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Reducing Water Stress in Green Bean using Glycinebetaine Application

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ABSTRACT

Water shortage represents a severe threat to the crop production sustainability. The present study aimed at investigating the possible role of exogenous glycinebetaine (GB) application (control, 5, 10, 15 and 20 mM/L) in improving the vegetative growth and yield of green bean (*Phaseolus vulgaris* L.) under different irrigation regimes 55, 70, 85 and 100% of irrigation requirements (IR). A field experiment was conducted, during the two seasons of 2017 and 2018 in split-plot design with three replications at the experimental farm, Faculty of Agriculture, Ain Shams University, Qalubia governorate, Egypt. The main plots were occupied by irrigation treatments and glycinebetaine concentrations distributed in the subplots.

The results of the experiment showed that the highest significant values vegetative growth characteristics (plant height, number of leaves per plant, number of branches per plant, total plant fresh weight) were obtained by the 100% irrigation level in the two seasons as compared to the other studied irrigation treatments. In contrast, the 85% irrigation level had positive effects on some yield characteristics (number of pods per plant, pods length, pods thickness, early yield and total yield) for green bean. Foliar application of glycinebetaine at 20 mM/l ameliorated the negative effects of water stress and produced the highest significant values of tested characteristics in the first and second seasons, Moreover, irrigation treatment of 85% IR and foliar application of 20 mM/L glycinebetaine produced the highest yield and fruit quality (pods number, pods length, pods thickness). In addition, the foliar spray of glycinebetaine improved water use efficiency under water stress conditions.

Keywords: Irrigation requirements, Glycinebetaine, Green Bean, Water shortage

Introduction

Egypt occupies the six ranks among countries with the production level of 215,000 tones (FAOSTAT, 2017). World green bean (*Phaselous vulgaris* L.) production is 4,310,733 metric Tons. Water management in green bean production is significant at all stages of plant development as a result of its influence on stand establishment, pod set and quality. Moreover, the crop must be provided with adequate water to ensure vigorous growth.

Since Egypt is located in the arid and semi-arid regions, it is an urgent need to increase the productivity of vegetable crops and to save the water resource. Plants grown in these regions face water stress conditions which reduce vegetative growth and fruit yield of vegetable crops (Souza *et al.*, 2004). In contrast, Ozbahce and Tari (2010) concluded that successful production of vegetables demands efficient water application.

It is known that bean is important source for protein in many developing countries. As much as 60% of bean production the developing world occurs under conditions of drought stress (Franca *et al.,* 2000).

In addition, the reproductive stage in legumes is the most sensitive stage to drought stress, whether it takes place during flowering stage, pods formation and grain filling. This is due to the water deficit causes falling or abortion of reproductive structures (Saucedo *et al.,* 2009). Moreover, the reduction of yield in dry common beans subjected to water stress was suggested to occur mainly by abscission of flowers and young pods. Reductions in harvest index were detected as a result of moderate moisture stress in common beans (Kellman, 2008).

Glycinebetaine (GB) is an amino acid derivative which is naturally synthesized in several plant species. Synthesis of glycinebetaine is promoted by salt and drought stress as it functions as a compatible solute regulating the intracellular osmotic balance. However, many important crop species, like potato or tomato are unable to accumulate glycinebetaine. It has also been revealed to

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stabilize extrinsic proteins and maintain the membrane structures (Abou El-Yazied, 2011). Furthermore, glycinebetaine protects both the photosystem II (PSII) complex and the ATP synthesis against the inhibiting effects of sodium chloride (Makela *et al.,* 1998). It also plays a protective role under stressful conditions (Wahid and Shabbir, 2005). In addition, Hussain *et al.* (2009) demonstrated that exogenous application of glycinebetaine was very effective in reducing the adverse effects of water scarcity on sunflower hybrid. Water stress reduced the leaf area index, leaf area duration and achene yield. The exogenous glycinebetaine application appreciably improved these attributes under water stress. The effects of water stress and foliar application of glycinebetaine were more pronounced when applied at flowering stage than at the budding stage.

Many investigators discovered that exogenous application of glycinebetaine increased the plant tolerance to environmental stress (Cha-um *et al.,* 2013; Chen and Murata, 2008). In support, Rezaei *et al.* (2012) concluded that drought stress treatments decreased the vegetative growth of tomato plants and exogenous application of GB at 10 mM alleviated the harmful effects of drought stress and significantly increased the vegetative growth. Furthermore, Gao *et al.* (2012) and Yan *et al.* (2012) mentioned that GB treatments increased the processing tomato yield for plants grown under drought stress conditions. Moreover, Ragab *et al.* (2015) revealed that drought stress decreased the yield of tomato (fruit number and weight) by the reduction in the plant growth and number of flowers per plant. While exogenous application of glycinebetaine significantly increased the number of flowers, fruits and fruit weight per plant by 86%, 115% and 125%, respectively.

Previous studies show that the reduction in bean productivity (number of pods per plant and seed biomass) due to heat stress was linked with reduced leaf water content (Omae *et al*, 2005). Under scarce water in semi-arid areas, irrigation water management aims to provide sufficient water to replenish depleted soil water in time to avoid physiological water stress in growing plants, using modern irrigation technologies such as a drip irrigation system (Saleh *et al*., 2018; Abdel-Mawgowd, 2006).

Hence, the present research aimed to reduce the caused hazards resulting from water fluctuations on seed yield and quality of green bean plants grown in flood irrigated clay loam in Egyptian Delta lands through enhancing the plant tolerance to irrigation water fluctuations by spraying with Glycinebetaine and chelated calcium.

Materials and Methods

Field experiment:

During the two successive spring seasons of 2017 and 2018, a field experiment was carried out at the experimental farm of Faculty of Agriculture, Ain Shams University, Qalubia governorate, Egypt to investigate the effect of using foliar application of glycinebetaine on vegetative growth, seed yield and quality of green bean (*Phaseolus vulgaris* L.) under irrigation at different percentages of available soil water depletion in clay loam soil. The investigated soil was a clayey one (*Vertic Torrifluvents*) and its physical and chemical properties (Table 1) were determined, before cultivation, by the standard methods outlined by Klute (1986) and Page *et al.* (1982).

Table 1: Some physical and chemical properties of the studied soil (0-30 cm).

Particle size distribution, %		**Soluble ions, mmol$_c$ L^{-1}**	
Sand	22.7	**Ca^{2+}**	3.04
Silt	25.1	**Mg^{2+}**	2.92
Clay	52.2	**Na^{+}**	1.12
Textural class	Clay	**K^{+}**	0.80
Field capacity, %	40.3	**HCO_3^-**	2.49
Wilting point, %	3.91	**Cl^-**	1.38
$CaCO_3$, g kg^{-1}	8.95	**SO_4^{2-}**	2.06
OM, g kg^{-1}	10.05	**Total macronutrient, %**	
CEC, cmol$_c$ kg^{-1}	41.63	**N**	0.126
pH (1:2.5)	7.04	**P**	0.018
EC$_e$, dS m^{-1}	0.46	**K**	1.291

Carbonate ions were not detected.

Applied Treatments

Seeds of "Nebraska" green bean cultivar were sown on the first week on March 2017 and 2018 seasons. The area of the experimental plot was 16.8 m^2 consisted of four rows, each row was 6 m length and 0.7 m width. The plant distance was 7 cm apart on one side, an alley (1.5 m wide) was left as boarder between each two irrigation treatment.

The experiment was laid out in split-plot design with three replications. The main plots were assigned to the irrigation treatments (55, 70, 85 and 100% of IR) and the subplots were occupied by the glycinebetaine concentrations (control, 5, 10, 15 and 20 mM/L). All plants received the recommended doses of N, P and K fertilizers according to the Egyptian Ministry of Agriculture (Anonymous, 2003), *i.e.* ordinary superphosphate while the Calcium super-phosphate (15 % P_2O_5) at 300 kg / fed was banded on rows at two times, the first (200 kg) was added during the soil preparation and the second one (100 kg) was carried out at flowering period. Ammonium nitrate (33% N), at 250 kg/fed and potassium sulphate (48% K_2O) at a rate of 100 Kg/fed were applied as soil application after three weeks from sowing with first irrigation and as well as after one month from the first addition. Other cultural managements were followed according to the recommendations of the Egyptian Ministry of Agriculture.

The Experimental Design and Treatments:

Irrigation Treatments

The total seasonal applied water volumes of 55(I1), 70(I2), 85(I3) and 100% (I4) of irrigation water requirement (IR) in the experimental area were, 1656, 2108, 2559 and 3011 m^3/fed, respectively, during the tested season of 2017 and 1635, 2080, 2525 and 2971 m^3/fed, respectively, during the tested season of 2018. Table (2) show the measured climatic factors during the experimental period; these data collected from automated weather station allocated at the experimental location. The irrigation water requirement was calculated according to Food and Agricultural Organization (FAO), Penman- Monteith (PM) procedure (Allen *et al.,* 1998). The first step was to calculate the potential evapotranspiration as following equation:

$$ET_o = \frac{0.408\Delta\,(R_n - G) + \gamma \frac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34\,u_2)}$$

Where: ET_o = The daily reference evapotranspiration (mm day^{-1}), Rn = Net radiation at the crop surface (MJ m^{-2} day^{-1}), G = Soil heat flux density (MJ m^{-2} day^{-1}), T = Mean daily air temperature at 2 m height (°C), U_2 = Wind speed at 2 m height (m s^{-1}), e_s = Saturation vapor pressure (kPa), e_a = Actual vapor pressure (kPa), Δ = The slope of vapor pressure curve (kPa $°C^{-1}$), γ = The psychometric constant (kPa $°C^{-1}$).

The second step was to obtain values of irrigation requirements (ET_{crop}) as following (Doorenbos and Pruitt, 1977):

$$IR = ET_o * K_c * LR * 4.2 \quad \ldots\ldots\ldots \quad (m^3/\ fed/\ day)$$

Where:

IR = Irrigation requirement for crop [m^3/ feddan/ day].
K_c = Crop coefficient [dimensionless].
ET_o = Reference crop evapotranspiration [mm/day].
LR = leaching requirements (was assumed 20% of irrigated water)

Glycinebetaine Treatments:

Glycinebetaine (MW 117.18) of Sigma Aldrich was used at five concentrations, i.e., 0 (control, spraying with distilled water), 5, 10, 15 and 20 mM/L, applied after 25 and 45 days from sowing as foliar application. The experiment was laid out in a split- plot design with three replicates.

Table 2: Average climatic data of Qalubia governorate 2017 and 2018 seasons.

Date	Solar radiation Dgt [MJ/m2]	Wind speed [m/sec]	Temperature [°C]	Relative humidity [%]	Dew Point [°C]	ETo [mm]
Week			**From march to June 2017 season**			
1	13.06	0.19	17.71	64.71	10.27	2.09
2	14.14	0.54	18.91	49.14	6.90	2.60
3	12.73	0.23	17.46	56.57	7.93	2.14
4	14.67	0.17	20.34	55.29	9.51	2.51
5	14.47	0.24	20.83	54.29	9.93	2.69
6	12.70	0.31	21.46	54.43	10.77	2.61
7	18.86	0.24	22.03	52.86	9.91	3.41
8	19.03	0.30	23.54	43.43	8.11	3.56
9	18.02	0.19	23.87	47.00	10.16	3.40
10	18.78	0.21	27.07	43.29	10.67	3.61
11	18.39	0.27	27.21	45.57	12.61	3.79
1 2	16.70	0.37	24.96	47.86	11.74	3.56
1 3	19.87	0.27	28.11	40.43	11.19	4.06
1 4	21.40	0.14	29.20	42.71	13.61	4.17
1 5	21.05	0.27	29.06	47.29	14.87	4.31
1 6	20.69	0.17	29.39	49.14	16.36	4.24
1 7	21.40	0.20	28.77	53.00	16.91	4.36
1 8	20.36	0.24	31.36	53.86	19.49	4.44
Week			**From march to June 2018 season**			
1	12.47	0.21	19.91	50.71	7.73	2.09
2	12.33	0.18	21.54	49.28	8.46	2.19
3	12.04	0.11	20.00	55.71	9.61	2.07
4	13.30	0.25	22.64	35.28	3.64	2.50
5	13.57	0.37	20.58	53.42	9.71	2.64
6	15.79	0.31	20.91	54.14	9.89	2.90
7	15.14	0.14	23.95	42.42	8.73	2.79
8	14.51	0.22	24.58	43.00	9.69	2.89
9	15.17	0.15	22.75	52.28	10.59	2.87
10	12.43	0.21	28.07	43.71	12.07	2.83
11	17.89	0.34	25.35	46.85	11.30	3.66
1 2	18.70	0.25	27.12	47.14	13.03	3.81
1 3	17.93	0.17	31.10	36.85	12.37	3.73
1 4	19.78	0.27	26.90	53.00	15.67	4.03
1 5	21.00	0.25	29.15	47.00	15.06	4.34
1 6	20.91	0.24	30.51	42.71	14.40	4.34
1 7	21.40	0.25	30.44	46.71	16.49	4.54
1 8	21.17	0.25	29.41	49.42	16.17	4.40

Measurements were as follows:

Vegetative growth characteristics: five guarded plants were chosen at random from three replications (from the inner rows) at 60 days from sowing to measure plant height, leaf number, number of branches and total plant fresh weight.

Leaf chemical components: leaf chlorophyll reading (SPAD) was determined using the recently full expanded mature upper leaf of 21 plants in the middle row per plot. A digital chlorophyll meter, Minolta SPAD- 502, (Minolta Company, Japan) was used. The collected plant leaves were sampled, separated into two groups, one was kept fresh to determine proline content according to the method of Troll and Lindsley (1955) modified by Petters *et al*. (1997). The other group was oven dried at 70°C for 48 h, and digested by H_2SO_4/H_2O_2 mixture according to the method described by Chapman and Pratt (1961). Total nitrogen in leaves was determined using Kjeldahl method according to the procedure described by Chapman and Pratt (1961), total phosphorus was determined using

Spectrophotometer according to Watanabe and Olsen (1965) and total potassium in plant leaves was determined using Flame photometer as described by Jackson (1958).

Fruit yield: The green pods were harvested at three weekly dates, starting 60 days after sowing. A sample of ten plants from each plot were collected to assess weight, length and diameter were determined using random representative samples.

Fruit quality: Representative green pod samples (three plants from each plot) from each plot were selected for chemical analysis to determine the total protein percentage measured colorimetrically according to A.O.A.C. (2005), total soluble solids using hand refractometer, Ascorbic acid content (V.C) was determined in fruit tissues blended in 10 ml of 3% oxalic acid solution, then 10 ml of blended fruit juice was titrated against 2, 6 dichloro- phenol indophenol, (A.O.A.C., 1990) as well as, fiber (total dietary fiber), according to Nielsen (2010).

Water use efficiency (WUE): WUE was calculated according to FAO (1982) as follows; the ratio of crop yield (y) to the total amount of irrigation water used in the field during full season (IR):

$$WUE\ (kg/m^3) = Y\ (kg)/\ IR\ (m^3)$$

Statistical Analysis

Gomez and Gomez (1984) suggested that the obtained data were then statistically analyzed using SAS software package (SAS, 2000). The means that were significant were separated using Duncan's multiple range test at $P \leq 0.05$.

Results and Discussion

Vegetative Growth Characteristics:

Data shown in Table (3) obviously showed that reducing irrigation water amount significantly decreased all vegetative growth parameters; plant height, number of leaves per plant, number of branches per plant, total plant fresh weight. While, the highest significant values were obtained by the full irrigation treatment 100% IR (control). The lowest values were obtained with 55% IR treatment during the both seasons. In the same trend, Bhardwaj and Yadav (2012) and Mutava *et al.* (2015) stated that water plays a pivotal role in increasing the uptake of mineral elements from soil and translocation of photosynthetic assimilates from the leaves, which reflect on increment in the plant vegetative growth (Leilah, 2009). On the other hand, they reported that drought stress decreased the vegetative growth parameters. Such results are in agreement with those obtained by Leilah (2009). El-Dakroury (2008) found also that increasing irrigation level from 60% and up to 100% of irrigation water requirement significantly increased the vegetative growth criteria, i.e. plant height, number of branches, leaves and pods/plant as well as leaf area and dry weight of stem and whole plant. Iqbal *et al*. (2008) mentioned that many morphological traits in sunflower plants were negatively affected by moisture stress; these included decreases of leaf area, which can be the result of reducing the number of leaves and size of younger leaves and inhibition of the expansion of developing foliage. In addition.

With regard to the effect of foliar application of glycinebetaine on the above-mentioned characteristics, data indicated that foliar application of glycinebetaine ameliorated the vegetative growth parameters for green bean plants. Where, the highest significant values were obtained with foliar application of glycinebetaine at 20 mM/l treatment in the both tested seasons. While, the lowest values were noticed with control treatment (0 mM/l GB). Similar results were obtained by Abbas *et al.* (2010) which revealed that foliar application of glycinebetaine improved the vegetative growth of eggplant and this improvement may be due to the role of glycinebetaine in enhancing photosynthetic rate and stomatal conductance (Tasuku *et al.,* 2009). In addition, once glycinebetaine is translocated to plant organs, it acts as an osmoprotectant in the cells and enhances growth and reproduction (Chen and Murata, 2008). In addition, Abbas *et al*. (2010) found also that foliar application of glycinebetaine caused improvement in growth of two eggplant cultivars.

Table 3: Effect of irrigation treatment and foliar application of glycinebetaine and their interactions on vegetative growth characteristics of green bean plant during 2017 and 2018 seasons.

	Plant height (cm)		Number of branches/plant		Leaf number/plant		leaf fresh weight (g/plant)	
	2017	2018	2017	2018	2017	2018	2017	2018
Irrigation treatment								
55% IR (I1)	26.43 d	24.00 d	4.77 d	4.96 d	17.03 d	16.65 d	37.60 d	39.00 d
70% IR (I2)	32.18 c	31.74 c	6.86 c	6.16 c	18.89 c	18.37 c	49.53 c	48.83 c
85% IR (I3)	39.06 b	40.70 b	8.45 b	8.49 b	20.06 b	20.39 b	55.34 b	55.16 b
100% IR (I4)	50.44 a	48.45 a	11.38 a	10.34 a	25.13 a	23.51 a	77.24 a	67.89 a
Glycinebetaine Treatments								
20 mM/L (GB1)	39.83 a	39.24 a	9.05 a	8.85 a	20.46 b	20.96 a	69.58 a	74.28 a
15 mM/L (GB2)	37.89 b	36.74 b	8.13 b	7.51 b	20.95 a	20.37 b	55.37 b	53.60 b
10 mM/L (GB3)	36.36 c	35.35 c	7.69 c	7.61 b	20.47 b	19.72 c	52.65 c	49.01 c
5 mM/L (GB4)	35.68 d	35.26 c	7.45 c	6.99 c	20.35 b	19.35 d	50.59 d	45.27 d
Control (GB5)	35.39 d	34.52 d	7.00 d	6.48 d	19.17 c	18.24 e	46.43 e	41.46 e
Interaction								
I1*GB1	28.81 l	24.50 m	5.01 kl	5.34 j	17.61 j	17.57 ij	52.34 ef	56.59 ef
I1*GB2	29.08 kl	26.23 l	5.41 jk	4.74 k	18.09 i	17.68 hij	36.68 j	39.49 k
I1*GB3	27.53 m	25.15 m	4.34 m	5.44 j	17.41 j	17.24 j	35.00 j	36.52 l
I1*GB4	23.64 n	23.40 n	4.27 m	4.47 k	16.63 k	15.70 k	34.40 j	31.20 m
I1*GB5	23.11 n	20.73 o	4.81 lm	4.80 k	15.41 l	15.05 l	29.60 k	31.21 m
I2*GB1	35.60 h	34.76 i	7.67 g	7.76 g	18.33 i	18.32 g	64.00 d	67.50 c
I2*GB2	34.70 i	30.39 k	8.43 f	6.92 h	18.86 gh	17.79 ghi	51.28 efg	49.39 h
I2*GB3	29.47 k	33.10 j	6.18 hi	5.93 i	18.59 hi	18.25 g	46.17 h	44.50 ij
I2*GB4	29.06 kl	30.35 k	6.34 h	4.68 k	19.42 f	19.22 f	43.42 i	42.01 jk
I2*GB5	32.10 j	30.10 k	5.68 ij	5.51 ij	19.25 fg	18.26 g	42.76 i	40.75 k
I3*GB1	41.76 f	46.37 c	9.13 e	10.02 cd	19.60 f	19.96 e	80.33 a	77.70 b
I3*GB2	38.51 g	43.98 e	8.60 ef	8.00 g	21.35 d	22.60 c	53.74 e	56.88 ef
I3*GB3	41.41 f	37.74 g	8.42 f	9.36 e	20.61 e	21.62 d	48.71 g	55.82 fg
I3*GB4	35.54 h	39.34 f	8.51 f	8.13 g	20.44 e	19.65 ef	50.97 fg	44.97 i
I3*GB5	38.08 g	36.05 h	7.57 g	6.95 h	18.30 i	18.11 gh	42.93 i	40.45 k
I4*GB1	53.13 b	51.32 a	14.40 a	12.29 a	26.30 a	28.00 a	81.66 a	95.33 a
I4*GB2	49.27 c	46.38 c	10.08 d	10.37 bc	25.48 b	23.42 b	79.78 a	68.62 c
I4*GB3	47.05 e	45.41 d	11.83 b	9.72 de	25.25 b	21.79 d	80.72 a	59.19 e
I4*GB4	54.47 a	47.93 b	10.66 c	10.66 b	25.09 b	22.82 c	73.58 b	62.90 d
I4*GB5	48.28 d	51.22 a	9.96 d	8.67 f	23.55 c	21.54 d	70.43 c	53.42 g

Regarding the interaction among studied treatments, results demonstrated that green bean irrigated by 100% IR and treated with glycinebetaine at 20 mM/l, produced the highest significant values for vegetative growth parameters in the both tested seasons. While the highest significant values for total leaves area per plant were noticed with 100% IR and sprayed with glycinebetaine at 20 mM/l.

Pod Yield and Pod Parameters:

Data in Table (4) showed that increasing the amount of irrigation water from 55% to 85% IR significantly increased the early yield and total yield during the both tested seasons. The highest values were obtained by 85% IR treatment, followed by 100% IR treatment.

Such results agree with those obtained by Farooq *et al.* (2009) and Kahlaoui *et al.* (2011) which indicated that deficit irrigation water leading to a reduction in plant growth and decreasing the number of pods and pods quality per plant. Furthermore, Earl and Davis (2003) and Aldesuquy *et al.* (2012) suggested that plants grown in soil water deficit produced a small plant canopy and decreased photosynthetic pigments, carbohydrates accumulation and total nitrogen and protein, leading to a reduction in crop yield. The same findings were obtained by Cetin *et al.* (2002) and Patane and Cosentino (2010), they stated that the full irrigated treatment produced the highest fruit yield. Adding 80% of ET was quite enough to achieve the maximum productivity for green bean. The results are similar to those previously reported by El-Noemani *et al.* (2010). They noted that increasing the irrigation amount up to 100% of ET prompted the highest growth, although the maximum pod yield was achieved by 80% of ET. water deficit had a significant impact on plant growth Under stress conditions, leading to a decline in growth, leaf area development, and photosynthetic capacity (Bayuelo-Jimenez *et al*, 2003).

With respect to the effect of glycinebetaine on the above-mentioned characteristics, data showed that the highest significant values for number of flowers per plant and total yield were observed with foliar application of glycinebetaine at 20 mM/l treatment in two tested seasons. These results are in agreement with those obtained by Gao *et al.* (2012), Rezaei *et al.* (2012) and Yan *et al.* (2012) on tomato plants.

As for the interaction among studied treatments, data demonstrated that plants irrigated by 100% ET_o and treated with glycinebetaine at 20 mM/l produced the highest significant values of pods per plant, and there were no significant differences with other concentrations (10 number and 15 mM/l glycinebetaine). While, the maximum total yield were obtained with green bean irrigated by 80% ET_o and sprayed by glycinebetaine at 10 and 20 mM/l in the two tested seasons.

Moderate soil moisture was essential to ensure a uniform plant stand after germination and during the plant establishment stage. Green bean are extremely sensitive to both water deficit and excess water (Saleh *et al*, 2018). For example, water deficit during vegetative growth and flowering has the greatest negative impact on pod yield and pod quality (Boutraa and Sanders, 2000). Mainly during flowering and pod set, green bean are very sensitive to water stress. To insure proper moisture availability, the soil should not hold more than 60% of field capacity during the pod elongation and pod-filling period, according to Abd El-Aal *et al*. (2011)

Leaf chemical Composition

Table (5) show the effect of four different irrigation treatment and foliar application of glycinebetaine and their interaction on chemical composition. The highest contents of N, P, K and proline were at 55% of IR. On the contrary, the highest irrigation level (100% of IR) resulted in the lowest contents of N, P, K and proline.

Data presented in Table 5 show that irrigation treatment 55% gave the highest values of both chlorophyll reading and free proline as compared with the other studied irrigation treatments in the two seasons. On the contrary, irrigation 100% IR exhibited the lowest values of chlorophyll reading in the two seasons of study. In this respect, increased chlorophyll reading might be a reaction center or a photosystem II modification as Kerrlous (1997) pointed out on green bean.

Respecting the foliar application of glycinebetaine, the obtained data show that plants sprayed with 20 or 15 mM/L glycinebetaine produced the highest values of chlorophyll reading in the two seasons. However, plants that were not sprayed with glycinebetaine produced the highest values of free proline compared to other treatments. In this respect, the reduction of free proline due to the

Table 4: Effect of irrigation treatment and foliar application of glycinebetaine and their interactions on vegetative growth characteristics of green bean plant during 2017 and 2018 seasons.

	Pod number/plant		Pod length (cm)		Pods thickness (mm)		Total yield (ton/Fed.)		Early yield (kg/Fed.)	
	2017	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
Irrigation treatment										
55% IR (I1)	2.52 d	2.31 d	7.33 d	7.39 d	4.13 d	4.07 d	3.33 c	3.39 c	85.22 d	84.64 d
70% IR (I2)	3.50 c	3.21 c	9.45 c	8.92 c	5.31 c	5.22 c	4.21 b	4.04 b	103.89 c	105.46 c
85% IR (I3)	4.88 a	4.49 a	13.55 a	12.73 a	7.81 a	7.45 a	5.31 a	5.12 a	148.20 a	145.72 a
100% IR (I4)	3.82 b	3.75 b	10.26 b	10.25 b	6.04 b	6.20 b	4.17 b	4.14 b	119.87 b	120.72 b
Glycinebetaine Treatments										
20 mM/L (GB1)	4.13 a	4.14 a	11.28 a	10.96 a	6.42 a	6.63 a	4.66 a	4.65 a	125.23 a	124.23 a
15 mM/L (GB2)	3.83 b	3.54 b	10.98 b	10.60 b	5.83 b	5.63 b	4.29 b	4.23 b	116.48 b	117.84 b
10 mM/L (GB3)	3.65 c	3.23 c	9.63 c	9.47 c	5.77 b	5.56 bc	4.28 b	4.12 c	113.33 c	114.00 c
5 mM/L (GB4)	3.56 c	3.22 c	9.44 d	8.99 d	5.60 c	5.50 c	4.11 bc	4.07 c	112.27 d	110.14 d
Control (GB5)	3.24 d	3.06 c	9.41 d	9.08 d	5.50 d	5.35 d	3.94 c	3.78 d	104.17 e	104.46 e
Interaction										
I1*GB1	3.11 hi	2.74 fg	8.08 h	7.86 i	4.58 j	5.03 g	3.10 i	3.36 kl	93.82 l	92.66 k
I1*GB2	2.57 j	2.44 gh	7.73 i	8.11 i	4.06 l	3.80 ij	3.14 i	3.48 jkl	85.68 m	86.53 l
I1*GB3	2.35 j	2.31 hi	7.05 j	6.86 k	3.87 m	3.96 i	3.49 hi	3.12 m	84.85 mn	83.53 m
I1*GB4	2.35 j	1.99 i	6.90 j	7.46 j	3.81 m	3.67 j	3.74 gh	3.67 hij	83.30 n	81.86 m
I1*GB5	2.23 j	2.10 hi	6.90 j	6.63 k	4.33 k	3.87 ij	3.18 i	3.31 lm	78.46 o	78.62 n
I2*GB1	3.74 ef	3.96 c	10.96 d	9.59 e	5.96 f	5.95 e	4.87 c	4.81 cd	113.41 h	113.78 f
I2*GB2	3.89 e	3.26 d	9.80 e	9.17 f	5.31 h	5.41 f	4.43 cde	4.11 f	106.79 j	105.58 h
I2*GB3	fgh 3.38	2.81 ef	8.78 fg	8.69 gh	5.53 g	5.04 g	3.78 fgh	4.06 fg	102.99 k	107.85 g
I2*GB4	3.50 fg	3.18 de	8.63 g	8.55 h	5.07 i	5.02 g	4.24 efg	3.66 ij	101.78 k	101.16 i
I2*GB5	3.00 i	2.82 ef	9.06 f	8.60 gh	4.70 j	4.66 h	3.75 gh	3.55 ijk	94.48 l	98.92 j
I3*GB1	5.03 a	5.27 a	15.27 a	14.22 a	8.55 a	8.62 a	6.37 a	6.05 a	162.80 a	159.35 a
I3*GB2	5.30 a	4.26 bc	15.57 a	14.02 a	7.78 b	7.70 b	5.81 b	4.96 c	150.11 b	148.09 b
I3*GB3	4.62 bc	4.40 b	11.96 c	12.24 b	7.69 b	6.99 c	5.60 b	5.57 b	147.94 c	145.07 c
I3*GB4	4.95 ab	4.29 bc	12.17 c	12.17 b	7.66 b	6.99 c	3.98 efgh	4.61 d	146.37 c	144.02 c
I3*GB5	4.48 cd	4.22 bc	12.78 b	11.02 c	7.36 c	6.97 c	4.77 cd	4.39 e	133.80d	132.06 d
I4*GB1	4.66 bc	4.60 b	10.81 d	12.19 b	6.59 d	6.91 c	4.30 def	4.37 e	130.91 e	131.13 d
I4*GB2	3.54 fg	4.22 bc	10.83 d	11.11 c	6.17 e	5.59 f	3.76gh	4.37 e	123.35 f	131.16 d
I4*GB3	4.26 d	3.40 d	10.74 d	10.08 d	5.98 f	6.25 d	4.24 efg	3.74 hi	117.54 g	119.54 e
I4*GB4	3.43 fgh	3.42 d	10.04 e	8.93 fg	5.86 f	6.32 d	4.49 cde	4.35 e	117.62 g	113.54 f
I4*GB5	3.24 ghi	3.10 def	8.91 fg	8.93 fg	5.61 g	5.92 e	4.08efg	3.89 gh	109.93 i	108.22 g

Table 5: Effect of irrigation treatment and foliar application of glycinebetaine and their interactions on leaf chemical composition of green bean plant during 2017 and 2018 seasons

	Total chlorophyll (SPAD)		N (%)		P (%)		K (%)		Free proline (µmol/100g FW)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Irrigation treatment										
55% IR (I1)	48.26 a	48.26 a	3.46 a	3.36 a	0.37 a	0.30 a	2.62 a	2.06 b	260.83 a	263.76a
70% IR (I2)	35.78 b	34.43 b	2.78 b	2.87 b	0.33 b	0.25 b	2.56 ab	2.46 a	255.49 a	257.08 b
85% IR (I3)	33.80 c	33.78 c	2.39 c	2.38 c	0.32 c	0.22 c	2.54 b	2.49 a	248.07 a	251.01 c
100% IR (I4)	24.62 d	23.74 d	2.29 c	2.32 c	0.26 d	0.21 c	1.97 c	2.07 b	178.28 b	170.68 d
Glycinebetaine Treatments										
20 mM/L (GB1)	36.60 a	36.08 a	2.88 a	3.10 a	0.37 a	0.28 a	2.40 b	2.39 b	256.99 a	271.16 a
15 mM/L (GB2)	36.62 a	35.56 b	2.81 a	2.70 bc	0.32 b	0.24 b	2.56 a	2.46 a	242.90 ab	241.57 b
10 mM/L (GB3)	35.73 b	35.10 c	2.77 ab	2.78 b	0.31 bc	0.24 bc	2.47 ab	2.24 c	233.76 b	232.06 c
5 mM/L (GB4)	35.54 b	34.95 c	2.63 bc	2.63 c	0.30 c	0.23 c	2.43 b	2.21 c	229.05 bc	221.84 d
Control (GB5)	33.59 c	33.56 d	2.56 c	2.45 d	0.31 bc	0.22 d	2.26 c	2.05 d	215.63 c	211.53 e
Interaction										
I1*GB1	49.31 a	49.31 a	3.62 ab	3.91 a	0.39 a	0.35 a	2.42 e	2.53 b	299.20 a	301.40 a
I1*GB2	49.31 a	48.96 a	3.35 bcd	3.39 b	0.37 ab	0.30 b	2.86 a	2.01 f	264.21 abcd	265.47 de
I1*GB3	48.96 a	47.91 b	3.73 a	3.21 bc	0.36 bc	0.28 b	2.59 bcde	2.10 e	257.83 bcde	260.79 f
I1*GB4	47.56 b	48.96 a	3.41 abc	3.35 bc	0.34 cd	0.28 b	2.57 bcde	1.86 g	249.68 bcde	248.27 h
I1*GB5	46.15 c	46.15 c	3.17 cd	2.91 de	0.38 a	0.28 b	2.66 abcd	1.79 gh	233.22 de	242.87 i
I2*GB1	37.88 d	35.56 def	3.03 de	3.34 bc	0.38 a	0.29 b	2.63 bcde	2.52 b	250.26 bcde	297.08 b
I2*GB2	36.63 e	34.71 f	2.82 ef	2.77 ef	0.33 def	0.24 c	2.64 abcde	2.91 a	275.27 abc	262.43 ef
I2*GB3	35.88 ef	36.13 d	2.75 efg	3.13 cd	0.31 fg	0.24 cd	2.48 de	2.35 cd	257.41 bcde	254.56 g
I2*GB4	33.38 h	33.58 g	2.77 ef	2.66 fg	0.31 fg	0.25 c	2.61 bcde	2.43 c	249.16 bcde	240.55 i
I2*GB5	35.13 fg	32.16 i	2.54 fghi	2.45 ghij	0.33 def	0.22 def	2.41 e	2.11 e	245.35 cde	230.80 j
I3*GB1	34.71 g	35.13 ef	2.57 fghi	2.62 fgh	0.34 cde	0.25 c	2.53 cde	2.35 cd	287.71 ab	290.76 c
I3*GB2	35.84 ef	35.88 de	2.66 fgh	2.39 hijk	0.33 def	0.22 efg	2.75 abc	2.61 b	251.44 bcde	266.73 d
I3*GB3	33.58 h	31.88 i	2.26 ij	2.31 ijk	0.31 fg	0.22 fg	2.80 ab	2.56 b	241.23 cde	249.23 h
I3*GB4	36.13 e	33.38 gh	2.09 j	2.35 ijk	0.32 efg	0.21 fgh	2.45 de	2.39 cd	240.26 cde	231.43 j
I3*GB5	28.76 i	32.63 hi	2.36 hij	2.21 jk	0.30 g	0.19 i	2.18 f	2.53 b	219.70 ef	216.88 k
I4*GB1	24.50 j	24.30 j	2.30 ij	2.51 ghi	0.35 bcd	0.24 cde	2.02 fg	2.17 e	190.80 fg	195.42 l
I4*GB2	24.70 j	22.70 l	2.41 ghij	2.26 jk	0.24 hi	0.21 fgh	1.97 fg	2.30 d	180.67 g	171.63 m
I4*GB3	24.50 j	24.50 j	2.32 hij	2.45 ghij	0.26 h	0.21 fg	1.99 fg	1.97 f	178.56 g	163.67 n
I4*GB4	25.10 j	23.90 k	2.26 ij	2.17 k	0.23 hi	0.19 hi	2.06 f	2.16 e	177.09 g	167.09 n
I4*GB5	24.30 j	23.30 kl	2.17 j	2.21 jk	0.22 i	0.20 ghi	1.81 g	1.75 h	164.26 g	155.57 o

application of glycinebetaine was also reported by Heuer (2003) who found that addition of 1 mM glycinebetaine to salt-affected tomato plants reduced proline accumulation in leaves by 41% while 5 mM increased it by 30%, suggesting its interference in the process of osmotic adjustment by proline production.

Concerning the effect of the different tested interaction treatments such data show that the interactions among irrigation treatment and foliar application of glycinebetaine on chemical components were significant. The highest values of chlorophyll reading, N, P and K percentage were observed when plants were irrigated by 55 % from total irrigation requirement combined with spraying with 20 or 15 mM/L glycinebetaine. However, the highest values of proline content were observed by 55 % IR without spraying glycinebetaine.

Fruit quality:

Data presented in Table (6) presents the effect of deficit irrigation, foliar application of glycinebetaine and their interactions on green bean fruit quality characteristics, i.e., total soluble solids (TSS %), protein, fiber and vitamin C content.

Results displayed that decreasing irrigation water significantly increased TSS, protein, fiber and vitamin c content in green bean fruits, where the highest significant values were obtained by 55% ETo treatment. On the other hand, 100% ETo treatment produced the lowest significant values of chemical fruit percentage in the both tested seasons. Additionally, Branthome *et al.* (1994) and Patane *et al.* (2011) found that 100% irrigation treatment decreasing TSS, protein, fiber and vitamin C content in green bean fruits. Total fiber content was gradually increased by reducing irrigation water level from 100 to 55% of ET (Table 6). There were no differences among the four water levels on vitamin C.

Regarding the effect of glycinebetaine on chemical composition, 20 mM/L glycinebetaine had higher contents of protein and vitamin C and soluble sugar compared to control. 20 mM/L glycinebetaine recorded decrease in total fiber. Concerning the interactions between irrigation water level and foliar application of glycinebetaine on chemical composition, the plant irrigated at 55% of ET without foliar application of glycinebetaine recorded the highest contents of N, P, K, and protein.

Water use efficiency (WUE)

In Table 7 Data indicated the calculated values of WUE of green bean under different irrigation water levels and foliar application of glycinebetaine during the two studied seasons. Obtained results showed that 20 mM/L glycinebetaine foliar application gave the highest values, i.e. 2.02 and 2.06 in the first and second seasons respectively. Concerning the irrigation treatments, the treatment of 85% of IR gave the highest values, i.e. 2.01 and 2.07 in the first and second seasons respectively, without significant effect with the 55% of IR treatment. Data demonstrated that the treatment of 85% irrigation level combined with 20 mM/L glycinebetaine foliar application gave the highest values (2.49 and 2.40) in the first and second seasons, respectively, compared to the other treatments.

It is also noted that green bean productivity was affected by the volume of irrigation water. Most plant growth parameters and pod yield increased with increasing water application from 55 to 85% of ET, without any additional increase up to 100% of ET. The results under the controlled system of the presented study using drip irrigation supported the hypothesis that a greater amount of water produced a faster growth rate, but only up to a certain level (85% IR).

On the other hand, excessive water volume can also reduce the growth rate of plants. Moreover, too much water in the soil is detrimental to plant growth, due to oxygen deprivation in the plant's roots. Furthermore, additional water must reflect the same increase rate in yield, because of the additional costs, based on the law of diminishing returns (WUE). However, the soil type has to be taken into account as well. For instance, Abdel-Mawgoud (2006) recorded a linear correlation between green bean yield and water application up to 120% IR. He found that vegetative growth parameters and yield components responded positively to water increasing up to 120% IR in newly reclaimed sandy land. This was due to sandy soil having a small water holding capacity compared to silty clay soil. With irrigation, we maintained adequate conditions for optimal plant growth during all stages.

Consequently, high yields and better WUE can be achieved by using suitable cultivars and optimizing water management, because the optimum water requirements are variable between different cultivars. The application of glycinebetaine foliar application will be useful.

Table 6: Effect of irrigation treatment and foliar application of glycinebetaine and their interactions on fruit quality of green bean plant during 2017 and 2018 seasons.

	TSS (%)		Protein (g/100 g FW)		Fiber (g/100 g FW)		Vitamin C (mg/100 g FW)	
	2017	2018	2017	2018	2017		2018	2017
Irrigation treatment								
55% IR (I1)	3.17 a	3.08 a	3.50 a	3.47a	1.53 a	1.70 a	32.51a	32.03 a
70% IR (I2)	2.68 b	2.98 a	3.16 b	2.87 b	1.28 b	1.07 b	30.09 b	30.65 b
85% IR (I3)	2.21 c	2.39 b	2.22 c	2.18 c	1.12 c	1.02 bc	26.45 c	26.75 c
100% IR (I4)	1.75 d	2.00 c	1.72 d	1.89 d	0.86 d	0.93 c	19.82 d	19.15 d
Glycinebetaine Treatments								
20 mM/L (GB1)	2.80 a	2.90 a	3.10 a	3.17 a	1.31 a	1.22 a	29.48 a	30.81 a
15 mM/L (GB2)	2.52 b	2.74 ab	2.77 b	2.94 b	1.30 a	1.21 a	28.07 ab	27.81 b
10 mM/L (GB3)	2.36 bc	2.66 b	2.66 b	2.45 c	1.15 b	1.20 a	27.04 b	26.81 c
5 mM/L (GB4)	2.30 c	2.41 c	2.43 c	2.33 d	1.11 b	1.16 ab	26.50 bc	25.73 d
Control (GB5)	2.29 c	2.36 c	2.29 c	2.13 e	1.10 b	1.12 b	25.01 c	24.57 e
Interaction								
I1*GB1	3.69 a	3.34 a	4.45 a	4.73 a	1.83 a	1.86 a	36.82 a	36.61 a
I1*GB2	3.34 ab	3.22 ab	3.50 c	3.24 c	1.68 a	1.85 a	32.94 b	32.59 c
I1*GB3	3.10 bc	3.10 abc	4.08 b	3.28 c	1.53 b	1.64 b	32.16 bc	31.83 d
I1*GB4	2.98 bcd	2.75 bcde	2.97 de	2.90 e	1.17 c	1.79 a	31.32 bc	30.20 f
I1*GB5	2.75 cd	2.99 abcd	2.50 fg	3.17 cd	1.43 b	1.38 c	29.34 bcde	28.91 g
I2*GB1	2.98 bcd	3.34 a	3.63 c	3.51 b	1.38 b	0.90 efg	29.83 bcd	34.63 b
I2*GB2	2.69 cd	3.07 abc	2.98 de	2.77 ef	1.48 b	1.07 d	32.19 bc	31.03 e
I2*GB3	2.60 de	3.16 ab	3.10 d	2.53 g	1.22 c	1.25 c	30.25 bcd	30.14 f
I2*GB4	2.55 de	2.50 defg	3.01 de	2.58 fg	1.19 c	0.88 efg	29.18 bcde	29.01 g
I2*GB5	2.60 de	2.83 abcde	3.10 d	2.97 de	1.12 cd	1.23 c	29.01 cde	28.45 h
I3*GB1	2.63 de	2.71 bcdef	2.22 gh	2.30 hi	1.19 c	1.08 d	30.18 bcd	30.51 f
I3*GB2	2.21 ef	2.63 cdef	2.69 ef	3.28 c	1.17 c	0.95 def	27.01 de	28.34 h
I3*GB3	2.04 fgh	2.46 efg	1.99 h	2.06 j	0.89 ef	1.07 d	25.77 ef	26.76 i
I3*GB4	2.04 fgh	2.38 efgh	2.15 h	2.06 j	1.23 c	0.93 defg	25.94 ef	24.84 j
I3*GB5	2.13 fg	1.79 i	2.05 h	1.22 l	1.13 cd	1.07 d	23.35 fg	23.30 k
I4*GB1	1.90 fgh	2.20 fghi	2.09 h	2.13 ij	0.86 ef	1.04 de	21.07 gh	21.51 l
I4*GB2	1.83 fgh	2.03 ghi	1.92 h	2.46 gh	0.86 ef	0.96 def	20.14 gh	19.28 m
I4*GB3	1.70 gh	1.90 hi	1.49 i	1.92 jk	0.98 de	0.85 fg	20.00 gh	18.51 n
I4*GB4	1.63 h	2.03 ghi	1.57 i	1.77 k	0.87 ef	1.02 def	19.58 h	18.85 n
I4*GB5	1.70 gh	1.84 i	1.50 i	1.16 l	0.73	0.78g	18.33 h	17.60 o

Table 7: Effect of irrigation treatment and foliar application of glycinebetaine and their interactions on Water use efficiency of green bean plant during 2017 and 2018 seasons.

Irrigation treatment (A)	Glycinebetaine Treatments (B)					Mean B
	2017					
	20 mM/L	**15 mM/L**	**10 mM/L**	**5 mM/L**	**Control**	
55% IR	1.87 hi	1.90 ghi	2.11 e	2.26 c	1.92 g	2.01 B
70% IR	2.31 b	2.10 e	1.79 j	2.01 f	1.78 j	2.00 B
85% IR	2.49 a	2.27 bc	2.19 d	1.55 k	1.86 i	2.07 A
100% IR	1.43 m	1.25 o	1.41 m	1.49 l	1.36 n	1.39 C
Mean A	2.02 A	1.88 B	1.87 B	1.83 C	1.73 D	
	2018					
	20 mM/L	15 mM/L	10 mM/L	5 mM/L	Control	
55% IR	2.06 e	2.13 d	1.91 h	2.25 c	2.02 e	2.07 A
70% IR	2.31 n	1.98 e	1.95 gh	1.76 j	1.71 k	1.94 B
85% IR	2.40 a	1.96 fgh	2.21 c	1.83 I	1.74 jk	2.03 A
100% IR	1.47 l	1.47 l	1.26 n	1.46 m	1.31 n	1.39 C
Mean A	2.06 A	1.88 B	1.83 C	1.82 C	1.69 D	

Conclusion

The application of 80% of IR was enough to increase green bean productivity and improve pod quality and WUE. Thus, we can recommend, under less water availability, using application of foliar spraying of glycinebetaine due to gave higher pod nutritional quality and improvement of WUE and minimize problems of water shortages. Furthermore, green bean could be produced under clay soil and open field conditions with deficit irrigation strategy at 85% IR using foliar application of glycinebetaine at 20 mM/l to overcome the negative effects of water stress and improve the vegetative growth, flowering, fruit yield and quality.

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