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Influence of Glycinebetaine on Water Stress Tolerance of *Hibiscus sabdariffa* L. Plant

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ABSTRACT

Glycinebetaine is an amino component that accumulates in many plant species under drought stress. In order to evaluate the response of vegetative growth, yield components, quality and anthocyanin content of roselle plants to glycinebetaine foliar application under water stress, a field experiment was performed during the two successive seasons of 2017 and 2018 at the Experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt as split plot. The irrigation treatments were 50±5, 65 ± 5 and 80±5% of available soil moisture depletion were allocated in the main plot while glycinebetaine foliar spray (25 and 50 mM) and distilled water as control were distributed in the sub-plots. Results showed that 80% reduced vegetative growth characters, fruit number, fruit fresh weight, calyces fresh and dry weight, calyces yield per plant and per fed as well as seed yield. Glycinebetaine at 25 and 50 mM enhanced vegetative growth and yield characters, TSS, acidity and total anthocyanin. Irrigation at 65 % combined with 25 mM glycinebetaine recorded the highest branch number, fruit number, fruit fresh weight, calyces fresh and dry weights/plant (g), seed yield per plant and calyces yield /fed, photosynthetic pigments, TSS, acidity % and total anthocyanin content while reduced pH value. Foliar spray of 25mM GB with 65 % of available soil moisture depletion was found to be more beneficial for avoiding the effects of water stress on above-mentioned attributes, which could be a feasible technique for roselle production that increases the yield and quality as compared to the other studied treatments.

Keywords: Glycinebetaine, Water stress, Roselle, Anthocyanin, Calyces quality

INTRODUCTION

Hibiscus sabdariffa L., commonly known as roselle, is a member of the Family Malvaceae. It is an erect, mainly branched, annual, herbaceous sub shrub that grows generally in warm humid tropical and subtropical climates. The stems are reddish in color, the leaves are dark green to red, and flowers are red to yellow with dark centers. The calyces, which contain flavonoids, riboflavin, ascorbic acid, calcium, and iron, are consumed as a natural food dye (Morton, 1987). Additionally, roselle seeds are plentiful in protein and have been crushed into a meal for human utilization in Africa. In several countries, particularly in Africa, the extract from the calyces is utilized for preparing hot tea or cold drink (McKay *et al*., 2009) because of its elevated content of anthocyanins and organic acids (Hong and Wrolstad, 1990 and Gomez-Leyva *et al*., 2008) as well as flavour and colour additives in the production of jam, liquor, and jellies (Akindahunsi and Olaleye, 2003). Anthocyanins in addition to their colorful characteristics have antioxidant properties (Francis, 2000) which keep the body from damage by free radicals and lipid peroxidation (Tee *et al*., 2002 and Liu *et al*., 2002). Furthermore, roselle calyces have been utilized in folk medicines for several years and have been demonstrated to reduce blood pressure patients with hypertension and type II diabetes (Faraji and Tarkhani, 1999). The medicinal characteristics of roselle were further established to exhibit a wide range of therapies including, as a mild laxative and diuretic, for digestive and kidney functions, and healing sores and wounds (Morton, 1987). Furthermore, wool fabrics could be colored in the dyeing processes by using the dried and grinded hibiscus plant (Yılmaz and Bahtiyari, 2017).

Drought is one of the main environmental threat to agricultural production and distribution worldwide. Deficiency of water, the greatest essential component of life, restricts plant growth and crop productivity, mainly in arid regions further than any other single environmental factor (Boyer, 1982). Egypt is located in arid and semi-arid regions and currently facing water scarcity problem. Moreover, in the few coming decades water scarcity will increase due to the limitation of water resources, rapidly population increment and the expected impact of climate change. Drought stress has unfavorable influences on different plant species , e.g., water deprivation reduced shoot biomass, number of pods/plant and pod dry weight of bean plants (Xing and Rajashekar, 1999), achene yield/plant of sunflower (Iqbal *et al*., 2008) photosynthetic rate and leaf fresh weight of papaya seedlings (Mahouachi *et al*., 2012) and diminished growth and yield of peas (Osman, 2015) as well as basil plants (Mahmoud *et al*., 2017). In addition to, decreasing height and flower number while increased anthocyanin concentration of roselle plants (Evans and Al-hamdani, 2015).

One of the mechanisms for increasing plant tolerance to drought is the process of osmotic adjustment, which leads to lower osmotic potential via the net accumulation of osmotically active materials (Blum, 1989). Glycinebetaine (GB) is one of these compounds. Glycinebetaine, an amino acid derivative, is naturally found in microorganisms, plants, and animals (Sakamoto and Murata, 2002; Mäkelä, 2004). Glycinebetaine was formerly detected in sugar beet (*Beta vulgaris*) juice at the rate of 100 mmolkg^{-1} plant tissue or 0.2–0.3% (Mäck *et al*., 2007). It physiologically performs as an osmolyte to protect cells or as a catabolic source of methyl groups to accelerate numerous biochemical processes (Craig, 2004), protect the plant cells against osmotic inactivation and enhances the water retention of cells (Sakamoto and Murata, 2002; Mäkelä, 2004; Ashraf and Foolad, 2007). Application of glycinebetaine as a foliar spray has been utilized to enhance drought tolerance in a number of crops, e.g., bean (Xing and Rajashekar, 1999), sunflower (Iqbal *et al*., 2008),olive(Roussos *et al*., 2010), maize (Ali and Ashraf, 2011), papaya (Mahouachi *et al*., 2012) and pea (Osman, 2015). In all previously mentioned studies GB has been revealed as an effective ameliorating agent against water stress. Moreover, it has been also, used to ameliorate adverse effects of salt stress in cowpea, okra and eggplant plants (Abbas *et al*., 2010, Habib *et al*., 2012 and Manaf, 2016).

Although, some of the literature are available about water stress impacts on roselle (Evans and Al-hamdani, 2015 and Sakimin *et al*., 2017) information concerning the influence of exogenous glycinebetaine on physiological characteristics of roselle under water deficit environment is lacking. Therefore, we hypothesized that foliar application of GB could effectively minimize drought-induced harmful effects on roselle growth and yield. Thus, the main objective of this study was to determine whether exogenous application of GB could ameliorate the effects of water deficit on vegetative growth, yield components, calyces quality and total anthocyanin content of roselle plants.

MATERIALS AND METHODS

A field experiment was performed during the two successive seasons of 2017 and 2018 at Sakha Agricultural Research Station (31° 07' N Latitude, 30° 05' E Longitude), Kafr El-Sheikh Governorate, North Nile Delta of Egypt. The agro-meteorological data of Sakha Station during both growing seasons are displayed in Table (A).

Table A. Monthly mean values of agro-meteorological data of Sakha Station during 2017 and 2018 growing seasons.

Seasons	Months	Air temperature			Relative humidity			Wind speed	Pan evaporation	Rain
		Max. (^oC)	Min. (^oC)	Mean (^oC)	Max. (%)	Min. (%)	Mean (%)	Mean (km d^{-1})	Mean (mm d^{-1})	(mm $month^{-1}$)
2017	April	26.50	21.60	24.05	79.40	50.80	65.10	89.30	4.63	10.60
	May	30.60	25.80	28.20	77.70	45.60	61.65	106.50	6.59	0.00
	June	32.50	28.10	30.30	80.10	51.40	65.75	102.60	7.10	0.00
	July	34.20	29.00	31.60	84.40	57.60	71.00	80.90	6.44	0.00
	August	33.90	28.30	31.10	85.90	55.30	70.60	70.20	6.04	0.00
	September	32.50	25.90	29.20	86.30	50.30	68.30	85.70	5.37	0.00
	October	28.70	24.00	26.35	81.10	54.70	67.90	73.20	3.26	0.00
2018	April	27.80	20.00	23.90	80.90	43.90	62.40	74.00	5.32	0.00
	May	31.20	23.80	27.50	75.6	43.90	59.75	95.80	6.34	0.00
	June	32.60	25.3	28.95	75.50	48.00	61.75	98.60	7.71	0.00
	July	34.20	25.40	29.80	82.60	51.00	66.80	89.50	7.37	0.00
	August	33.90	25.20	29.55	51.40	82.40	66.90	76.00	6.42	0.00
	September	32.80	23.50	28.15	83.10	48.30	65.70	68.7	4.98	0.00
	October	29.50	20.60	25.05	82.50	49.60	66.05	57.90	3.24	0.00

Soil properties of the experiment site were determined before cultivation, soil chemical properties were determined by the method of Page *et al.,* (1982). Particle-size distribution was carried out using the pipette method according to Klute, (1986), soil field capacity and permanent wilting point were determined by utilizing pressure membrane method at 0.33 and 15 atm according to James, (1988). Soil bulk density was concluded according to Vomocil, (1957) and total porosity P% was computed using values of soil bulk density according to Black, (1965) as stated in Table (B).

Table B. Some chemical and physical soil properties of the experimental site as mean values of both growth seasons.

Soil depth (cm)	Field capacity (%)	Wilting point (%)	Bulk density (Mg m^{-3})	Total porosity (%)	Sand (%)	Silt (%)	Clay (%)	Texture class	EC_e (dS m^{-1})	pH 1:2.5
0-15	47.20	25.9	1.13	57.36	23.31	25.02	51.67	Clayey	2.02	8.44
15-30	41.10	23.8	1.19	55.09	22.78	24.86	52.36	Clayey	2.37	8.51
30-45	37.90	23.1	1.26	52.45	21.35	23.91	54.74	Clayey	2.84	8.65
45-60	34.70	21.3	1.34	49.43	21.47	23.31	55.22	Clayey	3.13	8.82
Mean	40.23	23.53	1.23		22.23	24.28	53.49	Clay	2.59	

Seeds of Karkade (*H. sabdariffa* L. cv. Sabahia 17 dark) were acquired from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Seeds were sown on 1st and 4th of April 2017 and 2018 respectively in hills at 40 cm distance on rows 60 cm apart in plot. The plot area was 42 m^2 (6m width x7 m length). Three weeks from seeds sowing, plants were thinned to one plant/hill. The experimental design was a split plot design with three replicates. The irrigation treatments were carried out at 50±5, 65 ± 5 and 85±5% of available soil moisture depletion (ASMD) were allocated in the main plot while glycinebetaine foliar spray was distributed in the sub-plots.

Plants were normally irrigated until 30 days from seed sowing then irrigation treatments were applied and after 30 days from applying irrigation treatments the first foliar application of GB treatments was begun while the second one was a month later. Different concentrations of GB (25 and 50 mM) prepared in 0.1% (v/v) Tween-20 and distilled water as a control were used as exogenous application. All agricultural practices were operated according to Agricultural Research Center recommendations.

Irrigation water applied

Soil moisture content was gravimetrically determined in soil samples, which were taken from consecutive depths of 15 cm to 60 cm. For irrigation timing, soil samples were taken regularly until it reaches the required level of allowable moisture. The amount of water applied at each irrigation for every treatment was determined on the basis of raising the soil moisture content to its field capacity plus 10% as leaching requirements.

Vegetative growth characters, were estimated after 132 days from sowing, as previously reported by El Sherif *et al*., (2011).The following growth criteria were recorded, using nine random plants from each treatment, plant height (cm), number of main branches/ plant, fresh and dry weight of plant (g).

Estimation of photosynthetic pigments, the fourth leaf from the top was picked after 132 days from sowing. Chlorophyll a, b and carotenoids were extracted and measured by using absorption proposed by Lichtenthaler and Buschmann, (2001). The pigment contents were calculated as mg/cm^2 fresh weight.

Yield components, in both seasons at harvest after 180 days from seed sowing that formerly reported by El Sherif *et al*., (2011) number of fruits/plant, fruit fresh weight (g/plant) fresh and dry weight of calyx per plant (g) and per fed (kg/g) and seed yield per plant (g)were taken.

Determination of calyces quality parameters, total soluble solids (TSS), pH and titratable acidity were determined only at the end of harvest. Ten gm of calyces and 40 ml of distilled water were macerated and homogenized by a blender as prescribed by Sakimin *et al*., (2017). The mixture was purified using cotton wool and the drops of the filtrate were located on the prism glass of refractometer to get the percentage of total soluble solids. The pH of the juice was evaluated using a glass electrode pH meter (pH 526 multical) while the acidity, as malic acid was assessed as described by Horwitz *et al*., (1970).

Total anthocyanins content (mg/g) in dried harvested roselle calyces was determined by the method described by Du and Francis, (1973).

Statistical analysis of variance (ANOVA) was investigated using COSTAT software. Differences among treatments means were performed by Duncan's multiple range test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Vegetative growth characteristics

Drought caused a significant reduction in the plant height by increasing water deficit (Table, 1). Exogenous application of glycine betaine (GB) as a foliar spray at 50 mM significantly enhanced plant height under non-stressed treatment while there were no significant differences among used GB concentrations on plant height in both growing seasons. Severe drought treatment significantly declined main branch number, and plants irrigated at 65 % of available soil moisture depletion (ASMD) gave the highest branch number followed by plants irrigated at 50 % of ASMD without significant differences in between. Plants irrigated at 65 % of ASMD combined with GB at 25 and 50 mM increased the main branch number without significant differences between both applied levels of GB in the two growing seasons. There were significant differences between well-watered plants and deficit water treatments in fresh and dry weights of roselle plants, decreasing the amount of applied water led to decrease plant fresh and dry weights, the highest values were recorded for plants irrigation at 50 % of ASMD. This result indicates that irrigated at 50 % of ASMD treatment had enough and abundant water for optimum growth and yield as compared to that irrigated at 65 and 80% of ASMD. Application of GB at 50 % of ASMD was effective on promoting fresh and dry weights of roselle under non-stressed conditions in both growing seasons. It was noticed that drought stress diminished plant height by reducing cell turgor, cell enlargement, and division that all reflected on decreasing vegetative growth. Cell growth is further sensitive to drought than cell division (Farooq *et al*., 2009).

Table 1. Effect of glycinebetaine as a foliar application on plant height, main branch number, plant fresh and dry weight of *Hibiscus sabdariffa* L. Plants under deficit irrigation during the two seasons (2017/2018)

Treatments		1st Season 2017				2nd Season 2018			
		Plant height (cm)							
		Glycinebetaine				Glycinebetaine			
		Control	25 mM	50 mM	Mean	Control	25 mM	50 mM	Mean
Irrigation	50 %	178.00c	182.00b	193.00a	184.33a	172.52c	176.39b	187.06a	178.65a
	65%	144.66f	166.66d	156.66e	156.00b	139.49f	160.70d	151.06e	150.41b
	80%	124.66h	133.66g	131.00g	129.55c	119.95h	127.97g	126.05g	124.66 c
	Mean	149.11 b	160.55a	160.22a		143.98 b	155.0a	154.72a	
		Main branch No.							
Irrigation	50 %	10.00 cd	11.33bc	12.66ab	11.33 b	9.00cd	10.33bc	11.33ab	10.22 a
	65%	12.66ab	13.00a	13.00a	12.88 a	11.66ab	12.00a	12.00a	11.89 a
	80%	8.66 d	9.00d	8.66d	8.77 b	7.66d	8.00d	7.66d	7.78 b
	Mean	10.44a	11.11a	11.44a		9.44 a	10.11a	10.33a	
		Plant fresh weight (g)							
Irrigation	50 %	1185.22c	1300.46b	1330.18a	1271.95a	1141.66c	1252.67b	1281.29a	1225.21a
	65%	1100.29f	1150d	1140.22e	1130.17b	1054.90f	1102.56d	1093.19e	1083.55b
	80%	700.16i	715.88h	730.00g	715.35c	670.52i	685.57h	699.09g	685.06c
	Mean	995.22c	1055.45b	1066.8a		955.69c	1013.60b	1024.52a	
		Plant dry weight (g)							
Irrigation	50 %	198.40 e	249.69b	259.25a	235.78a	190.43e	239.65b	248.83a	226.30a
	65%	204.93d	227.02c	210.24d	214.60b	195.63de	216.71c	200.70d	204.34b
	80%	139.27f	142.21f	145.356f	142.27c	131.65f	134.43f	137.40f	134.49c
	Mean	180.86b	206.30a	204.95a		172.57b	196.93a	195.64a	

Means designed by the same letter at each cell are not significantly different at the 5% level according to Duncan's multiple range test.

Yield components

Number of fruits and fruit fresh weight per plant were considerably declined in roselle plants subjected to severe water deficit treatment, irrigated at 80 % of ASMD (Table, 2). The highest fruit number and fruits fresh weight resulted from plants irrigated at 65 % then 50 % of ASMD, respectively without considerable differences between them in both growing seasons. Both applied GB levels boosted number of fruits per plant and fruit fresh weight per plant. Furthermore, irrigation at 65% of ASMD associated with 25 mM GB considerably improved number of fruits per plant and fruit weight per plant followed by that irrigation at 50 % of ASMD with 50 mM GB without significant differences among themselves.

Calyces fresh and dry weights per plant were significantly decreased under deficit irrigation treatment (Table, 2). The decline in roselle fresh and dry calyces weight resulted from applying deficit irrigation of plants at 80 % of ASMD due to reducing the fruit size and number of fruits per plant, which finally reflected on calyces fresh and dry weight. Foliar application of GB at 25 mM and 50 mM produced higher calyces fresh weight without significant differences in between as compared to control, while GB at 50 mM further enhanced calyces dry weight in the two growing seasons. Calyces fresh and dry weights per plant were noticeably increased due to exogenous application of GB under stress and non-stress conditions, the highest calyces fresh weight in this concern was recorded by the interaction between irrigation at 50 % of ASMD combined with 50 mM GB and irrigation at 65 % of ASMD associated with 25 mM GB without significant differences among themselves. Moreover, the highest dry weight of calyces was recorded for plants irrigated at 65% of ASMD with 25 mM GB in both growing seasons.

In the current study, water deficit treatment induced adverse effects on seeds dry weight (g/plant) and calyces dry yield kg /fed. (Table, 2) which were also, alleviated by the foliar application of GB at 25 mM on plants irrigated at 65% of ASMD. Moreover, GB significantly increased seeds dry weight (g/plant) and calyces dry yield (kg/fed). The highest seeds dry weight (g/plant) and calyces dry yield (kg/fed) were obtained from plants irrigated at 65 % of ASMD in the two growing seasons.

Table 2. Effect of glycinebetaine as a foliar application on fruit number, fruit fresh weight, calyces fresh and dry weights/plant (g) seeds dry weight (g/plant) and calyces dry yield (kg/fed.) of *Hibiscus sabdariffa* L. plants under deficit irrigation during the two seasons (2017/2018).

Treatments		1st Season 2017				2nd Season 2018			
		Fruits No.							
		Glycinebetaine				Glycinebetaine			
		Control	25 mM	50 mM	Mean	Control	25 mM	50 mM	Mean
Irrigation	50 %	53.66bc	61.66b	64.33ab	59.88a	52.11bc	59.88b	62.47ab	58.15a
	65%	61.33b	76.00a	54.00bc	63.77a	59.25b	73.42a	52.17bc	61.61a
	80%	30.00d	41.00cd	40.00cd	37.00 b	28.83d	39.40cd	38.44d	35.56 b
	Mean	48.33b	59.55a	52.7ab		46.73b	57.57a	51.02ab	
		Fruits fresh weight (g/plant)							
Irrigation	50 %	220.43d	250.47c	337.91a	269.60a	209.39d	237.92c	320.98a	256.09a
	65%	199.19e	353.53a	284.00b	278.90a	188.61e	334.76 a	268.92 b	264.09a
	80%	104.83h	150.96f	129.51g	128.43b	98.67h	142.08f	121.90g	120.88b
	Mean	174.82 b	251.65 a	250.47 a		165.55 b	238.25 a	237.26 a	
		Calyces fresh weight/plant (g)							
Irrigation	50 %	97.68cd	114.10b	131.04a	114.28a	95.53d	111.59b	128.16a	111.76a
	65%	89.90d	127.33a	105.32bc	107.52a	87.39e	123.77a	102.38c	104.51a
	80%	48.19f	57.06ef	64.64e	56.63b	46.74h	55.35g	62.70f	54.93 b
	Mean	78.59b	99.5a	100.34a		76.55b	96.90a	97.75a	
		Calyces dry weight/plant (g)							
Irrigation	50 %	15.50cd	15.82cd	18.91ab	16.74b	14.00 bc	14.33 bc	18.04a	15.46b
	65%	17.08bc	20.36a	18.95ab	18.80a	15.33b	19.37a	18.03a	17.58a
	80%	11.14e	12.42e	14.71d	12.76c	10.59e	11.80de	12.66cd	11.69c
	Mean	14.57c	16.20b	17.52a		13.30b	15.17a	16.25a	
		Seeds dry weight (g/plant)							
Irrigation	50 %	29.47d	29.45d	33.38c	30.76 b	28.47d	28.46d	32.25c	29.73b
	65%	32.99c	43.47a	39.65b	38.70a	31.74c	41.83a	38.16b	37.24a
	80%	23.10e	24.58e	24.92e	24.20 c	22.18e	23.60e	23.93e	23.23c
	Mean	28.52b	32.50a	32.65a		27.46b	31.29 a	31.44a	
		Calyces dry yield (kg/fed.)							
Irrigation	50 %	258.40cd	263.65cd	315.20ab	279.09b	233.32bc	238.88bc	300.80a	257.67b
	65%	284.65bc	339.43a	315.90ab	313.33a,	255.55b	322.90a	300.52a	292.99a
	80%	185.74e	207.10e	245.21d	212.68c	176.45e	196.75de	211.10cd	194.77c
	Mean	242.93 c	270.06 b	292.10 a		221.77b	252.84a	270.81a	

Means designed by the same letter at each cell are not significantly different at the 5% level according to Duncan's multiple range test.

The observed decline in plant growth and yield under water stress treatments is deemed a common response for water shortage conditions, which were earlier reported for roselle by Evans and Al-Hamdani, (2015) and Sakimin *et al*., (2017) and on many plants, e.g., *Phaseolus vulgaris, Carica papaya* and *Pisum sativum* (Xing and Rajashekar, 1999 Mahouachi *et al*., 2012 and Osman, 2015). However, exogenously applied GB showed a positive impact on improving roselle plants growth and yield under water stress treatments. Enhancing vegetative growth characters, fruit number, fruit fresh weight, calyces fresh and dry yields as well as seed yield by using GB were parallel to earlier studies in which it was shown that plants are able to utilize foliar-applied GB and to translocate it to approximately all plant parts, particularly developing organs (Mäkelä *et al*., 1996). Thus, foliar applications may raise the levels of GB in plants, which, counteracts growth inhibition induced by water stress that previously confirmed in literature. On *Phaseolus vulgaris* Xing and Rajashekar, (1999) revealed that glycine betaine-treated plants (10 mM) showed a slower reduction in leaf water potential, thus developing wilting symptoms were much later than the untreated plants and kept better water status through water stress treatment as well as, showed better ability to recover from wilting than the untreated plants, consequently increased all the growth characteristics and yield. Similarly, Iqbal *et al*., (2008) applied glycinebetaine at (0, 50 and 100 mM) on *Helianthus annuus* L. lines and the obtained results indicated that water stress-induced decline in achene yield/plant which was significantly diminished by the foliar application of GB at at100 mM as compared with 50 mM GB through, increased leaf water and turgor potentials when grown under water stress. Also., Roussos *et al*., (2010) stated that glycinebetaine had a positive impact on leaf water content, photosynthesis and yield of olive trees under both drought and well-irrigated conditions. Furthermore, the results of Mahouachi *et al*., (2012) on papaya seedlings under water deprivation conditions suggested that GB may modify abscisic acid, jasmonic acid, and proline accumulation via the control of stomatal movement and the high availability of compatible solutes, leading to enhancement of leaf water status and growth. Likewise, Osman, (2015) stated that GB foliar applied at 4 mM increased the growth and yield of pea plants which were decreased under drought stress at the vegetative growth stage.

Photosynthetic pigments

Roselle chlorophyll a and b concentrations exhibited no significant differences at the watering treatments and both applied glycinebetaine levels (Fig.1) Moreover, the interaction between irrigation treatments and glycinebetaine showed that the highest values for chlorophyll a and b were recorded from plants irrigated at 65% of ASMD combined with GB at 25 mM in both growing seasons. These findings are supported by the previous investigation on roselle (Evans and Al-hamdani, 2015) who stated that chlorophyll a and b were not significantly affected by the drought. Also, these results were not consistent with the results from Roussos *et al*., (2010) who stated that drought significantly enhanced chlorophyll a and chlorophyll b concentration of olive trees compared to irrigated trees. Both applied levels of Glycinebetaine improved chlorophyll a and b concentrations because GB protects the reaction center of photosystem II (PS II) by stabilizing proteins and membranes, guarding enzymes and increasing chlorophyll concentration (Mäkelä *et al*., 1998).

In relation to carotenoids, increasing drought stress from irrigation at 65 to 80 % of ASMD obviously enhanced carotenoids content (Fig.1). Furthermore, the highest carotenoids content was achieved from plants irrigated at 80 and 65 % of ASMD combined with (control) without any significant variation between both treatments. The importance of carotenoid determination is due to the role of carotenoids as non-enzymatic antioxidants, responsible for active oxygen species scavenging, that increased under drought stress, therefore carotenoids increment is a defense mechanism and significant way to increase plant resistance to drought stress. Increases in roselle carotenoids content due to water deficit treatments were in conformity with the previous results of Rivas *et al*., (2013) and Shafiq *et al*., (2015) in which water deficiency significantly increased the accumulation of carotenoids in *Moringa oleifera* and *Raphanus sativus* L. respectively. On the other hand, these observations are inconsistent to the previous study of Evans and Al-hamdani, (2015) who noticed that roselle carotenoids content was not significantly influenced by the drought.

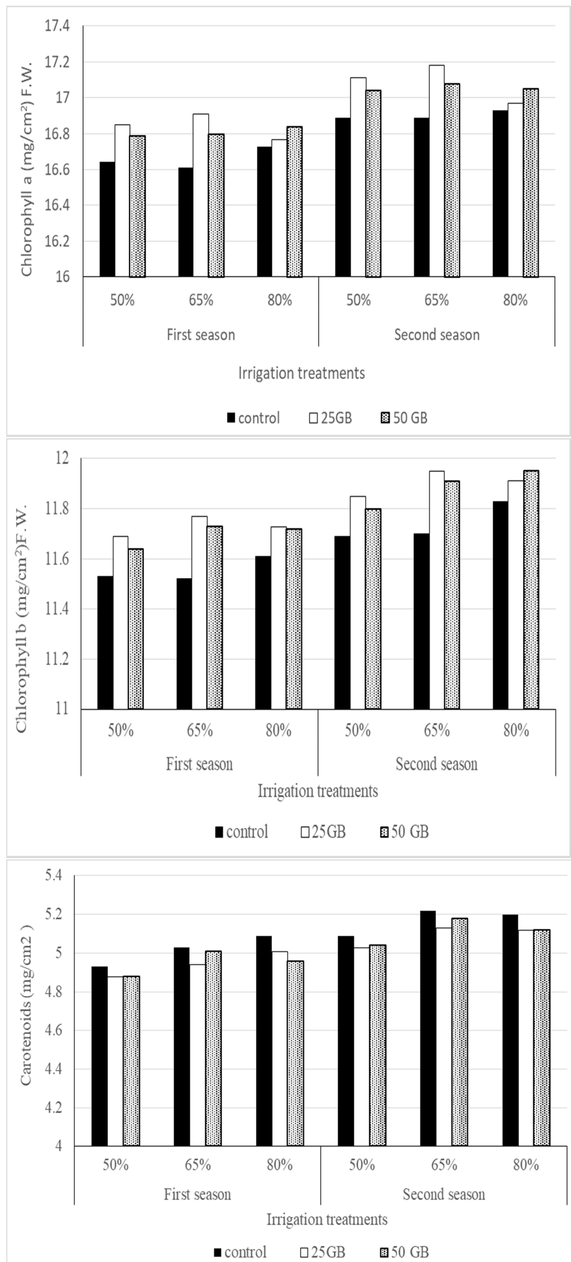

Fig. 1. Effect of glycinebetaine as a foliar application on chlorophyll a, b and carotenoids of *Hibiscus sabdariffa* L. plants under deficit irrigation during the two seasons (2017/2018).

Calyces quality parameters

pH value of roselle calyces was significantly influenced by decreasing soil water content (Table, 3). The highest pH value was obtained from irrigated plants at 80 % of ASMD and the same irrigation treatment combined with control, on the other hand, the lowest pH value as a quality indicator was achieved when plants were irrigated at 65% of ASMD and the same irrigation treatment combined with GB at 25 and 50 mM. Control plants had significantly higher pH values than both applied levels of GB in the first and second growing seasons. Similar findings were reported by Sakimin *et al*., (2017) who reported regulated deficit irrigated roselle plants had significantly higher pH values than well-watered plants. Lowering pH value as a result of irrigation at 65 % of ASMD and when was combined with both concentrations of GB is highly preferred because low pH value enhanced mineral availability when calyces of hibiscus were used as an alternative source of iron for anemia treatment and some other mineral deficiency diseases (Falade *et al*., 2005).

Titratable acidity percentage

Drought stress significantly increased titratable acidity percentage, the highest acidity percentages were achieved from plants irrigated at 80 and 65 % of ASMD without significant variation among themselves (Table, 3), on contrary, the lowest percentages were correlated with plants irrigated at 50 % of ASMD. Increases in acidity may be due to a corresponding decrease in pH value which was noticed especially when plants were irrigated at 65%. In addition, the interaction between drought and glycinebetaine were without significant differences when irrigated at 65 and 80 % of ASMD combined with both levels of GB. Exogenous application of GB at 50 mM noticeably enhanced acidity percentage followed by 25 mM GB without significant differences in between in both growing seasons.

Table 3. Effect of glycinebetaine as a foliar application on pH, titratable acidity %, TSS and total anthocyanins content (mg/g) of *Hibiscus sabdariffa* L. plants under deficit irrigation during the two seasons (2017/2018).

Treatments		1st Season 2017				2nd Season 2018			
		pH							
		Glycinebetaine				Glycinebetaine			
		Control	25 mM	50 mM	Mean	Control	25 mM	50 mM	Mean
Irrigation	50 %	3.77b	3.67e	3.61f	3.68b	3.89b	3.78e	3.73f	3.79b
	65%	3.69d	3.61f	3.62f	3.64c	3.81d	3.73f	3.74f	3.76c
	80%	3.82a	3.74c	3.68de	3.74a	3.95a	3.87c	3.81d	3.87a
	Mean	3.76a	3.67b	3.63c		3.88a	3.79b	3.75c	
		Titratable acidity %							
Irrigation	50 %	3.35d	3.79d	4.91c	4.02b	3.43d	3.88d	5.03c	4.11b
	65%	5.13c	6.70a	7.14a	6.32a	5.27c	6.87a	7.33a	6.49a
	80%	5.80b	7.14a	6.47a	6.47a	5.98b	7.36a	6.67a	6.67a
	Mean	4.76 b	5.88a	6.17a		4.89b	6.03a	6.34a	
		TSS (Brix)							
Irrigation	50 %	2.40d	2.93d	4.00c	3.11 c	2.47d	3.01d	4.12c	3.20c
	65%	4.80b	5.60a	5.06ab	5.15 a	4.94b	5.76a	5.21ab	5.30a
	80%	4.00c	4.00c	5.60a	4.53 b	4.12c	4.12c	5.76a	4.66b
	Mean	3.73c	4.17b	4.88a		3.84c	4.29b	5.03a	
		Total anthocyanin (mg/g)							
Irrigation	50 %	28.00d	23.00e	25.00e	25.33c	29.18c	23.97e	26.06d	26.40b
	65%	35.00c	39.00ab	39.00ab	37.67a	36.58b	41.00a	40.67a	39.41a
	80%	35.30c	38.00b	40.33 a	37.88 a	363.88b	393.16a	40.56a	38.76a
	Mean	32.78b	33.33b	34.78 a		34.05b	34.76ab	35.76a	

Means designed by the same letter at each cell are not significantly different at the 5% level according to Duncan's multiple range test.

Total soluble solids content

The highest values of total soluble solids (TSS) of roselle calyces which are considered quality indicator were obtained for plants irrigated at 65 and 65 % of ASMD associated with 50 mM GB as well as those irrigated at 80%of ASMD with the same GB level without significant differences among themselves (Table, 3). However, the lowest values were achieved from plants irrigated at 50 % of ASMD and 50 % of ASMD combined with control without spraying GB in both growing seasons.

Total anthocyanins content

Anthocyanins are the major phenolic compounds existing in *H. sabdariffa* calyces. Decreasing amount of applied water noticeably increased total anthocyanins content of roselle calyces (Table, 3). Anthocyanin was significantly higher in plants irrigated at 80 % of ASMD and 65 % of ASMD without significant variation in between as compared to normal irrigated plants. Foliar application of GB significantly enhanced the accumulation of anthocyanin pigments under water deficit conditions. The combined treatment of 80 % and 50 mM GB followed by plants irrigated at 65 % of ASMD associated with either 25 or 50 mM without significant differences among themselves recorded the highest values of total anthocyanins for the two successive seasons, respectively. Similar results were earlier reported by Evans and Al-hamdani, (2015) who demonstrated that anthocyanin was significantly elevated in the calyces of subjected plants to drought stress. Furthermore, this GB-induced increase in anthocyanin as phenolic content can be related to the findings of Ali and

Ashraf, (2011) who reported that foliar- applied glycinebetaine(0 or 30mM) induced increase in oil phenolic contents of maize plants under water deficit conditions.

CONCLUSION

Under the study condition especially when water becomes the most limiting factor in agriculture, it could be concluded that *H. sabdarifai* cv. Sabahia-17 plants could be irrigated at 65% of available soil moisture depletion with a foliar spray of GB at 25mM twice after one month from applying irrigation treatments then after a month later as alleviating factor of drought stress consequences. This treatment gave the highest values of main branch number, fruit number, fruit fresh weights, calyces dry weight (kg/plant), seed dry weight, calyces dry weight (kg fed.), chlorophyll a and b, TSS and acidity % in both growing seasons compared to all studied treatments. This observation may be important for the improvement of the medicinal properties of this plant under water stress conditions, especially in arid and semi-arid regions. Moreover, irrigation at 65% of ASMD saved a noticeable amount of irrigation water compared to irrigation at 50% of ASMD.

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تأثير الجليسين بيتايين على تحمل نبات الكركديه للإجهاد المائي

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الجليسين بيتايين مركب أميني يتراكم في العديد من الأنواع النباتية تحت ظروف الجفاف. لكى نحدد دور الجليسين بيتايين على النمو الخضري ومكونات المحصول والجودة ومحتوى الانثوسيانين لنباتات الكركديه تم إجراء تجربه حقليه خلال موسمي 2017 ,2018 بالمزرعة التجريبية بمحطة البحوث الزراعية بسخا - بمحافظة كفر الشيخ في قطع منشقه مره واحده حيث وضعت معاملات الري فى القطع الرئيسية وهي الري عند استنفاذ 50-65-80 % من الماء الميسر في حين كان الرش الورقي بالجليسين بيتايين بتركيزين هما 25 , 50 ملليمول بالإضافة لمعاملة الكنترول وهى الرش بماء مقطر في القطع تحت الرئيسية وقد أوضحت النتائج أن الري عند استنفاذ 80 % من الماء الميسر الري أدى لتقليل النمو الخضري وعدد الثمار والوزن الطازج للثمار ووزن السبلات الطازج والجاف ومحصول السبلات للفدان ومحصول البذور للنبات . استخدام الرش الورقي بالجليسين بيتايين بتركيزي 25 , 50 ملليمول أدى لتحسين النمو الخضري والمحصول ونسبة المواد الصلبة الذائبة الكلية ونسبة الحموضة ومحتوى الانثوسيانين. أدى الري عند استنفاذ 65 % من الماء الميسر مع الرش الورقي بالجليسين بيتايين بتركيز 25 ملليمول لزيادة عدد الافرع وعدد الثمار والوزن الطازج للثمار ووزن السبلات الطازج والجاف ومحصول السبلات للفدان ومحصول البذور للنبات وصبغات التمثيل الضوئي ونسبة المواد الصلبة الذائبة الكلية نسبة الحموضة ومحتوى الانثوسيانين بينما أدت لتقليل قيمة الرقم الهيدروجيني. الرش الورقي للجليسين بيتايين بتركيز 25 ملليمول مع الري عند استنفاذ 65 % من الماء الميسر كان أكثر فائدة في تقليل الأثر الضار للإجهاد المائي على الخصائص السابقة ه مما يعد تقنيه جيده في إنتاج الكركديه حيث أدى الى زيادة المحصول والجودة مقارنة بباقي المعاملات المدروسة.