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Morphological investigation of two wild *Crocus* species in Iran

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Abstract

In this study, the relationship between 16 quantitative and qualitative characters of flowers, leaves and corms in 25 samples of two species of wild *Crocus* plant *(Crocus. speciosus, Crocus. cancellatu*) and *Crocus. sativus* was evaluated. Results of simple correlation indicated positive and negative significant correlations between some important characteristics. In factor analysis, four independent factors could justify a total of 79.93 % of the total variance and cluster analysis based on four factors showed that *C. speciosus* is fully isolated from *C. cancellatus* and *C. sativus*, while *C. sativus* was separated at a distance of 12 from two wild species, but at the distance of 20 is similar to *C. cancellatus*.

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Introduction

Saffron is a valuable plant which belongs to the Iridaceae family, which has been used for 1600 years before Christ to dye, produce perfume and medicinal purposes (Molina *et al.*, 2005; Keifi & Beigi, 2011; Erol *et al.*, 2014). Designs of saffron has been observed in murals and pottery from the ancient civilization of Crete in the Minos and Knossos Palace at the Crete Island (Ebrahimzadeh *et al.*, 1998; Ozdemir *et al.*, 2006) . Saffron life cycle is similar in all producing countries. Flowering occurs in autumn and propagation is vegetative and the flowering forms are directly related to the size and quality of corm (Negbi *et al.*, 1989). *Crocus* genus is divided into two *Crocus* and *Crociris* subgenera, *Crocus* subgenera is divided into the *Crocus* and *Nudiscapus* groups (Grilli Caiola and Canini 2010; Robio Morago *et al.*, 2010). *Crocus* corms are in different forms and vary from a flattened and oval to spherical state. This organ tissue is very rich in starch. Corm is the rootstock of plant (Ebrahimzadeh *et al.*, 1998). Each *C. cancellatus* species is 1.5 – 3 cm in length, corm coverage is rough and violent network of fibers, which can be easily identified through the variety of cold autumn *Crocus*. The leaf color is seen grayish-green. This species of corm has been used in local food in some areas and it is sold as local vegetables (Ahouran *et al.*, 2012). In *C. speciosus* corm coverage is made of leather with a brown neck. The leaves appear before the flowers and leaves are dark green in color (Izadpanah *et al.*, 2014). *C. speciosus* corm is collected as *C. cancellatus* in the spring in Iran and Turkey, is sold in local markets, and is used as raw and cooked and these leaves are also used in the preparation of cheese in Turkey (Ozdemir and Kilinc, 2008). The use of genetic markers is as old as human history. The first man used the morphological markers to identify and distinguish different types of seeds, fruits and animals without even knowing it (Naghavi *et al.*, 2009). Izadpanah *et al.* (2009) studied genetic diversity of 39 accessions of *C. sativus* and two species using morphological and RAPD marker and concluded that stigma and style had significant positive correlation with the width of the sepals and petals at the level of five percent, and the length of sepals and petals at the level of one percent. Ozdemir *et al.* (2008) studied the morphology and taxonomy of *C. speciosus* in Turkey and reported that the style of the crop is above the stamen and divided into several branches. Kandemir (2010), with morphological and anatomical survey on two endemic and wild genera species of *Crocus*, concluded that the plant height is 12-15 cm and the number of leaves varied between 3-7 and determined the morphological characteristics of this species. Genetic and morphological close determination of *Crocus* is quite complex because of the lack of certain characteristics and high range and little research has been done on the morphological species of *Crocus*. Morphological information relates only to the Gahreman′s flora. The aim of this study was evaluation of morphological traits in two wild *Crocus* species and *C. sativus*.

Materials and methods

A total number of 24 samples of the species *C. cancellatus* and *C. speciosus* were collected from 24 regions of five Iran's provinces in the spring year of 2011. It started gathering the flowers in autum-2010 (October-November) with the aim to investigate their morphological traits. Sixteen morphological traits have been measured (Table 2). From each genotype, ten samples were used for morphological traits evaluation.

Data analysis and statistical calculations

The mean of traits was utilized in determining their simple correlation. Cluster analysis was performed by SPSS software (version 19) and cluster analysis was done by Ward's method.

Results and discussion

The minimum and maximum values, variation coefficients and mean of 16 traits have been shown in Table 2. The largest variation coefficients were related to tepal length, stigma length and flower weight. The highest mean of these traits was related to the genotypes belonging to *C. speciosus*. *C. sativus* was totally different from *C. speciosus* in the leaf number

(10.18), tepal length (5.18 cm), flower weight (0.99 g), flowering stem height (9.51 cm) and stigma length (10.86 cm), but it had no significant difference with *C. cancellatus*. In factor analysis, the above-mentioned traits had the highest effects on the first factor. Moreover, considering some traits as the number of tunics and the corm dry weight, *C. sativus* was different from *C. cancellatus*, but was similar to *C. speciosus* and regarding the other traits, no significant difference were observed between *C. sativus* and two wild species.

Table 1. Names, Collection site and habitat information of wild *Crocus* (*C. cancellatus* & *C. specious*) and *Crocus sativus*.

Sample No	Species	Collection Site
1	*C. speciosus*	Songhor-Kermanshah
2	*C. speciosus*	Sahne-Kermanshah
3	*C. speciosus*	Kangavar-Kermanshah
4	*C. speciosus*	Ravansar-Kermanshah
5	*C. speciosus*	Bistun-Kermanshah
6	*C. cancellatus*	Dehloran-Ilam
7	*C. cancellatus*	Meyme-Ilam
8	*C. cancellatus*	Margh-Golpaygan
9	*C. cancellatus*	Vanshan-Khansar
10	*C. cancellatus*	KhaneMiran-Arak
11	*C. cancellatus*	Shazand
12	*C. cancellatus*	Saki-Shazand
13	*C. cancellatus*	Muchan-Shazand
14	*C. cancellatus*	CheshmePahn-Ilam
15	*C. cancellatus*	Varche-Khomeyn
16	*C. cancellatus*	Kerk-Khomeyn
17	*C. cancellatus*	Kajarestan-Khomeyn
18	*C. cancellatus*	Azna
19	*C. cancellatus*	Khomeyn
20	*C. cancellatus*	Abbarik-Shazand
21	*C. cancellatus*	Hassan Abad-Shazand
22	*C. cancellatus*	Kudazr- Khomeyn
23	*C. cancellatus*	Tajmar-Shazand
24	*C. cancellatus*	Khorram Abad
25	*C. sativus*	Arak

Correlation coefficient

A positive and significant correlation was observed between the tepal length, stigma length, stamen length, flower weight and the flowering stem height. The number of tunics had a negative significant correlation with the flowering stem height, stigma length, tepal length, tepal thickness and the flower weight, and a positive correlation with the corm dry weight. tepal length showed a positive and significant correlation with stigma length, stamen length, flower weight and the flowering stem height. The highest correlation was observed between the stigma length with the flowering stem height and the flower weight (r=+0.90 and r=+0.97 respectively), the flower weight with the flowering stem height (r=+0.93) and the tepal length with the flower weight and stigma

length (r=+0.91 and r=+0.92 respectively) (Table 3). Izadpanah *et al.* (2009) studied morphological and molecular markers of 39 accessions of C. *sativus* and two wild species and concluded that stigma and style had significant positive correlation with the width of the sepals and petals at the level of five percent, and with the length of sepals and petals at the level of one percent. Their results were different from this results in this study. Stigma length had a significant positive correlation at the level of one percent with the length of the stamen, flower weight and flowering stem height. While the length of the petals had significant positive correlation at the level of five percent with the length of stigma, the length of the stamen, flower weight and flowering stem height and petals thickness had significant positive correlation at the level of five percent with the length of stigma, the length of the stamen, flower weight and flowering stem height.

Table 2. Traits variation range and variation coefficients.

Row	Trait	Unit	Minimum	Maximum	Mean	Variation coefficient (%)
1	Leaf length	cm	12.5	26.33	19.06	25.32
2	Leaf width	cm	1.80	2.73	2.14	26.32
3	Leaf number	Number	0.53	11.5	5.80	47.44
4	Leaf thickness	mm	0.47	5.5	0.67	19.67
5	Corm fresh weight	gram	2.11	8.87	4.78	49.48
6	No. of outer cover (tunics)	Number	3.8	11.1	6.88	39.28
7	Corm big diameter	mm	15.66	28.17	21.05	20.96
8	Corm small diameter	mm	1.83	24.46	19.35	21.71
9	Corm dry weight	gram	0.87	3.97	1.91	63.51
10	Petal thickness	mm	0.04	0.21	0.09	41.18
11	Tepal length	cm	3.41	5.72	6.19	90.48
12	Stigma length	cm	3.04	12.01	6.61	78.84
13	Stamen length	cm	1.57	4.02	2.49	24.63
14	Corolla thickness	mm	1.12	2.79	2.25	60.78
15	Flower weight	gram	0.16	1.16	0.49	70.07
16	Flowering stem height	cm	2.16	10.12	4.67	54.16

Factor analysis

In this analysis, four independent factors could justify a total of 79.93% of the total variance (Table 4). The first factor which justified 40.83% of the total variation of flowering stem height, stigma length, flower weight, petal length, length of the stamen and number of leaves per plant with positive coefficients (respectively, 0.97, 0.95 , 0.93, 0.95, 0.79, 0.80) justified most of the variance in this group. Since the characteristics were in one category and were associated with each other, selecting the one which is the highest value can be considered as representative of the characteristics to be considered in future studies.

The second factor is the big diameter of corm, the small diameter of corm and leaf length with positive coefficients (respectively, 0.92, 0.92, 0.76) and justified 19.63% of the total variance. These two factors together justified 60.47% of the total variance. The traits which played the key roles in the third factor were characteristics of Corm fresh weight and corm big diameter (with positive coefficients of 0.62, 0.66) that justified a total of 10.96% of the total variance. In the fourth factor, it was the thickness of the corolla and petals with positive coefficients (respectively 0.77, 0.64) that justified a total of 8.49% of the total variance. Factor analysis revealed that traits such as leaf length, number of leaves per plant, weight of corms, large and small diameter of corm, flower weight, flowering stem height, petal length and stamen are affecting characters in the diversity of these species.

Table 3. The correlation between 16 morphological traits in (*C. cancellatus* , *C. specious*) and *Crocus sativus*.

Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Trait	LL	WL	NL	LT	FO	NO	DO	SO	DW	PW	LP	LS	LF	CT	DL	WF
1	Leaf length	1															
2	Leaf width	0.116	1														
3	Leaf number	0.369	0.227	1													
4	Leaf thickness	0.321-	0.046	0.187	1												
5	Corm fresh weight	0.328	0.015-	*0.440	0.181	1											
6	No. of outer cover	0.212-	0.201-	**0.576-	0.026-	0.16	1										
7	Corm big diameter	0.642	0.005-	0.369	0.028-	**0.698	0.05	1									
8	Corm small diameter	**0.647	0.016	*0.442	0.116-	**0.620	0.023	**0.892	1								
9	Corm dry weight	0.018-	0.152-	0.159-	0.243	**0.611	**0.566	0.281	0.232	1							
10	Petal thickness	0.121-	0.152	0.364	0.244	0.001	*0.444	0.149-	0.154-	0.235-	1						
11	Tepal length	0.323	**0.559	**0.839	0.159	0.339	**0.608-	0.222	0.27	0.244-	0.365	1					
12	Stigma length	0.376	0.342	**0.814	0.17	0.359	**0.605-	0.205	0.209	0.272-	*0.427	**0.914	1				
13	Stamen length	0.394	0.337	**0.581	0.025-	0.291	0.348-	0.052	0.144	0.145-	0.059	**0.793	**0.827	1			
14	Corolla thickness	0.185-	0.282-	0.111-	0.131	0.033	0.112	0.022-	0.09-	0.022	0.257	0.241-	0.229-	0.353-	1		
15	Flowering stem height	0.301	**0.471	**0.728	0.192	0.241	**0.537-	0.107	0.103	0.243-	*0.413	**0.882	**0.906	**0.756	0.254-	1	
16	Flower weight	0.365	*0.403	**0.832	0.148	0.261	**0.631-	0.129	0.161	0.317-	*0.450	**0.925	**0.971	**0.798	0.263-	**0.932	1

Table 4. Factor analysis of the morphological characters of samples of the two wild.

	Factor	1	2	3	4
	Eigen values	6.53	3.14	1.75	1.35
	Variance (%)	40.83	60.47	71.43	79.93
Number	trait				
1	Leaf length	0.252	0.769	0.285-	0.201-
2	Leaf width	0.509	0.141-	0.025	0.372-
3	Leaf number	0.809	0.372	0.024	0.196
4	Leaf thickness	0.247	0.265-	0.661	0.330
5	Corm fresh weight	0.233	0.659	0.623	0.043
6	No. of outer cover	0.611-	0.039	0.488	0.257-
7	Corm big diameter	0.060	0.924	0.166	0.035
8	Corm small diameter	0.092	0.923	0.082	0.030-
9	Corm dry weight	0.302-	0.285	0.811	0.134-
10	Petal thickness	0.484	0.215-	0.030-	0.646
11	Tepal length	0.955	0.166	0.011	0.052-
12	Stigma length	0.954	0.175	0.015-	0.026
13	Stamen length	0.792	0.125	0.010-	0.357-
14	Corolla thickness	0.267-	0.007	0.056	0.774
15	Flower weight	0.939	0.041	0.020	0.053-
16	Flowering stem height	0.972	0.111	0.070-	0.004

Cluster analysis

Cluster analysis was done based on the characteristics of the four important factors. At a distance of 12 samples were divided into five groups (Fig 1). The first group included *C. cancellatus* sample from Muchan, Kajarestan, Abbarik, Tajmar and Dehloran samples that these genotypes were approximately at the same latitude. In the second and third group, *C. cancellatus* samples which included Varche, Kerk, Kudazr and Khorram Abad samples which had similar latitude and weather condition were collected. In the third group, there are *C. cancellatus* of Margh, Vanshan and samples of Azna, Khomein, Arak and Shazand and Meyme. The fourth group includes species of saffron (*C. sativus*) which was separated from the other two species. Domesticated saffron at

distance of 20 was similar to *C. cancellatus*, but distinct from the *C. speciosus*. The fifth Group included *C. speciosus* samples. The main characteristics of the separation of samples in this group rather than other groups were the number of leaves per plant, weight of the flower and the Flowering stem height which were different with the rest of the samples. Climatic conditions of these samples were different with other samples.

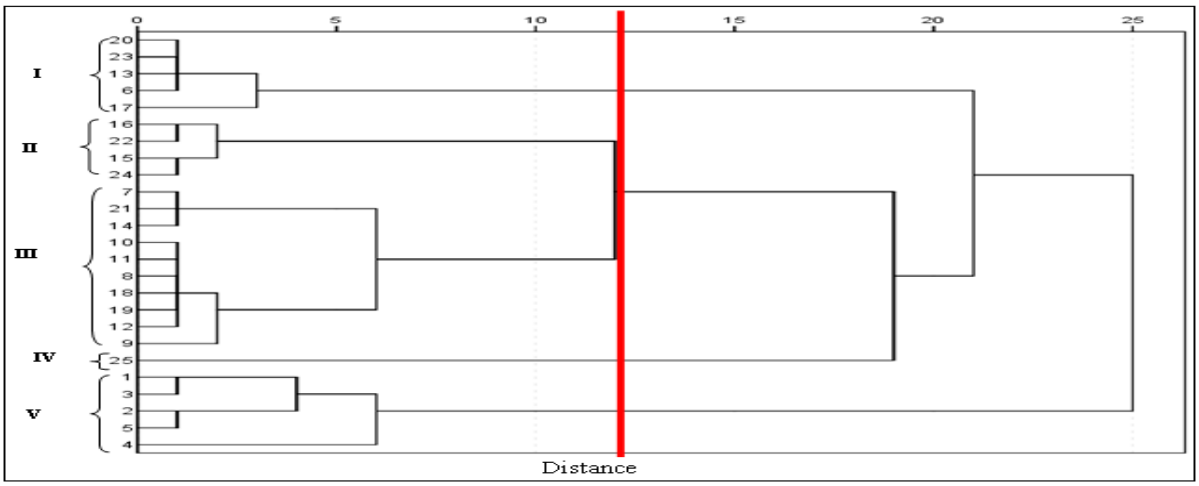

Fig. 1. Dendrogram of 25 *Crocus* samples using morphological traits, using Ward method (number of samples according to Table 1).

Conclusion

In this study, using morphological markers, the genetic diversity of 25 sampels of two wild species of *Crocus* from Iran (*C. cancellatus* and *C. speciosus*) with domesticated saffron were studied. The morphological characteristics of the samples were separated by species and climatic conditions. In the cluster analysis, *C. speciosus* was separated from *C. cancellatus* and *C. sativus* , while *C. sativus* at a distance of 12 were separated from two wild species, but at a distance of 20 was similar to *C. cancellatus*.

References

Ahouran M, Hosseini R, Zarghami R. 2012. Corms as a Source of Explants for the Successful Clonal Propagation of *Crocus cancellatus*. Journal of crop science and biotechnology **15,** 41-57.

Ebrahimzadeh H, Radjabian T, Karamian R, Abrishamchi P, Saboora A. 2006. Iranian saffron at Research. Ettelaat Publishers. 644. (in Farsi).

Ebrahimzadeh H, Saboora A, Ghaffari SM. 1998. Chromosomal studies on four Iranian *Crocus* species (Iridaceae). Iranian Journal of Botany **7,** 179-192.

Erol O, Kaya HB, Şik L,Tuna M, Can L, Tanyolac MB. 2014. The genus *Crocus*, series *Crocus* (Iridaceae) in Turkey and 2 East Aegean islands: agenetic approach. Turkish Journal of Biology **38,** 48-62.

Grilli Caiola M, Canini A. 2010. Looking for Saffron (*Crocus sativus* L.) parents. Functional Plant Science and Biotechnologhy **4,** 1-14.

Izadpanah F, Kalantari S, Hassani ME, Naghavi MR, Shokrpour M. 2014. Variation in Saffron (*Crocus sativus* L.) accessions and *Crocus* wild species by RAPD analysis. Plant Systematic and Evolution **300,** 1941-1944.

Izadpanah F. 2009. Primery evaluation of (*Crocus sativus* L.) accessions and *Crocus* wild species by morphological and RAPD markers. M. S. thesis,

University of Tehran, Iran.

Keify F, Beiki A. 2012. Exploitation of random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) markers for genetic diversity of saffron collection. Journal of Medicinal Plants Research **6(14),** 2761-2768.

Kandemir N. 2010. A Morphological and anatomical investigation about two rare and endemic *Crocus* taxa (Iridaceae) from Southern Anatolia. EurAsian Journal of BioSciences **4,** 54-62.

Molina RV, Valero M, Navarro Y, Guardiola JL, Garcı´a-Luis A. 2005. Temperature effects on flower formation in saffron (*Crocus sativus* L.). Scientia Horticulturae **103,** 361–379.

Negbi M, Dagan B, Dror A, Basker D. 1989. Growth, flowering, vegetative reproduction, and dormancy in the saffron (*Crocus sativus* L.). Israel Journal of Botany **38,** 95–113.

Naghavi MR, Ghareyazie B, Hosseini Salkadeh B. 2009. Molecular Markers. University of Tehran Press.

Ozdemir C, Kilinc M. 2008. Morphology and anatomy of three subsp. of *Crocus speciosus* BIEB. Bangladesh Journal of Botany **37(2),** 97-103.

Ozdemir C, Baran P, Akyol Y. 2006. The Morphology and Anatomy of *Crocus flavus* Weston subsp. *flavus* (Iridaceae). Turkish Journal of Biology **30,** 175-180.

Rubio Moraga A, Trapero-Mozos A, Gemez-Gemez L, Ahrazem O. 2010. Intersimple sequence repeat markers for molecular characterization of *Crocus cartwrightianus* cv. *Albus*. Industrial Crops and Products **32,** 147-15.