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STUDIES ON THE VARIABILITY OF MORPHOLOGICAL AND QUALITY CHARACTERS IN SAFFRON *(CROCUS SATIVUS)*

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Abstract
Saffron is an aromatic herb appreciated for its olfactory qualities, but also for its medicinal contributions, having multiple pharmaceutical properties. The cultivation of saffron in Romania is rather little known, the scientific data obtained being of interest for farmers who want to start such a culture. In this paper two populations of saffron were studied, the bulbs being of the same origin and age, grown in two different localities in western Romania: Izvin and Cicir. Regarding the quality characteristics, the two cultures belong to quality categories 1 and 2, and analyses were determined on moisture, colouring power (crocin), aromatic power (safranal) and bitterness power (picrocrocin). From a quantitative point of view, the variability of morphological characters, the manifestation of morphological characters, the coefficients of corallance on characters such as: number of bulbs, total diameter of bulbs, diameter of dominant bulb, length of leaflet, leaf length, leaf width, number of leaves dominant bulb, petal length, petal width, petal number, stamen length, stigma length were determined.

Keywords: Crocus sativus, quantitative parameters, qualitative parameters, variability

1. INTRODUCTION

Saffron is nicknamed the "golden spice", not because it is golden in colour, but rather, because saffron is the only spice worth its weight in gold. The history of saffron is not well documented. Many people think of *Crocus sativus* as a mutation of *Crocus cartwrightianus*, a wild species native to Greece that was selected and domesticated in Crete in the late Bronze Age.

Saffron is a perennial plant consisting of an underground, rounded, protruding bulb, 3 - 5 cm in diameter, enclosed in one or more brownish-brown fibrous tunics. It is a monocotyledonous bulbous plant belonging to the family *Iridaceae*, order *Liliaceae* and subfamily *Crocoideae*. The scientific name is *Crocus sativus*, the bulb is also called cormus or corm. Saffron is a sterile tripoid plant that does not grow wild, it is a reverse-growing plant (safran-marie.fr, sativus.com) .

Due to its folkloric popularity as a medicinal herb that cures a hundred diseases, modern medicine is detecting the mystery of saffron, studying its properties and taking advantage of the various potentials it offers to the medical world. As a result, over a hundred chemical components have been isolated and are available for commercial or medical applications (http://www.safran-marie.fr). The pharmacological effects of saffron are: antidepressant (Wang et al., 2010), anticonvulsant (Hosseinzadeh et al, 2005), in the treatment of memory disorders (Abe et al, 2000), in the treatment of tremor (Amin et al., 2015), respiratory (Boskabady et al., 2012), anticancer

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(Abdullaev et al., 1992), anti-inflammatory and analgesic (Moallem et al., 2014), on the reproductive system (Hosseinzadeh et al., 2008), protective (Bandegi et al., 2014), gastrointestinal (Al-Mofleh et al., 2006), on overweight (Gout et al., 2010), immunological (Boskabady et al., 2011), antidiabetic (Xiang et al., 2006), dermatological (Khorasani et al., 2008), antiparasitic (Yousefi et al., 2014).
Mature and larger bulbs have developed more flowers and daughter bulbs. Thus, one of the main goals of saffron production is to produce larger bulbs. It has been concluded that bulb size is an important factor in determining the presence or absence of flowers, even if the bulb does not reach the original size and produce flowers (Turhan et al., 2007). Environment, bulb origin and climatic conditions have a significant impact on flower number and stigma yield significantly altering the quantitative and qualitative traits of saffron (Amirnia et al., 2013).

2. MATERIALS AND METHODS

The biological material is saffron (C*rocus sativus*), the planting material has the same origins, the bulbs come from Holland. The study was carried out in two locations with different natural conditions: the locality of Izvin in Timiș county (Figure 1) and the locality of Cicir in Arad county.
The experiment was carried out for two years, the plots had an area of 2,200 m2, planting was done at 20-40 cm between rows and 10-15 cm between plants per row.
There were also differences between the two locations in terms of soil structure and content, with the soil in Izvin being more clayey and nutrient-poor, while in Cicir the soil is loamy and nutrient-rich.
The part of the saffron plant that is edible and valued in gastronomy is part of the female sexual organ, the stigma. The quality of saffron is quantified according to the level of three defining elements: aroma - determined by the concentration of safranal, colouring power - determined by the concentration of crocin and bitterness - determined by the concentration of picrocrocin. The spice is divided into 3 quality categories, determined by spectrophotometric analysis according to ISO 3632 1,2:2010/2011, where category I is the upper category, while category III is the lower category. The edible part of the plant, the stigmas were dried for 2 hours in a steamer at 45 ºC.
Samples for quality determination were taken from the harvested material, with two samples for each location: P1 and P2 from Izvin, P3 and P4 from Cicir.
Deterioration by biometric measurements on saffron plants was carried out:
- on the root part of the plants, examining the following characters in both populations studied: total bulb diameter, total number of bulbs, dominant bulb diameter, leaflet length (underground stem);
- on the aerial part of the plants, examining the following characters in both populations studied: leaf length, leaf width, leaf number of dominant bulb;
- on the flowers, examining on dry plants, independently of the other characters examined: petal length, petal width, petal number and stamen length in the Izvin population and stigma length in both populations, also independently of the other characters.
The data obtained from the biometric measurements were statistically processed, determining the estimated values of the mean, standard deviation of the mean and coefficient of variability and correlation coefficients. (Ciulca, 2006).

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(a) ***(b)***

Figure 1. Saffron plant from Izvin culture(a); Saffron plant from Cicir culture (b) (original)

3. RESULTS AND DISCUSSIONS

Total bulb diameter showed values between 5 and 6 cm, with a higher mean value in the Cicir population, the variability within them being small, and the variability of the Izvin population falling into the medium category.

The total number of bulbs in the clump in the Cicir population is double the mean value found in the Izvin population, with high variability in both cases. In the Izvin population, the dominant bulb is larger, both falling into the medium variability category (Figure 2). The length of the leaflet value depends strongly on the planting depth. For this reason the mean value of this characteristic is three times higher in the Izvin population than in the Cicir population, with the variability falling into the medium category. The leaves of the Izvin population are twice as long as those of the Cicir population. The coefficient of variability of the Izvin population is low, while the Cicir population has a coefficient in the medium category. Leaf width is somewhat correlated with leaf length, with both populations having medium variability. Regarding the number of leaves of the dominant bulb, the two populations are similar with a coefficient specific to the mean variability.

Determinations of floral characters were carried out on dried flowers only in the Izvin population. Petal length and stigma length are characters with low variability, while petal width and stamen length have medium variability. For the number of petals there is no variability, as saffron flowers consist of 6 petals. In the Cicir population, measurements were made only on stigmas, which are shorter than in the Izvin population.

For these characters, the differences in the behaviour of biological material may be due to differences in soil quality, the applied cultivation technology being slightly different, criteria also observed by Turhan. et al (2007) and Amirnia (2013).

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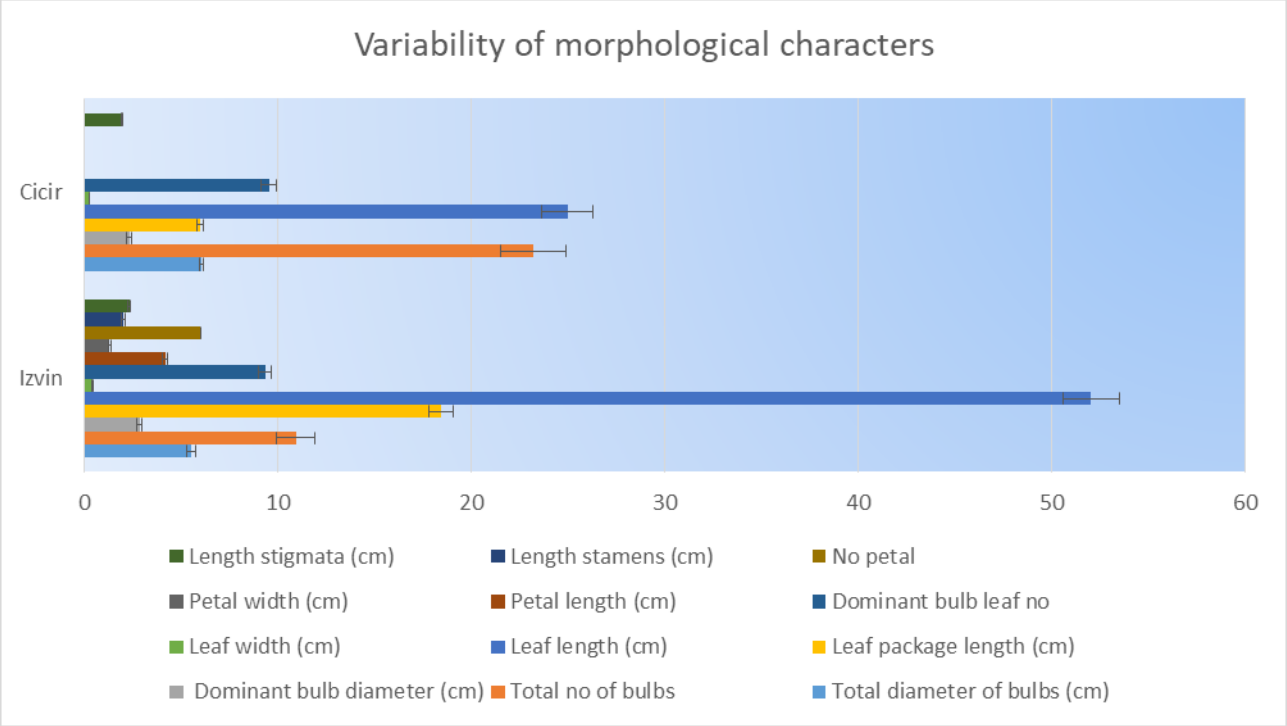

Figure 2. Variability of saffron morphological characters in the two studied populations

The two safflower crops were compared by means of mean values of morphological characters (Table 1). Total bulb diameter showed values ranging from 5.36 cm in the Izvin population to 6.05 cm in the Cicir population. By applying statistical calculation it is observed that there are no significant differences between the mean of the experiment and the two populations. The number of bulbs in the clump in the Izvin population is on average 10.93 bulbs, and in the Cicir population 23.20 bulbs determining very significant differences from the control. The means calculated for the diameter of the dominant bulb were 2.31 cm in the Cicir population and 2.86 cm in the Izvin population, with no statistical assurance compared to the experimental mean. Leaflet length averaged 5.387 cm for the Cicir plants and 18.43 cm for the Izvin plants, more than three times longer. Applying the statsitic calculation it appears that there are very significant differences between the two populations. Plants from Cicir had an average leaf length of 24.96 cm and those from Izvin had an average leaf length of 52.03 cm, between the two populations the differences are very significant. In the case of leaf width there was a very significant difference from the mean of the experiment, the mean of Cicir population was 0.26 cm, while the mean of Izvin population was 0.42 cm. The number of leaves of the dominant bulb in the Cicir population averaged slightly higher (9.53 cm) than in the Izvin population (9.33 cm) so compared to the population average there are no differences with statistical assurance. Determinations on stigma length were made in dry condition (Figure 3), the mean of the Cicir population was 2.13 cm while the Izvin population had a mean of 1.94 cm, so the differences from the mean were significant.

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Table 1. Results regarding the manifestation of morphological characters

Character	Population						Experiment average		
	Izvin			Cicir					
	Average	% to control	Difference from control/ significance	Average	% to control	Difference from control/ significance	Average	% to control	Difference from control/ significance
Bulbs diameter (cm)	5.63	97.29	-0.16	6.05	104.43	0.26	5.79	100	control
	LSD $_{5\%}$=0.79 cm; LSD $_{1\%}$=1.30 cm; LSD $_{0.1\%}$ =2.44 cm								
Bulbs number	10.93	64.06	-6.13^{000}	23.20	135.93	6.13***	17.06	100	control
	LSD $_{5\%}$=0.69; LSD $_{1\%}$=1.14; LSD $_{0.1\%}$ =2.14								
Dominant bulb diameter (cm)	2.86	110.56	0.27	2.31	89.43	-0.27	2.58	100	control
	LSD $_{5\%}$=0.60 cm; LSD $_{1\%}$=1.00 cm; LSD $_{0.1\%}$ =1.88 cm								
Leaflet length (cm)	18.43	151.05	6.23***	5.87	48.12	-6.33^{000}	12.20	100	control
	LSD $_{5\%}$=0.80 cm; LSD $_{1\%}$=1.33 cm; LSD $_{0.1\%}$ =2.49 cm								
Lef length (cm)	52.03	135.15	13.53***	24.96	64.84	-13.53^{000}	38.50	100	control
	LSD $_{5\%}$=2.15 cm; LSD $_{1\%}$=3.56 cm; LSD $_{0.1}$% =6.68 cm								
Leaf width (cm)	0.42	123.52	0.08***	0.26	76.47	-0.08^{000}	0.34	100	control
	LSD $_{5\%}$=0.02 cm; LSD $_{1\%}$=0.03 cm; LSD $_{0.1\%}$ =0.07 cm								
Dominant bulb leaf number	9.33	98.93	-0.10	9.53	101.06	0.10	9.43	100	control
	LSD $_{5\%}$=0.98 cm; LSD $_{1\%}$=1.63 cm; LSD $_{0.1\%}$ =3.06 cm								
Stigmata length (cm)	1,94	90,93	-0,19^{0}	2,32	109,06	0,19*	2,13	100	control
	LSD $_{5\%}$=0.16 cm; LSD $_{1\%}$=0.29 cm; LSD $_{0.1}$% = 0.50 cm								

The correlations between the studied morphological characters was established for each population separately, and a comparison of the correlation coefficient values was made. For flower characters, only the Izvin population was studied.

In the Izivn population, the correlation coefficients had low values, which shows that there are no important relationships between the morphological characters of the plants (Table 2). The highest value of correlation coefficients is present for the link between leaflet length and leaf length (0.805 - highly significant). Also very significant is the link between bulb clump diameter and number of bulbs in the clump (0.720 - distinctly significant). Other correlations worth considering are those between the number of bulbs in the clump and the diameter of the dominant bulb (0.496) and that between the diameter of the dominant bulb and the number of leaves of the dominant bulb (0.448).

Table 2. Results regarding the values of correlation coefficients for the realities between characters for Izvin population

Character	Numbar of bulbs	Diameter of dominant bulb	Leaflet length	Leaf length	Leaf width	Number of leaves of dominant bulb
Bulb diameter	0.720**	0.202	0.178	0.510	0.339	0.158
Bulb number		0.496	0.281	0.184	0.155	0.161
Diameter of dominant bulb			0.380	0.298	0.083	0.448
Leaflet length				0.805***	0.245	0.176
Leaf length					0.173	0.331
Leaf width						0.122
$r_{5\%}$ =0.514 $r_{1\%}$= 0.641 $r_{0.01\%}$ = 0.760						

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The Cicir population does not have very high correlation coefficient values either (Table 3). There are high values, but for other relationships, compared to those found for the Izvin population. The highest value of 0.631 (significant) was established for the correlation between bulb clump diameter and leaflet length. A value approximately equal to this (0.627 - distinctly significant) was also found for the correlation between the number of bulbs in the clump and leaf width. The number of bulbs in the clump was also correlated with leaf length (0.595 – significant). Significant correlation is also found for the correlation between bulb length and leaf length (0.517). As a result, in the Cicir population, leaf size is dependent on the number of bulbs in the clump. There are also relationships between leaf length and leaf width (0.481), and between bulb clump diameter and other characters: number of bulbs in the clump (0.486), leaf length and number of leaves of the dominant bulb (0.416 in both cases). We consider significant only the correlations that leaves are longer in plants with higher numbers of bulbs in the clump and longer leaflet.

Table 3. Results regarding the values of correlation coefficients for the realities between characters in Cicir population

Character	Numbar of bulbs	Diameter of dominant bulb	Leaflet length	Leaf length	Leaf width	Number of leaves of dominant bulb
Bulb diameter	0.486	0.031	0.631*	0.416	0.167	0.416
Bulb number		0.054	0.293	0.595*	0.627**	0.126
Diameter of dominant bulb			0.126	0.100	0.391	0.000
Leaflet length				0.517*	0.031	0.181
Leaf length					0.481	0.272
Leaf width						0.054
$r_{5\%}$ =0.514 $r_{1\%}$= 0.641 $r_{0.01\%}$ = 0.760						

The correlation study for flower characters was performed only for the Izvin population flowers. Measurements were made on dry flowers so that these characters could not be correlated with other plant characters. From the data in Table 4, there is a very strong relationship between stigma length and petal width (0.509), which is also the most important correlation. Petal dimensions, width and length, are correlated with stamen length (0.580 and 0.587 respectively, both with significant assurance).

Table 4. Results regarding the values of correlation coefficients for the realities between flower characters in Izvin population

Character	Petal width	Stamen length	Stigma length
Petal length	0.392	0.587*	0.000
Petal width		0.580*	0.509
Stamen length			0.288
$r_{5\%}$ =0.514 $r_{1\%}$= 0.641 $r_{0.01\%}$ = 0.760			

Table 5 shows the characteristics analysed for the four saffron samples. The three quality characteristics analysed - crocin, safranal, picrocrocin - are influenced by the weather conditions during the harvesting period, so that rain, dew and clouds can negatively influence the quality category, the influence of cultivation conditions are also mentioned by A. Giorgi (2017) (Giorgi et al., 2017). Also, if saffron is not harvested at the optimal time, in the morning before the flower opens, the crocin may be negatively influenced, as stigmas suffer slight discoloration when exposed

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to direct sunlight, the importance of saffron quality control is also supported by Moratalla-Lopez (2020) (Moratalla-Lopez et al., 2020).
The 4 samples analysed belong to quality categories I and II according to ISO standards (ISO 3632 1,2:2010/2011) - quality I (CI) includes samples P2 and P3, and quality II (CII) includes P1 and P4. Significant differences in humidity content are observed for P3 (3.79%). Crocin has the highest value in P3 (216.10), safranal is in high quantities in P1 (68.28), while in the case of picrocrocin the values are very similar, P1 having the highest value (88.20). The differences that occur may be due to harvesting and drying conditions, but also to sampling.

Table 5. Quality categories of analyzed saffron

Samples	Humidity (%)	Crocin Colouring power	Safranal Aromatic power	Picrocrocin Bittering power	Quality categories
P1	8.43	163.93**	68.28	88.20	CII
P2	6.62	171.59*	39.69	80.64	CI
P3	3.79	216.10	46.12	85.78	CI
P4	7.30	161.67**	49.77	85.31	CII

* does not comply with the values of category I; ** does not comply with the values of category II; *** does not comply with the values of category III

4. CONCLUSIONS

The variability of morphological characters showed significant fluctuations and results in both populations studied.
In the case of Izvin population, it was observed that there are no significant links between the morphological characters of the plains, which is a consequence of the way of culture technologies implementation.In this population, the correlation coefficients were significant in terms of value, if we refer to aspects such as the link between the length of the leaflet and the length of the leaf; to the diameter of the bulb clump and the number of bulbs in the clump, to the number of bulbs in the clump and the diameter of the dominant bulb and last but not least that between the diameter of the dominant bulb and the number of leaves of the dominant bulb.
Within the Cicir population the same common element appears, which creates both a differentiation and a correlation, namely the characteristic of the technology of culture implementation. Here again we find that there are not very high values of the correlation coefficients, but we find significant differences from the Izvin population in characteristics such as: diameter of the bulb clump and length of the leaflet, number of bulbs in the clump and leaf width, length of the leaflet and leaf length, as a result in the Cicir population leaf size depends on the number of bulbs in the clump.
Regarding the quality and characteristics of the stigmas, we can conclude that factors such as weather conditions during the harvesting period and the optimal time of harvesting may have an undesirable impact on the quality characteristics of the crop.
Between the two populations it is noticeable that the number of bulbs is double in the Cicir population compared to the Izvin population, also the total diameter of the bulbs is larger in the Cicir population. But at the same time, in the Izvin population, the dominant bulb has a larger diameter compared to the dominant bulb in the Cicir population. Regarding the length of leaflet in Izvin population is three times longer than in Cicir population. As for stigma length, those from Cicir are shorter than those from Izvin, and the differences are significant. These differences may be due to differences in soil structure and composition, but also to the cultivation technologies used.

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