Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.134

IMPACT OF TYROSINE AND GLUTAMINE ACIDS ON YIELD AND SOME CHEMICAL CONSTITUENTS OF *HIBISCUS SABDARIFFA* PLANTS UNDER DIFFERENT SOWING DATES

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(Date of Receiving-28-11-2020; Date of Acceptance-05-03-2021)

ABSTRACT

Two field experiments were carried out at experimental farm of Faculty of Agriculture, Zagazig University, Egypt, during the two summer consecutive seasons of 2018 and 2019. To study the impact of sowing dates and some amino acids on the roselle (*Hibiscus sabdariffa* L.) yield in terms of number of fruits per plant, dry sepal's yield per plant and per feddan as well as its chemical constituents in terms of total chlorophyll (SPAD) and anthocyanin (mg/100g as dry weight) contents. A split-plot experiment was carried out in the field based on a randomized complete block design with three replications. The main plot was four sowing dates (15th April, 1st May, 15th May and 1st June) and the sub-plot was five amino acids treatments (tap water as control, tyrosine and glutamine acids each at 100 or 200 ppm concentrations). The results showed that the early in sowing from June1 to April 15 increased fruit number per plant, dry sepals yield per plant (g) and per feddan (kg) as well as total chlorophyll content and anthocyanin content. Also, all the values of the studied parameters were significantly increased when the roselle plants sprayed with glutamine acid at 200 ppm compared to control. In final, planting roselle seeds in 15th April and sprayed with 200 ppm glutamine four times per season manifests to enhance yield and is adequate for fulfilling the highest anthocyanin contents in sepals of roselle under Sharkia Governorate conditions.

Keywords: Roselle, sowing date, amino acids, yield components, chlorophyll, anthocyanin

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an ideal multi-function model for medicine, fiber, feed and food (Dutt *et al.,* 2009) and it grows productively in the sub-tropics and tropics regions (Begum *et al.,* 2015). It is belongs to the Malvaceae family. The tender or young leaves are consumed as a green vegetable and the main product of this crop is the sepals and epi-sepals that are used in preparation of drinks (Ottai *et al.,* 2006). In addition, Lin *et al.,* (2007) has revealed benefits of roselle extract that works as appetizer, sexual stimulator, cancer-protective, cathartic, refrigerant and anti-cough.

Succeeded cultivation of any crop is determined by sundry factors. Sowing date is one of the serious aspects for production system of several crops. Optimum planting or sowing date encloses suitable development and growth of plant resulting maximum yield of crop and economic utilize of land (Islam *et al*., 2010). Optimization of roselle planting date is a stellar approach to improve both plant yield and economic benefit (Seghatoleslami *et al.*, 2013). Many researchers have studied the influence of sowing date on yield and anthocyanin content of roselle (Nnebue *et al*., 2014; Apeyuan *et al*., 2017; El-Bakhshwan and El-Kouny, 2018 and Motlagh *et al*., 2018).

However, the regulatory impact of amino acids on plant evolution could be stated by the notion that several amino acids e.g. tyrosine and glutamine are hydroxyl phenyl amino acids that are utilized to build hormones as well as neurotransmitters (Walter and Nawacke, 1978). Exogenous application of amino acids has been utilized as stand by ways of polyamines synthesis in plants for direct or indirect improvement of physiological activities under various plantations (Boras and zidan, 2011). Many researchers have studied the impact of amino acids on yield and chemical constituents of medicinal crops (Hadi *et al*., 2011; Wahba *et al*., 2015; Helaly and Ibrahim, 2019 and El Shayeb *et al*., 2020).

Therefore, in this study the enhancement impacts of sowing dates as well as tyrosine and glutamine in different concentrations, via foliar application, were tabulated on the fruit and sepals yields and some chemical constituents of roselle (*Hibiscus sabdariffa*, L.) plants.

MATERIALS AND METHODS

Sowing date was applied every 15 days with the dates of 15th April, 1st May, 15th May and 1st June. Different tyrosine (Tyr) and glutamine acids (Gln) concentrations of 100 and 200 ppm of each beside tap water as control were tested. The treatments were arranged in a split-plot in randomized complete blocks design with three replicates. Sowing dates were randomly arranged in the main plots and different amino acid types were randomly distributed in the sub plots. Two field experiments were carried out at experimental farm of Faculty of Agriculture, Zagazig University, Egypt, during the two summer consecutive

Table A. Physical and chemical properties of experimental soil (average of the two seasons)

Physical analysis												Soil texture
Clay (%)		Silt (%)	Fine sand (%)			Coarse sand (%)						Clay
41.39		19.26	15.62			23.73						
Chemical analysis												
pH	E C m.mohs /cm	Organic mater (%)	Soluble cations (meq./ L)				Soluble anions (meq. /L)			Available (ppm)		
			Mg^{++}	Ca^{++}	K^{+}	Na^{+}	Cl^{-}	HCO_3^{-}	SO_4^{--}	N	P	K	
7.82	0.98	0.58		2.7	1.5	1.6	3.9	4.5	1.7	3.5	17	8.3	71

Table 1. Impact of sowing date (S), amino acid concentration (A) and their interaction (S×A) treatments on *Hibiscus sabdariffa* number of fruits/plant during 2018 and 2019 seasons

Planting date	Amino acids concentration (ppm)					
	Control	Tyrosine		Glutamine		Mean (S)
		100	200	100	200	
Number of fruits/plant						
2018 season						
15th April	97.00	130.33	150.33	144.67	157.67	**136.00**
1st May	96.00	111.67	129.33	118.33	136.33	**118.33**
15th May	91.33	99.67	126.67	117.67	130.67	**113.20**
1st June	78.67	96.33	124.00	113.33	127.00	**107.87**
Mean (A)	**90.75**	**109.50**	**132.58**	**123.50**	**137.92**	
L.S.D. at 5 %	**(S)= 2.61**	**(A)= 2.72**		**(S×A)= 5.51**		
2019 season						
15th April	105.00	139.00	153.67	142.33	162.33	**140.47**
1st May	102.67	119.33	141.00	127.33	143.67	**126.80**
15th May	96.00	103.67	132.00	129.00	134.33	**119.00**
1st June	78.33	97.00	126.67	109.67	129.33	**108.20**
Mean (A)	**95.50**	**114.75**	**138.33**	**127.08**	**142.42**	
L.S.D. at 5 %	**(S)= 2.67**	**(A)= 2.60**		**(S×A)= 4.45**		

seasons of 2018 and 2019.These experiments made to study impacts of sowing dates and amino acids on yield and production of anthocyanin and chlorophyllin a dark cultivar of *Hibiscus sabdariffa* plants.

The plot area was 10.80 m^2 (3.00 × 3.60 m) included six ridges. Each ridge was 60 cm wide and 3 meters in length. The seeds were sown in hills on one side of the ridge, and hills were spaced 50 cm, a part. Amino acid treatments were exercised at 30, 50, 70 and 90 days after sowing as foliar application. Each experimental unit received five letters solution utilizing spreading agent (Super Film at a rate of 1ml /l). The untreated plants (control) were sprayed with tap water.

The physical and chemical properties of the used experimental soil are presented in Table A, according to (Chapman and Pratt, 1978). Seed of roselle plant were sown at the before time above-mentioned sowing dates during the two seasons (2018 and 2019) and promptly irrigated. After 20 days from sowing, seedlings were thinned to be one plant/hill.

The source of glutamine acid ($C_5H_{10}N_2O_3$) and tyrosine acid ($C_9H_{11}NO_3$) were Techno Gene Company (TGC), Dokky, Giza, Egypt. All recommended agricultural practices of growing roselle plants were done when always necessary. All experimental units were fertilized with 200 kg calcium superphosphate (15.5 % P_2O_5), 200 kg ammonium sulphate (20.5 % N) and 100 kg potassium sulphate (50 % K_2O) per feddan (0.42 hectare). Phosphorus fertilizer was applied during soil preparation. While, nitrogen and potassium fertilizers were divided into three equal doses and were added to the soil at 50, 70 and 90days after sowing.

Data Recorded

Table 2. Impact of sowing date (S), amino acid concentration (A) and their interaction (S×A) treatments on *Hibiscus sabdariffa* dry sepals yield/plant (g) and /feddan (kg) during 2018 and 2019 seasons

Planting date	Amino acids concentration (ppm)					
	Control	Tyrosine		Glutamine		Mean (S)
		100	200	100	200	
Dry sepals yield/plant (g)						
2018 season						
15th April	37.08	49.72	68.18	51.67	70.68	**55.47**
1st May	35.02	38.51	42.70	40.66	54.31	**42.24**
15th May	31.52	34.13	39.14	36.72	44.27	**37.16**
1st June	27.81	31.40	35.29	34.58	36.19	**33.05**
Mean (A)	**32.86**	**38.44**	**46.33**	**40.91**	**51.36**	
L.S.D. at 5 %	**(S)= 0.85**	**(A)= 1.10**		**(S×A)= 2.14**		
2019 season						
15th April	40.07	56.32	70.23	67.83	71.67	**61.23**
1st May	36.89	40.20	55.94	43.49	67.54	**48.81**
15th May	30.91	34.15	42.88	33.95	43.80	**37.14**
1st June	26.89	30.89	33.77	33.25	35.11	**31.99**
Mean (A)	**33.69**	**40.39**	**50.71**	**44.63**	**54.53**	
L.S.D. at 5 %	**(S)= 0.71**	**(A)= 0.59**		**(S×A)= 1.27**		
Dry sepals yield/feddan (kg)						
2018 season						
15th April	519.17	696.13	954.47	723.43	989.47	**776.53**
1st May	490.33	539.09	597.75	569.92	760.39	**591.37**
15th May	441.28	477.87	548.01	514.08	619.73	**520.37**
1st June	389.29	439.55	494.01	484.12	506.71	**462.74**
Mean (A)	**460.02**	**538.16**	**648.56**	**572.73**	**719.07**	
L.S.D. at 5 %	**(S)= 11.94**	**(A)= 15.41**		**(S×A)= 29.98**		
2019 season						
15th April	561.00	788.50	983.30	949.60	1003.40	**857.15**
1st May	516.50	562.80	783.20	608.90	945.60	**683.38**
15th May	432.80	478.10	6000.30	475.30	613.20	**519.92**
1st June	376.50	432.80	472.70	465.50	491.50	**447.81**
Mean (A)	**471.70**	**565.52**	**709.87**	**624.83**	**763.42**	
L.S.D. at 5 %	**(S)= 10.00**	**(A)= 8.26**		**(S×A)= 17.79**		

Yield components: at harvesting stage (150-180 days after sowing), number of fruits /plant and dry sepals yield per plant (g/plant) were determined, and then total dry sepals yield per feddan (kg/feddan) was calculated.

Chemical constituents: Total chlorophyll (SPAD unit) was determined in fresh leaves of roselle by utilizing SPAD- 502 meter as demonstrated by Markwell *et al.,* (1995).The anthocyanin content (mg/100 g) in roselle dried sepals was determined colorimetrically according to the method presented by (Abou-Arab *et al*., 2011) and adopted by Francis (2000) for *Hibiscus sabdariffa*.

Statistical Analysis

Collected data were analyzed according to Gomez and Gomez (1984). Least significance difference (L.S.D.) was utilized to differentiate means at the at 5 % level of probability. The means were compared utilizing computer program of Statistix version 9 (Analytical software, 2008).

Table 3. Impact of sowing date (S), amino acid concentration (A) and their interaction (S×A) treatments on *Hibiscus sabdariffa* leaf total chlorophyll content (SPAD unit) during 2018 and 2019 seasons

Planting date	Amino acids concentration (ppm)					
	Control	Tyrosine		Glutamine		Mean (S)
		100	200	100	200	
Total chlorophyll content (SPAD)						
2018 season						
15th April	40.223	42.153	44.643	43.097	45.840	**43.191**
1st May	40.717	41.787	42.467	42.183	43.150	**42.061**
15th May	40.043	40.290	41.703	41.980	43.167	**41.437**
1st June	39.430	39.563	42.270	40.620	42.130	**40.803**
Mean (A)	**40.103**	**40.948**	**42.771**	**41.970**	**43.572**	
L.S.D. at 5 %	**(S)= 0.337**	**(A)= 0.369**		**(S×A)= 0.739**		
2019 season						
15th April	41.523	43.623	45.630	42.567	44.807	**43.630**
1st May	40.470	41.067	42.507	41.823	43.773	**41.928**
15th May	39.887	39.677	40.957	41.243	42.073	**40.767**
1st June	38.553	39.880	40.443	40.913	41.950	**40.348**
Mean (A)	**40.108**	**41.062**	**42.384**	**41.637**	**43.151**	
L.S.D. at 5 %	**(S)= 0.250**	**(A)= 0.333**		**(S×A)= 0.646**		

Table 4. Impact of sowing date (S), amino acid concentration (A) and their interaction (S×A) treatments on *Hibiscus sabdariffa* anthocyanin content (mg/100g as dry weight) 2018 and 2019 seasons

Planting date	Amino acids concentration (ppm)					
	Control	Tyrosine		Glutamine		Mean (S)
		100	200	100	200	
2018 season						
15th April	11.240	12.230	13.910	12.413	14.167	**12.792**
1st May	11.787	12.487	12.927	12.523	14.630	**12.871**
15th May	10.957	12.107	12.093	12.430	13.700	**12.257**
1st June	10.203	10.630	12.700	12.293	13.673	**11.900**
Mean (A)	**11.047**	**11.863**	**12.908**	**12.415**	**14.042**	
L.S.D. at 5 %	**(S)= 0.247**	**(A)= 0.200**		**(S×A)= 0.434**		
2019 season						
15th April	12.140	13.550	15.920	13.760	15.710	**14.216**
1st May	12.360	12.510	13.267	14.183	15.773	**13.619**
15th May	10.973	11.853	12.140	12.520	14.377	**12.373**
1st June	11.233	11.103	12.757	12.980	13.363	**12.287**
Mean (A)	**11.677**	**12.254**	**13.521**	**13.361**	**14.806**	
L.S.D. at 5 %	**(S)= 0.304**	**(A)= 0.328**		**(S×A)= 0.659**		

RESULTS AND DISCUSSION

Yield components

Results under discussion in Tables 1 and 2 that, sown roselle seeds in 15th April significantly increased number of fruits per plant, dry sepals yield per plant (g) and dry sepals yield per feddan (kg)compared to the others sowing dates under study. Moreover, delaying the sowing date led to a significant decrease in the yield components. Furthermore, the decreases in dry sepals yield per feddan were about 40.41 and 47.76% for 1stJune, 32.99 and 39.34 % for 15th May lower the 15thApril in first and second seasons, respectively.These resulted are in line with those found by Nnebue *et al*., (2014), Khattak *et al*., (2016) and Apeyuan *et al*., (2017)on roselle plant. In addition, Motlagh *et al*., (2018) reported that delayed sowing date significantly reduced number of bolls, calyx dry weight, calyx yield and biomass yield.

It is quite clear from data in Tables 1 and 2suggest that,utilizing glutamine acid at 200 ppm gave the highest values of yield components of roselle (number of fruits per plant, dry sepals yield per plant and per feddan) compared to control in both seasons. Generally, increasing Tyr or Gln acids concentrations from 100 to 200 ppm gradually improved yield components of *Hibiscus sabdariffa* plant. The application of amino acids can encourage the performance of various plants (Hendawy *et al*., 2012, Radkowski and Radkowska, 2018 and Helaly and Ibrahim, 2019). Similarly, Al-Mohammad and Al-Taey (2019) on arugula plant indicated that spraying of tyrosine showed significant enhancement yield parameters compared to the controls. Also, Noroozlo *et al*., (2019) pointed out that foliar application of glutamine amino acid can have beneficial impacts on lettuce fresh yield compared to control.

The data given in Tables 1 and 2 show that combination between sowing dates and amino acid concentrations significantly enhanced yield components of roselle compared to the control (sprayed with tap water and planted in 1st June) in both seasons. However, the best interaction treatment in number of fruits per plant, fresh and dry sepals yield per plant (g) and dry sepals yield per faddan (kg) were that of sowing at 15th April combined with glutamine at 200 ppm. In conclusion, as mentioned above, both sowing date and amino acids concentrations (each at 200 ppm) increased yield of roselle plant, in turn; they together might maximize their impacts leading to more fruits as well as higher sepals yield per plant and per feddan.

Moreover, Badawy *et al*., (2013) reported that October sowing date to *Lallemantia iberica* plant had a pronounced impact on seeds yield/plant, mucilage contents and essential and fixed oil yield/plant. Furthermore, El Shayeb *et al*., (2020) stated that the highest values in number of inflorescences per coriander plant, fruit yield per plant and per feddanof coriander was obtained when Gln acid was used as foliar spray at 100 ppm concentration compared to the other ones under study.

Chemical constituents

Data recordedin Tables3 and 4suggest that total chlorophyll content (SPAD) and anthocyanin content (mg/100g s dry weight) of roselle plant was gradually decreased with delaying sowing date (except that of anthocyanin content during first season) in both seasons. However, maximum chemical constituents' values were noticed at the earliest sowing (15th April), while minimum values were observed in roselle plants sown on 1st June. The increases in total chlorophyll content were about 5.53 and 7.54% for 15th April, 4.06 and 6.56 % for 1st May over the 1st June in 1st and 2nd seasons, respectively. The prospective cause for the differences in chlorophyll and anthocyanin pigments due to sowing dates is that early sown roselle plants had a longer period for vegetative growth, which produced darker colors. These results are in harmony with those reported by Motlagh *et al*., (2018) on roselle; they reported that anthocyanin was significantly reduced by delaying in sowing date.

From data presented in Tables 3 and 4 it is clear that each Tyr and Gln acids at any concentration (100 or 200 ppm) significantly increased roselle chemical constituents compared to control (sprayed with tap water) in both seasons. However, total chlorophyll content (SPAD) and anthocyanin content (mg/100g s dry weight) was gradually increased with increasing tyrosine and glutamine acids concentrations. In addition, the highest values in this concern were achieved with Gln acid at 200 ppm compared to control and the other ones under study. The increases in anthocyanin content were about 27.11 and 26.80 % for Gln at 200 ppm as well as 16.85 and 15.79% for Tyr acid at 200 ppm over the control in 1st and 2nd seasons, respectively. Many researchers pointed out those amino acids have various roles in plant metabolism, and exogenous application of amino acids may have benefits and stimulation effects on plant growth which reflected on chlorophyll and anthocyanin (Helaly and Ibrahim, 2019).

Data tabulated in Tables 3 and 4 reveal thatthe best combination treatment in roselle chlorophyll and anthocyanin contents was that of sowing at 15th April combined with Gln at 200 ppm. However, combination between sowing dates and amino acid concentrations significantly increased total chlorophyll content and anthocyanin content compared to the control (sown in 1st June andsprayed with tap water) in the two seasons, in most cases. In the same time, as mentioned above, both sowing date and tyrosine or glutamine concentrations (at 200 ppm) increased yield components of roselle plant, in turn; they together might maximize their impacts leading to more chlorophyll and anthocyanin contents. Moreover,

Jahanshahi *et al*., (2014)on *Anethum graveolens* demonstrated that chlorophyll content decreased due to late seed sowing and enhanced a little bit when more vermicompost was applied.

CONCLUSION

From above mentioned findings, it could be concluded that, the sowing date of 15th April combined with glutamine acid at 200 ppm as foliar spraying is suitable for increase the yield components and total chlorophyll as well as anthocyanin contents of *Hibiscus sabdariffa* L. plant under Sharkia Governorate conditions.

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