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Responses of Calyx Phytochemical Characteristic, Yield and Yield Components of Roselle (*Hibiscus sabdariffa* L.) to Different Sowing Dates and Densities

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Abstract
In order to evaluate the effects of sowing date and intra rows spacing on yield, yield components and some phytochemical characteristics in Roselle, a field experiment was conducted as a split plot layout based on randomized complete block design with three replications in 2013 and 2014 growing seasons. Five sowing dates were considered as the main plots (11^{th} and 30^{th} of March, 14^{th} of April, 5^{th} and 22^{th} of May) and three intra rows spacing were considered as the subplots (50, 75 and 100 cm). The results indicated that delayed sowing date significantly reduced plant height, number of bolls, number of branches per plant, calyx dry weight, calyx yield, seed yield and biomass yield. Plant height, calyx yield, seed yield and biomass yield were increased by reducing intra rows spacing. Reducing the intra rows spacing caused decrease in number of branches, number of bolls, seed weight, calyx and plant dry weights. Anthocyanin and vitamin C were significantly reduced by delaying in sowing date. Although sowing dates had no significant effect on total phenol but antioxidants were influenced by sowing date. Furthermore, intra rows spacing did not caused any significant effect on phytochemical traits of Roselle. Based on the obtained results, it seems sowing hibiscus tea in the late of March to mid-May and 50 cm intra rows spacing, under hot and dry climate condition would be suitable for increase the final yield of Roselle.

Keywords: Anthocyanin, Antioxidant, Calyx, Row spacing, Vitamin C.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is an annual herbaceous shrub of the Malvaceae family. The main origin of the Roselle is unknown while some researchers believe that this crop was originated from India (Mat Isa et al., 1985) and Saudi Arabia (Abu-Tarboush et al., 1996). Roselle may has been domesticated in western Sudan before 4000 BC (Wilson and Menzel, 1964). Roselle is a short day and self-fertile crop which is sensitive to cold and freezing (Duke, 2006). Its flowering begins with 12-12.5 hours duration of day and the best growth conditions occur in hot areas with high humidity and average temperature in the range of 25-35^{o}c (Hacket and Carolene, 1982).

The economical part of Roselle is the fleshy calyx surrounding the boll, which have various phytochemical components. The color of the calyx plays an important role in determining the quality of the crop. Calyx is used as a raw material for making

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healthy drink, syrup, jelly, tea, and vinegar (Singh et al., 2006). Flower and fleshy fruit of Roselle are used in the pharmaceutical industry to sooth the symptoms of bronchitis and cough. Calyxes are used to cure high blood pressure, diarrhea, mouth abscess and many other diseases. Many phytochemical constituents and diverse medicinal have been attributed to this plant. Roselle pigments, representing a potent source of anthocyanin holds promise as a large commercial potential source of natural food colorants (red-violent) (Hendry and Houghton, 1995). Roselle contains higher amount of ascorbic acid when compare to orange (*Citrus sinensis* L.) and mango (Mangifera indica L.) (Wonget et al., 2002).

The sowing date determination, which is closely linked to the environmental factors, is known as an important decision, because it determines the start of different phonological stages and the length of the plant growing period during its life cycle (Srivastava et al., 2016). Plant density is the most important influencing factors for development and production of medicinal plants and is an important factor in achieving optimal yield as well (Omidbeigi and Hasani Malayeri, 2007). It has been reported that by increasing plant density in Okra (*Abelmoschus esculentus* L.), plant height and fruit yield were increased and diameter of stem and number of lateral stem were decreased (Maurya et al., 2013). In a study on the effect of sowing date and plant density on yield and yield components of Roselle in Birjand, Iran, it was reported that the delay in sowing date from 4th of May to 14th of June significantly decreased boll number per plant, boll number and calyx yield per unit area, calyx dry yield and single-plant biomass by 43.6, 39.5, 44.9, 45.7 and 47.3%, respectively. They added as the density increased from 8 to 13.3 plants/m^2, boll number per plant, calyx weight per boll and plant single-biomass were decreased significantly by 29.8, 24.4, 39.1, 55.4 and 33.6%, respectively, but boll number/m^2 was increased by 18.2% (Moosavi, 2012). Mir et al. (2011), in a study on the effects of plant density and sowing date on Roselle in Zabol, Iran, reported that delay in sowing date decreased plant height, number of bolls, calyx yield and biomass yield, and by increasing plant density plant height, number of bolls, calyx yield and biomass yield were increased. Ekwu and Nwokwu (2012) reported that the earliest sowing date of Okra (*Abelmoschus esculentus* L.), 15th of May, compared with 15th of June and 15th of July produced the tallest plants, greater number of branches and leaves and highest number of pods in 50 × 75 cm compared to 50 × 25 cm and 50 × 50 cm raw spacing. Castro et al. (2004) evaluated the calyx yield of Roselle in four planting dates (18th of October, 15th of November and 18th of December of 2001, and 15th of January, 2002) in the city of Lavras in Brazil and observed that there was not any clear trend among studied planting seasons on calyx yield. In addition, it has been reported that anthocyanin composition and antioxidant activity are different in different varieties of Roselle (*Hibiscus sabdariffa* L.) and have been changed during maturity stages (Keisha et al., 2009). Tabasi et al. (2013) reported that plant species, plant density, and maturation stage of plant can affect ascorbic acid, soluble sugars, and lycopene contents in plant organs. In a study on the effects of temperature on antioxidant activity, total phenolics and agronomic traits of two thyme species, significant differences between the two species for essential oil percentage, antioxidant activity and total phenolics was reported (Rahimi and Ramezani, 2017). Asadi-Sanam et al. (2018) showed that delay in sowing date of Purple Coneflower (*Echinacea purpurea* L. Moench) produced the highest level of antioxidant activity and the effect of sowing date and density on plant phenol was significant.

Therefore, the aim of the present study was to determine the effect of sowing date and intra rows spacing on yield, yield components and calyx phytochemical characteristics of Roselle in Jiroft region, Iran.

Materials and methods

Location of experiment

A field experiment was conducted at the Research Station of Faculty of Agriculture, University of Jiroft (latitude: 28°40´ N; longitude: 57°44´ E; elevation: 650 m) during 2013 and 2014 growing seasons. Experimental site was located in southeast of Kerman province, Jiroft county, Iran. The climate of the area is arid and semiarid. Information about total precipitation, average of maximum and minimum temperatures (^{0}C) of the experimental site is presented in Table 1.

The soil texture was sandy with low fertility. The physical and chemical soil characteristics of the experimental site are presented in Table 2.

Table 1. Annual rainfall, average maximum and minimum temperatures in Jiroft, Iran 2011-2014

Year	Minimum Temperature (^{0}C)	Maximum Temperature (^{0}C)	Annual rainfall (mm)
2011	16.56	33.62	59.9
2012	17.55	33.49	107.8
2013	16.77	32.48	231.0
2014	15.55	36.2	153.1

Table 2. Main physical and chemical characteristics of the studied soil

Soil analysis	2013	2014
Physical		
Clay (%)	12	12
Silt (%)	22	27
Sand (%)	66	61
Chemical		
Organic carbon (%)	0.40	0.48
Total N (%)	0.046	0.07
Available P (ppm)	22.3	25.2
Available K (ppm)	141	210
pH	8.7	7.4
EC (dS m^{-1})	1.8	2.1

Experimental design

A split plot experiment based on Randomized Complete Block Design with three replications was conducted.

Five sowing dates (11th of and 30 th of March, 14th of April, 5th and 22th of May) were considered as the main plots and three intra rows spacing (100, 75 and 50 cm) were considered as sub-plots.

Agronomic practices

The land lied fallow for three years before the onset of experiment. Seed bed was prepared using plough and disk in autumn 2012. Plots were designed with 6 m long and 3 m width and 1 m apart from each other. Between blocks, 2 m alley was kept. According to the soil analysis, triple super phosphate (100 kg.ha^{-1}) and potassium sulfate (150 kg.ha^{-1}) were incorporated into the soil at the same time before seed sowing. One third of urea fertilizer (out of 200 kg.ha^{-1}) was incorporated into the soil before seed sowing and two third were applied as top dress at the vegetative and flowering stages. Seed sowing was performed by hand on middle of the furrows. Seedlings were thinned at the four to six leaf stages. The irrigation was done after seed sowing once every four days until plant establishment and then with weekly irrigation until maturity stage. Weeds were removed by hand during growing seasons. Harvesting the bolls, calyxes and seeds were done on 1th of December, in both studied years (2013 and 2014).

Data collection on yield, yield components and morphological traits

At maturity stage, five plants from each plot were randomly selected and plant height, number of bolls, number of branches, calyx dry weight and seed weight per plant were recorded. Finally calyx, seed and biological yields were measured by harvesting 6 m^2 of the central part of each plot.

Antioxidant capacity

The antioxidant capacity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method

according to the method described by Brand-Williams et al., (1995).

The antioxidant capacity was calculated using the following equation (1):

$$\% Rs = a\text{-}b/a \times 100 \quad (1)$$

Rs = radical-scavenging; a= control; b=sample

The amount of total phenolic compounds in the extracts was determined with the Folin- Ciocalteu reagent according to the method of Gao (2000) using Gallic acid as a standard.

The anthocyanin content was determined by using the method described by Wanger (1976). The vitamin C content was determined by using titration method (Arya, 2000).

Statistical analyses

All data were subjected to analysis of variance (ANOVA) using SAS 9.3 software (SAS, 2011). When F test indicated statistical significance at $p < 0.01$ or $p < 0.05$, the least significant difference (LSD) was used to separate the means.

Results

Plant height and number of branches

According to the results of the combined analysis of variance, plant height and the number of branches were significantly affected by sowing date and intra row spacing ($p \leq 0.01$), but the effect of year was not significant (Table 3). The highest (180 cm) and the lowest (131 cm) plant height were obtained on 11th of March and 22nd of May sowing dates, respectively (Table 4). Intra row spacing had a significant effect on plant height and number of branches. Longest (151 cm) and shortest (142 cm) plant height were recorded in 50 and 100 cm intra row spacing, respectively (Table4). Highest (8.4) and lowest (7.2) number of branches were observed in 100 and 50 cm intra row spacing, respectively (Table 4).

Table 3. Analysis of variance (mean of squares) for studied traits of Roselle grown in different sowing dates and intra row spacings.

Source of variance	df	Plant height	branches per plant	Number of bolls per plant	Calyx dry weight per plant	Seed weight per plant	Plant dry weight	Calyx yield	Seed yield	Biomass yield	Antioxidants	Total Phenol	Anthocyanin	Vitamin C
Year	1	11.61ns	3.61ns	3483.32*	77.82	15.04	11581.08*	449683ns	2014446*	25.61*	12.55ns	0.152ns	0.090ns	4.44ns
Year * Block	2	268.38^{ns}	6.47*	208.05ns	11.30	8.03	837.29ns	72518ns	25717ns	1.53ns	5.37ns	0.813ns	0.052ns	26.23**
Sowing date	4	6192**	96.25**	8348.53**	216.33**	426.04**	51005.53**	455510**	1039377**	150.44**	180.22*	2.29ns	2.56**	91.02**
Error (a)	16	125.54	1.73	71.19	4.47	7.28	2533.41	16398	12317	5.43	6.66	1.60	0.090	8.390
Intra row spacing	2	3877**	11.72**	4612.32**	91.37**	763.14**	25431**	1066557**	267043**	353.13**	9.90ns	0.285ns	0.057ns	11.65ns
Year* Intra row spacing	2	31.37ns	6.68ns	40.14ns	5.87	0.0001ns	67.36na	18296ns	19.91ns	0.01ns	0.002ns	0.088ns	0.004ns	1.81ns
Year*Sowing date	4	783.47ns	2.25ns	58.17ns	1.18	0.47	1108.98ns	5845ns	19326ns	0.79ns	0.011ns	0.18	0.006	5.56ns
Sowing date* Intra row spacing	8	163.29ns	3.62ns	193.54ns	2.19	12.37	979.62ns	10772ns	14363ns	5.50ns	10.80ns	0.38ns	0.41*	13.06ns
Year* Sowing date * Intra row spacing	40	62.32ns	2.17ns	9.55ns	1.41	0.0001ns	145.99ns	4156ns	801.08ns	0.012ns	0.001ns	0.16ns	0.004ns	1.99ns
Error (b)		365.80	10.90	101.49	172.61	10.60	1716.57	17279	29739	4.93	13.87	1.34	0.059	6.25
Coefficient of Variation (%)		11.97	18.09	13.18	12.19	16.00	15.85	16.55	16	15.23	4.31	8.92	9.45	10.07

ns, * and ** are no significant, significant at 5 and 1% probability levels, respectively.

Table 4. Means comparison for plant height, number of branches, number of bolls, calyx dry weight, seed weight and plant dry weight of Roselle grown in different sowing dates and intra row spacings.

Treatment		Plant height (cm)	Number of branches per plant	Number of bolls per plant	Calyx dry weight (g plant^{-1})	Seed weight (g plant^{-1})	Plant dry weight (g plant^{-1})
Year	2013	159.39a	7.74a	70.19b	16.10a	19.92a	249.89b
	2014	160.11a	8.14a	82.63a	17.96a	20.74a	272.58a
Sowing date	11th of March	179.74 a	9.91 a	102.27 a	21.21a	26.40a	322.75a
	30th of March	170.40 b	9.60 a	88.78b	19.20 b	24.33 b	299.47b
	14th of April	162.16 c	9.36 a	79.11c	17.57 c	18.81c	266.27b
	5th of May	155.68 c	5.44 b	65.66d	14.55 d	16.63d	225.81c
	22nd of May	130.75 d	5.37 b	45.23e	12.62 e	15.17 d	191.89c
Intra row	100	150.53b	8.42 a	86.80a	18.52a	25.00 a	289.51a
	75	156.26 b	8.18 a	79.75b	17.46a	20.67b	262.86b
	50	172.45 a	7.23 b	62.69c	15.11 b	15.13 c	231.34c

Means followed by similar letters in each column are not significantly according to LSD test ($p \leq 0.05$).

Number of bolls

The combined analysis of variance of data indicated that the year ($p \leq 0.05$), sowing date ($p \leq 0.01$), and intra row spacing ($p \leq 0.01$) had significant impacts on the number of bolls per plant (Table 3). Highest and lowest number of bolls per plant were obtained on 10th of March and 22nd of May sowing dates, respectively (Table 4). The number of bolls per plant increased by increasing the intra row spacing from 50 to 100 cm (Table 4).

The calyx dry weight

The combined analysis of variance showed that the sowing date ($p \leq 0.01$) and the intra row spacing ($p \leq 0.01$) had significant effect on dry weight of calyx per plant. The effect of year was not significant (Table 3).

By decreasing the intra row spacing, in comparison with 100 cm, the dry weight of calyx per plant decreased by 6% and 22% in 50 and 75 cm intra row spacings, respectively, (Table 4).

Seed weight

The combined analysis of variance showed that the effect of sowing date ($p \leq 0.01$) and intra row spacing ($p \leq 0.01$) were significant on seed weight per plant. However, the year did not have a significant effect on seed weight (Table 3). The results showed that the maximum seed weight per plant was obtained in sowing date of 11th of March and the intra row spacing of 100 cm, while seed weight was decreased by postponing the sowing date and decreasing the intra row spacing (Table 4).

Dry weight per plant

The combined analysis of variance showed that the effects of year ($p \leq 0.05$), sowing date ($p \leq 0.01$), and intra row spacing ($p \leq 0.01$) were significant on biomass weight per plant (Table 3). The biomass weight per plant in the first year (2013) decreased by 9% compared to the second year (2014). By postponing the sowing date from 11th of March to 22nd of May, plant dry weight declined. Moreover, the maximum plant dry weight was shown in the intra row spacing of 100 cm that increased by 10% and 25% in the intra row spacing of 75 cm and 50 cm, respectively (Table 4).

Calyx yield

The combined analysis of variance showed that the effect of sowing date ($p \leq 0.01$) and intra-row spacing ($p \leq 0.01$) were significant on calyx yield. However, the year did not have a significant effect on calyx yield (Table 3). Furthermore, the combined analysis of variance demonstrated that the maximum calyx yield (990 kg.ha^{-1}) was obtained in 11th of March sowing date, and the minimum calyx yield was recorded in 5th of May and 22nd of May sowing dates with 681 and 594 kg.ha^{-1}, respectively. The results revealed that the calyx yield was increased by 24% and 61% in intra row spacing of 50 cm compared with the intra row spacing of 75 cm and 100 cm (Table 5).

Seed yield

The combined analysis of variance indicated that the effect of year ($P \leq 0.05$), sowing date ($p \leq 0.01$), and the intra row spacing ($p \leq 0.01$) were significant on seed yield (Table 3). The maximum seed yield was achieved on 11th of March and decreased along with the delay in the sowing date. The seed yield rose respectively by 9.5% and 20% in the intra row spacing of 50 cm in comparison with the intra row spacing of 75 cm and 100 cm (Table 5).

Biomass yield

The combined analysis of variance showed that the effect of year ($p \leq 0.05$), sowing date ($p \leq 0.01$), and the intra row spacing ($p \leq 0.01$) were significant on biomass yield (Table 3). The biomass yield in the second year (2014) increased by 7.5% compared to the first year (2013) of the experiment. The maximum biomass yield was obtained on 11th of March (17.8 t.ha^{-1}) sowing date, which was not significantly different from the sowing date of 30th of March, whereas the minimum biomass yield was observed on 22nd of May (10.67 t.ha^{-1}) sowing date. In the intra row spacing of 50 cm, the biomass yield increased by 58.6 % and 32.7% in comparison with the intra row spacing of 75 cm and 100 cm (Table 5).

Antioxidant content

Among the studied treatments, only sowing date had a significant effect on antioxidant contents ($p \leq 0.01$). The maximum calyx antioxidant was achieved on 5th of May (Table 6). Nevertheless, the year, the intra row spacing, and other interaction effects were not significant (Table 3).

Table 5. Means comparison for plant calyx yield, seed yield and biomass yield of Roselle grown in different sowing dates and intra row spacings

Treatment		Calyx yield (kg ha^{-1})	Seed yield (kg ha^{-1})	Biomass yield (t ha^{-1})
Year	2013	723a	994b	14.05b
	2014	864a	1089a	15.11a
Sowing date	11th of March	990 a	1338 a	17.87a
	30th of March	839 b	1233b	16.64a
	14th of April	809 b	989c	14.87b
	5th of May	681 c	874 d	12.85c
	22nd of May	594 c	766e	10.67d
Intra row spacing (cm)	100	608 c	948c	11.55c
	75	789 b	1038b	13.83b
	50	983 a	1137a	18.32a

Means followed by similar letters in each column are not significantly according to LSD test ($p \leq 0.05$).

Table 6. Means comparison for antioxidants, total phenols, anthocyanin and vitamin C contents of calyx in Roselle plants grown in different sowing dates and intra row spacings

Treatment		Antioxidants (%)	Total phenol (mg g^{-1})	Anthocyanin (mg g^{-1})	Vitamin C (mg $100ml^{-1}$)
Year	2013	86.70a	13.02a	2.61a	25.05a
	2014	85.95a	12.94a	2.54a	24.60a
Sowing date	11th of March	81.67d	12.69a	2.41b	26.01 a
	30th of March	85.27c	12.51a	2.86a	26.43 a
	14th of April	86.64 bc	13.24a	3.01a	25.44 a
	5th of May	90.17a	13.30a	2.54b	25.36 a
	22nd of May	87.88 b	13.17a	2.06c	20.88 b
Intra row spacing (cm)	100	86.08a	13.09a	2.53a	24.12a
	75	85.91a	12.91a	2.59a	25.04a
	50	86.98a	12.94a	2.61a	25.31a

Means followed by similar letters in each column are not significantly according to LSD test ($p \leq 0.05$).

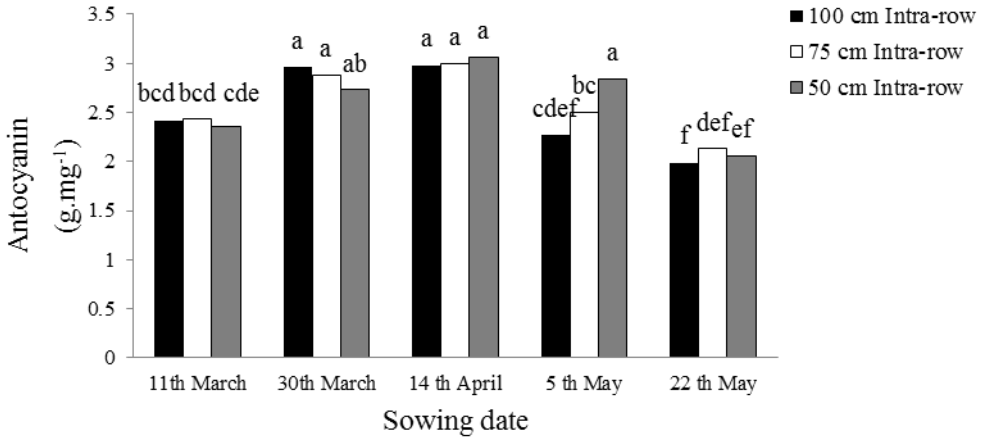

Fig. 1. Effects of sowing date and intra row spacing on the anthocyanin contents of Roselle's calyx

Total phenol content

The effect of studied treatments on the total phenol content of calyx was not significant (Table 3). However, the amount of phenolic compounds increased by delaying sowing date (Table 6).

Anthocyanin content

The combined analysis of variance showed that the sowing date ($p \leq 0.01$) and the interaction effect of sowing date and intra rows spacing ($p \leq 0.5$) had significant effect on the anthocyanin contents of Roselle's calyx (Table 3). The results revealed that the maximum anthocyanin contents was related to the sowing dates of 30th of March and 14th of April, and the minimum anthocyanin contents was belonged to the sowing date of 22nd of May (Table 6). The interaction of the sowing date and intra rows spacing had a significant effect on the anthocyanin contents of calyx. The highest anthocyanin contents was detected in 30th of March and 14th of April sowing dates, and the lowest anthocyanin contents was obtained in 22nd of May sowing date at all studied intra rows spacing (Fig. 1).

Vitamin C content

The combined analysis of variance showed that the sowing date ($p \leq 0.01$) had a significant effect on the vitamin C content of Roselle's calyx (Table 3). The highest vitamin C was obtained in the sowing date of 30th of March (26.42 mg.100 ml^{-1}) and the lowest was detected in 22nd of May sowing date (20.88 mg. 100 ml^{-1}) (Table 6).

Discussion

In the present study, increase in plants height at higher plant densities might be attributed to the competition for light and other growth resources among the plants in the closer intra row spacing. These results are in agreement with the findings of Faramarzi et al. (2012) in cotton (*Gossypium hirsutum* L.) and Patane and Sortino (2010) in Kenaf (*Hibiscus cannabinus* L.). Furthermore, reduced competition for light and other resources as well as reduced overlapping from adjacent Roselle plants within the population could enable the plants to utilize their energy for maximum branching and, subsequently, the production of larger leaf area. Similar results were also reported by Maurya et al. (2013) and Kajidu et al. (2015).

The current results are in the same line with Patane and Sortino (2010) who reported that late sowing from 28th of May to 30th of June reduced pod number in Kenaf plant (*Hibiscus cannabinus* L.).

The number of bolls per plant increased by increasing the intra row spacing from 50 to 100 cm. It has been reported that pod weight per plant of okra (*Abelmoschus escuelentus* L.) decreased by increasing plant density (Dikwahal et al., 2007). Higher number of Roselle bolls in 90 cm compared with 30 cm row spacing has been reported previously (Kajidu et al., 2015). It appears that by increasing plant density, the number of bolls in each plant was reduced due to the competitive factors such as light, water, and nutrients.

It was observed that the number and the weight of bolls per plant and the weight of calyx per plant decreased by postponing Roselle planting date from 11th of March to 25th of April and were increased by increasing plant density from 4 to 8 plants.m^{-2} (Mir et al., 2011).

In agreement with the obtained result in current study, in another experiment performed on the effect of plant density on Roselle yield by El Naim et al. (2012), the decreased numbers of bolls, calyx weight, and seed weight were observed by increasing plant density. In addition, they reported that high plant density led to lower calyx weight and lower seed number per boll (El Naim et al., 2012).

It seems that decreasing number of bolls, calyx dry weight, plant seed weight, and biomass dry weight as a result of postponing sowing date can be attributed to the high temperature during the growth period, shorter vegetative growth period, the exposure of vegetative growth stages to the hot weather in early summer and severe moisture stress. These conditions decreased leaf area and plant photosynthesis which would ultimately reduce total biomass production. Regarding the different densities in this study, it give the impression due to the long vegetative growth of Roselle, less space and nutrient elements were given to the plant in the intra row spacing of 50 cm with doubled plant density in comparison with the intra row spacing of 100 cm. Thus, this intensified the competition among plants to reduce the number of bolls, calyx weight, and seed weight and dry weight of biomass per plant.

In an experiment on the effect of plant density on Roselle yield, the decreased numbers of bolls, calyx weight, and seed weight were observed by increasing plant density (El Naim et al., 2012). In addition, they reported that high plant density led to lower calyx weight and lower seed number per boll (El Naim et al., 2012). Moosavi (2012) and Mir et al. (2011) reported that delay in sowing date decreased the calyx yield of Roselle. These results are in close

conformity with the findings of Faramarzi et al. (2012) and Patane and Sortino (2010).

Our results are in agreement with those of Mir et al. (2011), Rafieiolhossaini et al. (2010), and Danalatos and Archontoulis (2010) who found that late sowing reduced biological yield in Roselle (*Hibiscus sabdariffa* L.), German chamomile (*Matricaria chamomilla* L.), and Kenaf (*Hibiscus cannabinus* L.) plants. Early planting led to boosting biomass yield via increasing the growth period of the plant, more development of leaf area, and production of abundant sub-branches. Furthermore, biomass yield was enlarged along with the increase in plant density so that the maximum biomass yield was obtained in the intra row spacing of 50 cm and its minimum in the intra row spacing of 100 cm.

In the present study, according to the measurement of calyx phytochemical characteristic such as antioxidant activity of Roselle's calyx, the sowing date 5th of May showed 2.5%, 3.8%, 4.9%, and 9.4% more antioxidant activities than those of 22nd of May, 14th of April, 30th of March and 11th of March. It seems that Roselle calyx could be considered as a good source of natural antioxidants in the diet. It seems higher temperatures and water deficits in the delayed sowing dates are the main reason for increasing phenolic compounds. The results showed that intra rows spacing did not have any significant effect on the phenol contents of calyx.

It has been reported that the different environmental conditions such as temperature, sun light, altitude, type of soil, and accessible elements (Kazemzade et al., 2010), the time of germination (Danhue, 2000), time and duration of flowering (Tumen et al., 1996), genetics (Eckhart et al., 1992), plant competition (Stahl-biskup, 1991) and pests and diseases invasions (Escarre et al., 1999) can affect the synthesis of secondary metabolites components.

It seems delaying in sowing dates reduced the length of flowering period which may affect the anthocyanin contents of Roselle's calyx.

Tabasi et al. (2013) stated that plant species, plant density, and maturation stage of plants can affect the amount of ascorbic acid, soluble sugars, and lycopene content of plant. Tabasi et al. (2013) added that sunlight is an important factor in the quality of plant products. In general, decreasing plant density resulted in more penetration of light into the plant canopy, more photosynthesis, and higher ascorbic acid, lycopene, and carotenoids content synthesis. In the present study, the sowing date affected the length of different stages of the plant (vegetative and reproductive stages) and the amount of vitamin C in the calyx.

Conclusion

According to the obtained results of the present study, it can be concluded that postponing the sowing date led to reductions in number of bolls, dry boll, calyx weight, seed and calyx yield. Although reduction in intra row spacing from 100 to 50 cm caused increase in seed and calyx yield, but it resulted in reduction in the number of bolls, boll dry weight, calyx dry weight, and seed weight per plant. The sowing date of 11th of March produced the highest seed and calyx yield. Our results revealed that studied qualitative criteria such as antioxidants, total phenol, anthocyanin, and vitamin C content declined by delay in sowing dates from 10th of March to 22nd of May but intra row spacing did not have any significant effect on studied qualitative criteria.

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