Journal of Applied Sciences Research 1(2): 137-146, 2005

Increasing the Active Constituents of Sepals of Roselle (*Hibiscus sabdariffa* L.) Plant by Applying Gibberellic Acid and Benzyladenine

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Abstract: Two pot experiments were carried out during two successive seasons. The experiments aimed to study the effect of folair application of benzyladenine (BA) and/or gibberellin (GA_3) at 100 and/or 200 mg/L in absence and presence of Fe-EDTA on the quantity and quality of roselle sepals. All the treatments significantly increased the number of fruits/ plant, fresh weight of fruits/plant, fresh and dry weights of sepals/plant as compared with those of the untreated plants. The most effective treatment was 100 mg/L GA_3 + 100 mg/L BA in presence of Fe-EDTA. The various treatments increased significantly the total anthocyanin contents of roselle sepals, while they mostly decreased titratable acidity. The most suitable harvest age for roselle sepals is around 50 – 60 days after beginning of flowering, where the sepals of the differently treated roselle plants recorded the highest amount of anthocyanins and acid contents which declined if being left later. The different treatments increased markedly the total soluble sugars and elements composition (K, Ca, Mg, P and Fe) of roselle sepals and decreased Na content as compared with those of the control. The different treatments increased the activities of PAL and TAL enzymes, total phenol, K, Ca, Mg, P and Fe in shoot systems, while Na contents decreased.

Key words: *Hibiscus sabdariffa*, Roselle, GA_3, BA, Anthocyanin Acidity

INTRODUCTION

Hibiscus sabdariffa L. (roselle); a member of family Malvaceae; is cultivated in Egypt for multipurpose uses. It is well known in Egypt with the name of "Karkadeh", its purplish sepals (calyx and epicalyx) are the most important economic parts of the plant which is used in food (Jam and Jelly) and cosmetic industries as a source of natural colouring agent[15]. Kalt *et al*[20] reported that the pigments (anthocyanin) which are responsible primarily for red colour were delphinidin 3 - glucoside and cyanidin-3 glucoside.

In the meantime karkadeh is considered a very popular beverage and valuable medicinal plant due to its effect on lowering and/or adjusting the blood pressure without producing any side effect[16]. Also, it has a favorable effect on the functions of stomach. It possesses a high intestinal antiseptic action and can be used to resist various infections of intestinal diseases[33]. Obiefuna, *et al*[30] added that *Hibiscus sabdariffa* flowers can be used to relax the pain muscles of the uterus and intestine. The extract also proved experimentally to have highly antibacterial properties. Tanaka *et al*[40] also stated that protocatechuic acid (a simple phenolic compound) detected in *Hibiscus sabdariffa* could be used to fight pyrexia and liver disorders. Also, it has been demonstrated that this compound is an effective agent in reducing the carcinogenic action of diethylnitrosamine in the liver.

The hormonal regulation of anthocyanin synthesis has been investigated by Kwack *et al*[24] and Chung *et al*[9]. Gibberellins and cytokinins play a vital role in enhancing phenylalanine ammonia layase (PAL) and tyrosine ammonia lyase (TAL) synthesis and in turn increased anthocyanin contents, HeeOck, *et al*[19] postulated that, there was a close physiological relationship between them. Montero *et al*[27] stated that GA_3 treatment increased anthocyanin content in addition to the enhancement of PAL and TAL activities in *Fragaria ananossa* fruits. El – Meleigy[15] found that BA and/or phenylalanine enhanced the biosynthesis of anthocyanin content in roselle leaves and calyex. Deikman and Hammer[12] showed that exogenous application of cytokinins increased anthocyanin pigments and PAL synthesis in *Arabidopsis thaliana* plants.

The present work is designed to study the influence of foliar spraying of GA_3 and/or BA in absence and presence of Fe-EDTA on the economic yield of sepals,

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total anthocyanins and titratable acidity of sepals extracts during flower development and to find out the most suitable harvest age. Carbohydrates fractions and mineral contents of shoots and sepal extracts were recorded.

MATERIALS AND METHODS

The experimental plant used in this investigation was roselle (*Hibiscus sabdariffa* L.) deep red sepals cultivar. Seeds obtained from Medicinal and Aromatic Research Station Agricultural Research Centre, Ministry of Agriculture, Egypt The growth regulators used in the present work were gibberellic acid (GA_3) and benzyladenine (BA). They were supplied from Sigma Chemical Company, St. Louis, MO, USA.

The experiment was carried out on two successive seasons. Homogenously sized lots of roselle seeds (*Hibiscus sabdariffa* L) were sown in pots (50 cm in diameter) containing equal amounts of homogenous soil (2 clay : 1 sand). Phosphorus (3 g/pot) in the form of triple phosphate was added after sowing. After 15 days from sowing thinning was done, so as five uniform plants were left in each pot. Three plants from each pot were used through the experimental period and the remaining two were grown for studying the effect of different treatments on the quantity and quality of the harvested yield. The pots were divided into 2 sets, each composed of 45 pots. The pots of the first set were divided into 9 groups, each group composed of 5 pots. The plants of the 9 groups were sprayed with H_2O (control), 100, 200 mg/L GA_3, 100, 200 mg/L BA, 100mg/L GA_3+100 mg/L BA, 200 mg/L GA_3+100 mg/L BA, 100 mg/L GA_3+200 mg/L BA and 200 mg/L GA_3 +200 mg/L BA, respectively.

The plants of the second set were also, divided into the same 9 groups as in the first set, the plants of the first group sprayed with Fe – EDTA only, while the plants of the rest groups were treated with both Fe – EDTA and the previous variable treatments of growth regulators. The treatments were imposed as foliar spray and were carried out twice after 60 and 75 days from sowing. Roselle plants of the different treatments were taken after 90 and 120 days from sowing and referred to stage A and B. Stage A was at the vegetative growth, while B was at the beginning of flowering and the plants were then left to harvesting.

The activities of phenyl alanine ammonialyase (PAL) and Tyrosine ammonialayse (TAL) enzymes, the phenol contents were estimated in the fresh shoots. Titratable acidity and mineral ion contents (K, Na, Ca, Mg and Fe) were also determined at stages A and B.

The data of yield components were determined; soluble sugars, K, Na, Ca, Mg, P and Fe were estimated in the yielded sepals. The total anthocyanins and titratable acidity of roselle sepals in the differently treated and untreated plants were determined after 130 day from sowing. Samples were collected at 6 stages during the flowering development (10 days intervals) starting from the flower appearance to find out the most suitable harvest age at which the highest concentration of anthocyanin and titratable acidity were obtained.

Chemical analysis: The extraction and assay of phenylalanine ammonia lyase (PAL, EC, 4.3.1.1) and tyrosine ammonia lyase (TAL, EC, 4.3.1), enzymes activities were carried out according to the method adopted by Beaudoin-Egan & Thorpe[5]. The method applied for alcohol soluble phenolic compounds determination was carried out according to Danial and George[11] and recommended by AOAC[2]. Anthocyanins were estimated according to Tibor & Francis (1967). The titratable acidity of plant tissues and sepals was determined as citric acid by the method of titration against alkali[1].

The extracted soluble sugars were analyzed using HPLC according to the method of Oefner[31] , Aminex Hp x -87H column, 300 x 7.8 mm at 0.6 ml/min flow rate at 85ᴱC, detection: RI 16 x standard of galactouronic acid (Gal.), glucose (Glu.), rhamnose (Rham.) and mannitol (Mannit.) were used. K, Na, Ca, Mg, P and Fe were determined by Atomic Absorption Spectrometr (FMD_3) according to Chapman and Pratt[8].

The results were statistically analyzed using the Least Significant Difference(LSD) at 5% and 1% levels of probability[38].

RESULTS AND DISCUSSIONS

Yield components. The foliar application of GA_3, BA or mixture of both significantly increased the yield components of roselle plants represented by the number of fruits, fresh weight of fruits, fresh and dry weight of sepals per plant over the control plants in absence and presence of Fe-EDTA(Fig. 1) . Maximum increases were much more pronounced in response to the combined effect GA_3 and BA (each at 100 mg/L) in presence of Fe-EDTA. Similar results were obtained in different plant species by Balraj *et al*[4] and Kaushal and Rana[21].

It has been found in the present work that treatment of roselle plant with BA has a more pronounced effect on yield components than GA_3. in this respect, Talaat and Youssef[39] reported that BA foliar treatment of *Hibiscus sabdariffa* plants increased significantly the number of fruits per plant, dry weight of fruits and sepals/plant.

It is worthy to mention here that the combined effect

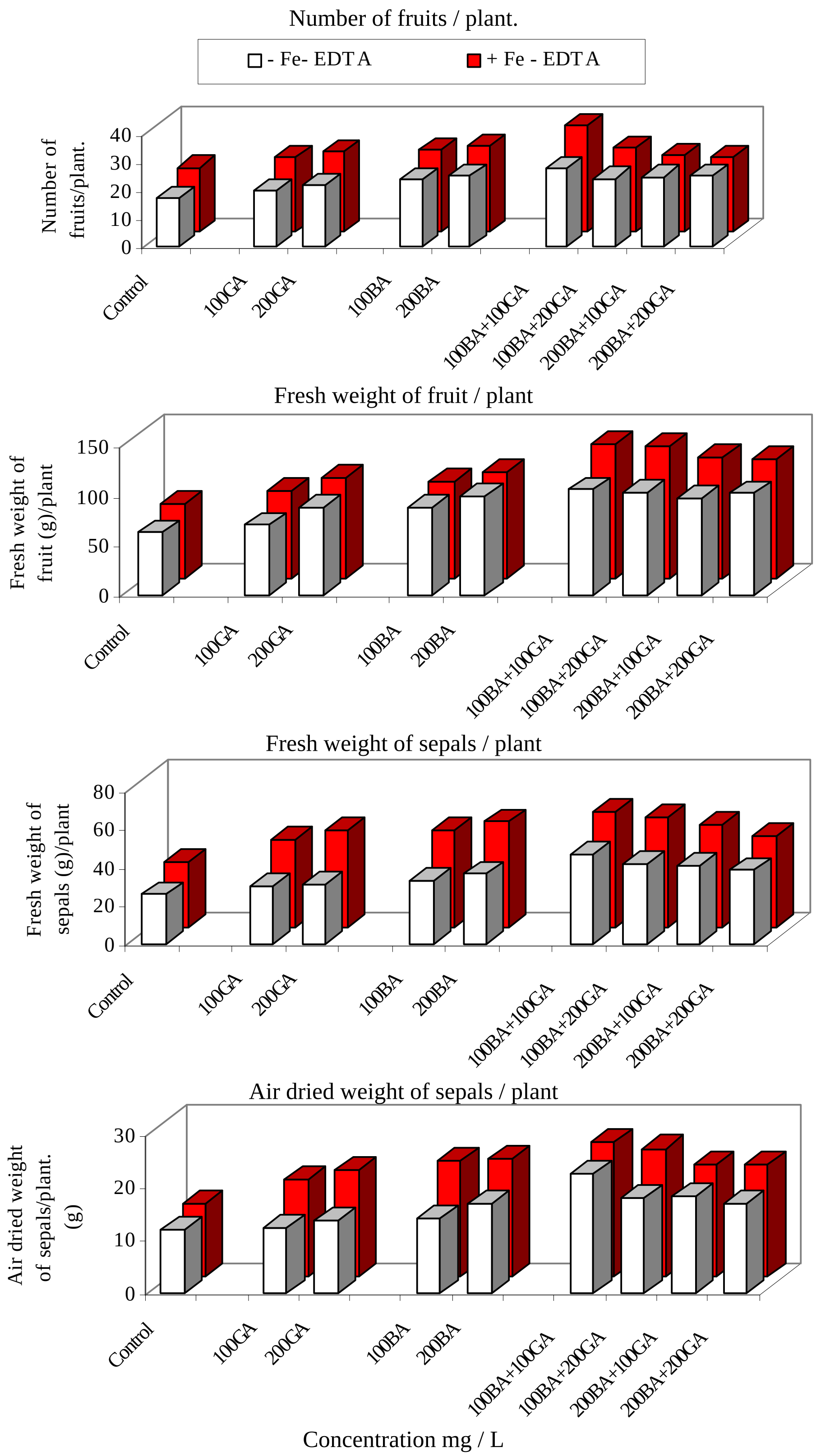

Fig. 1: Effect of gibberellin, benzyladenine or mixture of both on the yield components at harvisting of *Hibiscus sabdariffa* L. plants in absence (-) and presence (+) of Fe - EDTA.

of 100mg/L of each of BA and GA_3 on increasing the number of fruits was much higher than the effect of single hormone treatment. In this connection, Bondok *et al*[7] found that the number of flowers, number of bolls and seed cotton yield increased in response to promalin treatment.

The increase in the yield parameters of roselle plant in response to GA_3 and/or BA treatments relative to untreated plants might be result from breaking the apical dominance of roselle plants leading to the increase in flowering branches and consequently the number of fruits and their weights. This might be due to the increase of the assimilates and their translocations from the leaves to the fruits where the fruit weight increased.

The addition of Fe-EDTA to BA and/or GA_3 increased the yield components as compared with the same treatments in absence of Fe-EDTA. In this respect, Naguib and Ali[29] found that application of Fe increased significantly the yield components of roselle plants and pointed to the role of Fe^{+3} in chlorophyll biosynthesis and in turn on increasing the photosynthetic rate which is followed by an accumulation of soluble sugars and soluble nitrogen in the plant tissues. The excess of these metabolites were mobilized to the different yield components.

Activities of PAL and TAL enzymes. The PAL and TAL activities were increased in response to the application of the different treatments on roselle plants as compared with those of the untreated control plants throughout the experimental period. Such increase was much more pronounced by applying 100 mg/ L GA_3 + 100 mg/ L BA (Table 1). Similar results were obtained by Maksoud and Dadoura[25], Montero *et al*[27] and Ohlsson and Berglund[32] on *Helianthus annuus*, strawberry fruits and periwinkle plants, respectively.

In the present investigation, it could be deduced that there has been a close relationship between the higher activities of PAL and TAL and the higher levels of anthocyanins and phenol contents of roselle plants. PAL is one of the key enzymes in controlling anthocyanin biosynthesis from phenylalanine. Also, TAL, another enzyme involved in phenols synthesis[44]. The PAL enzyme catalyzes the elimination of ammonia from phenylalanine to give trans- cinnamic acid. This reaction is the first step of phenyl propanoid pathway in plants which results in the diversion of L-phenylalanine into secondary metabolism with subsequent production of anthocyanins, flavonoids and other phenolic derivatives[41].

Total phenol contents. Application of various concentrations of GA_3 and/or BA in absence and presence of Fe-EDTA induced high significant increases in the total phenol contents of roselle shoots (Table 2).

The effects of GA_3 on total phenols have been observed by Zhou and Tan[50] on apple fruits. The increased accumulation of phenolic compounds observed after GA_3 treatments may depend on the increase of PAL and TAL activities. PAL activity is known to be correlated with synthesis various phenolic compounds[32]. However, the effects of cylokinins on phenol contents were observed by Wassel[48] who reported that cytokinins increased phenol contents in cotton plants. These increases in phenol contents by application of GA_3 and/or BA may be attributed to the increase in carbohydrate synthesis[34].

Total anthocyanin and total acidity of roselle sepals. The total anthocyanins of sepals of the differently treated and untreated plants increased by progress of the flowers age reaching their maximum peak levels when the flowers were 60 days old and then slightly declined later when the seeds become fully mature (Table 3).

The application of the different concentration of GA_3 and/or BA significantly increased the anthocyanin contents. The maximum increase was recorded in response to 100 mg/L of both GA_3 + BA. The addition of Fe – EDTA to the different treatments markedly increased the total anthocyanin contents compared with those of the corresponding treatments in absence of Fe – EDTA (Table 3). Similar results were obtained by Khater[22] and Kwack *et al*[24] who showed that the GA_3 foliar treatment increased anthocyanin contents of *Hibiscus sabdariffa* L. and *Ajuga reptans*, respectively. El-Meleigy[15] and Talaat and Youssef[39] found that BA significantly increased anthocyanin contents of *Hibiscus sabdariffa* L. plants. Moreover, Thakur *et al*[42] stated that, the mixture of GA_3, BA and GA_{4+7} increased the anthocyanin contents by strawberry fruits. Also, foliar treatment of Fe – EDTA increased total anthocyanin contents in *Hibiscus sabdariffa* L. plant[29].

The increases in anthocyanin contents in roselle sepals resulted from the different treatments may be attributed to the increase in PAL and TAL activities in roselle shoot (Table 1) concomitantly with increase in sucrose contents[34]. Dube *et al*[14] confirmed this result in leaf disks of Indian almond treated with sucrose through inducing anthocyanins synthesis and phenylalanine ammonia lyase activity. Weiss *et al*[49] reported that anthocyanins are synthesized as a result of activities of the key enzyme PAL and chalcone synthase within the phenyl propanoid/ flavonoid pathway which can be induced by GA_3 in *Petunia hybride* corolla. Tucker[44] suggested that phenylalanine was catalyzed to form cinnamic acid and ammonia through PAL enzyme.

Table 1: Effect of gibberellic acid, benzyladenine and mixture of both on PAL and TAL enzyme activities of shoots of *Hibiscus sabdariffa* L. plants during their growth (stages A and B)in absence (-) and presence (+) of Fe-EDTA.

Treatment		PAL activity (µg t.cinnamic acid/g f. wt./min.)				TAL activity (µg p-coumaric acid/g f. wt./min.)			
BA (mg/L)	GA_3 (mg/L)	Stage							
		A		B		A		B	
		-	+	-	+	-	+	-	+
0.0	0.0	1.34	1.57	2.87	3.08	3.91	3.83	7.27	7.56
0.0	100	3.24	3.48	4.15	5.00	9.21	8.38	11.15	13.15
	200	4.67	6.10	5.62	6.81	10.67	14.42	14.52	20.06
100	0.0	4.71	5.23	7.38	7.91	11.94	13.62	21.79	22.48
200	0.0	7.61	7.71	7.89	8.29	20.38	23.48	20.76	25.50
100	100	9.86	10.55	15.13	15.66	36.46	40.00	47.21	47.54
	200	8.47	9.12	9.56	9.36	24.12	29.47	36.43	32.09
200	100	9.56	9.57	9.71	10.15	25.54	34.59	29.18	35.87
	200	8.10	8.49	8.44	9.48	22.87	24.82	23.83	29.68
LSD	5%	0.109	0.105	0.130	0.146	2.488	2.426	1.459	2.536
	1%	0.150	0.145	0.179	0.201	3.427	3.343	2.011	3.494
LSD	5%	0.099		0.133		2.295		1.935	
	1%	0.133		0.178		3.080		2.596	

Table 2: Effect of gibberellic acid (GA_3), benzyladenine (BA) or mixture of both on phenol contents (mg/100 g dry weight) of shoots of *Hibiscus sabdariffa* L. plants during their growth (stages A and B) in absence (-) and presence (+) of Fe-EDTA.

Treatment		Stage			
BA (mg/L)	GA_3 (mg/L)	A		B	
		-	+	-	+
0.0	0.0	188.84	209.67	99.43	201.51
0.0	100	197.11	334.99	187.11	265.60
	200	292.28	370.62	280.60	289.20
100	0.0	206.74	284.75	164.22	194.51
200		280.60	356.60	233.52	285.25
100	100	597.95	677.97	359.07	400.64
	200	594.26	593.22	359.07	396.78
200	100	393.72	492.15	342.74	287.82
	200	587.57	491.15	257.68	276.16
L.S.D	5%	6.160	4.906	3.652	8.324
	1%	8.488	6.760	5.031	11.469
L.S.D.	5%	5.549		5.999	
	1%	7.446		8.050	

Cinnamic acid is known to be the parent compound for anthocyanin and lignin biosynthesis. Moreover, El-Meleigy[15] found that roselle plants accumulate anthocyanins by applying phenylalanine.

The behavior of the acid contents of roselle sepals in response to BA and/or GA_3 followed a pattern more or less similar to that of total anthocyanins which showed a gradual increase along the duration of flowers development reaching its peak level after 60 days from flower formation followed by a marked decline at later ages. The untreated plants showed an increase in the titratable acidity than the differently treated plants in absence and presence of Fe-EDTA (Table 4). The lowest acidity value is recorded in the sepals of roselle plants with 100 mg/L of the combined treatments of GA_3 and BA in the presence of Fe – EDTA. Similar results were obtained in different plant species in response to BA or GA_3 application[15,36,37]. In addition, Naguib and Ali[29] stated that application of Fe-EDTA on *Hibiscus sabdariffa* L. reduced the acidity values of sepals as

Table 3: Effect of gibberellic acid (GA_3), benzyl adenine (BA) or mixture of both on anthocyanin Contents (mg /100g air dry weight) of sepals of *Hibiscus sabdariffa* L. plants during flower development in absence (-) and presence (+) of Fe-EDTA

Treatment		- Fe-EDTA Sepals age (days)							+ Fe-EDTA Sepals age (days)						
BA mg/L	GA_3 mg/L	10	20	30	40	50	60	70	10	20	30	40	50	60	70
0.0	0.0	11.0	18.1	31.53	43.0	71.3	70.0	69.8	13.9	25.6	39.4	59.0	98.4	98.0	96.3
0.0	100	21.0	42.8	53.10	64.0	97.8	97.5	97.5	23.5	42.7	60.6	91.2	132.1	155.4	155.4
	200	36.4	56.0	69.72	97.5	113.2	112.8	111.8	38.6	62.2	81.0	125.8	185.8	218.5	208.5
100	0.0	32.1	52.4	69.33	83.5	108.3	106.3	106.2	36.1	43.2	61.6	93.2	156.1	149.6	148.8
200		38.2	53.3	68.72	82.2	134.5	133.2	131.5	28.8	45.5	63.5	135.5	183.5	220.9	218.4
100	100	41.4	63.4	76.87	109.0	220.9	221.4	221.4	45.9	67.6	81.6	164.4	210.6	263.9	260.2
	200	37.7	59.5	69.76	91.7	139.1	149.7	149.6	38.8	56.5	75.0	103.2	149.0	146.1	146.1
200	100	29.7	44.2	65.78	82.5	164.3	208.4	207.4	39.4	54.3	71.3	113.3	151.3	161.3	152.8
	200	37.3	54.7	76.87	93.1	174.6	166.9	164.6	28.3	45.8	83.5	139.3	177.2	173.5	170.3
LSD	5%	0.927	3.379	0.108	1.505	0.479	0.774	0.690	0.537	0.391	0.761	0.756	0.722	0.826	0.517
	1%	1.269	4.626	0.149	2.062	0.656	1.061	0.946	0.736	0.536	1.043	1.035	0.989	1.131	0.709

Table 4: Effect of gibberellic acid (GA_3), benzyl adenine (BA) or mixture of both on titratable acidity (mg citric / 100g dry weight) of sepals of *Hibiscus sabdariffa* L. plants during flower development in absence (-) and presence (+) of Fe-EDTA.

Treatment		- Fe-EDTA Sepals age (days)							+ Fe-EDTA Sepals age (days)						
BA mg/L	GA_3 mg/L	10	20	30	40	50	60	70	10	20	30	40	50	60	70
0.0	0.0	9.60	13.52	16.00	18.32	24.16	18.88	17.28	8.96	12.52	14.72	16.00	18.80	17.60	16.00
0.0	100	9.60	10.88	12.16	14.48	20.90	16.24	15.36	8.96	10.24	13.64	15.92	18.36	15.60	14.08
	200	8.96	10.16	12.44	13.76	21.24	14.24	14.72	8.32	8.96	12.42	14.36	17.52	16.38	13.20
100	0.0	7.68	8.80	10.20	16.30	18.00	14.60	14.08	8.84	11.52	12.16	16.12	17.90	16.96	14.08
200		8.96	10.24	14.08	16.68	20.48	17.60	13.20	9.60	11.92	12.16	12.80	17.11	14.60	12.80
100	100	8.30	12.80	12.80	15.36	16.30	12.68	10.28	8.96	10.88	11.90	14.24	14.70	12.060	9 . 6 0
	200	8.68	12.80	14.80	17.92	18.80	16.90	11.52	6.40	7.68	10.24	12.84	16.80	13.68	11.52
200	100	6.40	11.14	15.64	18.00	18.72	16.14	12.80	8.90	11.52	12.16	16.00	16.42	14.60	13.44
	200	7.68	11.24	15.64	17.28	17.44	15.32	12.16	8.32	8.96	12.80	14.72	16.80	13.32	12.16
LSD	5%	0.196	0.196	0.671	0.599	0.819	0.144	0.162	0.094	0.054	0.094	0.810	0.260	0.133	0.597
	1%	0.268	0.268	0.919	0.821	1.122	0.197	0.223	0.129	0.074	0.129	1.109	0.356	0.182	0.817

Table 5: Effect of gibberellic acid (GA_3),benzyladenine(BA)and both of them on soluble sugars (g /100g air dry weight)of water extract of sepals of *Hibiscus sabdariffa* L. plants in absence (-) and presence (+) of Fe-EDTA at 60 days from flower appearance.

Treatment		- Fe-EDTA Sepals age (days)					+ Fe-EDTA Sepals age (days)				
BA mg/L	GA_3 mg/L	Gal.	Gluc.	Rham.	Mannit.	Total	GAL.	Gluc.	Rham.	Mannit.	Total
0.0	0.0	35.60	6.04	1.00	0.11	42.75	46.60	6.57	0.65	0.047	53.84
0.0	100	42.64	7.17	1.14	0.03	50.98	47.36	6.65	1.92	0.52	56.49
	200	48.46	6.22	1.32	0.05	56.05	52.85	9.29	1.41	0.04	63.59
100	0.0	46.57	6.62	2.39	0.42	62.93	45.74	6.53	2.64	0.42	55.17
200		51.53	6.86	1.27	0.02	59.68	52.21	7.17	1.29	0.00	60.67
100	100	52.99	7.23	2.29	0.42	62.93	56.95	8.05	2.92	0.42	68.34
	200	50.66	6.94	1.83	0.39	59.82	46.49	8.21	1.43	0.09	56.22
200	100	53.01	6.55	2.51	0.00	62.07	55.72	7.45	1.21	0.02	64.40
	200	51.98	7.40	1.03	0.03	60.44	51.44	7.87	2.50	0.39	62.20

compared with the control.

The continuous increase in the acid contents of the early stages of the developing sepals of the differently treated and untreated roselle plants may be attributed to the increased catabolic activities[15], while the decrease in the acid values in the sepals of the differently treated plants below those of the control, at the same stage, might be due to the shift of sugars and certain catabolic

Table 6: Effect of gibberellic acid (GA_3), benzyladenine (BA) and mixture of both on element contents (mg/100g air dry weight) in water extract of sepals of *Hibiscus sabdariffa* L. plants in absence (-)and presence (+) of Fe-EDTA

Treatment		Fe – EDTA											
BA (mg/)	GA_3 (mg/L)	-						+					
		Na	K	Ca	Mg	P	Fe	Na	K	Ca	Mg	P	Fe
0.0	0.0	99	1930	2160	640	1354	32	116	2230	2500	870	1561	46
0.0	100	76	2190	2500	650	1746	62	80	2530	2500	870	1777	48
	200	84	2340	2500	870	1237	73	86	2830	2660	980	1470	100
100	0.0	78	2130	2500	870	1772	46	73	2500	2630	870	1872	55
200		76	2810	2750	870	1549	73	82	2960	2840	980	1920	64
100	100	75	3150	3130	1017	1746	95	72	3230	3250	1640	1958	117
	200	79	2500	2750	870	1536	91	85	2880	2840	1420	1659	96
200	100	75	2580	2910	980	1518	82	87	3130	3250	1530	1735	99
	200	83	2910	2500	870	1630	86	87	3200	3000	1090	1641	115
L.S.D	5%	1.7	15.1	15.1	16.2	21.0	4.6	3.1	15.4	19.2	17.2	51.0	5.9
	1%	2.3	20.7	20.7	22.3	28.8	6.3	4.3	21.2	26.3	23.5	69.9	8.1

Table 7: Effect of gibberellic acid (GA_3), benzyladenine (BA) and mixture of both on element contents (mg/100g dry weight) of shoots of *Hibiscus sabdariffa* L.plants during their growth in absence (-) and presence (+) of Fe-EDTA.

Treatment		Fe – EDTA											
BA (mg/)	GA_3 (mg/L)	-						+					
		Na	K	Ca	Mg	P	Fe	Na	K	Ca	Mg	P	Fe
						Stage A							
0.0	0.0	144.2	1750	1753	327	910	23.9	112.5	1800	1350	434	840	28.4
0.0	100	106.5	2025	1990	440	1200	36.7	88.7	2050	2100	500	968	40.0
	200	109.2	2125	1940	440	1496	47.9	97.5	2250	2030	572	954	67.4
100	0.0	111.5	1937	2090	540	1276	32.9	88.7	2037	2116	536	1290	73.7
200		115.0	2025	2040	650	1006	93.9	93.0	2100	2280	720	1224	98.1
100	100	89.0	2257	2225	710	1222	109.7	87.2	2300	2290	781	1274	124.8
	200	106.5	2250	2190	540	964	82.0	87.5	2237	2200	670	1198	112.4
200	100	89.3	1987	1800	435	1156	73.7	87.5	2087	2100	563	1164	97.0
	200	99.5	1862	1965	440	1112	80.6	97.5	1982	2130	665	1134	106.0
L.S.D.	5 %	0.66	28.7	14.9	12.3	15.1	2.17	0.64	27.7	237	9.7	22.3	0.92
	1 %	0.91	39.4	20.4	16.8	20.7	2.97	0.87	38.0	324	13.3	30.6	1.3
						Stage B							
0.0	0.0	115.5	1953	1915	545	1218	13.0	90.7	1898	1650	540	850	80.1
0.0	100	83.7	21.37	2050	708	1302	14.9	80.0	2150	1750	602	1010	80.1
	200	81.75	2344	2175	980	1354	42.6	76.6	2238	1970	655	1498	101.1
100	0.0	82.5	2100	2140	599	1298	31.0	77.5	2150	2500	645	1482	168.9
200		83.0	2325	2180	730	1142	86.9	81.7	2025	2000	820	1160	151.7
100	100	80.7	2300	2370	874	1406	89.3	72.0	2325	2380	820	1596	181.1
	200	85.0	2475	2282	783	1116	40.5	79.0	2213	2230	740	1472	135.2
200	100	89.2	2413	2265	820	1226	36.7	85.5	2282	2880	655	1410	168.4
	200	83.5	2225	2295	940	1252	26.5	83.5	2050	2380	765	1330	129.2
LS D	5 %	1.4	29.6	25.7	20.9	22.8	1.4	0.94	45.2	31.9	25.9	28.4	0.32
	1 %	1.9	40.5	35.2	28.6	31.3	1.9	1.3	61.9	43.8	35.5	38.9	0.43

intermediates to the developing seeds where they incorporated into the biosynthesis of oils and carbohydrates[34]. Moreover, the decline in the acid contents in the sepals of the differently treated and untreated roselle plants at the latest stages of sepal development might be attributed to the increased rate of catabolism more than anabolism

In conclusion the most suitable harvest age for roselle sepals is ranged between 50–60 days after the appearance of flowers. In general, at this time the sepals of the differently treated roselle plants contain the highest amounts of anthocyanins and acids represented by citric acid as compared with those of the control sepals.

Total soluble carbohydrates of sepals. It has been also shown, in this investigation that the different treatments used (GA_3 and/or BA in absence and presence of Fe–EDTA) had significant effects on the total soluble sugar levels of roselle sepals at harvesting as compared with those of the control (Table 5). The most effective treatment is 100 mg/L GA_3 +100 mg/L BA in presence of Fe–EDTA. Similar results were obtained in different plant species by Vadigeri *et al*[45] and Kumar *et al*[23] In addition, Fe–EDTA caused a significant increase in total and soluble carbohydrates in sepals of *Hibiscus sabdariffa* plants[29].

Concerning the soluble carbohydrate fractions, the application of different treatments led to a marked increase in the galactouronic acid, glucose and rhaminose fractions above the control. The most effective treatment was 100 mg/L of both GA_3 and BA in presence of Fe–EDTA. Muller and Franz[28] isolated distinct sugar fractions from the flower buds of *Hibiscus sabdariffa* L. The fractions were rhaminose, arabinose, galactouronic acid (24%) and glucose. They suggested that these sugars might be involved in decreasing the blood pressure, relaxation of rat uteri, inhibition of taenia motility and bacterial growth in aqueous extract.

Mineral composition. Application of various concentrations of GA_3 and/or BA in absence and presence of Fe-EDTA increased K, Ca, Mg, P and Fe, while Na ion contents decreased compared to those of control plants (Tables 6,7) in shoot and sepals of roselle plants, this may be due to the translocation of those elements from shoots to sepals. These results are in agreement with those of Belakbir[6] and Arun *et al*[3] who found that GA_3 treatments induced increases of P, Ca, Mg and Fe contents in pepper and rose plants respectively. Shahin[35] stated that Kinetin foliar application increased P, K, Ca but decreased Na contents on anna plants.

The influence of GA_3 and BA on the mechanism of ions uptake may be related to their effects on membrane permeability and rate of ion entry through the membrane, or enhance their translocation to the shoot[46]. The positive effect of GA_3 or BA may presumably be due to the activation of ATPase of plasma membrane and consequently the stimulation of K^+ carriers bound to plasma membrane[10]. They also increased the rate of water uptake by roots as well as the transpiration and consequently increase the uptake and translocation of mineral ion contents which were driven by transpiration[18] Furthermore, Kinetin altered membrane composition[26] its selectivity[13] and increased membrane fluidity[47]. The decreased in Na ion contents in response to GA_3 and/or BA in roselle shoots could be attributed to the effect of those hormones in the selective action of plasma membrane[17].

The foliar spray of Fe-EDTA induced marked increases in the contents of K, Mg, Ca, P and Fe in roselle shoots and decreased the Na contents as being compared with those of the untreated control. The similar results were obtained by Naguib and Ali[29] found that Fe–EDTA increased N, P, K and Fe in *Hibiscus sabdariffa* L. sepals.

ACKNOWLEDGEMENT

The authors wish to express their deepest appreciation and gratitude to Prof. Dr. Mostafa, H.A.M., Professor of Plant Physiology, department of Botany, National Research Centre for his guidance throughout this work.

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