A	63.52	D	57.83	A
0 mM/l		73.19	C	43.95	D	73.83	C	43.95	D
5 mM/l		74.66	B	46.28	C	74.95	B	47.00	C
10 mM/l		76.22	A	51.94	B	76.77	A	53.31	B
20 mM/l		76.38	A	53.14	A	76.90	A	54.09	A

Values followed by the same letter (s) within column are not significantly different (P<0.05)

Respecting the effect of foliar application of GB on the above mentioned parameters, results showed that foliar spraying of 10 and 20 mM/l GB treatments gave the highest significant values of LRWC, while 20 mM/l GB treatment only for IWUE in the both seasons. Similarly, plants treated by GB under water stress treatment, showed a slower decrease in leaf water potential, thus developing wilting symptoms much later than untreated plants. In addition, GB treated plants showed better ability to recover from wilting than untreated plants<sup>41</sup>.

Regarding the interactions between DI treatments and foliar application of GB, using 100% ET<sub>0</sub> (control) with 10 mM/l GB treatment gave the highest significant values of LRWC, but using 55% ET<sub>0</sub> with 10 or 20 mM/l GB treatments had the highest significant values of IWUE.

## Conclusion

It was concluded that, tomato plants could be produced under sandy soil conditions using DI strategy at 85% ET<sub>0</sub> with foliar spraying by 10 mM/l GB to overcome the negative effects of water stress and improve the vegetative growth, fruit yield and quality.

## Abbreviations

- GB	Glycine betaine	- ET	Evapotranspiration
- DI	Deficit irrigation	- ET <sub>c</sub>	Crop evapotranspiration
- LRWC	Leaf relative water content	- ET <sub>o</sub>	Reference evapotranspiration
- IWUE	Irrigation water use efficiency	- kp <sub>c</sub>	Class A pan evaporation
- WUE	Water use efficiency	- mM/l	Millimolar/liter
- TSS	Total soluble solids	- F.Wt.	Fresh weight
- D.Wt.	Dry weight	- T.Wt.	Turgid weight
- ABA	Abscisic acid		

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