Ralli PE *et al*. (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 2, Article number 13556
DOI:10.15835/nbha52213556
Research Article

Corm size and planting density affect the yield and longevity of saffron (*Crocus sativus* L.)

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Abstract

The effect of the corm size of saffron (*Crocus sativus* L.) and the planting density was determined on a field study for six years. The objective of the present study was to determine the impact of the corm size and planting density on flower and stigma yields. Two corm sizes were used: corms with a diameter up to 2.5 cm and corms with a diameter greater than 2.5 cm, and five planting density: 22, 27, 33, 44 and 67 corms/m^2 with planting distances 30, 25, 20, 15 and 10 cm on the plant row, respectively. The results showed that the year (growing season) and the interaction of year (growing season) with corm size affected all the characteristics that were studied. Over the six-year study period, there was a gradual increase in flowering from the first to the third year, followed by a decline in the fifth and sixth years. The yield of larger corms (greater than 2.5 cm) was higher during the first three years, while smaller corms (less than 2.5 cm) produced higher yields in the fourth through sixth years. On the contrary, the planting density, did not significantly affect the characteristics studied over the six years of cultivation and therefore did not affect the stigma yield of saffron. Therefore, corm size together with the environment are important factors that can affect the productivity and the longevity of the crop and should be considered when planting a new saffron cultivation.

Keywords: corm size; flower yield; longevity; planting distance; stigma yield

Introduction

Saffron (*Crocus sativus* L.) is widely considered to be the most important cultivated species of Iridaceae family and is widely dispersed around the world (Gresta *et al*., 2008a). Saffron is a species that is sterile triploid and it is propagated through corms (Gresta *et al*., 2008a; Agayev *et al*., 2009; Nemati *et al*., 2019). *C. sativus* is considered to be derived from the wild species *Crocus cartwrightianus* Herbert, which is still found in Greece and especially in the Cyclades islands (Chichiriccò, 1984; Heywood and Zohary, 1995; Mathew, 1999; Grilli Caiola, 2004, 2005; Nemati *et al*., 2019). The main product that is produced from *Crocus* species is the dried stigmas which are particularly rich in crocetin, crocin, picrocrocine, and safranal that is found in *Crocus sativus* and some allies, among other carotenoid secondary metabolites (Fernández, 2004; Kumar *et al*., 2009). Saffron is used as a food colorant and also as a spice and in addition it has many interesting medicinal properties

Received: 12 Dec 2023. Received in revised form: 23 Mar 2024. Accepted: 21 May 2024. Published online: 12 Jun 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

including its analgesic, sedative, anti-spasmodic, carminative, diaphoretic, antioxidant, has a cytotoxic effect on cancer cells, and is anticonvulsant and exhibits antidepressant properties (Sampathu *et al*., 1984; Zhang *et al*., 1994; Abdullaev and Frenkel, 1999; Abdullaev, 2002; Akhondzadeh *et al*., 2005; Kumar *et al*., 2009; Mohtashami *et al*., 2021).

C. sativus is a species that is known to be well adapted to the Mediterranean climate and especially to the cycle warm–cool–warm. The production of progeny corms starts during or right after the flowering that happens during autumn (Kumar *et al*., 2009). Flowers are also initiated at the end of summer in August and beginning of September when there is relatively high temperature (Negbi *et al*., 1989; Benschop, 1993). The optimal temperature for flower initiation is 23-27 °C and the flower emergence requires temperatures below 15-17 °C (Plessner *et al*., 1989; Molina *et al*., 2004, 2005). Corms from saffron have a lifespan of one year and the mother corm provides the flower and the clonal reproduction in the next annual cycle (Rees, 1988). Vegetative growth happens when there is soil moisture during winter and spring period while during the summer the corms are in the state of dormancy (Gresta *et al*., 2008a; Kumar *et al*., 2009). In order for the crop to grow in autumn there is a need for soil moisture (Negbi *et al*., 1989; Gresta *et al*., 2009; Yau *et al*., 2006).

The most important yield components of saffron are the number of flowers per area (Agayev *et al*., 2009). Mother corm size is critical to the production of saffron and in order for saffron corms to produce flowers there is a need to be above a certain size (Negbi *et al*., 1989; Benschop, 1993; Kumar *et al*., 2009). During the cultivation most farmers use corms with a diameter 2.5-3 cm (~8-16 g) (Kumar *et al*., 2009). In the literature different researchers used a wide range of corm sizes as Negbi *et al*. (1989) found that in order for corms to produce flowers they had to be larger than 3.2 g while other researchers found that corms larger than 2.5 cm [~7.3 g] should be used for planting in order to ensure flower production (Plessner *et al*., 1989; Tammaro, 1999) since 2.5 cm is considered the smallest size below which corms need to be placed in a nursery for vegetative reproduction (Gresta *et al*., 2008a). In another study it was found that corms smaller than 6.9 g did not flower (MacGimpsey *et al*., 1997) and more recent publications revealed that small corms (below 6 g) usually do not flower in the first year and also exert a negative effect on the flowering of coming years when saffron field is used for several years in one planting time (De Juan *et al*., 2009; Koocheki *et al*., 2016a, b, 2019a). On the other hand, as the corm size increases there was an increase in flower production (Negbi *et al*., 1989; De Mastro and Ruta, 1993; De Juan *et al*., 2009; Poggi *et al*., 2010; Koocheki *et al*., 2019a, 2019b; Esmaeilian *et al*., 2022). Also, the effect of corm size is not permanent, and it was reported that the higher yield that larger corms can produce can be lost after three years of cultivation (Çavuşoğlu and Erkel, 2009). The size of the corms can affect also the size of the progeny corms as Gresta *et al*. (2008b) found that bigger corms gave higher weight of progeny corms by 16.6% and De Juan *et al*. (2003) found higher rates of multiplication for progeny corms from bigger mother corms. From all these studies it is obvious that corm size affects the productivity and the production of progeny corms, however, most of them were annual experiments or limited to maximum three years and there are no reports for longer periods as saffron can be grown up to six or more years in the same field.

Although saffron yield is influenced by many agronomic, biological, and environmental factors that can exert a significant influence on production, production is strictly influenced by factors such as corm storage conditions (De Mastro and Ruta, 1993; Gresta *et al*., 2008 a,b), climatic conditions (Tammaro, 1999), sowing time (Gresta *et al*., 2008b), cultural techniques (annual or perennial), crop management (irrigation, fertilization, and weed control), and diseases (Gresta *et al*., 2008a). Since saffron remains in the same fields for several years, the growth and yield are affected by variations in the corm size distribution of the daughter corm populations in different years. Additionally, climate conditions and the climate change that our planet is facing can have a significant impact on saffron yield. Also, climate change created several problems in many countries the last years since there was low rainfall during corm formation and/or high temperature during flowering which reduced the flowering period and subsequently yield of stigma (Bukhari, 2020; Jones, 2023). Halevy

(1990) believes that temperature can be the most important regulator of saffron flowering. On the other hand, precipitation has a great effect on growth of roots. Changing precipitation patterns along with increasing evaporation caused by warming will increase water requirement of the plant and limiting plant yield. Due to strong correlation between saffron behavior, temperature, and precipitation, climate extreme events and future climate change seem to influence saffron cultivation and yield (Kouzegaran *et al*., 2020). Results of the later study indicated important role of changes in temperature and precipitation extreme events in saffron yield and future saffron yield was predicted based on the yield-extreme indices model.

Another important growing parameter is the planting density as it can affect the productivity and the yield components of the crop and especially the number of flowers per m^2 (Douglas *et al*., 2014; MacGimpsey *et al*., 1997). Planting density depends on size of the corms, the number of years that the field is used, and the planting arrangements (Alonso *et al*., 2006). In the different countries that saffron is growing there are different densities that are used. According to a comparative study by Kafi and Showket (2007) the number of corms sown per unit of land in Kashmir (India) is much lower than Khorasan (Iran). For instance, 40-50 corms are sown in 1 m^2 of saffron farm in Kashmir compared with 150-250 corms/m^2 in Khorasan. In addition, only a single corm per hill is sown in Kashmir (at a depth of 10-15 cm) in contrast to 3-15 corms per hill (at 15-20 cm depth) in Khorasan (Husaini *et al*., 2010). In Castile-La Mancha (Spain) and Sardinia (Italy), where production period lasts 3 to 4 years, planting takes place at a depth of 15 to 20 cm and the distance between corms is 3,3 to 15 cm. In Castile-La Mancha (Spain) the normal planting density is 60 corms/m^2. In Sardinia, where corms are more expensive, planting density varies between 10 and 50 corms/m^2. In Western Macedonia (Greece), planting density is at average. In Greece the density that is used is in rows that are 10-20 cm apart and on the row 3,5-15 cm from one corm to another and are planted at a depth of 20-25 cm (Alonso *et al*., 2006). Therefore, the flowering and the production ability were studied over a range of planting densities and also the effect of corm sizes which is an important characteristic for the productivity of a saffron crop and the longevity of the crop.

The hypothesis that was tested was whether larger corms (above 2.5 cm in diameter) compared with smaller corms (below 2.5 cm in diameter) can contribute to high yield for the whole duration of the cultivation which can be up to six years. In addition, it was tested whether different planting densities (five treatments: 22, 27, 33, 44 and 67 corms/m^2 with planting distances 30, 25, 20, 15 and 10 cm on the plant row, respectively) can affect stigma yield of the saffron over a period of six years of cultivation.

Materials and Methods

Study site and crop management

A field experiment was established for six successive years (growing seasons) (from 2009 up to 2014) at the Farm of the Hellenic Agricultural Organization-DIMITRA (ELGO-DIMITRA) in Thessaloniki in northern Greece (40.536401, 22.999239). The soil at the experimental site has a sandy loam (SL) texture with pH 7.75, organic matter content 1.20%, electrical conductivity 0.799 mS/cm, $CaCO_3$ 3.1%, P (Olsen) 46.76 ppm, and K 210 ppm (0-30 cm depth). The previous crop at the site was durum wheat (*Triticum turgidum subsp. durum* L.). Seedbed preparation was conducted before the planting of the experiment and included moldboard plough, disk harrow, and cultivator. Before planting, diammonium phosphate (20-10-0) was incorporated into the soil at a rate of 80 and 40 kg/ha of nitrogen and P_2O_5, respectively, according to the standard practice of farmers. The experiment was conducted in the same field for six consecutive years. Before planting a net with a mesh diameter of 2.5 cm was placed at a depth of 25 cm, in order to avoid corm loses from moles and other animals. Hand weeding and tilling were used when it was necessary for weed control. During the six years the standard crop management practices were applied that are used by the farmers in Greece (Goliaris, 1999). The weather data (rainfall, temperature) were recorded daily with an automatic weather station which was close to the experimental site and were reported as mean monthly data for the six years of the experimentation and are given in Table 1.

Table 1. Weather data (mean monthly temperature and rainfall) for the area where the experiments took place

Month / Year	Mean monthly temperature (°C)							Rainfall (mm)						
	2009	2010	2011	2012	2013	2014	30-years average	2009	2010	2011	2012	2013	2014	30-years average
January	7.1	5.9	6.5	3.8	7.9	8.6	5.2	86.8	10.0	19.6	35.8	0.0	27.6	29.3
February	8.1	7.9	7.7	5.6	8.9	9.8	6.4	2	106.6	22.4	9.2	97.2	49.0	31.5
March	10.1	10.3	9.3	11.0	10.7	11.8	9.6	43.4	32.4	30.6	32.8	36.4	76.6	31.5
April	13.9	15.0	13.3	15.1	15.3	14.4	13.9	21.6	19.4	22.2	52.6	11.0	76.6	38.3
May	20.3	19.9	18.2	19.7	22.0	19.3	19.3	18.6	49.0	128.6	59.6	7.4	15.4	44.4
June	23.9	23.9	23.8	26.6	24.8	23.5	24.5	48.4	77.6	28.0	7.4	16.2	19.0	32.4
July	27.5	26.8	27.6	29.7	26.9	26.0	26.7	6.0	31.4	2.4	0.0	70.2	0.0	30.5
August	26.0	28.8	26.6	28.0	27.7	18.6	26.0	74.0	4.4	13.0	4.6	11.8	89.2	24.0
September	21.6	22.6	23.9	23.3	22.8	21.1	21.7	37.6	12.0	46.8	42.4	25.4	83.2	29.4
October	17.4	14.8	14.7	20.3	17.1	12.8	16.3	22.0	155.2	26.6	12.4	7.4	65.0	42.0
November	12.4	14.9	8.6	14.8	13.8	8.4	10.3	26.8	15.2	12.4	3.4	23.2	211.8	60.9
December	10.3	7.6	7.4	9.0	6.4	8.6	6.5	63.8	15.6	15.7	0.0	33.4	27.6	50.7
Mean annual temperature	16.6	16.5	15.6	17.2	17.0	15.2	15.5							
Total annual rainfall								451.0	528.8	368.3	260.2	339.6	713.4	439.7

Field experiment and measurements

Saffron corms about 30 kg were collected during June 2009 from farms in the area of Kozani (Northern Greece) where saffron is traditionally growing. The corms were planted at a depth of 15 cm with five different distances on the row on 29 August 2009 in four replicates of five rows in each plot (15 cm apart). The five different distances that were used were 30, 25, 20, 15, and 10 cm on the row and resulting in 5 densities (D1-D5) of 22, 27, 33, 44 and 67 corms/m^2, respectively. In addition, corms were separated into two different sizes above 2.5 cm in diameter (S2) and below 2.5 cm in diameter (S1).

The planting positions of individual corms in each plot were marked. From the beginning of autumn, the flowering was recorded and also they were hand-picked daily. The flowers from the original mother corm position were counted, weighed and the fresh stigmas were separated from the other parts of the flower and weighed. The stigmas from the flowers were then dried in shade conditions, at room temperature for 48-72 h and weighted again. The duration of flowering was recorded during the experiment from the beginning of flowering to the end of flowering in each plot, the number of flowers that were produced per corm and per m^2, fresh weight of flowers per m^2, fresh weight and dry weight of stigma produced per m^2, length of stigmas as well as other descriptors for characterization and evaluation (Bioversity International, 2015). All the management practices including planting of corms, weeding, harvesting and separating the different flower parts were done by hand (Figure 1).

Figure 1. The parts of *Crocus sativus* (1): corm and tunics, (2): leaves, (3): perianth tube, (4): flower, (5): stamens, (6): anthers (7): stigma and flowers harvest (bottom middle)

Statistical analysis

The experimental data for fresh weight of the whole flower (g/m^2), fresh weight of stigma (g/m^2), number of flowers/m^2, dry weight of flower (g/m^2) and dry weight (yield) of stigma (g/m^2) were analyzed with the ANOVA method according to a mixed linear model. The statistical analysis involved the effects (main and interactions) of three factors: "Corm Size" with 2 levels (S1<2.5 cm and S2>2.5 cm), "Planting Densities" with 5 levels (coded as D1-D5), and "Year" with 6 levels (2019-2014, coded as Y1-Y6). More specifically, the experiment was set up according to a Randomized Complete Block Design (4 blocks) with a split-split plot arrangement. The two levels of "Corm Size" were considered as the main plots, the levels of "Planting Densities" were considered as the sub-plots, and the 6 levels of "Year" were considered as the sub-sub plots, since "Year" is a factor with repeated measures. Therefore, a combined analysis over years was carried out using the aforementioned design (Gomez and Gomez, 1984; Steel *et al*., 1997). Preliminary statistical analyses showed that within the methodological frame of mixed linear models some basic assumptions of the ANOVA method were not fulfilled. So, prior to ANOVA and the multiple comparisons of the mean values, the data for the 5 measured plants' characteristics were, where appropriate, $\log_{10}(X)$ or $\log_{10}(X+1)$ transformed in order to achieve normality and homoscedasticity of the corresponding models' residuals. Data presented in tables (mean values) are in their original scales. Where appropriate, all pair-wise differences among the mean values of the examined effects were tested with the protected Least Significant Difference (LSD) criterion. All mean values were estimated from the corresponding linear models. The ANOVA method was used mainly for estimating the correct standard errors of the differences among mean values compared with the LSD criterion. The significance level of all hypotheses testing was preset at a=0.05 ($P\leq0.05$). All statistical analyses were performed using the IBM SPSS Statistics v.23 software package. One of the authors developed and programmed a special SPSS syntax code and an MS-Excel worksheet for performing the statistical analysis, testing the effects of the proposed model and computing the LSD criterion values.

Results

The weather conditions varied significantly during the six years of the experiment (Table 1). In some years, rainfall was significant during autumn and throughout the year, while in others, it was quite low (Table 1). This can affect the growth stages of saffron and the yield. The annual rainfall ranged from 260.2 mm to 528.8 mm during the six years, indicating high variation in annual rainfall. Similarly, the mean annual temperature ranged from 15.2°C to 17.2°C during the six years, which can significantly affect the growth and development of crop plants, especially saffron.

The factor 'Year' was found to have a significant effect on all the characteristics that were studied. Furthermore, the interaction between the factors 'Year' and 'Corm Size' was found to have a significant effect on most of the characteristics (Table 2). However, neither the main effect of the factor 'Planting Density' nor its interactions with the other two factors were found to be statistically significant. Additionally, there was no significant three-way interaction among the factors 'Corm Size', 'Density', and 'Year' for all the measured plant characteristics (Table 2).

Table 2. Analysis of variance results (significance of the effects) for testing the effects (main and interactions) of Corm Size (S), Planting Density (D), and Year (Y) (growing season) on the log transformed values of the measured plants' characteristics

	Plants' characteristics					
Effects	**Flower fresh weight yield**	**Stigma fresh weight yield**	**Stigma length**	**Number of flowers per m²**	**Flower dry weight yield**	**Stigma dry weight yield**
Size (S)	NS[a]	NS	NS	NS	NS	NS
Density (D)	NS	NS	NS	NS	NS	NS
D×S	NS	NS	NS	NS	NS	NS
Year (Y)	*[b]	*	*	*	*	*
Y×S	*	*	NS	*	*	*
Y×D	NS	NS	NS	NS	NS	NS
Y×D×S	NS	NS	NS	NS	NS	NS

[a] "NS" Stands for not statistically significant effect at the a=0.05 level

[b] "*" Stands for statistically significant effect at the a=0.05 level

The duration of flowering over the six years (2009-2014) of the experiment and the variability of the mean number of flowers per m^2, over the five densities for the two corm sizes are described in Table 3 and also in the supplementary Figures S1-S6 and A1-A12. During the first year, flower picking started on 27 October 2009, two months after planting, and finished on 23 November 2009, and lasting 27 days (Table 3, Figures S6, A1 and A7). During the second year the flowering started on 9 November 2010 and finished on 2 December 2010, lasting for 23 days (Table 3, Figures S6, A2 and A8). In the third year, flowers were harvested from 8 November 2011, until 8 December 2011, lasting 30 days (Table 3, Supplementary Figures S6, A3 and A9). During the fourth year, there was a reduction in flowering duration as flowering started on the 6 November 2012, and finished on 25 November 2012, lasting 19 days, almost half the duration of flowering from the previous years (Table 3, Supplementary Figures S6, A4, and A10). In the fifth year, the situation was similar to the fourth year, as flowering started on 5 November 2013, and finished on 23 November 2013, lasting for 18 days (Table 3, Supplementary Figures S6, A5 and A11). Finally, during the sixth year, there was a significant reduction in the duration of flowering, starting on 1 November 2014, and finishing on 7 November 2014, lasting only six days (Table 3, Supplementary Figures S6, A6 and A12). Additionally, there was a shift in the peak of flowering during the six years of the study, and the peak of flowering was observed in the third year. Corms with a greater size showed the highest number of flowers, while during the fourth year, smaller corms showed the highest number of flowers (Table 3, Supplementary Figures S6, A1-A12).

Table 3. Timetable of the period of flowering over the six years (growing seasons) of the study

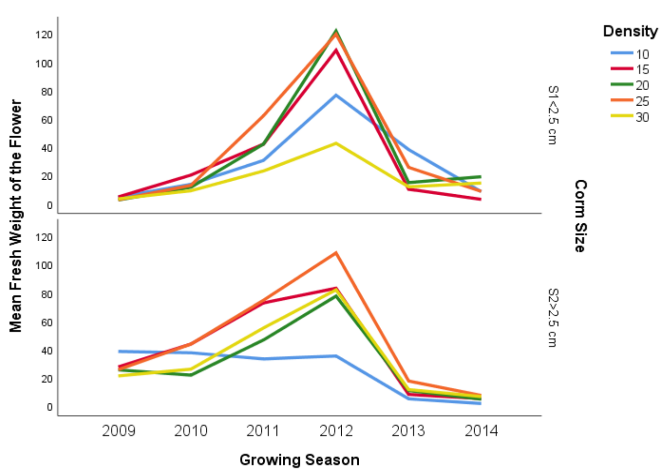
Year	Beginning of flowering	Peek of flowering	End of flowering	Duration of flowering (days)
2009	27-10-2009	7-11-2009	23-11-2009	27
2010	9-11-2010	16-11-2010	2-12-2010	23
2011	8-11-2011	23-11-2011	8-12-2011	30
2012	6-11-2012	14-11-2012	25-11-2012	19
2013	5-11-2013	13-11-2013	23-11-2013	18
2014	1-11-2014	4-11-2014	7-11-2014	6

There was an increase in the yield of fresh weight of flowers, fresh weight of stigmas, and the number of flowers per m^2 during the first four years, followed by a significant decline up to the sixth year (Table 4, Figures 2, 3 and 4, Supplementary Figures S1, S2, S3, S1.1, S2.1, S3.1).

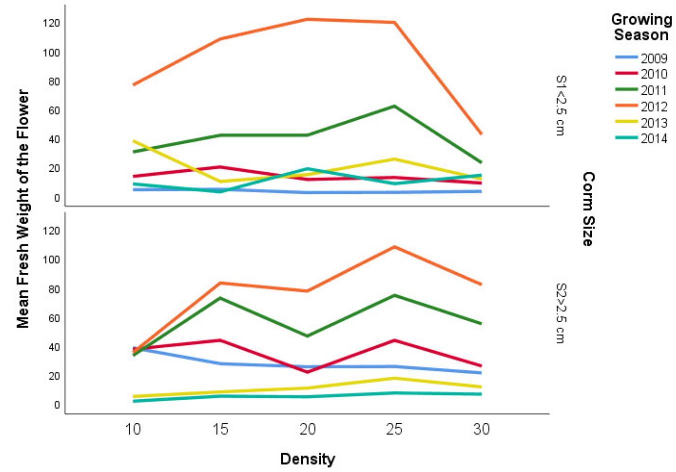
Table 4. Mean values of the original data of fresh weight of flower, stigma, and number of flowers/m^2 for the two different corm sizes (S1<2.5 cm and S2>2.5 cm) during the six years (growing seasons) of cultivation (Y1:2009, Y2:2010, Y3:2011, Y4:2012, Y5:2013 and Y6:2014)

Size × Year	^{z} Fresh weight of the flower (g/m^2)	^{z} Fresh weight of stigma (g/m^2)	^{z} Number of flowers/m^2
S1Y1	3.58a	0.33a	13ab
S1Y2	13.36c	1.25bc	35c
S1Y3	38.76d	3.09d	122d
S1Y4	93.76f	7.68f	229e
S1Y5	21.05bc	1.96c	66c
S1Y6	10.88bc	0.99bc	29bc
S2Y1	27.73d	2.63d	82d
S2Y2	34.58de	3.17d	90d
S2Y3	56.49de	4.59de	169de
S2Y4	76.98ef	6.39ef	193de
S2Y5	11.29ab	1.06ab	35bc
S2Y6	5.08a	0.45a	13a

^{z} Mean values in the same column followed by the same letter (or letters) are not statistically significant at a=0.05 according to the LSD criterion. Multiple comparisons were performed on the log transformed data. Data are averaged over the 5 planting densities and each mean value was computed from n=20 measurements.

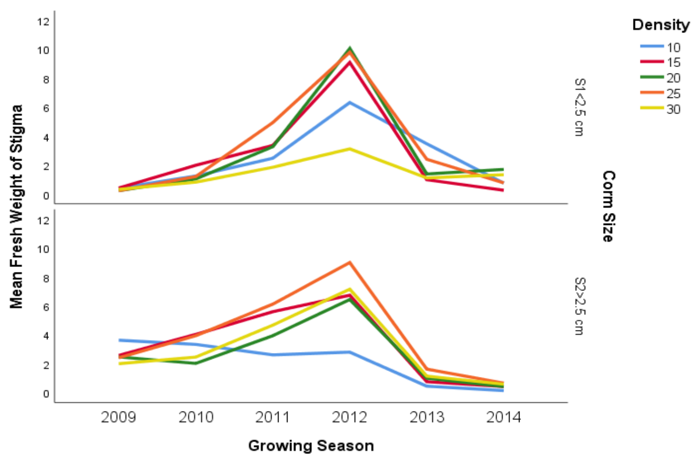


a)

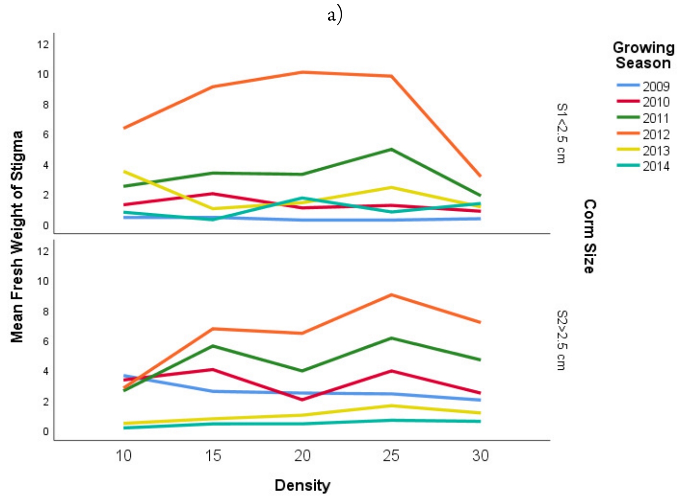


b)

Figure 2. Mean fresh weight of the flower (g/m^2) relative to year (growing season), planting density, and corm size (a) and relative to planting density, year (growing season), and corm size (b)



a)



b)

Figure 3. Mean fresh weight of stigma (g/m^2) relative to year (growing season), planting density, and corm size (a) and relative to planting density, year (growing season), and corm size (b)

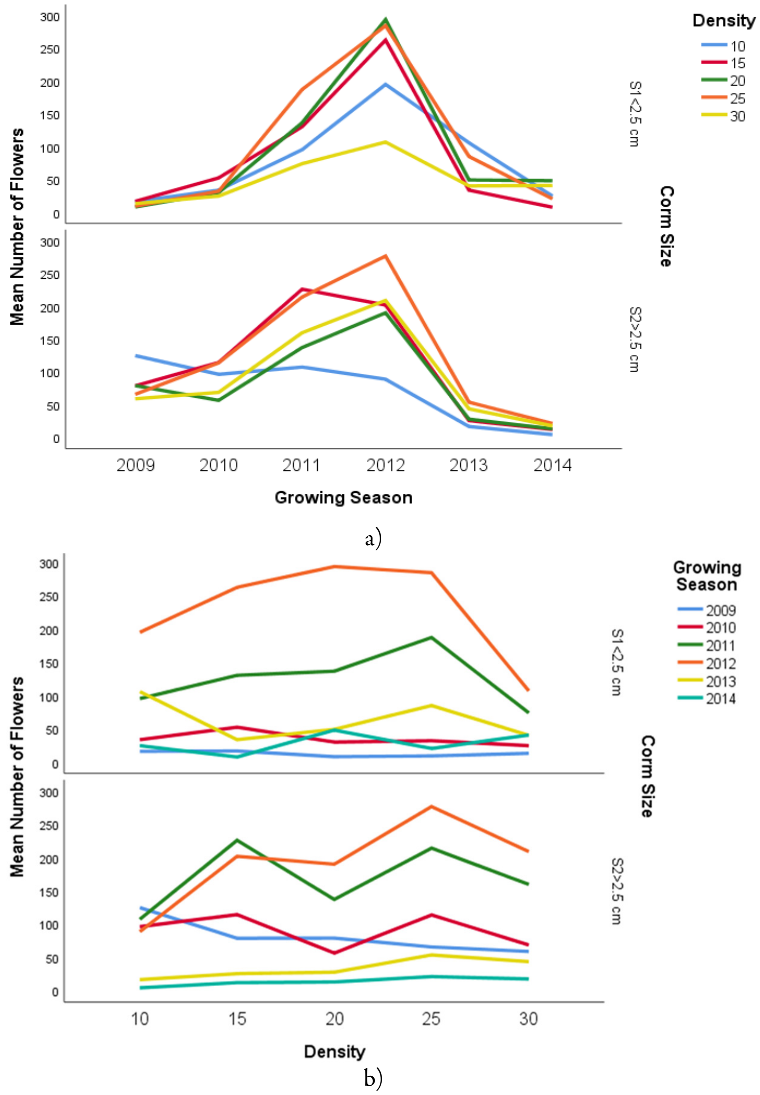


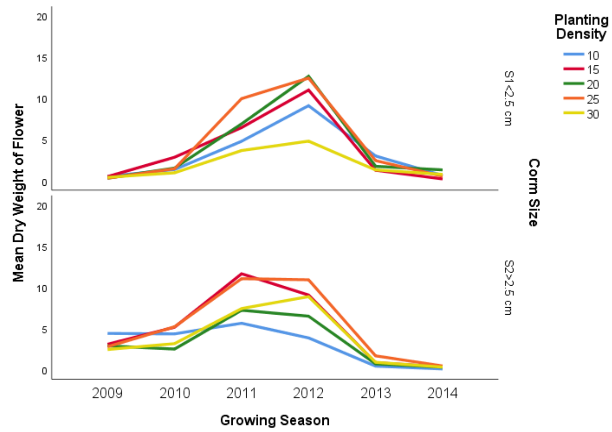
Figure 4. Mean number of flowers/m² relative to year (growing season), planting density, and corm size (a) and relative to planting density, year (growing season), and corm size (b)

The highest yield of fresh weight of the flowers and stigma, and the highest number of flowers were found in the fourth year for the S1 and S2 size, with yields of fresh weight of the flowers of 93.76 g/m² and 76.98 g/m², respectively, yields of fresh weight of stigmas of 7.68 g/m² and 6.39 g/m², respectively, and numbers of flowers of 229 and 193 flowers per m², respectively. A similar trend was found in the case of the dry weight of flowers, and the dry yield of stigmas increased from year one up to the fourth year for the S1 treatment, and for the treatment S2, it reached its highest point in the third year (Table 5, Figures 5 and 6, Supplementary Figures S4, S5, S4.1, S5.1). However, these traits decreased gradually during the fifth and sixth years.

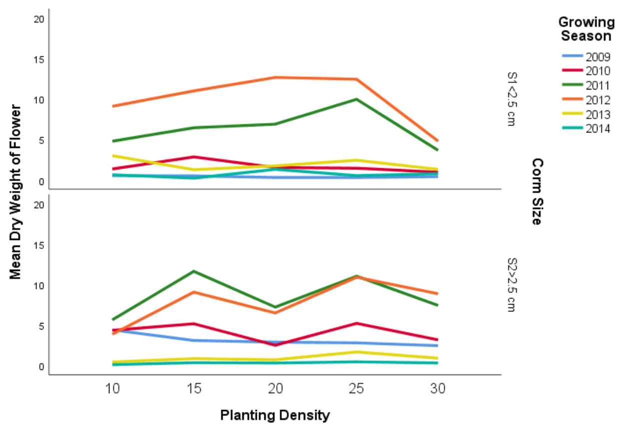
Table 5. Means values of the original data of dry weight of flower and dry weight (yield) of stigma for the two different corm sizes (S1<2.5 cm and S2>2.5 cm) during the six years (growing seasons) of cultivation (Y1:2009, Y2:2010, Y3:2011, Y4:2012, Y5:2013 and Y6:2014)

Size × Year	[z] Dry weight of flower (g/m^2)	[z] Dry weight (yield) of stigma (g/m^2)
S1Y1	0.44ab	0.06a
S1Y2	1.63c	0.21ab
S1Y3	6.17ef	0.76de
S1Y4	10.00g	1.31f
S1Y5	2.05c	0.33b
S1Y6	0.72b	0.12ab
S2Y1	3.15d	0.48c
S2Y2	4.10de	0.55cd
S2Y3	8.60fg	1.05ef
S2Y4	7.80ef	1.09e
S2Y5	1.01b	0.18ab
S2Y6	0.34a	0.06a

[z] Mean values in the same column followed by the same letter (or letters) are not statistically significant at a=0.05 according to the LSD criterion. Multiple comparisons were performed on the log transformed data. Data are averaged over the 5 planting densities and each mean value was computed from n=20 measurements.

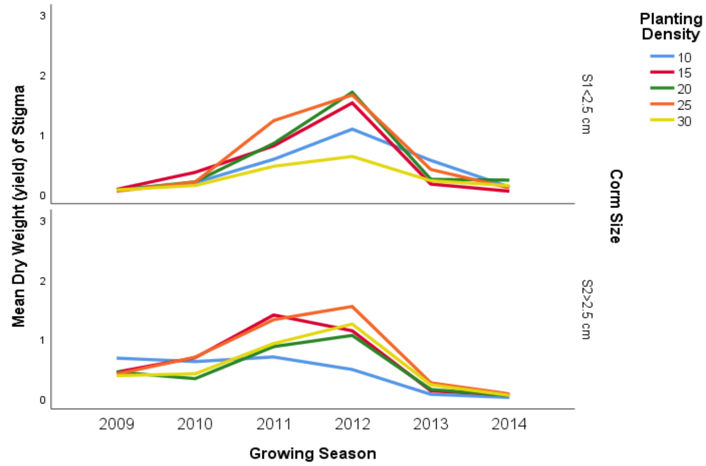


a)

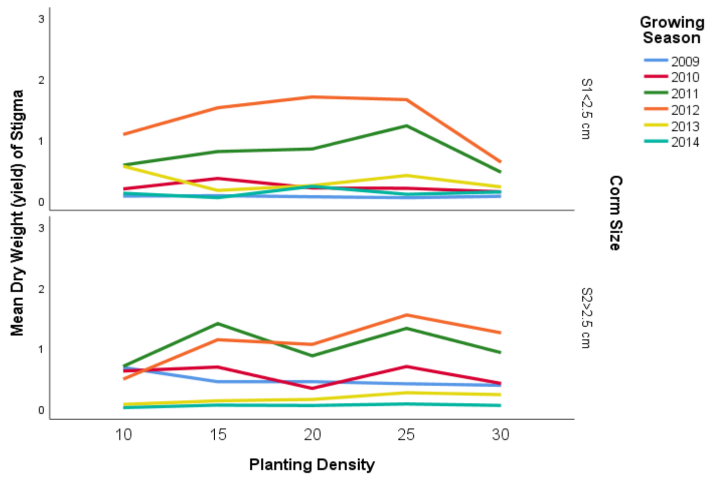


b)

Figure 5. Mean dry weight of the flower (g/m^2) relative to year (growing season), planting density, and corm size (a) and relative to planting density, year (growing season), and corm size (b)



a)



b)

Figure 6. Mean dry weight of stigma (g/m^2) relative to year (growing season), planting density, and corm size (a) and relative to planting density, year (growing season), and corm size (b)

During the first three years, there was a significant difference in all of the above traits between the different corm sizes. In the S2 group (>2.5 cm), the yield of fresh and dry weight of flowers and stigmas, as well as the number of flowers per m^2, were higher (Table 4 and 5). However, during the last three years, there was an opposite trend. The higher number of flowers and fresh and dry weight of yield (Tables 4 and 5, Supplementary Figures S1-S5) were found in the smaller corm size group, S1 (<2.5 cm). Additionally, during each year, there was a difference in the number of flowers. In the first year, the higher number of flowers per m^2 was found eight days after the initiation of flowering in the S2 group, while in the case of S1, the difference was lower on the same day (Table 4, Figures 2, 3 and 4, Supplementary Figures S3, S3.1, S6, A1, and A7). For the second year, there was a difference between the two different corm sizes. In the corm sizes S2, there was a higher number of flowers per m^2, and there was a small increase in the number of flowers per m^2 in both S1 and S2 corms on the sixth day (Table 4, Figures 2, 3 and 4, Supplementary Figures S3, S3.1, S6, A2, and A8).

The trend was different in the third year as the number of flowers per m^2 was higher during the third day and increased again on the 13th day, after which it was similar in both corm size groups (Table 4, Figures 2, 3, and 4, Supplementary Figures S3, S3.1, S6, A3, and A9). In the fourth year, there was a different trend as the number of flowers was higher in the smaller corms and decreased more rapidly in the larger corms. The eighth day after the beginning of flowering was the most productive for both corm sizes (Table 4, Figures 2, 3, and 4, Supplementary Figures S3, S3.1, S6, A4, and A10). During the fifth year, the trend was similar to the fourth year, with the number of flowers per m^2 higher in S1 compared with S2, and the higher number of flowers per m^2 was found eight and 12 days after the initiation of flowering in S1, while in the case of S2, the increase was similar on the eighth day, but the difference was lower on the 12th day (Table 4, Figures 2, 3, and 4, Supplementary Figures S3, S3.1, S6, A5, and A11). Finally, in the sixth year, flowering was quite small, and the number of flowers per m^2 was higher in S1 compared with the S2 treatment (Table 4, Figures 2, 3, and 4, Supplementary Figures S3, S3.1, S6, A6, and A12).

Despite the fact that the interaction between corm size and year of cultivation influenced most of the studied characteristics, planting density did not significantly affect the studied characteristics during the six years of cultivation, and therefore did not affect the saffron yield (Table 2). Regarding the number of flowers during flowering at the first corm size S1 (<2.5 cm), there was an increase in the mean number of flowers up to the fourth year, reaching up to 186 flowers per m^2, while at the second corm size S2 (>2.5 cm), it reached up to 155 flowers per m^2. During the first year, the highest number of flowers per m^2 was recorded at the higher planting densities, with the S1 corms at a density of 15 cm between plants (or 44 corms/m^2) and the S2 corms at a density of 10 cm between plants (or 67 corms/m^2). However, in the last years (the fourth and the fifth year) of cultivation, the lower densities of 20 to 25 and 25 to 30 cm between plants (or 33, 27, and 22 corms/m^2) gave the highest number of flowers per m^2 for the S1 and S2 corm sizes, respectively (Figures 2, 3 and 4). Similar trends were found in the other studied characteristics (Figures 2, 3, 4, 5 and 6).

Discussion

Flowering in saffron begins 60-90 days after planting and depends on weather conditions and planting time (Sampathu *et al*., 1984; Alonso *et al*., 2006; Gresta *et al*., 2008a, 2009; Kumar *et al*., 2009). In the present study, flowering started 60 days after planting and lasted almost 30 days during the first year. The duration was similar in the second and third years but decreased during the following three years. However, the duration of flowering was not studied over a long period of time to determine how flower production evolves during successive years (growing seasons), especially since saffron corms remain in the same field for several years. Indeed, it appears that annual corm multiplication leads to high competition between replacement corms, especially in corms older than four years. This increased competition can ultimately result in a decrease in the weight of these corms. Consequently, this reduction in weight leads to a decline in flowering, as the smaller corms may not have the capacity to produce flowers effectively. It was found that flowering duration fluctuated and is crucial for the crop as the highest stigma yield was found during the fourth year. Several studies have examined the effects of corm size and planting density separately or for less than six years (growing seasons) (Plessner *et al*., 1989; Negbi *et al*., 1989; De Mastro and Ruta, 1993; Tammaro, 1999; Gresta *et al*., 2008b; Çavuşoğlu and Erkel, 2009; De Juan *et al*., 2009; Kumar *et al*., 2009; Renau-Morata *et al*., 2012; Koocheki *et al*., 2019a, 2019b). However, no studies have determined the effect of corm size at different planting densities for the entire six years that a saffron crop can last as reported in the present study.

It was found that the stigma yield and other studied characteristics, such as the fresh weight of the flower and stigma and the number of flowers per m^2, increased from the first to the fourth year, followed by a decline up to the sixth year. The highest yield of fresh weight of flowers and stigma and the highest number of flowers were found in the fourth year for both sizes of corms (Table 4). A similar trend was observed for the dry weight of flowers and the dry yield of stigmas, with an increase from the first to the fourth year, followed by a gradual reduction during the fifth and sixth year (Table 5). The effect of corm size at planting on saffron stigma yield has been evaluated in several studies (Rees, 1992; Le Nard and De Hertogh, 1993). Also, Koocheki *et al*. (2019a) concluded that larger mother corms (6-8 g) had more preference in one and two-year-old fields than in three-year-old fields. In other studies, it was found that corm size determines whether the corm will flower or not. If the corm does not reach a certain size (above 2.5 cm in diameter), usually does not flower in the first year, it only produces leaves and has a negative effect on the flowering in the following years, especially when the field is used for many years after the initial planting (Gresta *et al*., 2008a; De Juan *et al*., 2009; Koocheki *et al*., 2016 a,b).

However, the interactive effect between corm size and planting density has not been studied, particularly for longer periods up to six years, during which saffron cultivation can last in many countries (Alonso *et al*., 2006). In the present study, it was found that there was no interaction between planting density and corm size, and between planting density and year. It was also determined that using larger corms for planting increased the number of flowers per corm (Negbi *et al*., 1989; De Mastro and Ruta, 1993; Çavuşoğlu and Erkel, 2005). In particular, from other studies it was found that there was a positive correlation between the production of saffron per flower with the average weight of the corms planted (Iqbal *et al*., 2012; Douglas *et al*., 2014). Additionally, other studies found that corms with a weight above 8 g have a high rate of flowering during the first three years and produced in the first year 3-5 kg, during the second year 11 kg and during the third year can reach up to 20 kg dry saffron per hectare (Sadeghi, 1994). From the present study it is obvious that the hypothesis that was tested whether larger corms can have higher stigma yield seems that it is not correct as the effect of corm size at planting lasted only for three years and then the smaller corms showed to have higher stigma yield compared with the larger corms. Probably, this is due to the higher number of replacement corms that the larger mother corms produced when the field age reached to more than four years. Then the competition between replacement corms can reduce their mean weight and finally their flowering ability.

In most cases farmers are using corm sizes with a diameter of 2.5-3.0 cm as these have been found to yield the highest stigma and flower yields (Plessner *et al*., 1989; Tammaro, 1999; Kumar *et al*., 2009). In addition, when the corms are larger, they reach their maximum stigma yield earlier than smaller corms and the yield of flowers is much higher which was also found in the present study (De Juan *et al*., 2009). Despite this initial advantage, the benefit of using larger corms can be lost after several years of cultivation, as seen in the fourth year of the present study and reported by Çavuşoğlu and Erkel (2009). The size of the mother corm also has a significant effect on vegetative development and daughter corm production, with larger mother corms producing more daughter corms than smaller ones (Negbi *et al*., 1989; De Mastro and Ruta, 1993; De Juan *et al*., 2003). However, daughter corm yield decreases after three years in large corms and increases in small corms (Çavuşoğlu and Erkel, 2009). Most experiments investigating the effect of corm size have been conducted for up to three years (Negbi *et al*., 1989; Çavuşoğlu and Erkel, 2009) or have not considered longer periods (Plessner *et al*., 1989; Tammaro, 1999; Kumar *et al*., 2009), even though saffron crops can be maintained for up to six years or more in some countries (Goliaris, 1999; Alonso *et al*., 2006). In the present study, the reduction in yield due to corm size was most pronounced in the fifth and sixth years of cultivation, consistent with other studies that have reported reductions as early as the fourth year (Temperini *et al*., 2009; Douglas *et al*., 2014). The variation between years is attributed to both environmental fluctuations and the density and size of the daughter corms. It is possible that large mother corms produce more replacement corms, leading to competition for water and nutrients among them, resulting in a decrease in flower yield when the field reaches five and six years of age. The last years of saffron production are of no commercial interest, as they yield very little with marginal production. Additionally, by this stage, the effect of the initial sowing size or density has already been lost, and we are left with a population of shallow, small corms that are unable to flower. The observed differences in maximum stigma yield between corm sizes may be attributed to competition from larger corms, which reduce yield and plant growth after the third year. Additionally, daughter corm populations increase over time, and higher densities can lead to increased competition for environmental resources, potentially affecting saffron yield in subsequent years (Andabjadid *et al*., 2015). Also, the yield decline is likely due to different temperature and rainfall over the six years, as well as plant pathogens such as *Rhizoctonia* spp. that can spread quickly after many years of cultivation (Gupta *et al*., 2021).

Planting density was investigated in this study, and there were no statistically significant differences in the studied characteristics. However, the effect of planting density on saffron crop yield and other traits has been poorly studied over several years. Oromí (1992) studied two planting densities, 57 and 72 corms per m^2, but no conclusion was reached. Other researchers recommended an optimal plant density of 50 corms per m^2 with row and plant distance (20×10) and (30×10) and corms weighing above 8 g for maximum yield of 4-5 tons ha^{-1} (Alavi-Shahri *et al*., 1994). An important yield increase was observed during the first flowering period when planting density increased to 40-100 corms per m^2 (ITAP, 1998). Similarly, Tammaro (1999) found that the optimal density per hectare is about 590,000-620,000 corms. In this study, the optimum density depended on corm size and the year of cultivation, and there was no interaction between density and corm size on stigma yield. However, the combined effect of corm size and planting density was not determined. Additionally, this study determined the effect of several planting distances (five), providing new information.

The planting density did not affect the characteristics during the six years of cultivation, which contradicts the findings of other studies (McGimpsey *et al*., 1997; Douglas *et al*., 2014) that reported an impact on crop yield, possibly due to different weather conditions or cultivation duration. Rezvani-Moghaddam *et al*. (2013) also found that dense planting of saffron using larger mother corms is a good strategy for increasing stigma yield in the first and second years (growing seasons). Similarly, Koocheki *et al*. (2019a) concluded that larger mother corms (6-8 g) had more preference when corm density was lower and planting of at least 100 corms per m^2 in one and two-year-old fields had more preference while the best density for three-year-old fields was planting of 75 corms per m^2. Planting saffron at a higher density can lead to increased competition between plants for resources such as nutrients, water, and sunlight. This competition can result in reduced flower production and smaller corms. Conversely, planting at a lower density can allow each plant to have more access to resources, potentially leading to more flower production and larger corms. Moreover, longer-term experiments are needed to assess the effect of planting density on stigma yield for crops that remain in the field for several years. The present study reported a much higher stigma yield compared with previous studies (Koocheki *et al*., 2019a; Esmaeilian *et al*., 2022), which may be attributed to management practices, such as weed control and better environmental conditions, such as temperature, rainfall and soil properties including organic matter and P deficiency which are crucial for saffron corm growth and yield. The soil at the experimental site has a sandy loam (SL) texture with pH 7.75, organic matter content 1.20%, electrical conductivity 0.799 mS/cm, $CaCO_3$ 3.1%, P (Olsen) 46.76 ppm, and K 210 ppm (0-30 cm depth). Stigma yield depends on the soil fertility as the organic matter content, which plays an important role in maintaining physical, chemical, and biological properties of soil (Micheni *et al*., 2004). According to Koocheki *et al*. (2019b), organic matter positively affected the leaf development, stigma, and daughter corm yield. Finally, the soils characterized by a loam and sandy-loam texture, not very calcareous, with a sub-alkaline and neutral pH, low electrical conductivity, a content of organic matter between 5.46 and 8.67 g kg^{-1}, and a content of active lime between 21.25 and 26.25 g kg^{-1}, are favourable for the growth, stigma, and daughter corm production (Cardone *et al*., 2020).

Furthermore, the average saffron yield in Greece over a five-year period is 7 kg/ha, which is relatively high compared with other countries (Alonso, 2006), such as Iran where saffron yield has been recorded as relatively low (3.53 kg/ha) (Agricultural statistics, 2018). Generally, saffron yield can vary from 1.5 to 15.0 kg/ha depending on planting density, plantation age, and climatic conditions during the crop season (Negbi, 1999).

Conclusions

The year (growing season) and the interaction of year (growing season) with size of corm affected all the studied characteristics, such as fresh and dry weight of flowers and stigmas (saffron) and the number of flowers per m^2 and consequently the yield. Over the six-year study period, there was a gradual increase in flowering from the first to the third year, followed by a decline in the fifth and sixth years. The yield of larger corms (S2, greater than 2.5 cm) was higher during the first three years, while smaller corms (S1, less than 2.5 cm) produced higher yields in the fourth through sixth years. In contrast, planting density did not have a significant effect on the studied characteristics or the saffron yield over the course of the six-year study period.

Authors' Contributions

All authors made significant contributions to the manuscript. P.R. conducted the experiments and wrote the manuscript, G.M. was responsible for the statistical analysis, review and editing, C.D. was responsible for conducting the experiments, writing, and also reviewing the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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