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EFFECT OF VERNALIZATION AND GIBBERELLIN TREATMENTS ON EARLY FLOWERING, MATURITY, YIELD AND CHEMICAL COMPOSITION IN *Hibiscus subdariffa* PLANT

Kamal E. Attia

Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, ARC., **Egypt**.

***Corresponding author:** kattia200099@yahoo.com **Received:** 10 Jan. 2021 **; Accepted:** 15 Feb. 2021

ABSTRACT: Roselle (*Hibiscus sabdariffa*, L.) is medicinal plant species producing anthocyanin, which are flavonoids are water-soluble natural pigments. Two field experiments were conducted at the Experimental Farm of EL-Quassasin Horticultural Research Station, Ismailia Governorate, Egypt during 2019 and 2020 summer seasons. That, to define an appropriate vernalization types and gibberellin rates as well as their combinations for achieving an early flowering and shortening of plant life, a better plant growth, fruits and sepals yield and quality of roselle. Vernalization treatments were seven types: without vernalization, vernalization at 4 or 10ºC for one week, 4 or 10ºC for two weeks and 4 or 10ºC for three weeks. Gibberellin treatments were three rates: control, 100 and 200 ppm. The result of the research revealed that the early flowering date and the shortest duration from sowing to reach the harvest was observed from the pre-sowing cold treatment of seeds at 10°C for three weeks followed by two weeks with no significant difference between them compared to the other vernalization treatments under study. The same two treatments produced the highest values concern fruit number and weight per plant, sepals yield per plant and per feddan as well as anthocyanin content. Using GA_3 at 200 ppm reduced number of days to flowering and harvest compared to the other rates and control. The tallest plants, more branches and fruit number per plant, heaviest fruits and sepals per plant and higher chlorophyll and anthocyanin contents were obtained by the treatment of 200 ppm GA_3 compared to the other ones under study. The combined application of 10°C for two weeks with GA_3 at 200 ppm produced better responses and further enhancement in plant growth, yield components, pigments and early harvest date. Therefore, it can be recommended to apply it to the roselle plant under the same conditions.

Key words: *Hibiscus sabdariffa*, vernalization, Gibberellin, growth, yield, chlorophyll, anthocyanin.

INTRODUCTION

Hibiscus sabdariffa, L. is one of the greatest remarkable medicinal plants of Family Malvaceae, which yielded red sepals (calyx and epicalyx). In addition to Roselle, in English-talking areas it is named as Rozelle, Indian sorrel, Red sorrel, Sorrel, Guinea sorrel, Jamanica sorrel, Jelly okra, Queensland jelly plant and Lemon bush (**Mahadevan *et al.*, 2009**). Roselle plants are sown for its sepals, seeds and leaves. Nutritionally young roselle leaves are consumed as a green vegetable and contain several nutrients such as P, Ca, Mg and K (**Delgado-Vargas and Parcedes-Lopez, 2003**). Moreover, the roselle seed is a valuable food resource on account of its fiber and valuable micro-nutrients, protein and calorie (**Akanbi *et al.*, 2009**). Also, it contains edible fixed oil (about 17 to 20%) which is similar in its characteristics to seed oil of cotton (**Wahba *et al.*, 2001 & Ottai *et al.*, 2006**). Sepals extract (anthocyanin) is also a potential source of natural colorant to replace red synthetic coloring agents for carbonated soft drinks, juices, jams, sauces, jellies, wines, chutneys, and preserves foods (**Wong *et al.*, 2000 & Falusi *et al.*, 2014**). The red varieties of roselle have cyclooxygenase inhibitory and antioxidant activities. In addition, roselle participates in cosmetic and pharmaceutical industries **(Al-Ansary *et al.*, 2016**).

Vernalization (cold treatment) accelerates growth, flowering, yield and active ingredients in many annual ecotypes as reported by **Sakr *et al.* (2013)** on sugar beet, **El Sherif and Khattab (2016)**

on roselle, **Wu *et al*. (2016**) on garlic and **Zheng *et al*. (2018**) on turnip, which on the other hand offer a strong earliness in flowering under normal conditions. Furthermore, vernalization of pea seeds at 2, 5 and 10°C before sowing pointed out that an improve in all yield parameters as well as the highest values in this regard were achieved by 10°C treatment before sowing (**Abd Alla**, **2000**). Environmental conditions (exposing seeds to cold treatment) greatly affect yield components of onion plant (**Ami *et al*., 2013**).

Plant growth regulators are organic compounds that play a serious role in various molecular and physiological processes of plants. It regulates the cell differentiation, cell division as well as shoots and root growth and senescence of plants (**Lone *et al*., 2005**). The treatment of gibberellic acid increased chickpea number pods per plant, harvest index and seed yield per plant as well as seed carbohydrate protein and contents (**Mazid and Naz, 2017**). Hormone application (gibberellins) was the preferable treatment to improve flower quality of *Allmanda* plant. Moreover, treated plant with GA_3 recorded the highest values in chlorophyll fluorescence and carotenoid contents (**Hossain and Amaninah, 2018**).

The aim of this study was to achieve early flowering and shortening of the plant's life, taking into account the resulting high quantity and quality yield, by exposing the seeds to vernalization treatments combined with different gibberellin rates.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm of EL-Quassasin Horticultural Research Station, Ismailia Governorate, Egypt, during the two consecutive summer seasons of 2019 and 2020. This study was done to investigate the effect of vernalization types, gibberellin rates and their interactions on flowering time, plant growth and yield components as well as leaves pigments and sepal's anthocyanin content of roselle plants. Roselle seeds (red dark variety) were obtained from the Department of Medicinal and Aromatic plants, Hort. Res. Inst., Agric. Res. Center, Giza, Egypt.

Vernalization treatments were made for the seeds by placing 200 seeds in a plastic bag and putting an amount of water on the seeds that is sufficient to wet the seeds only. Then the roselle seeds were cooled at 4º C and 10 º C for one, two- and three-weeks during 19th April, 26th April and 3rd May, respectively, in both seasons. The control seeds had not been refrigerated. Moreover, gibberellin acid (GA_3) was added to the plant as foliar spray at different rates (0.0, 100 and 200 ppm) during the vegetative period in three equal rates after 30, 50 and 70 days from sowing date.

The roselle seeds were sown on 10th May during both seasons. Seeds were sown then immediately irrigated. The plot area was 9 m^2 (1.80 × 5.00) m included three rows; each row was 60 cm apart and five meters in length. The seeds were sown on row in hills on one side. The distances between hills were 50 cm. After three weeks from planting, seedlings were thinned to be one plant per hill. The physical and chemical properties of the experimental soil site are shown in Table 1, according to **Chapman and Pratt (1978)**.

Table 1. Physical and chemical characteristics of the utilized experimental farm soil (average of two seasons)

Physical analysis			Soil texture
Clay (%)	Silt (%)	sand (%)	Sandy
20.78	9.40	69.69	

Chemical analysis												
pH	E.C. (dsm^{-1})	Soluble cations (m.mol/l)					Soluble anions (m.mol/l)			Available (ppm)		
		Ca^{++}	Mg^{++}	Na^{+}	Zn^{++}	Mo^{++}	Cl^{-}	HCO_3^{-}	SO_4^{--}	N	P	K
7.78	1.31	1.81	0.93	0.33	1.12	1.30	3.01	1.14	0.87	117	42	68

All roselle plants received normal agricultural practices whenever they needed. All plants were fertilized with nitrogen as ammonium sulphate (20.5%N) at the rate of 200 kg/feddan (4200 m^2) and phosphorus fertilization as calcium super phosphate (15.5% P_2O_5) at the rate of 200 kg/feddan as well as potassium as potassium sulphate (48 %K_2O) at the rate of 100 kg/feddan.

Recorded Data

Flowering and harvest dates

The number of days from sowing date until flowering was recorded. Also, the number of days from sowing date to the end of harvest was noticed.

Growth parameters

Plant height (cm) and number of branches per plant were recorded after 75 days from sowing date in both seasons.

Yield components

Fruit number per plant, fresh weight of fruits per plant (g), seed and dry sepals yield per plant (g) and sepals yield per feddan (kg) were tabulated.

Chemical constituents

Chlorophyll a and chlorophyll b contents (mg/100g as fresh weight) in roselle leaves were determined after 75 days from sowing according to **Mazumder and Majumder (2003)**. The anthocyanin content (mg/100 g) in dried roselle sepals was determined cholorimetrically as described with the method pointed out by **(Abou-Arab *et al*., 2011)** and adopted by **Francis (2000)** for *Hibiscus sabdariffa*.

Experimental design and statistical analysis

The layout of this experiment was a split-plot design with three replicates. Vernalization types were assigned to the main plots and Gibberellin rates to the sub plots. The combination treatments between main factor and sub factor were 21 treatments. Collected data were analyzed as stated by **Gomez and Gomez (1984)**. Least significance difference (L.S.D.) was utilized to differentiate means at the at 5 % and 1 % levels of probability. The means were compared utilizing computer program of Statistix version 9 (**Analytical software, 2008**).

RESULTS AND DISCUSSIONS

Flowering and harvest dates

Data listed in table 2 suggest that, vernalization treatments of 4º C for three weeks as well as 10º C for two or three weeks enhanced earlier flowering and harvest dates when compared to the other periods of vernalization in both seasons. Furthermore, non-vernalized treatment (control) led to a delay in flowering and harvest time compared to the vernalization treatments under study. In addition, the earliest flowering and harvest dates were significantly obtained with 200 ppm GA_3 treatment compared to the other rate under study and control (unsprayed plants), in most cases, in the two seasons. In general, vernalized roselle seeds at 4 or 10ºC for two or three weeks recorded the earliest flowering and harvest date compared to the other interaction treatments under study. Moreover, exposure seeds of sugar beet for a prolonged period of cold (vernalization) before the sowing enhance flowering (**Reeves *et al*., 2007**). Also, **Zheng *et al*. (2018**) reported that vernalization effectively promoted flowering in Tibetan turnip. Likewise, Increasing GA_3 concentration up to 400 ppm significantly reduced number of days to flowering of carrot plant (**Ghoname *et al*., 2011**). In addition, **Pramanik *et al*. (2018**) indicated that gibberellins stimulate vegetative growth of tomato to flowering, and seed development along with an interaction of different environmental factors viz., light and temperature.

Plant growth parameters

Table 3 reveals that increasing vernalization temperature degree from 4 to 10 ºC as well as exposure periods from 1, 2 to 3 weeks gradually increased roselle plant height and number of branches per plant in both seasons. Generally, the tallest plants and more branches per plant were produced when roselle seeds vernalized for three weeks at 10 ºC compared to the other types under study. The similar trend regard enhance in plant growth parameters were achieved with 200 ppm gibberellin rate compared to the lowest rate 100 ppm and control in the two consecutive seasons. In most cases, all interaction treatments highly significantly increased plant height and branch number per plant compared to control (un-vernalized seeds without GA_3 foliar spray) in 2019 and 2020 seasons. The best interaction treatment in this connection was 10 ºC for three weeks vernalization type plus 200 ppm gibberellin rate.

Collectively, vernalization clearly encourages stem elongation of canola by raising gibberellins synthesis and specially the GAs biosynthesis (**Zanewich, 1993**). Similarly, **El Sherif and Khattab (2016)** demonstrated that roselle plants produced from seeds vernalized at 12 ºC significantly increased the plant height and number of branches compared to control. Also, gibberellins are plant growth regulator that stimulate cell elongation and cause plants to grow taller. The GA_3 affects various growth and biochemical traits (**Qudassi *et al*., 2014**).

Table 2. Effect of vernalization types (ver.), gibberellin rates (GA) and their interaction on the number of days from sowing seeds until flowering and the number of days from sowing seeds to harvest of *Hibiscus subdariffa* L. plant during the two seasons of 2019 and 2020

Vernalization type	1st season				2nd season			
	Gibberellin rates (ppm)				Gibberellin rates (ppm)			
	0.0	100	200	$M_{Ver.}$	0.0	100	200	$M_{Ver.}$
	The number of days from sowing seeds until flowering							
Control	98.0	96.0	94.0	**96.0**	99.0	96.0	96.0	**97.0**
1 week - 4º	91.0	91.0	88.0	**90.0**	91.0	90.0	89.0	**90.0**
1 week - 10º	91.0	90.0	88.0	**89.7**	90.0	89.0	89.0	**89.3**
2 weeks - 4º	83.0	82.0	79.0	**81.3**	84.0	82.0	80.0	**82.0**
2 weeks - 10º	81.0	79.0	78.0	**79.3**	82.0	79.0	79.0	**80.0**
3 weeks - 4º	81.0	79.0	79.0	**79.7**	83.0	80.0	79.0	**80.7**
3 weeks - 10º	80.0	79.0	79.0	**79.3**	81.0	79.0	79.0	**79.7**
M_{GA}	**86.4**	**85.1**	**83.6**		**87.1**	**85.0**	**84.4**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**1.5**	**1.2**	**3.1**		**1.4**	**1.2**	**3.1**	
LSD. at 1%	**2.1**	**1.6**	**4.2**		**2.0**	**1.6**	**4.1**	
	The number of days from sowing seeds to harvest							
Control	188.0	186.0	184.0	**186.0**	189.0	186.0	186.0	**187.0**
1 week - 4º	181.0	181.0	178.0	**180.0**	181.0	180.0	179.0	**180.0**
1 week - 10º	181.0	180.0	178.0	**179.7**	180.0	179.0	179.0	**179.3**
2 weeks - 4º	173.0	172.0	169.0	**171.3**	174.0	172.0	170.0	**172.0**
2 weeks - 10º	171.0	169.0	168.0	**169.3**	172.0	169.0	169.0	**170.0**
3 weeks - 4º	171.0	169.0	169.0	**169.7**	173.0	170.0	169.0	**170.7**
3 weeks - 10º	170.0	169.0	169.0	**169.3**	171.0	169.0	169.0	**169.7**
M_{GA}	**176.4**	**175.1**	**173.6**		**177.1**	**175.0**	**174.4**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**1.4**	**1.2**	**3.1**		**1.5**	**1.2**	**3.1**	
LSD. at 1%	**2.0**	**1.6**	**4.2**		**2.0**	**1.6**	**4.1**	

Table 3. Effect of vernalization types (ver.), gibberellin rates (GA) and their interaction on plant height and number of branches of *Hibiscus subdariffa* L. plant during the two seasons of 2019 and 2020

Vernalization type	1st season				2nd season			
	Gibberellin rates (ppm)				Gibberellin rates (ppm)			
	0.0	100	200	$M_{Ver.}$	0.0	100	200	$M_{Ver.}$
	Plant height							
Control	145.7	155.3	161.3	**154.1**	141.7	147.3	155.0	**148.0**
1 week - 4º	162.3	166.3	171.3	**166.7**	158.3	161.3	166.3	**162.0**
1 week - 10º	174.3	178.3	180.3	**177.7**	164.7	135.3	172.3	**157.4**
2 weeks - 4º	176.3	184.7	185.7	**182.2**	170.0	175.3	180.0	**175.1**
2 weeks - 10º	184.3	191.7	198.3	**191.4**	181.0	186.0	191.7	**186.2**
3 weeks - 4º	189.3	195.3	202.0	**195.6**	185.3	193.3	195.3	**191.3**
3 weeks - 10º	**192.7**	**203.0**	**206.7**	**200.8**	**186.7**	**196.3**	**203.7**	**195.6**
M_{GA}	**175.0**	**182.1**	**186.5**		**169.7**	**170.7**	**180.6**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**0.8**	**0.9**	**2.3**		**12.9**	**8.0**	**21.2**	
LSD. at 1%	**1.1**	**1.2**	**3.1**		**18.0**	**10.8**	**28.6**	
	The number of branches/ plant							
Control	8.3	11.0	13.3	**10.9**	9.7	11.7	13.7	**11.7**
1 week - 4º	12.3	14.7	16.0	**14.3**	13.0	15.3	16.7	**15.0**
1 week - 10º	14.3	16.3	19.3	**16.7**	15.3	18.3	21.0	**18.2**
2 weeks - 4º	15.3	18.3	21.3	**18.3**	17.3	20.0	23.0	**20.1**
2 weeks - 10º	17.3	20.7	22.0	**20.0**	19.3	23.0	24.3	**22.2**
3 weeks - 4º	23.7	24.3	26.7	**24.9**	21.7	24.0	27.7	**24.4**
3 weeks - 10º	**22.7**	**25.3**	**28.3**	**25.4**	**24.3**	**27.3**	**31.0**	**27.6**
M_{GA}	**16.3**	**18.7**	**21.0**		**17.2**	**20.0**	**22.5**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**1.1**	**0.9**	**2.4**		**0.6**	**0.4**	**1.1**	
LSD. at 1%	**1.6**	**1.2**	**3.2**		**0.8**	**0.5**	**1.5**	

Yield components

Data of both seasons in Tables 4 and 5 indicate that, roselle plants produced from seeds vernalized at 10ºC for two or three weeks highly significantly increased number of fruits per plant, fresh weight of fruits per plant and seed yield per plant as well as dry sepals yield per plant and per feddan compared to the other vernalization types under study in both seasons. In the other words, roselle seeds having no vernalization application produced the lowest values in this concern. Using GA_3 as foliar spray three times/season at 200 ppm rate highly significantly increased yield components of roselle plant compared to the other rates under study. The increases in sepals yield per feddan were about 49.47 and 54.67% for 200 ppm rate, 37.64 and 38.13 % for 100 ppm rate over the control in 1st and 2nd seasons, respectively.

Table 4. Effect of vernalization types (ver.), gibberellin rates (GA) and their interaction on number and fresh weight of fruits/plant and seeds yield/plant of *Hibiscus subdariffa* L. plant during the two seasons of 2019 and 2020

Vernalization type	1st season				2nd season			
	Gibberellin rates (ppm)				Gibberellin rates (ppm)			
	0.0	100	200	$M_{Ver.}$	0.0	100	200	$M_{Ver.}$
	Number of fruits/ plant							
Control	12.3	14.7	16.3	**14.4**	13.3	16.0	17.7	**15.7**
1 week - 4º	16.7	22.7	28.7	**22.7**	18.3	24.7	30.0	**24.3**
1 week - 10º	27.3	37.0	45.0	**36.4**	30.0	41.0	47.3	**39.4**
2 weeks - 4º	32.0	45.7	54.3	**44.0**	36.3	51.3	57.7	**48.4**
2 weeks - 10º	41.3	56.3	69.0	**55.6**	47.0	65.0	75.0	**62.3**
3 weeks - 4º	40.0	47.3	55.3	**47.6**	43.7	52.7	58.7	**51.7**
3 weeks - 10º	44.3	69.3	68.3	**60.7**	49.0	66.3	72.0	**62.4**
M_{GA}	**30.6**	**41.9**	**48.1**		**34.0**	**45.3**	**51.2**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**2.3**	**1.0**	**2.7**		**2.4**	**0.8**	**2.2**	
LSD. at 1%	**3.2**	**1.4**	**3.7**		**3.4**	**1.1**	**2.9**	
	Fresh weight of fruits/plant							
Control	117.3	128.3	135.7	**127.1**	121.7	132.3	138.7	**130.9**
1 week - 4º	135.7	165.3	177.3	**159.4**	140.0	172.3	185.7	**166.0**
1 week - 10º	146.3	177.7	192.0	**172.0**	151.7	187.7	208.0	**182.4**
2 weeks - 4º	174.7	231.3	261.0	**222.3**	181.7	236.0	267.7	**228.4**
2 weeks - 10º	187.7	264.0	294.0	**248.6**	192.3	277.7	306.0	**258.7**
3 weeks - 4º	178.3	234.3	261.7	**224.8**	185.3	238.0	270.0	**231.1**
3 weeks - 10º	182.3	266.7	293.7	**247.6**	187.3	276.7	308.3	**257.4**
M_{GA}	**160.3**	**209.7**	**230.8**		**165.7**	**217.2**	**240.6**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**3.8**	**1.9**	**5.0**		**2.8**	**1.8**	**4.8**	
LSD. at 1%	**5.3**	**2.6**	**6.8**		**4.0**	**2.4**	**6.5**	
	Seeds yield/plant							
Control	9.2	13.1	14.6	**12.3**	10.1	13.5	15.7	**13.1**
1 week - 4º	10.8	15.1	17.8	**14.6**	11.2	15.9	16.2	**14.4**
1 week - 10º	16.9	20.9	23.7	**20.5**	17.6	21.6	24.3	**21.2**
2 weeks - 4º	19.7	24.5	27.9	**24.0**	20.4	25.8	28.9	**25.0**
2 weeks - 10º	24.8	29.1	31.4	**28.5**	25.8	30.7	32.9	**29.8**
3 weeks - 4º	20.3	24.8	28.2	**24.4**	21.5	26.3	29.1	**25.6**
3 weeks - 10º	25.1	29.4	31.7	**28.7**	26.0	30.8	33.0	**29.9**
M_{GA}	**18.1**	**22.4**	**25.0**		**18.9**	**23.5**	**25.7**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**0.36**	**0.26**	**0.70**		**0.79**	**0.33**	**0.87**	
LSD. at 1%	**0.51**	**0.36**	**0.94**		**1.11**	**0.44**	**1.17**	

Table 5. Effect of vernalization types (ver.), gibberellin rates (GA) and their interaction on yield of dry sepals per plant and per feddan of *Hibiscus subdariffa* L. plant during the two seasons of 2019 and 2020

Vernalization type	1st season				2nd season			
	Gibberellin rates (ppm)				Gibberellin rates (ppm)			
	0.0	100	200	$M_{Ver.}$	0.0	100	200	$M_{Ver.}$
	Dry weight of sepals (g)/plant							
Control	17.8	16.4	16.6	**16.9**	17.5	16.5	15.7	**16.6**
1 week - 4º	20.4	23.0	23.0	**22.1**	20.6	23.1	25.9	**23.2**
1 week - 10º	16.7	20.0	20.5	**19.1**	16.8	20.9	22.9	**20.2**
2 weeks - 4º	20.5	28.7	32.1	**27.1**	20.8	27.8	31.8	**26.8**
2 weeks - 10º	18.3	31.6	36.2	**28.7**	17.8	32.3	36.5	**28.9**
3 weeks - 4º	20.7	29.1	32.0	**27.3**	20.6	27.7	32.1	**26.8**
3 weeks - 10º	16.8	31.9	35.9	**28.2**	16.5	31.9	36.9	**28.4**
M_{GA}	**18.7**	**25.8**	**28.0**		**18.6**	**25.7**	**28.8**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**0.6**	**0.4**	**1.0**		**1.0**	**0.6**	**1.6**	
LSD. at 1%	**0.9**	**0.5**	**1.4**		**1.4**	**0.8**	**2.2**	
	Yield of dry sepals (kg)/fed.							
Control	213.0	196.6	198.8	**202.8**	209.8	197.7	188.5	**198.7**
1 week - 4º	245.2	275.5	275.4	**265.4**	246.6	277.7	310.9	**278.4**
1 week - 10º	200.7	240.0	245.5	**228.7**	201.1	251.1	274.6	**242.3**
2 weeks - 4º	246.1	344.1	385.6	**325.2**	249.5	333.0	381.6	**321.4**
2 weeks - 10º	220.0	379.4	434.2	**344.5**	214.1	387.6	438.1	**346.6**
3 weeks - 4º	248.2	349.6	384.2	**327.3**	246.6	332.4	385.2	**321.4**
3 weeks - 10º	201.6	382.8	430.5	**338.3**	198.1	383.2	442.9	**341.4**
M_{GA}	**225.0**	**309.7**	**336.3**		223.7	309.0	346.0	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**7.5**	**4.6**	**12.1**		**11.8**	**7.3**	**19.2**	
LSD. at 1%	**10.6**	**6.2**	**16.4**		**16.6**	**9.8**	**25.9**	

Roselle plants presented highest number of fruits per plant, fresh weight of fruits per plant and seed yield per plant in plants sprayed with 200 ppm GA_3 from seeds vernalized at 10ºC for two or three weeks. The same interaction treatment produced the highest values in dry sepals yield per plant and per feddan compared to the other interaction treatments under study. Generally, as mentioned just before, both vernalization and gibberellin rate treatments (each alone) increased plant growth of roselle plants, in turn, they together might maximize their influences leading to better results in roselle fruit number per plant and dry sepals yield per plant and per feddan compared to untreated seeds and plants. Since, **Ami *et al.* (2013)** pointed out that yield and quality of onion seeds were significantly impacted by vernalization. Maximum seeds /umbel and seed yield per plot were produced by the plants that were vernalized in 5±10ºC. **El Sherif and Khattab (2016)** found that the highest sepals yield per roselle plant was obtained from the vernalization treatment of seeds at 12 ºC for 1 week. **Mathieu *et al.* (2020)** reported that heat increased the flowering % of non-vernalized chicory plants by 25% but decreased that of vernalized plants by 65%. However, the best values of umbel weight and number of umbels per plant and also the highest seed yield per flowering orders and total seed yield of carrot plant were achieved with 400 ppm GA_3 treatment (**Ghoname *et al.*, 2011**).

Chemical constituents

From data presented in Table 6 it is clear that, roselle seeds cold pretreatment at 10ºC highly significantly increased chlorophyll a and chlorophyll b contents in the roselle leaves as well as anthocyanin content in the roselle sepals compared to the other vernalization types under study in both seasons. The increases in anthocyanin content were about 15.41 and 14.57% with 10ºC for two weeks, 14.79 and 14.89 % 10ºC for three weeks over the control in 1st and 2nd seasons, respectively. The highest values in this connection were obtained by the treatments of 200 ppm gibberellin rate which produced the highest values in chlorophyll a (1.517 and 1.504), chlorophyll b (0.701 and 0.683) and anthocyanin contents (10.66 and 10.48) in first and second seasons, respectively. Generally, all

interaction treatments significantly increased plant height and branch number per plant compared to control (un-vernalized seeds without GA_3 foliar spray) in both seasons. The best interaction treatment in this connection was 10 ºC for two weeks vernalization type plus 200 ppm gibberellin rate, in most cases. In the same time, **Zhang *et al.* (2006)** revealed that the concentration of chlorophyll of rice seedlings from sand treatment were also significantly higher after the exposure to low temperature condition. **Haroun *et al.* (2011)** reported that vernalization treatment significantly enhanced the concentration of chlorophyll a and b compared to control plants (un-vernalized tomato seeds). Moreover, **Al-Whaibi *et al.* (2012)** concluded that plant growth regulators (GA_3) enhanced the photosynthetic pigments of *Eruca sativa* plant. Also, **Hossain and Amaninah (2018)** suggested that chlorophyll fluorescence yield and carotenoid contents were also highest in *Allamanda* flowers treated with GA_3.

Table 6. Effect of vernalization types (ver.), gibberellin rates (GA) and their interaction on chlorophyll percentage in leaves and anthocyanin content in sepals of *Hibiscus subdariffa* L. plant during the two seasons of 2019 and 2020

Vernalization type	1st season				2nd season			
	Gibberellin rates (ppm)				Gibberellin rates (ppm)			
	0.0	100	200	$M_{Ver.}$	0.0	100	200	$M_{Ver.}$
Chlorophyll A percentage								
Control	1.409	1.432	1.448	**1.430**	1.407	1.417	1.429	**1.418**
1 week - 4º	1.443	1.458	1.476	**1.459**	1.437	1.453	1.469	**1.453**
1 week - 10º	1.468	1.491	1.518	**1.492**	1.462	1.482	1.497	**1.480**
2 weeks - 4º	1.479	1.497	1.514	**1.497**	1.471	1.479	1.512	**1.487**
2 weeks - 10º	1.509	1.550	1.592	**1.550**	1.500	1.525	1.565	**1.530**
3 weeks - 4º	1.488	1.513	1.515	**1.506**	1.481	1.508	1.510	**1.500**
3 weeks - 10º	1.511	1.548	1.558	**1.539**	1.502	1.542	1.546	**1.530**
M_{GA}	**1.472**	**1.498**	**1.517**		**1.466**	**1.487**	**1.504**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**0.002**	**0.001**	**0.003**		**0.001**	**0.002**	**0.005**	
LSD. at 1%	**0.003**	**0.002**	**0.005**		**0.002**	**0.003**	**0.007**	
Chlorophyll B percentage								
Control	0.523	0.640	0.660	**0.608**	0.498	0.604	0.638	**0.580**
1 week - 4º	0.610	0.669	0.698	**0.659**	0.581	0.623	0.665	**0.623**
1 week - 10º	0.652	0.681	0.688	**0.674**	0.637	0.668	0.674	**0.660**
2 weeks - 4º	0.669	0.699	0.707	**0.692**	0.663	0.678	0.684	**0.675**
2 weeks - 10º	0.687	0.710	0.725	**0.708**	0.680	0.685	0.703	**0.689**
3 weeks - 4º	0.683	0.702	0.706	**0.697**	0.668	0.693	0.701	**0.687**
3 weeks - 10º	0.684	0.706	0.720	**0.703**	0.697	0.702	0.713	**0.704**
M_{GA}	**0.644**	**0.687**	**0.701**		**0.632**	**0.665**	**0.683**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**0.003**	**0.001**	**0.003**		**0.009**	**0.006**	**0.015**	
LSD. at 1%	**0.004**	**0.002**	**0.005**		**0.013**	**0.008**	**0.021**	
Anthocyanin content (mg/g)								
Control	9.37	9.60	9.67	**9.54**	9.27	9.43	9.50	**9.40**
1 week - 4º	9.60	9.90	10.33	**9.94**	9.57	9.73	10.07	**9.79**
1 week - 10º	10.20	10.47	10.63	**10.43**	9.90	10.20	10.43	**10.18**
2 weeks - 4º	10.27	10.57	10.70	**10.51**	10.03	10.23	10.43	**10.23**
2 weeks - 10º	10.43	11.13	11.47	**11.01**	10.20	10.97	11.20	**10.79**
3 weeks - 4º	10.27	10.43	10.60	**10.43**	10.10	10.20	10.47	**10.26**
3 weeks - 10º	10.50	11.10	11.20	**10.93**	10.33	10.80	11.27	**10.80**
M_{GA}	**10.09**	**10.46**	**10.66**		**9.91**	**10.22**	**10.48**	
	Ver.	**GA**	**Inter.**		**Ver.**	**GA**	**Inter.**	
LSD. at 5%	**0.14**	**0.06**	**0.16**		**0.13**	**0.08**	**0.21**	
LSD. at 1%	**0.19**	**0.08**	**0.22**		**0.18**	**0.11**	**0.28**	

Conclusion

Vernalization treatment at 10 ºC for two weeks as well as gibberellin at 200 ppm rate contributed to an increase in total roselle sepals yield per feddan with significant differences with the other interactions under study. Also, vernalized roselle seeds before sowing at this type as well as foliar spray with GA_3 at this rate led to stimulate growth, increasing yield and enhancing the biochemical constituents of roselle plant. Generally, it can be recommended to apply it to *Hibiscus sabdariffa* plant under Ismailia Governorate conditions.

REFERENCES

Abd Alla, M. S. A. (2000). Physiological studies on the role of some growth substances for early production of pea. Ph. D. Thesis, Hort. Dept., Fac. Agric. Moshtohor, Zagazig University, Egypt.

Abou-Arab, A.A.; Abu-Salem, F.M. and Abou-Arab, E.A. (2011). Physico-chemical properties of natural pigments (anthocyanin) extracted from roselle calyces (*Hibiscus subdariffa*). J. Am. Sci., 7: 445-456.

Akanbi, W.B.; Olaniyan, A.B.; Togun, A.O.; Hupeju, A.E.O. and Alaniran, O.A. (2009). The effects of organic fertilizer on growth, calyx yield and quality of Roselle (*Hibiscus sabdariffa* L.). Am. Eurasian J. Sustain. Agric., 3: 652-657.

Al-Ansary, A.M.F.; Abd-El Hamied, R.N.; Ottai, M.E.S. and El-Mergawi, R.A. (2016). Gamma irradiation effect on some morphological and chemical characters of Sudani and Masri roselle varieties. Int. J. Chem. Tech. Res., 9(3): 83-96.

Al-Whaibi, M.H.; Siddiqui, M.H.; Al-Munqadhi, B.M.A.; Sakran, A.M.; Ali, H.M. and Basalah, M.O. (2012). Influence of plant growth regulators on growth performance and photosynthetic pigments status of *Eruca sativa* Mill. Journal of Medicinal Plants Research, 6(10): 1948-1954.

Ami, E.J.; Islam, M.D.T. and Farooque, A.M. (2013). Effect of vernalization on seed production of onion. Agriculture, Forestry and Fisheries, 2 (6): 212-217.

Analytical Software (2008). Statistix Version 9, Analytical Software, Tallahassee, Florida, USA.

Chapman, D.H. and Pratt, R.F. (1978). Methods Analysis for Soil, Plant and Water. Univ. of California Div. Agric. Sci. 16: 38.

Delgado-Vargas, F. and Parcedes-Lopez, O. (2003). Natural Colorants for Food and Nutraceutical Uses. CRC Press, LLC: Boca Raton, FL, p.327.

El Sherif, F. and Khattab, S. (2016). Effect of seed vernalization temperature, duration and planting date on growth and yield of *Hibiscus sabdariffa* plants. Life Sci. J., 13(6): 52-60.

Falusi, O.A.; Dangana, M.C.; Daudu, O.A.Y.; Oluwajobi, A.O.; Abejide, D.R. and Abubakar, A. (2014). Evaluation of some Rossele (Hibiscus sabdariffa L.) germplasm in Nigeria. Inter. J Biotech. Food Sci., 2: 16-20.

Francis F.J. (2000). Anthocyanins and betalains composition: composition and applications. Cereal Foods World, 45: 208-213.

Ghoname, A.A.; Saleh, S.A.; Riad, G.S.; Fawzy, Z.F. and El-Abd, S.O. (2011). Enhancement of carrot seed yield and quality by root dipping in gibberellic acid. Journal of Applied Sciences Research, 7(12): 1821-1827.

Gomez, N.K. and Gomez, A.A. (1984). Statically Procedures for Agricultural Research. 2nd Ed., John wiley and sons, New York. USA, 680.

Haroun, S.A.; Shukry, W.M.; Abbas, M.A. and Mowafy, A.M. (2011). Growth and physiological responses of *Solanum lycopersicum* to atonik and benzyl adenine under vernalized conditions. Journal of Ecology and the Natural Environment, 3 (9): 319-331.

Hossain, A.B.M. and Amaninah, M. (2018). Flower induction, chlorophyll fluorescence and carotenoid of *Allamanda* SP. as affected by gibberellic acid and aluminum sulfate. Global J. Biol. Agric. Health Sci., 7 (1): 6-12.

Lone, M.T.; Haripriya, K. and Maheshwari, U.T. (2005). Influence of plant growth regulators on growth and yield of chilli (*Capsicum annuum* L.) cv. K-2. Crop Research, 29 (1): 111-113.

Mahadevan, N.; Shivali, P. and Kamboj, P. (2009). *Hibiscus subdariffa* Linn. - An overview. Nat. Prod. Radiance, 8(1): 77-83.

Mathieu, A.; Périlleux, **C.; Jacquemin, G.; Renard**, **M.; Lutts, S. and Quinet**, **M. (2020).** Impact of vernalization and heat on flowering induction, development and fertility in root chicory (*Cichorium intybus* L. var. *sativum*). Journal of Plant Physiology, 254: 153-172.

Mazid, M. and Naz, F. (2017). Effect of macronutrients and gibberellic acid on photosynthetic machinery, nitrogen-fixation, cell metabolites and seed yield of chickpea (*Cicer arietinum* L.). J. Sci., 1(4): 120–129.

Mazumdar, B.C. and Majumder, K. (2003). Methods of Physiochemical Analysis of Fruits. Daya Publishing House Delhi, India.

Ottai, M.E.S.; Aboud, K.A.; Mahmoud, I.M. and El-Hariri, D.M. (2006). Stability analysis of roselle

cultivars (*Hibiscus sabdariffa* L.) under different nitrogen fertilizer environments. World J. Agric. Sci., 2: 333-339.

Pramanik, K.; Pradhan, J. and Sahoo, S.K. (2018). Role of auxin and gibberellins growth, yield and quality of tomato: A review. The Pharma Innovation Journal, 7(9): 301-305.

Qudassi, S.; Khan, F.; Mazid, M.; Khan, T.A.; Sh. Ansari and M. Arshad (2014). Quality status of nutrients, vitamins and hormones under pathological threats in tomato: Basic scenario. Medi. Chem. Anal., 4(1): 12–21.

Reeves, P.A.; He, Y. and Schmitz, R.J. (2007). Evolutionary conservation of the flowering locus C-mediated vernalization response: Evidence from the sugar beet (*Beta vulgaris* L.). Genetics, 176 (1): 295-307.

Sakr, M.M.; Gowayed, S.M.H.; Kadasa, N.M.S.; Azab, E.S.I. and Harabawy, A.S.A. (2013). The relationship between sowing dates and vernalization treatments and growth characters and some chemical components of *Beta vulgaris* L. cv. Pleno. Life Sci. J., 10(3): 1846-1849.

Wahba, E.H.; Mohamed, M.A.; Eraki, M.A.; Mazrou, M.M.; Afify, M.M. and Mahfoz, S. (2001). Growth and chemical components of roselle in relation to the irrigation and transparent, calcium carbonate. Egypt J. Hort., 28: 485-504.

Wong, P.K.; Yusof, S.; Ghazali, H.M. and Cheman, Y.B. (2000). Physicochemical characteristics of Roselle (*Hibiscus sabdariffa* L.). Nutr. Food Sci., 32: 68-73.

Wu, C.; Wang, M.; Dong, Y.; Cheng, Z. and Meng, H. (2016). Effect of plant age and vernalization on bolting, plant growth and enzyme activity of garlic (*Allium sativum* L.). Scientia Horticulturae, 201: 295–305.

Zanewich, K.P. (1993). Vernalization and gibberellin physiology of winter canola. M. Sc. Thesis, University of Lethbridge Lethbridge, Alberta, Canada.

Zhang, S.; Hu, J.; Liu, N. and Zhu, Z. (2006). Pre-sowing seed hydration treatment enhances the cold tolerance of direct-sown rice. Seed Science and Technology, 34(3): 593-601.

Zheng, Y.; Luo, L.; Liu, Y.; Yang, Y.; Wang, Ch.; Kong, X. and Yang, Y. (2018). Effect of vernalization on tuberization and flowering in the Tibetan turnip is associated with changes in the expression of FLC homologues. Plant Diversity 40: 50-56.