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Influence of different irrigation regimes and planting times on the quality and quantity of calyx, seed oil content and water use efficiency of roselle (*Hibiscus sabdariffa* L.)

A. Zand-Silakhoor [a], H. Madani[b,✉], H. Heidari Sharifabad[c], M. Mahmoudi[d] and G. Nourmohammadi[e]

[a]Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[b]Department of Agronomy, Arak Branch, Islamic Azad University, Arak, Iran. Email:

[c]Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[d]Soil and Water Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization. Sari, Iran

[e]Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran.
✉Corresponding author: madani.au@yahoo.com; h-madani@iau-arak.ac.ir

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ABSTRACT: This study was carried out to examine the physiological traits, quality of calyx extract and seed oil content of roselle (Hibiscus sabdariffa L.) as affected by irrigation regimes and planting dates. The growth period from seed sowing to calyx harvesting was shortened as planting time was delayed. Stem diameter and plant height were decreased by drought stress or late planting, but calyx yield, total phenolic content, total anthocyanin content, vitamin C, and calyx water use efficiency increased under mild drought condition. In addition, antioxidant activity and calyx water use efficiency were significantly increased by late planting. It was suggested that an increase in calyx harvest index in delay in planting would be due to better photosynthesis activity and higher assimilate use efficiency because of the increase in sink capacity. Seed oil content decreased considerably due to drought stress and delay in planting date. These findings suggest that mild drought stress improves the quality and quantity of calyx and water use efficiency.

Keywords**:** *Antioxidant activity; Harvest index; Phenolic compounds; Sowing date*

RESUMEN: ***Influencia de diferentes regímenes de riego y tiempo de siembra en la calidad y cantidad del cáliz, el contenido de aceite de las semillas y la eficiencia del uso del agua de la roselle (*****Hibiscus sabdariffa** ***L.).*** Este estudio se llevó a cabo para examinar los rasgos fisiológicos, la calidad del extracto de cáliz y el contenido de aceite de semillas de roselle (*Hibiscus sabdariffa* L.) afectado por regímenes de riego y fecha de siembra. El período de crecimiento desde la siembra de la semilla hasta la cosecha del cáliz se acortó debido a que se retrasó el tiempo de siembra. El diámetro del tallo y la altura de la planta disminuyeron por el estrés por sequía o la siembra tardía, pero el rendimiento del cáliz, el contenido fenólico total, el contenido total de antocianinas, la vitamina C y la eficiencia del uso del agua del cáliz aumentaron en condiciones de sequía leve. Además, la actividad antioxidante y la eficiencia del uso del agua del cáliz aumentaron significativamente en la siembra tardía. Se sugirió que el aumento del índice de cosecha del cáliz en la demora en la siembra se debería a una mejor actividad de fotosíntesis y una mayor eficiencia en el uso de asimilación debido al aumento de la capacidad de hundimiento. El contenido de aceite de las semillas disminuyó considerablemente debido al estrés por sequía y al retraso en la fecha de siembra. Estos hallazgos sugieren que el estrés por sequía leve mejora la calidad y cantidad del cáliz y la eficiencia del uso del agua.

PALABRAS CLAVE: *Actividad antioxidante; Compuestos fenólicos; Fecha de siembra; Índice de cosecha*

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1. INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an annual medicinal and edible plant belonging to the Malvaceae family and cultivated in South-East Asia as well as other arid and semi-arid regions (Gendy *et al.,* 2012) with a temperature of about 25 to 35 °C. The different useful parts of this plant, including stem fiber, leaves, seeds and flowers have been used as vegetables, oil and drinks. Roselle is generally planted for its red calyx which contains vitamin C, anthocyanin and other phenolic compounds (Da-Costa-Rocha *et al.,* 2014). Many environmental agronomic factors influence the growth, yield and quality of roselle plants (Xu *et al.,* 2019).

Drought stress is the most important obstacle in achieving a high crop yield throughout the world. Furthermore, saving irrigation water and improving crop yields are two related and important global issues. Proper planting date and practices of irrigation management are some effective techniques for maximizing the use of limited water resources (Keshavarz Mirzamohammadi *et al.,* 2021). It has reported that increasing intervals between irrigation and drought condition resulted in a significant decrease in yield and yield components but increase in active constituents of calyx such as vitamin C and total phenols in the roselle plant (Hewidy *et al.,* 2018). However, another researcher reported an increase in flowering and dry yield (Begum *et al.,* 2015). In addition, Seghatoleslami *et al.* (2013) determined that drought stress had no significant effect on the height of the roselle plant, number of branches or calyx yield. In another study, mild drought stress following an increase in irrigation intervals led to an increment in anthocyanin due to carbohydrate accumulation which reshapes into secondary metabolites (El-Boraie *et al.,* 2009). Fallahi *et al.* (2017a) found that drought conditions in light soil did not affect stem diameter or the plant height of roselle but instead calyx yield and number of flowers increased under mild drought stress. Khalil and Yousef (2014) defined that drought stress led to a reduction in biological yield, calyx yield and quality of roselle.

Maximum production potential due to agronomic practices such as time of sowing have been related to high yield. An appropriate sowing date promotes plant growth and development, resulting in higher biological yield and better economic use of the land. The optimum sowing date ensures that the susceptible growth stage of the plant does not coincide with harmful environmental conditions. The roselle is short day plant with a critical photoperiod of 12-12.5 hours for flowering. Long days at the wrong developmental stage lead to losses in yield (Ghayour *et al.,* 2020). Morphological traits and yield components like stem diameter, plant hight and branch numbers are influenced by planting due to a longer growth period from the first planting date (Asadpour *et al.,* 2020). The results of Seghatoleslami *et al.* (2013) for roselle (*Hibiscus sabdariffa* L.) highlighted a significant correlation between plant dry matter and sowing date and the wrong sowing date brought about loss on economic yield by affecting yield components. Asadpour *et al.* (2020) showed that a delayed sowing date decreased the grain yield of maize (*Zea mays* L.). In a study on the effect of five sowing dates for rice, Basyouni Abou-Khalifa (2010) concluded that the greatest grain yield was achieved with the sowing dates of May 10 and April 30 (stage of milky and maturity, respectively). It was reported that a delay in sowing date from mid-May to mid-July led to a 60% flower yield loss and 58% reduction in the yield of calyx (Ghayour *et al.,* 2020). Parsa Motlagh *et al.* (2018) stated that a delay in sowing from 30th March to 22nd May resulted in lower plant height, harvest index and calyx yield per unit. Also, Alizade Moradi *et al.* (2018) showed that a delay in sowing time led to losses in flower yield and biological yield but increased the water use efficiency of the calyx and flower.

A study on the effect of irrigation and planting date on the quality of the calyx and water use efficiency is still rare. Changing irrigation schedule, water deficit technique and planting date have been widely used in to improve the harvest index and water use efficiency of plants. Given the importance of determining desirable irrigation regimes and sowing dates for the cultivation of a newly-introduced plant to this region, the present study aimed to examine the influence of different irrigation regimes and panting dates on yield quantity and quality and water use efficiency of roselle (*Hibiscus sabdariffa* L.), and to determine the exact quantity of irrigation water to be applied on roselle plant grown.

2. MATERIALS AND METHODS

2.1. Site study

This study was carried out at the farm station of Azad Islamic University, Karaj, Iran to study the effect of water deficit stress and sowing date on the quantitative and qualitative yield of Roselle (*Hibiscus sabdariffa* L.) during the two consecutive growing seasons of 2017 and 2018. This experiment included nine treatments which were included interactions among three irrigation regimes (well-watered, mild and drought stress) and three planting dates (4th June, 18th June and 2nd July). The climate in this province is semi-dry with an annual rain fall of about 250 mm which mostly occurs in autumn and winter between November and April. Average minimum and maximum temperatures and precipitation in both years of the present study are shown in Figure 1. The soil type of the field was sandy-loam and low in organic matter (0.28%) with total N (0.031%) as measured by the Kjeldahl method (Beljkas *et al.,* 2010), available P (35 mg kg^{-1}), using the Olsen procedure (Olsen and Sommers, (1982) and available K (225 mg kg^{-1}) after extraction with ammonium acetate, with pH and EC of 6.96 and 0.82 dS m^{-1}, respectively.

2.2. Experiment design

The nine treatments were employed in split plot with three replicates in a randomized complete block design (RCBD). Irrigation regimes were distributed among the main plots and included three irrigation levels (30, 45 and 60% depletion of available water, labelled control, mild and drought stress, respectively) (Keshavarz Mirzamohammadi *et al.,* 2021). Planting date treatments were arranged in subplots. Roselle (*Hibiscus sabdariffa* L.) seeds were directly sown in prepared plots (divided into four rows at 60 cm apart and 5 cm between the plants on each row) on 4th June, 18th June and 2nd July in both years. After germination, the seedlings were thinned to reach optimum density. The seedlings were irrigated when needed until they completely developed. Soil moisture content in each plot was monitored daily using a time domain Reflectometry (TDR). Irrigation water needed prior to irrigation (V_w in m^3) was estimated based on the soil water content (θ_i) (by TDR) and effective rooting depth (*D*, 0.3m here) according to the following equation:

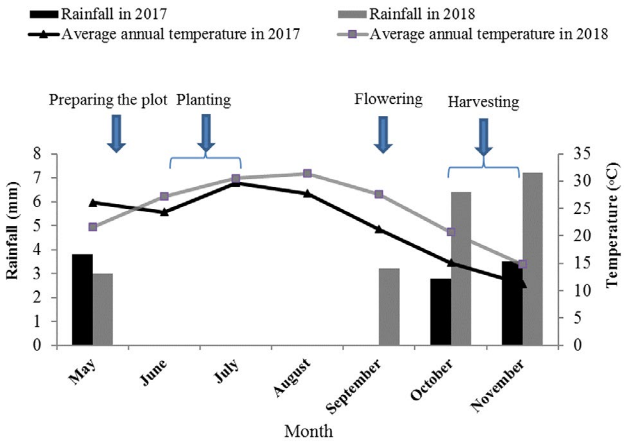

FIGURE 1. The average temperature and the total rainfall in the months of the growing season in 2017 and 2018.

$$V_w: (\theta_{F.C} - \theta_i) \times D \times A$$

Where, $\theta_{F.C}$ is the volume of soil moisture at field capacity and *A* is the main plot area (m^2). Data on volumetric water content were collected daily prior to setting the experiment calibrates before seed sowing and during the growing season to calculate the time of irrigation. The amount of water irrigation applied was according to the soil deficit to bring it back to field capacity. All plots were fertilized uniformly, during soil preparation in the spring of each year.

2.3. Data collection

The leaves and calyxes were harvested within four weeks after flowering. The fresh calyxes of each plot were washed with water, air-dried at room temperature, and weighed. The seeds were removed manually, air dried at room temperature and the whole calyces were used for the analysis of chemical properties. The extraction of dried calyxes with water at a ratio of 1:10 was conducted in a water bath (50 °C, 30 min) (Chumsri *et al.,* 2008). The total phenolic and anthocyanin contents of calyx extract were assessed according to the Folin-Ciocalteu reagent method (Temraz and El-Tantawy, 2008) and Krizek *et al.* (1993), respectively. Vitamin C was measured spectrophotometrically (Hitachi, U-1800, Tokyo, Japan) by measuring Fe^{2+} complexes with 2, 2-dipyridyl (Sarker and Oba, 2018) and reading the

Table 1. Analysis of variance (mean squares) of physiological traits, yield, water use efficiency and harvest index of roselle (*Hibiscus sabdariffa* L.) in irrigation and sowing date treatments

Source of variation	d.f	Plant height	Stem diameter	Biological yield	Calyx yield	Total phenolic content	Total anthocyanin content	Vitamin C	Antioxidant activity	Seed oil content	Calyx water use efficiency	Calyx harvest index
Replication	2	818.92^{ns}	0.02 ^{ns}	0.0034 ^{ns}	0.05 *	0.01 ^{ns}	15.59 ^{ns}	37.3 ^{ns}	5.25 ^{ns}	1.072 ns	0.10 *	2.80 *
Irrigation	2	4637.37**	1.52 **	34.42 **	0.28 *	1.53 *	206.92 **	36580 **	1294.4 **	81.35 **	0.247 **	2.41 *
Main error	4	144.81	0.054	0.039	0.0043	0.17	4.92	299.9	2.48	1.75	0.010	299.3
Sowing date	2	831.59 **	0.071 ^{ns}	2.58 **	0.13 *	0.022 ^{ns}	1.03 ^{ns}	1393 ^{ns}	140.48 *	3.40 *	0.24 *	1393.7 ^{ns}
Irrigation × sowing date	4	51.81 ^{ns}	0.068 ^{ns}	0.19 ^{ns}	0.023 ^{ns}	0.012 ^{ns}	18.37 ^{ns}	412.27 ^{ns}	49.45 ^{ns}	0.75 ^{ns}	0.040 ^{ns}	412.2 ^{ns}
Error	12	107.57	0.049	0.13	0.051	23.9	10.03	377.27	23.96	0.85	0.043	0.72
CV%		6.51	9.048	2.71	9.6	9.6	9.46	5.78	6.43	6.86	10.51	8.56

^{ns} – not significant, *: significant at $P \leq 0.05$ and **: significant at $P \leq 0.01$

absorbance of the sample solution at 525 nm. Antioxidant activity was measured according to Sarker and Oba (2018) by means of the diphenyl-picrylhydrazyl (DPPH) radical degradation method.

Harvesting was done at the physiological maturity stage on the first of October until the first of November (in both years) by harvesting the four middle rows. The traits examined included plant height, stem diameter, biological yield and calyx yield. Biological yield was determined after oven drying at 75 °C for 72 hours to constant weight. Then the dried biological part of plant was weighed by a digital 0.01-precision scale. Stem diameters and plant height were measured manually from the ground level with a caliper and a barcoded height stick (1 mm resolution), respectively. Therefore, they were considered to be precise and accurate. For this purpose, five individual plants from each plot were measured, and the averaged stem diameter and plant height were used as the plot-level. Seed oil content was measured by Inframatic 8620 Percor, England. Calyx water use efficiency (WUE_{calyx}) and calyx harvest index (HI_{calyx}) were calculated by dividing the calyx yields by the volume of applied water and biological yield, respectively.

2.4. Statistical analysis

Analyses of variance (ANOVA) were performed using the SAS software (SAS Institute Inc. ver. 9.2). Two years' data were analyzed by combined years because the Bartlett test was not significant for all traits measured. Differences among mean values for the roselle plant responses to the level of irrigation regimes and planting date were analyzed with the least significant difference (LSD) test at a significance of $\alpha \leq 0.05$. Principal Component Analysis (PCA) based on biplot (SAS 9.1) and coefficient of correlation were applied to consider the visualization of similarities or differences and interrelationships by acute and obtuse angles among all parameters. Clustering analysis (S-PLUS ver. 6.1 software, Insightful Corporation, USA) aimed at classifying objects based on the minimum variance linking method and similarity of input data for the existing parameters using Ward's hierarchical approach and Euclidean distance to organize data into groups.

3. RESULTS AND DISCUSSION

The results showed that the different irrigation regimes differed among the studied traits (Table 1). Sowing date treatments differed significantly in terms of plant height, biological yield, calyx yield, antioxidant activity, seed oil content and calyx water use efficiency. However, the two-way interactions of irrigation regimes × sowing date treatments was not significant in any traits (Table 1). The results of the cluster analysis showed that all the treatments were divided into three separate groups, so that the interaction of severe drought + 4th June, severe drought + 18th June, and severe drought + 2nd July (T_7, T_8 and T_9, respectively) treatments were placed into one group and well-watered + 4th June, well-watered

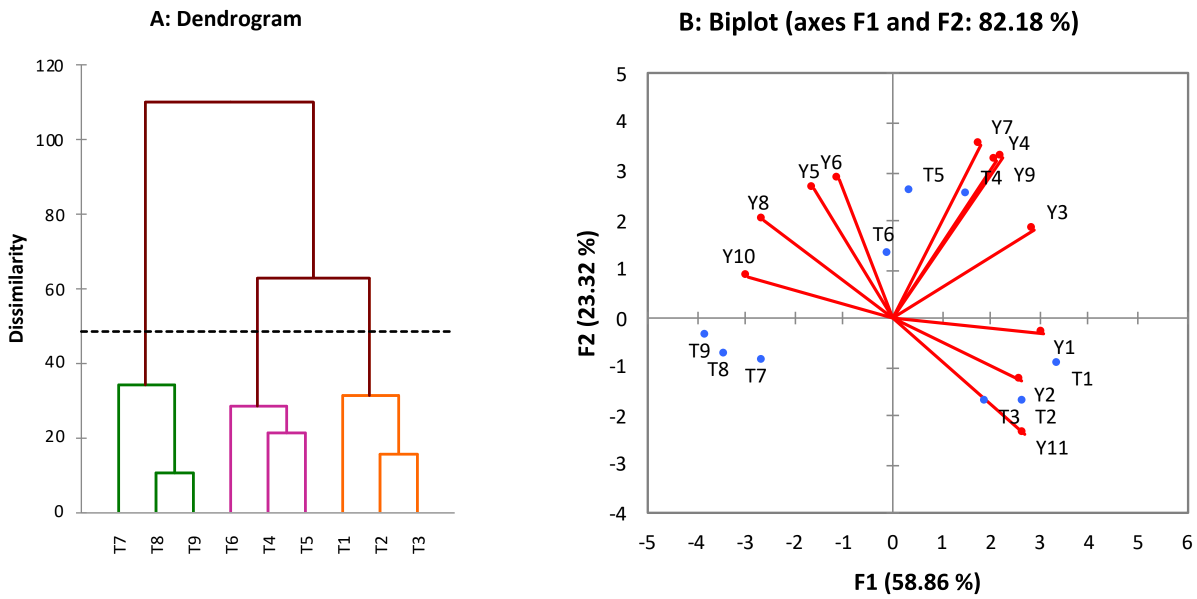

FIGURE 2. The results of dendrogram based on cluster analysis (A) and (B) biplot of first and second components based on principal component analysis. T1: well-watered+4th June, T2: well-watered+18th June, T3: well-watered+2nd July, T4: mild drought+4th June, T5: mild drought+18th June, T6: mild drought+2nd July, T7: severe drought+4th June, T8: severe drought+18th June, T9: severe drought+2nd July. Y1: plant height, Y2: stem diameter, Y3: biological yield, Y4: calyx yield, Y5: calyx water use efficiency, Y6: calyx harvest index, Y7: total phenolic content, Y8: total anthocyanins content, Y9: vitamin c, Y10: antioxidant activity, Y11: seed oil content.

+ 18th June and well-watered + 2nd July (T_1, T_2 and T_3, respectively) treatments were placed in the other group. In addition, based on the results of the cluster analysis, it was found that mild drought + 4th June, mild drought + 18th June and mild drought + 2nd July (T_4, T_5 and T_6) treatments were more closely related to all studied traits and were placed in another group (Figure 2). The results of the principal component analysis showed that the first and second components presented the highest relative variance with 58 and 23%, respectively, and accounted for a total of 82% of the total variance. Based on the biplot obtained from the first and second components, it was observed that the TPC, calyx yield, vitamin c and biological yield had the highest correlation with T5 and T4 treatments. Also, T1, T2 and T3 treatments showed a strong relationship with plant height, stem diameter and seed oil content (Figure 2).

3.1. Plant height and stem diameter

The data related to plant height (Tables 2 and 3), the well-watered irrigation (181.2 cm) and first sowing date (4th June) (168.7 cm) showed maximum value for plant height. The well-watered plants measured significantly higher stem diameters (2.85 cm) than the mild and severe drought stress by 14.03 and 28.7%, respectively. Our results are in agreement with those found by Karami *et al*. (2016) for soybean cultivars [*Glycine max* (L.) Merr.], which indicated that drought stress reduced plant growth, plant height and stem diameter due to disturbance in photosynthesis and low carbohydrate production. In addition, prolonged growth periods allowed the crops to use growth resources like light, water and nutrients which finally increased the growth of the crops. Similar findings with respect to drought stress and sowing date have been reported by Apeyuan *et al*. (2017) and Rah Khosravani *et al*. (2017) for roselle (*Hibiscus sabdariffa* L.) and maize (*Zea mays* L.) hybrids, respectively.

3.2. Biological yield

A decrease of 14.69 ton.ha^{-1} to 11.40 ton.ha^{-1} in biological yield of roselle was recorded due to decreasing the irrigation supply from control to severe drought stress. However, there were no significant difference between well-watered and mild drought stress treatments (Table 2). This may be related as stress conditions reduce the biological yield production because of limitations in the water and essential fertilizer uptake by the plants (Seghatoleslami *et al.,* 2013). Reductions in total dry matter of rice (*Oryza*

Table 2. Morphological and physiological traits of roselle (*Hibiscus sabdariffa* L.) from three different irrigation treatments.

Irrigation	Plant height	Stem diameter	Biological yield	Calyx yield	Total phenolic content	Total anthocyanin content	Vitamin C	Antioxidant activity	Seed oil content	Calyx water use efficiency	Calyx harvest index
	(cm)	(cm)	(ton.ha^{-1})	(ton.ha^{-1})	(mg.100 g^{-1} DW)	(mg.100 g^{-1} FW)	(mg.100 g^{-1} FW)	(%)	(%)	(kg.kg^{-1})	(%)
Well-watered	181.2±16. a	2.85±0.18 a	14.69±0.48 a	1.37±0.16 b	2.33±0.22 ab	28.11±3.14 b	342.1±23 b	63.71±5 c	16.88±1.2 a	1.79±0.17 b	9.32±0.84 b
Mild drought stress	160.6±16 b	2.45±0.25 b	14.88±0.69 a	1.52±0.23 a	2.77±0.25 a	35.00±3.24 a	395.7±19 a	77.04±7 b	12.21±1.1 b	2.11±0.24 a	10.19±1.25 a
Severe drought stress	135.8±11 c	2.03±0.25 c	11.40±0.50 b	1.16±0.09 c	1.94±0.25 b	37.33±3.24 a	268.7±19 c	87.64±3 a	11.27±0.7 b	2.02±0.29 a	10.24±0.72 a

DW – dry weight; FW – fresh weight. Means donated by similar letter(s) indicate no significantly ($p \leq 5\%$) by Least Significant Difference test. Values represent the mean of three replicates ± SD.

Table 3. Morphological and physiological traits of roselle (*Hibiscus sabdariffa* L.) from three different sowing date treatments

Sowing date	Plant height	Biological yield	Calyx yield	Antioxidant activity	Seed oil content	Calyx water use efficiency
	(cm)	(ton.ha^{-1})	(ton.ha^{-1})	(%)	(%)	(Kg.kg^{-1})
4th June	168.7±24.8 a	14.24±1.7 a	1.48±0.23 a	71.57±12.7 b	14.14±3 a	1.81±0.17 b
18th June	159.4±24.1 ab	13.55±1.8 b	1.33±0.24 b	78.62±12.1 a	13.27±2.5 ab	1.97±0.30 ab
2nd July	149.5±20.6 b	13.18±1.5 b	1.24±0.10 b	78.20±8.8 a	12.95±2.6 b	2.14±0.23 a

Means followed by similar letters indicate no significance ($p \leq 5\%$) by Least Significant Difference test. Values represent the mean of three replicates ± SD.

sativa L.) under drought conditions seemed to be attributed to the reduction in water availability, which reduced cell division, lowered LAI and plant height and finally resulted in lower dry matter and biological yield (Aghdasi *et al.,* 2018a). The authors stated that an increased allocation of photosynthates to the plant root relative to the shoots was another reason for the reduction in biological yield. Regarding sowing dates, a significant maximum biological yield (14.24 ton.ha^{-1}) was achieved when the plants were sown on 4th June with the minimum biological yield (13.18 ton. ha^{-1}) in the case of the crop sown on 2nd July. However, there were no significant difference between 18th June and 2nd July. These results are in accordance with those of Keshavarz and Khodabin (2019) and Aghdasi *et al*. (2018b). Higher biological yield with early sowing was mainly due to a greater number of effective branches per m^2, more plant height and greater stem diameter (some data not show). Another reason for this result is probably due to the fact that the first sowing dates resulted in longer growing periods, and therefore, greater total rainfall received during the growing periods. An adequate irrigation supply would eventually yield the higher growth rate. These results are in line with Rah Khosravani *et al.* (2017). There was a significant positive correlation between calyx yield, total phenolic content, vitamin C and seed oil yield with biological yield. However, biological yield had a negative correlation with total anthocyanin content and antioxidant activity (Table 4). In a similar study on roselle, Fallahi *et al*. (2017b) concluded that biological yield had a positive effect on the number of capsules per plant and plant height which may be attributed to genetic effects rather than environmental ones and these traits may be good selections for improving seed and calyx yield in roselle.

3.3. Calyx yield

An increase from 1.37 ton.ha^{-1} to 1.52 ton.ha^{-1} in calyx yield was recorded for roselle with an increase

Table 4. Coefficient of correlation matrix based on all studied traits

	1	2	3	4	5	6	7	8	9	10	11
1- Plant height	1										
2-Stem diameter	0.59 **	1									
3-Biological yield	0.73 **	0.63 **	1								
4-Calyx yield	0.35 ns	0.16 ns	0.75 **	1							
5-Total phenolic content	0.35 ns	0.36 ns	0.64 **	0.42 *	1						
6-Total anthocyanin content	-0.76 **	-0.57 **	-0.50 **	-0.082 ns	-0.14 ns	1					
7-Vitamin C	0.54 **	0.48 *	0.87 **	0.68 **	0.76 **	-0.24 ns	1				
8-Antioxidant activity	-0.79 **	-0.59 **	-0.77 **	-0.51 **	-0.25 ns	0.74 **	-0.43 *	1			
9-Seed oil content	0.71 **	0.63 **	0.56 **	0.27 ns	-0.02 ns	-0.75 **	0.24 ns	-0.84 **	1		
10-Calyx water use efficiency	-0.58 **	-0.28 ns	-0.16 ns	0.24 ns	0.081 ns	0.52 **	-0.03 ns	0.34 ns	-0.48 **	1	
11-Calyx harvest index	-0.35 ns	-0.50 **	-0.068 ns	0.59 **	-0.11 ns	0.49 **	-0.03 ns	0.14 ns	-0.26 ns	0.58 **	1

ns – not significant, *: significant at $P \leq 0.05$ and **: significant at $P \leq 0.01$

drought severity from well-watered to mild drought stress but severe drought stress significantly decreased calyx yield to 1.16 ton.ha^{-1} (Table 2). It was demonstrated that well-watered irrigation is needed to guarantee high crop yield but in the case of medicinal plants a mild drought stress could be beneficial to the quality and quantity of these plants (keshavarz *et al.,* 2018). Increases in roselle calyx may be attributed to the mild water stress imposed. This was in agreement with the reports of Fallahi *et al*. (2017a), who claimed that the calyx yield of roselle increased under mild drought stress compared to full irrigation. El-Dissoky *et al*. (2020) studied the effect of irrigation frequency on the calyx yield of the roselle plant and found that mild drought stress improved the calyx production but severe drought condition reduced the calyx yield. Stress conditions have been shown to decrease the number of flowers and calyx (depending on the time of the stress severity), because the flowering phase involves several processes that are vulnerable to stress conditions (Jasim *et al.,* 2020). From Table 3, it is clear that first sowing dates (4th June) registered maximum calyx yield (1.48 ton. ha^{-1}) while the lowest calyx yield (1.24 ton.ha^{-1}) was recorded for the third sowing date (2nd July). Given the favorable growth conditions such as temperature and the sun light on 4th June, plants produced more assimilates and yielded greater dry matter. Due to a shortened vegetative phase, flowering occurred when summer temperatures were high and the flowers aborted. Previous research has reported that optimal planting time leads to better developed plants with higher LAI than those sown later (Rah Khosravani *et al.,* 2017). Accordingly, flowering abortion would be expected to increase during drought stress as it decreases the flux of photosynthate supply from source leaves to the vegetative tissues. Also, drought stress may change the concentration of ABA in the plants, and thereby induce flower abortion in drought-stressed crops (Salih, 2019).

3.4. Total phenolic content (TPC)

The highest TPC (2.77 mg.100 g^{-1} DW) was recorded for plants treated with mild drought stress, while the lowest TPC (1.94 mg.100 g^{-1} DW) was recorded for severe drought stress treatment. Although, mild drought stress increased TPC, there was no significant difference between well-watered and mild drought stress treatments (Table 2). However, a greater increase in drought severity, to 55% FC, lowered the TPC of roselle. TPC, as a group of non-enzymatic antioxidant compounds with carbon skeleton, by osmotic regulation of plant cells serves to respond to drought conditions, stabilize proteins, prevent lipid peroxidation, and act as an osmolyte in the permeability of cell membranes (Keshavarz Mirzamohammadi *et al.,* 2021). Under severe drought conditions, TPC decreases, which can be because of an increase in the production of oxygen radicals, peroxidation of chlorophyll pigments, degradation of genes related to the chlorophyll biosynthetic path, reduction in the photosynthetic level and increasing the allocation of carbon to the root.

3.5. Total anthocyanin content (TAC)

The effect of irrigation treatment was significant for the TAC of roselle (Table 1). On average, TAC increased by 19.68 and 24.69% compared to the well-watered treatment when the plants were treated with mild and severe drought stress, respectively (Table 2). However, mild and severe stress were statistically at the same level. As documented in many papers (An *et al*., 2020; Cirillo *et al*., 2021), the total anthocyanin content of roselle was increased by drought stress to reduce excess light availability and provide useful protection to leaves without significantly reducing photosynthesis efficiency. This shows a mechanism to modulate light absorption and reduce photo-oxidative injury. It has been reported that anthocyanin has the potential to reduce photo-oxidative damage in leaves, both by scavenging reactive oxygen species and protecting chloroplasts from excess high-energy quanta (He *et al.,* 2020). The influence of drought stress on TAC is in agreement with the findings of Fallahi *et al*. (2017a) and Hinojosa-Gómez *et al*. (2020) on roselle, which stated that the improvement in the plants' qualitative traits under simultaneous drought stress, such as anthocyanins, has been related to improved secondary metabolism.

3.6. Vitamin C

Vitamin C content was affected significantly by irrigation levels (Table 1) and the amount of these compounds under mild drought stress (395.7 mg 100 g^{-1}) was higher than well-watered irrigation (13% on average). In contrast, severe drought stress sharply decreased the vitamin C content by 21 and 32% compared to the well-watered and mild stress, respectively. Changes in vitamin C under drought stress have been shown to depend on stress severity, drought duration and cultivar (Dolatabadian *et al*., 2010). Mataa *et al*. (2020) showed that the concentrations of vitamin C, TPC, and TAC showed similar trends and were reduced in severe drought stress. Therefore, it is possible that in this study, the concentration of other antioxidants, such as superoxide dismutase and catalase (not measured in this study), can be increased in roselle under drought stress.

3.7. Antioxidant activity

In this investigation, antioxidant activity (DPPH) was significantly increased with the increase in the severity of drought stress in the order: control < mild < severe drought stress (Table 2). In general, with delay in planting date, the percentage of antioxidant activity in the plant increased and 4th June exhibited the lowest (71.57%) antioxidant activity for septal extract, whilst the highest antioxidant activities were observed on 18th June and 2nd July (78.62 and 78.20, respectively) (Table 3). Free radicals are generated under stress conditions and the antioxidant capacity of plants includes enzymatic and non-enzymatic compounds, free radical species are oxidized by releasing electrons to free radicals (Keshavarz, 2020). A significant decrease was observed for total anthocyanin content (y = -0.76**) and antioxidant activity (y = -0.79**) with the increase in plant height (Table 4). This is due to the difference in red (R): far red (FR) ratio perceived by the plants grown at different irrigation regimes, planting time and neighbor detection (Khattak *et al.,* 2016). The plants develop a strategy to grow taller in order to capture more photosynthetic radiation at the expense of physiological changes (Smith, 1982).

3.8. Seed oil content

On average, the seed oil content (% of seed dry matter) of the plants under mild and severe drought treatment was significantly low (12.21 and 11.27%, respectively) compared to 16.88% in the seeds of the well-watered plants (Table 2). Delay in planting time significantly decreased the seed oil content in fruits compared to on-time sowing dates, where the highest values for seed oil content were recorded on 4th June (14.14%) with an average of 6.15 and 8.41% higher than 18th June and 2nd July (Table 3). As previously explained, water stress conditions affected plant growth and development, leading to an effect on seed oil content. The optimal water supply throughout the growing period could enhance oil content in seed oil plants, while drought stress reduces it (Soheili-Movahhed *et al*., 2019). A reduction in seed oil content might be attributed to the polyunsaturated fatty acid oxidation by free radicals under drought conditions (Keshavarz, 2020). The reduction in seed oil content when drought stress occurred during the growing season is supported by various reports (Keshavarz *et al.,* 2020). The quantity of seed oil content is mainly determined by water balance, soil fertilizers, sun light and other environmental factors throughout the growth period. The high temperature and consequently changes in water balance in the seed filling stage could likely be the reason for lower oil content on 2nd July compared to 4th June. Asadpour *et al*. (2020) and Yazdanpanah *et al*. (2018) reported that high seed oil content in roselle (*Hibiscus sabdariffa* L.) was related to lower temperature and water availability at the seed filling phase.

3.9. Calyx water use efficiency (WUE_{calyx}) and calyx harvest index (HI_{calyx})

The highest water use efficiency for calyx (2.02 $kg.kg^{-1}$) was obtained with severe drought treatment and the lowest (1.79 $kg.kg^{-1}$) was with the well-watered treatment (Table 2). The results showed that water use efficiency increased when the sowing date was delayed to after June 4th. Based on our results (Table 3) the highest use efficiency was observed on 2nd July and it was higher by 15.42 and 7.94% than the first and second planting dates, respectively. However, there were no significant difference between the first and second planting dates. Based on harvested calyx yield and the amount of irrigation, severe drought stress was determined to be the best irrigation regime in terms of the WUE_{calyx}. As previously mentioned, the biological yield of roselle was decreased by drought stress. In our study, since the average amount of irrigation water was reduced among the drought stress or delayed planting treatments, higher calyx yield compared to biological yield led to the greater WUE_{calyx}.

A limited supply of carbohydrates for dry matter production leads to a reduction in biological yield. Previous research has reported that higher values for HI_{calyx} in roselle (*Hibiscus sabdariffa* L.) under drought conditions might be due to higher air temperature and heat stress, which could negatively affect biological yield and plant growth, resulting in earlier flowering due to shortened vegetative stages and increased economical yield/biological yield ratio (Javadzadeh, 2018). In fact, drought conditions reduced biological yield more than calyx yield. As shown in Table 2, calyx yield is higher under mild drought conditions. It was thought that the increase in calyx under mild drought conditions resulted from the increase in assimilate partitioning to flower due to a shortened vegetative phase. On the hypothesis that short distance translocation of is limited to first/second node (Liu *et al.,* 2020), the improvement in HI_{calyx} means the increase in sink demand to source. So, we can conclude that the increase in calyx yields improved HI_{calyx} by increasing sink demand, therefore, the use efficiency of assimilates was increased under drought conditions.

CONCLUSIONS

Plant height and stem diameter decreased with decreasing water availability; while TAC, antioxidant activity, WUE_{calyx} and HI_{calyx} increased with decreasing water availability. However, in term of biological yield and calyx yield, there was no significant difference between well-watered and mild drought stress. Sowing date, plant height, biological yield, calyx yield and oil seed content decreased with delayed planting while antioxidant activity and WUE_{calyx} increased. In brief, the planting date on 2nd July that improved WUE_{calyx} (probably due to reducing irrigation supply) was a good strategy to increase calyx production and WUE. These results suggest that the delay in on-time planting date from 4th June to early July (2nd July) is possible to improve calyx WUE in arid and semi-arid regions like Karaj.

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