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Saffron (*Crocus sativus* L.) yield parameter assessment of abiotic stressed corms stored in Low Temperature

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Abstract

Saffron (*Crocus sativus* L. / Iridaceae family) is the most expensive spice in the world. It has been cultivated in Morocco for centuries and has represented a traditional staple for culinary, medical and cosmetic uses. This study aims to optimize saffron yield parameters (saffron morphology, flowering, and corm yield) focusing on its performance in the semiarid regions of eastern Morocco using low-temperature storage, salt and drought stresses. Corms obtained from salt and drought stresses and stored in cold storage at 4 °C for 7 and 14 days (with a control kept at room temperature) were cultivated in open fields at the Experimental Station of the Faculty of Sciences of Oujda. The number of flowers formed in the yield of spice saffron per corm and daughter corm parameter depended on storage temperature and cold storage duration. Flowers from the corm that were cold-stored for 14 days formed earlier than other treatments. The flowers' number, fresh stigma yield decreased with the increase of the duration of cold- storage. Similarly, length and leaf number showed the same decrease with the increase of the cold storage period. The diameter of produced daughter corms was the largest in control. However, the boost of cold storage time increases the number of small daughter corms per plant. Cold storage induces precocious dormancy. Overall, no benefit resulted from cold- storage of corms; and corms from stressed plants had no effect on the studied parameters.

1. Introduction

Saffron (Crocus sativus L.) is an autumn-flowering geophytic plant, which is one of the most known medicinal and aromatic plant species in the world and is commonly known as the “Golden Condiment” [1]. In 2015, the saffron plantation in Morocco was conducted in a surface area of around 1600 ha with an average yield of 3.5t, making Morocco the fourth saffron producer in the world [2].

Specific morphological characteristics and low water requirement are the most important factors that allow the plant to grow in arid and semi-arid regions [3,4]. This may be valuable for eastern Morocco, which is characterized by rarefaction of water resources. Saffron is acclimated to hillsides, plateaus and mountain valleys ranging in altitudes between 600 and 1700 m [5].

Being sterile, saffron reproduces exclusively by corms [6]. Therefore, the selection and pre-treatment of corms are crucial for saffron's production. Among several environmental factors (light, moisture, temperature, salinity, and drought) affecting geophytes development, temperature is considered as the predominant factor in the control of growth and flowering of bulbs [7]. The influence of bulbs storage temperatures was investigated previously by various authors [7-9]. These authors showed the determining effect of storage temperatures on the physiological development of the bulbs.

In general, the optimal temperature for the initial organogenesis ranges from 15 to 21 °C, while the development of a flower stalk and well-formed flowers requires a low positive temperature [7]. Nowdays, the effect of salt and drought stressed corms on the growth and development of saffron is not well examined. We undertook the study of the effect of storage conditions on saffron morphology, flowering and corm yield using corms from plants pre-exposed to salt and drought stresses.

2. Experimental details

2.1 *Experimental site*

The experiment was conducted in an open field at the Experimental Research Station of the Faculty of Sciences of Oujda, located at 661 m altitude and 34 ° 39 '06-71" north and 01 ° 53 '58-80" West (GPS Back Track Bushnell). The semiarid climate is characterized by temperate winters. The climatic data of the experimental station are presented in Table 1.

Table 1. The climatic data of the experimental station* (2014).

Month	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Mai	Jun	July	Aug
Précipitation (mm)	26.9	7	41	41	37	37.4	19.1	12.8	18.5	9.8	0	0.7
Absolute minimum T °C	13.4	11	6.2	-0.4	1.6	2.8	3	4.4	5.7	12	13.8	16.6
Absolue maximum T °C	41	35.9	29.9	22.2	25	25.6	27.3	35.5	34.5	45	42.8	40.2

*The data were obtained from the City of Oujda State Meteorological Service,

2.2 *Growing substrate*

The substrate was prepared by mixing equal volume proportion of sand, field soil and peat and was filled in pots (12 cm * 15 cm). The composition of the substrate was performed: pH, electric conductivity and organic material (Table 2).

Table 2. Substrate analyses results of the experimental substrate samples.

pH	E.C. (mS/cm)	Organic material (%)
7.25	0.73	0.83

2.3 *Plant material*

The corms used in this research were obtained from corms that were exposed to drought (100 %, 60% and 40% of reference evapotranspiration of Oujda city ET0) and salt stress (0, 1, 3 and 5 g/l of NaCl) throughout a period of two years (2013-2015) in the Experimental Research Station of the Faculty of Sciences. Corms were kept in aerated bags and room temperature in a dark area until the cold storage treatment.

2.4 *Treatments used*

Each of the latter stressed corms (salt and drought) was treated with three levels of cold storage as follows:

T0: control was kept at room temperature 20 °C in darkness.

T1: corms were treated for 7 days at 4 °C in darkness.

T2: corms were treated for 14 days at 4 °C in darkness.

Corms with minimum 30 mm diameter were shown on 25th September 2015. Irrigation was made up based on ET0 of Oujda city.

2.5 *Experimental design*

The adopted experimental design is split-plot with 3 replicates and 60EU, the whole-plot indicates the type of stress (drought or salt) and that the storage time factor is the split-plot factor.

2.6 *Studied parameters*

The effects of cold storage and stress were determined by the assessment of different plant traits, namely:

- The first-last flowering date: determined by daily plots monitoring.
- The total harvest period: flowering period, as defined by Elzinga et al. [10] is the number of days between the first and last flowering.
- The flowers' number: flowers were picked up and counted early in the morning every day.
- The stigma weight (g): the flowers of each treatment were harvested early in the morning. Afterwards, we proceeded with the separation of stigmas, then the weight was obtained by weighing the fresh stigmas.
- The length of leaves (cm): growth in length of the aerial part (leaves) was evaluated every month with a scale in millimeters (mm) from the leaf base to the top.
- The number of leaves: counted each month for each tuft.

• The number and diameter of corms: at the end of the cycle, plants have been dug up, corms rid of topsoil, cleaned and de-tunicates then the number and diameter of corms were determined. The Caliber of corms was determined by using a caliper. Two sizes were distinguished: the large caliber: Ø> 2.5cm and the small one: Ø <1.5cm.

2.7 *Statistical analysis*

The values of different parameters were expressed as the mean ± standard deviation (x ± S.D). SPSS statistical analysis software was used for analysis of variance, ANOVA and Duncan's multiple range tests were utilized to separate means in 0.05 confidence level.

3. Results and Discussion

3.1 Harvest period

The first flowering date and harvest period are presented in Table 3. These parameters appear to be linked to two factors:

- The degree of stress: salt and drought stresses influenced the flowers' emergence. Flowers from stressed corms were delayed and the harvest period was shortened. Previous studies reported the delaying effect of flowers' emergence due to salinity [11].
- The cold storage: flowers from cold-stored corms for 14 days were consistently precocious up to a week in contrary to the salt and drought stresses (Figure 1 & 2). These results are in accordance with previous studies [12], reporting precocious flowers production from cold-stored corms. Molina et al [13] revealed the effect of low-temperature storage on precocity and extension of the saffron flowering period.

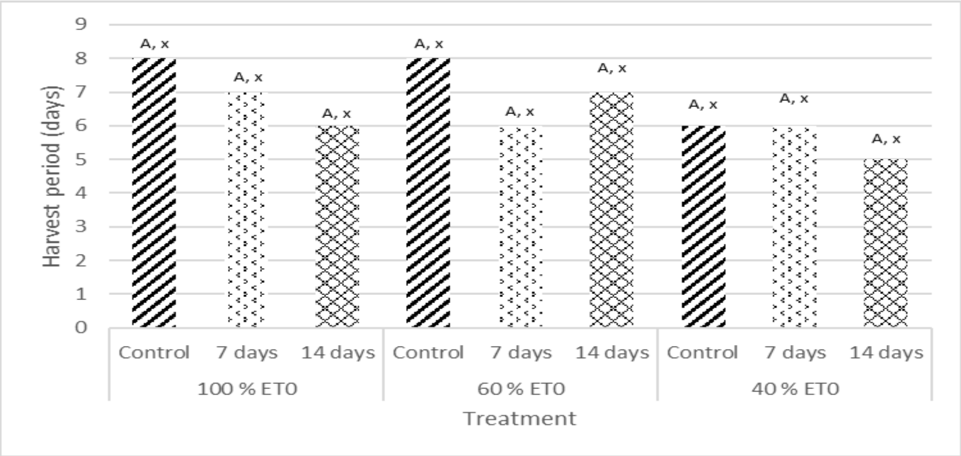

Figure 1: Effect of cold storage and drought stress level of the corms on harvest period. (Mean values with different higher-case-letters (A and B) (only for degree of drought stress) and lower-case-letters (x and y) (only for the cold storage) differ significantly at P<0,05.)

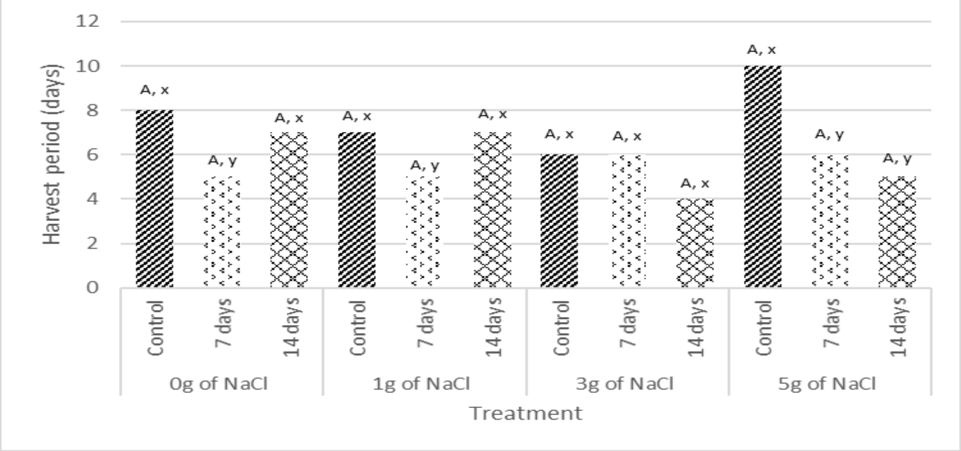

Figure 2: Effect of cold storage and salt stress level of the corms on harvest period. (Mean values with different higher-case-letters (A and B) (only for degree of salt stress) and lower-case-letter (x and y) (only for the cold storage) differ significantly at P<0,05.)

Table3. The effect of cold storage and the abiotic stress level of the corms on flowering dates

Treatment		Days of pre-planting cold storage of Corms at 4°C	First-last flowering Date																			
			October					November														
			26	27	28	29	30	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Drought stress	100% ET0	Control																				
		7 days																				
		14 days																				
	60% ET0	Control																				
		7 days																				
		14 days																				
	40% ET0	Control																				
		7 days																				
		14 days																				
Salt stress	0g of NaCl	Control																				
		7 days																				
		14 days																				
	1g of NaCl	Control																				
		7 days																				
		14 days																				
	3g of NaCl	Control																				
		7 days																				
		14 days																				
	5g of NaCl	Control																				
		7 days																				
		14 days																				

3.2 *Fresh stigma yield and number of saffron flowers*

The fresh stigma yield and the number of flowers per corm decreased with the increase of the cold storage period (Table 4 & 5). By fixing the stress factor, higher values of saffron flowers number (0.57 and 0.46 flower /corm) and fresh stigma yield (0.29 and 0.28g/0.5m²) were observed in the control respectively for salt and drought stress, and smallest values in cold-stored corms at 4 °C for 14 days (Figure 3 & 4). Thus, the cold storage revealed a negative effect on the number of flowers and fresh stigma yield. Similarly, Amooaehaie [14] and Cavusoglu [12] revealed that the effect of the increase of the cold storage duration was the decrease of number and sizes of flowers. Molina [13] revealed a close relation between the low-temperature storage effect and the developmental stage of the saffron corms, the conditions, and the duration of storage. However, there were no significant differences between the various stresses treatment.

3.3 *Number and length of leaves*

The length and leaf number decreased with the increase of the cold storage duration (Table 4 & 5). When fixing stress factor, the number and length of leaves were the lowest in cold-stored corms at 4 °C for 14 days with 23.7 leaves/corm, 11.9 cm/length of leaves for drought stress and 25 leaves/corm, 12.3 cm / plant for salt stress (Figure 5). However, the cold storage period showed a moderate effect on the studied parameters, salt and drought stresses had no effect on the number and length parameters. These results corroborate with previous studies [12] on the negative effect of cold storage on the length of leaves contrary to the leaf numbers. The author reported a positive effect of cold storage on the leaf's number. Sano [15] found that the number of Iris leaves that were formed before the flower bud formation was reduced by the increase of the duration of the low-temperature treatment.

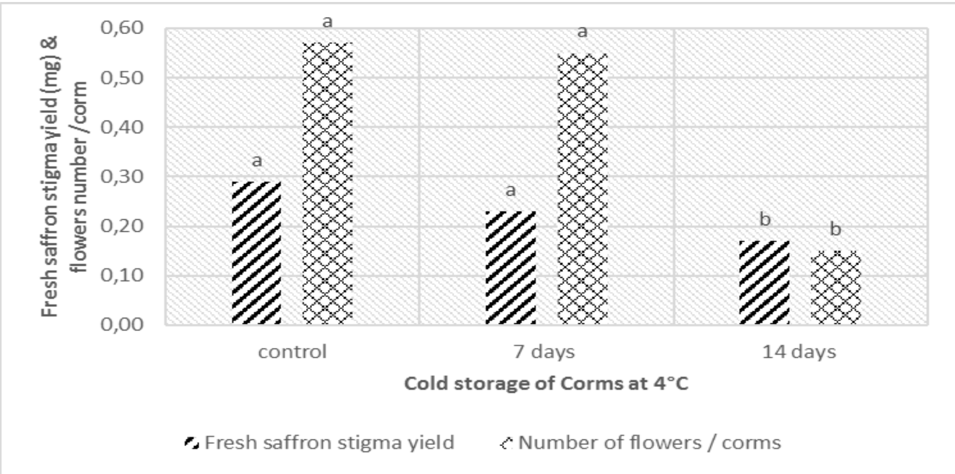

Fig 3: The effect of cold storage on fresh saffron stigma yield and number of flower for corms from salt stress

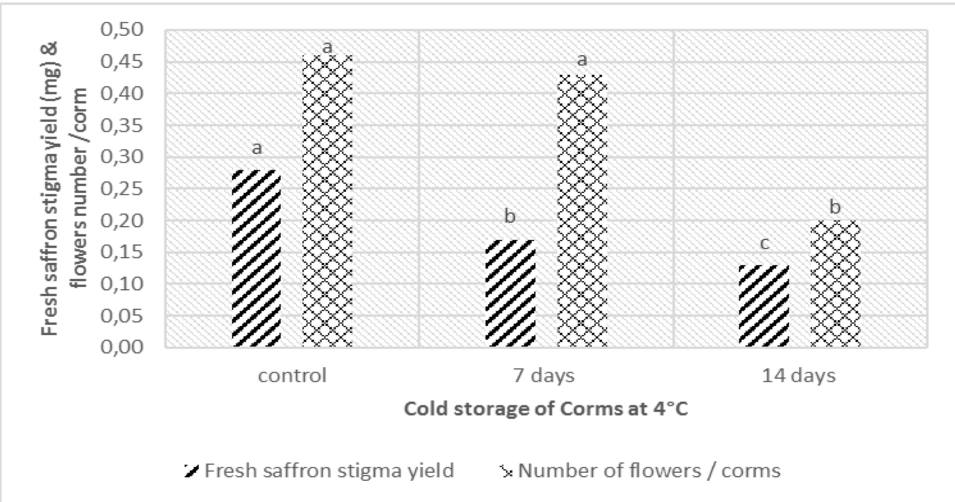

Figure 4: The effect of cold storage on fresh saffron stigma yield and number of flower for corms from drought stress.

Table 4: The effect of cold storage and the salt stress level of the corms on fresh stigma yield, number of flowers, number and length of leaves.

Treatment	Cold storage of Corms at 4°C	Fresh saffron stigma Yield (g/0.5m²)		Number of flowers / corm		Number of leaves /corm		length of leaves (cm)	
		average	**± SD**	**average**	**± SD**	**Average**	**± SD**	**average**	**± SD**
	Control	**0.28**$^{A. x}$	0.008	**0.6**$^{A. x}$	0.18	**30.3**$^{A. x}$	2.14	**14.0**$^{A. x}$	2.94
0g/l of NaCl	7 days	**0.17**$^{A. y}$	0.003	**0.6**$^{A. x}$	0.05	**30.9**$^{A. x}$	2.45	**13.6**$^{A. x}$	2.30
	14 days	**0.15**$^{A. z}$	0.005	**0.1**$^{A. y}$	0.01	**29.0**$^{A. x}$	2.22	**13.6**$^{A. x}$	1.86
	Control	**0.30**$^{A. x}$	0.009	**0.6**$^{A. x}$	0.10	**30.8**$^{A. xy}$	2.05	**15.0**$^{A. x}$	1.72
1g/l of NaCl	7 days	**0.24**$^{A. y}$	0.005	**0.7**$^{A. x}$	0.10	**32.0**$^{A. x}$	5.10	**14.6**$^{A. x}$	2.00
	14 days	**0.20**$^{A. z}$	0.006	**0.3**$^{A. y}$	0.20	**27.0**$^{A. y}$	2.28	**12.8**$^{A. y}$	1.17
	Control	**0.30**$^{A. x}$	0.006	**0.7**$^{A. x}$	0.20	**29.0**$^{A. x}$	1.97	**14.4**$^{A. x}$	1.29
3g/l of NaCl	7 days	**0.27**$^{A. y}$	0.008	**0.3**$^{A. y}$	0.17	**24.0**$^{A. xy}$	2.10	**11.8**$^{A. y}$	1.14
	14 days	**0.21**$^{A. z}$	0.004	**0.3**$^{A. y}$	0.10	**22.7**$^{A. y}$	2.66	**11.5**$^{A. y}$	2.23
	Control	**0.29**$^{A. x}$	0.010	**0.4**$^{A. x}$	0.20	**29.7**$^{A. x}$	3.59	**13.8**$^{A. x}$	1.5
5g/l of NaCl	7 days	**0.24**$^{A. y}$	0.005	**0.6**$^{A. x}$	0.11	**26.3**$^{A. xy}$	2.14	**13.2**$^{A. x}$	1.19
	14 days	**0.15**$^{A. z}$	0.008	**0.1**$^{A. y}$	0.02	**21.2**$^{A. y}$	3.50	**11.2**$^{A. x}$	2

Mean values in the same column with different letter (x and y) (only for the cold storage) differ significantly at P<0,05.

Table 5: The effect of cold storage and drought stress level of the corms on fresh stigma yield, number of flowers, number and length of leaves.

treatment	**Cold storage of Corms at 4°C**	**Fresh saffron stigma yield (g/0.5m²)**		**Number of flowers / corms**		**Number of leaves /corm**		**length of leaves (cm)**	
		average	± SD	average	± SD	average	± SD	average	± SD
	Control	**0.30**$^{A.\ x}$	0.042	**0.5**$^{A.\ x}$	0.21	**29**$^{A.\ x}$	2.86	**14.0**$^{A.\ x}$	2.04
100 % ET0	7 days	**0.17**$^{A.\ y}$	0.018	**0.4**$^{A.\ x}$	0.24	**31**$^{A.\ x}$	4.73	**13.2**$^{A.\ x}$	4.03
	14 days	**0.12**$^{A.\ z}$	0.033	**0.4**$^{A.\ x}$	0.20	**25**$^{A.\ y}$	1.81	**11.2**$^{A.\ y}$	2.43
	Control	**0.29**$^{AB.\ x}$	0.027	**0.5**$^{A.\ x}$	0.36	**30.5**$^{A.\ x}$	4.31	**13.5**$^{A.\ x}$	2.26
60 % ET0	7 days	**0.21**$^{AB.\ y}$	0.011	**0.5**$^{A.\ x}$	0.30	**30**$^{A.\ x}$	3.25	**13.2**$^{A.\ x}$	3.11
	14 days	**0.14**$^{AB.\ z}$	0.031	**0.1**$^{A.\ y}$	0.16	**24**$^{A.\ y}$	2.76	**12.9**$^{A.\ x}$	2.50
	Control	**0.27**$^{B.\ x}$	0.011	**0.4**$^{A.\ x}$	0.10	**28.5**$^{A.\ x}$	1.22	**13.8**$^{A.\ x}$	1.34
40 % ET0	7 days	**0.15**$^{B.\ y}$	0.011	**0.4**$^{A.\ x}$	0.35	**25**$^{A.\ y}$	1.08	**13.5**$^{A.\ x}$	1.66
	14 days	**0.13**$^{B.\ y}$	0.024	**0.1**$^{A.\ y}$	0.17	**22**$^{A.\ y}$	2.22	**11.6**$^{A.\ y}$	1.87

Mean values in the same column with different higher-case-letters (A and B) (only for degree of drought stress) and lower-case-letter (x and y) (only for the cold storage) differ significantly at $P<0.05$.

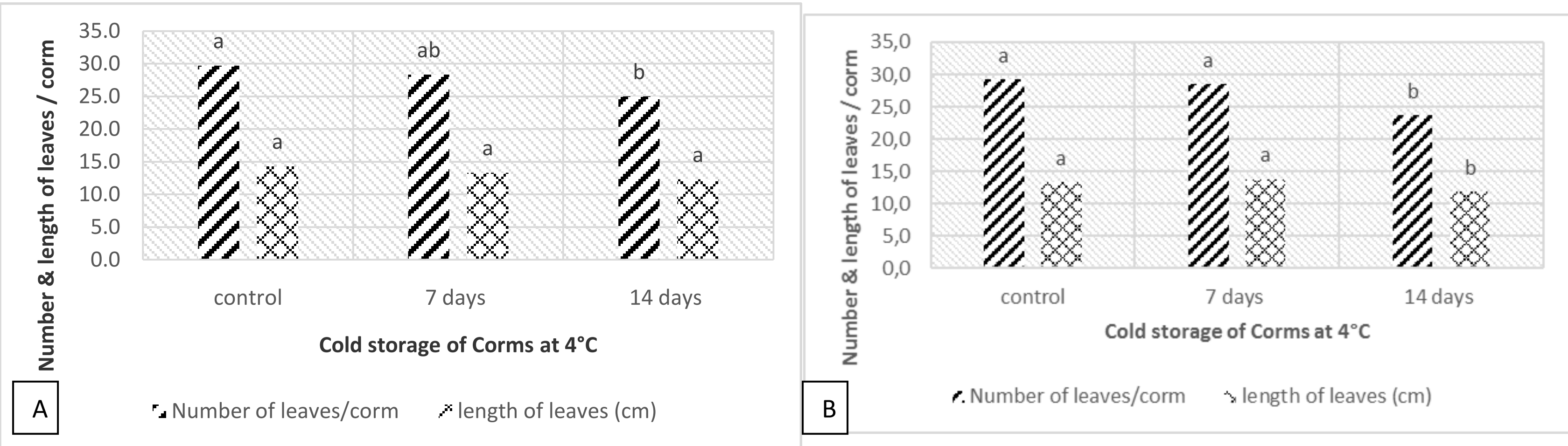

Figure 5: The effect of cold storage with fixing salt (A) and drought (B) stress factors on number and length of leaves.

3.4 *Daughter corm yield parameter*

The daughter corm yield parameters presented in table 6 revealed that cold storage had a negative influence on daughter corm yield, but no impact of storage duration has been revealed. Salt and drought stresses didn't show any effect on these parameters. The largest diameter (≈2 cm) per daughter corm value was found in control. However, an increase of cold storage duration increased the number of small (<1.5 cm) daughter corms per plant. The highest values in corms were recorded in cold-storage for 14 days (table 6) with 4.8-5.7 daughter corms/plant. Cavusoglu [12] reported negative effects of cold storage on daughter corms' weight and diameter on saffron. However, Hopinks [16] showed the effect of low temperature on Gladiolus small corm which consequently is reflected by the decrease of small corms. These results revealed the role cold storage in increasing the number of daughter corms. Unfortunately, the size was too small for cultivation. Nevertheless, an increasing number of corms, regardless of its size, remained interesting for propagation material for the nursery.

Table 6: The effect of cold storage and stress level of the corms on Daughter corm yield parameters

treatment	Cold storage of Corms at 4°C	Number of daughter corms /plant		Diameter of daughter corm (cm)	
		average	± SD	**average**	±SD
	Control	$4.5^{A. x}$	0.85	$2.0^{A. x}$	0.4
100 % ET0	7 days	$3.7^{A. x}$	1.46	$1.5^{A. x}$	0.7
	14 days	$4.8^{A. x}$	1.27	$1.3^{A. x}$	0.6
	Control	$3.7^{A. y}$	0.97	$2.0^{A. x}$	0.3
60 % ET0	7 days	$4.4^{A. x}$	1.59	$1.6^{A. x}$	0.5
	14 days	$5.1^{A. x}$	1.69	$1.4^{A. x}$	0.4
	Control	$4.0^{A. x}$	0.76	$1.7^{A. x}$	0.4
40 % ET0	7 days	$3.6^{A. y}$	0.87	$1.8^{A. x}$	0.3
	14 days	$5.6^{A. x}$	0.87	$1.4^{A. x}$	0.6
	Control	$4.5^{A. x}$	1.2	$1.8^{A. x}$	0.5
0g/l of NaCl	7 days	$4.8^{A. x}$	0.8	$1.6^{A. x}$	0.5
	14 days	$4.8^{A. x}$	0.6	$1.3^{A. x}$	0.4
	Control	$5.4^{A. y}$	0.9	$2.0^{A. x}$	0.3
1g/l of NaCl	7 days	$4.6^{A. x}$	0.7	$1.9^{A. x}$	0.4
	14 days	$4.3^{A. x}$	1.4	$2.1^{A. x}$	0.4
	Control	$4.0^{A. y}$	0.4	$1.6^{A. x}$	0.4
3g/l of NaCl	7 days	$4.5^{A.xy}$	0.4	$1.8^{A. x}$	0.4
	14 days	$5.0^{A. x}$	0.4	$1.5^{A. x}$	0.4
	Control	$4.2^{A.x}$	0.7	$1.7^{A. x}$	0.4
5g/l of NaCl	7 days	$3.4^{A. y}$	0.9	$1.6^{A. x}$	0.4
	14 days	$5.7^{A. x}$	0.7	$1.4^{A. x}$	0.4

Mean values in the same column with different letter (x and y) (only for the cold storage) differ significantly at P<0.05.

Table 7:The effect of cold storage and stress level of the corms on dormancy.

Treatment		Cold storage of Corms at 4°C	dormant date																				
			April																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Salt stress	0g/l of NaCl	Control																					
		7 days				vegetative activity											Dormancy						
		14 days																					
	1g/l of NaCl	Control																					
		7 days																					
		14 days																					
	3g/l of NaCl	Control																					
		7 days																					
		14 days																					
	5g/l of NaCl	Control																					
		7 days																					
		14 days																					
Drought stress	100 % ET0	Control																					
		7 days																					
		14 days																					
	60 % ET0	Control																					
		7 days																					
		14 days																					
	40 % ET0	Control																					
		7 days																					
		14 days																					

3.5 *Vegetative cycle*
The results presented in Table 7 showed the effect of cold storage on the undesirable shortening saffron cycle. The field observations showed early dormancy of 14 days cold stored corms whose shortcut up to 17 days has been observed. However, corms from stressed plants had no effect on the saffron cycle. The shortening of the vegetative cycle has been demonstrated for several years.
Klippart [17] proposed to reduce the time between sowing and flowering in cereals, by exposing grains to cold temperature.

Conclusion
Studies concerning the effect of cold storage and salt or drought stresses on saffron are rare. The present results on the effect of corm cold storage of saffron showed that the flowers' number, fresh stigma yield, leaf, length, and plant height decreased with the increase of the duration of cold-storage. Furthermore, the increase of the cold storage period increased the number of small daughter corms per plant and induces precocious dormancy. These results confirmed no cold storage requirement for saffron as mostly found in geophytes. However, corms from salt and drought stressed plants had no effect on the studied parameters, confirming the ability of saffron to adapt to abiotic stresses.

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