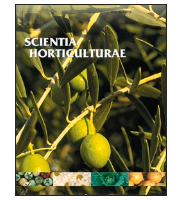
Scientia Horticulturae 288 (2021) 110393

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Comparing annual and biennial crop cycle on the growth, yield and quality of saffron using three corm dimensions

Loriana Cardone [a,*], Vincenzo Candido [b], Donato Castronuovo [a,*], Michele Perniola [b], Nunzia Cicco [c]

[a] School of Agricultural, Forest, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano, 10, 85100, Potenza, Italy
[b] Department of European and Mediterranean Cultures, Environment, and Cultural Heritage, University of Basilicata, Via Lanera, 20, 75100, Matera, Italy
[c] Institute of Methodologies for Environmental Analysis, National Research Council, C.da S. Loja, 85050, Tito Scalo (PZ), Italy

A R T I C L E I N F O

Keywords:
Crocus sativus, medicinal plant, stigma yield, daughter corm yield, crocin
picrocrocin

A B S T R A C T

Saffron (*Crocus sativus* L.) is a geophyte plant belonging to the Iridaceae family and it is appreciated for its red dried stigmas used as cooking spice and flavouring agent. Effects of crop cycle length and mother corm dimension, as well as their interaction, have been evaluated on the flowering (morphological traits of flowers, days to flowering, flowering interval and flower production), quantitative traits (stigma and daughter corm yield), vegetative development (leaf and daughter corm traits) and qualitative characteristics (coloring, bittering and aromatic powers) of spice. A two-year field study (2017–2019) was conducted to compare annual and biennial crop cycle of saffron using three corm dimensional classes (D1: 2.0–2.5 cm, D2: 2.6–3.5 cm and D3: 3.6–4.5 cm) according a split-plot design with 3 replications. The results showed that the corms of D3 class, planted in annual crop cycle, produced flowers with the highest stigma length (42.2 mm), and dry weight of stigmas (7.4 mg), stamens (11.4 mg) and tepals (40.7 mg). The highest number of flowers per m^2 (311.8) and stigma yield (20.7 kg ha^{-1}) were found when corms belonging to D2 class were planted in biennial crop cycle, meanwhile the highest daughter corms production (35.9 t ha^{-1}) was obtained when corms belonged to D3 class were planted in annual crop cycle. Number of daughter corms per m^2 with a horizontal diameter from 3.1 to 4.5 cm and weight from 10.1 to > 25 g decreased as increasing the crop cycle length. In biennial crop cycle, corms of D1 class produced more daughter corms belonged to 3.1–3.5 cm diameter class and to 15.1–20 g weight class compared to D2 and D3 classes. Regarding to the spice quality, coloring and bittering powers were positively influenced by biennial crop cycle. According to International Standardization Organization (ISO 3632) references, the maximum values of color (306.3 $A_{1\ cm}^{1\ \%}$ 440 nm) and taste (116.2 $A_{1\ cm}^{1\ \%}$ 257 nm) were reached in spice obtained from "biennial crop cycle x D2 class" interaction. No significant effect of all experimental factors on aromatic power was found. It was concluded that the evaluation of combination between crop cycle length and corm dimension is necessary in the saffron management in order to achieve the optimum yield of stigmas and corms, to improve the qualitative traits of spice and to enhance the by-products as corms of D1 class.

1. Introduction

Saffron is known as the most world expensive spice because it requires manual labor during the harvesting and processing of *Crocus sativus* L. flowers. It is used mainly in food, medicinal and cosmetic sectors due to its presence of bioactive compounds which play an important role in human health (Bagur et al., 2018; Mzabri et al., 2019).

Saffron is widely cultivated in different environments, especially in Iran, India, Afghanistan, Morocco, Greece, Spain and Italy, over a total area of about 122 thousand hectares (Cardone et al., 2020a). *C. sativus* belongs to Iridaceae family and since it is a sterile plant, it propagates vegetatively only through daughter corms during every annual biological cycle (Grilli Caiola and Canini, 2010). The biological cycle of saffron lasts about 240 days and involves five major stages: sprouting (September-October), flowering (October-November), leaf development (October-April), growth and multiplication of daughter corms (November-April), and dormancy (May-August) (Lopez-Corcoles et al., 2015).

* Corresponding authors.
E-mail addresses: loriana.cardone@unibas.it (L. Cardone), donato.castronuovo@unibas.it (D. Castronuovo).

https://doi.org/10.1016/j.scienta.2021.110393
Received 17 November 2020; Received in revised form 19 June 2021; Accepted 25 June 2021
Available online 9 July 2021
0304-4238/

The crop cycle length of saffron is very different in each cultivation country and it can be annual or pluriannual: 1 year [Italy (Abruzzo, Tuscany and some regions of South Italy)], 3–4 years [Spain and Italy (Sardinia and Sicily)], 5–6 years (Morocco), 6–8 years (Iran), 10–12 years (Greece) and 10–15 years (India) (Husaini et al., 2010; Cardone et al., 2020a).

Generally, saffron corms are lifted maximum after 3 years to avoid the loss of flower and spice production due to overcrowding, competition for water and nutrients, or diseases presence (Temperini et al., 2009; Branca and Argento, 2010). Instead, in annual crop cycle, the corms are uprooted every year at the beginning of the summer, during the senescence phase, and replanted from the middle of August to middle of September (Cardone et al., 2020a). The positive aspect of annual crop cycle is the possibility to select the corms for dimension, separating those not able to flower with a horizontal diameter less of 2.5 cm, and to check and eliminate the corms with diseases caused by fungi (*Fusarium oxysporum* f. sp. *gladioli, Rhizoctonia violacea, Penicillium corymbiferum*), and the ones damaged by nematodes and rodents (Nehvi et al., 2010; Ahrazem et al., 2010).

The choice of the crop cycle length plays a critical role in stigma and daughter corm yield. It depends mainly on the corm geographical origin and, climatic and pedologic conditions of cultivation site. It has been recommended to plant annually in rainy site using "Abruzzo" corm origin (Gresta et al., 2009), while for more years in dry site using "Sardinia" corm origin (Cardone et al., 2019), or on soil with sandy texture (Shajari et al., 2020).

Some authors evaluated how the crop cycle length influenced the saffron productive parameters (European Saffron White Book, 2006; Branca and Argento, 2010). The highest number of flowers and stigma yield were recorded when the two-year crop cycle was adopted, whereas in the three-year treatment they decreased and declined drastically after four years (Temperini et al., 2009).

Many studies showed the positive correlation between the spice yield and corm dimension (Amirnia et al., 2014; Khorramdel et al., 2015; Bayat et al., 2016; Fallahi et al., 2017) and some authors investigated this aspect focusing the attention on the influence both of the mother corm dimension and crop cycle length on saffron yield. In this regard, Branca and Argento (2010) suggested a biennial crop cycle using corms weighting more of 40 g to obtain the highest flower number and dry stigma yield. Douglas et al. (2014) reported that the stigma fresh weight and flower number per corm were higher in a three year management cycle, but the highest production of large and medium progeny corms was obtained in a two year management cycle using corms weighting 38–53 g.

Although there are many information available in literature regarding the influence of crop cycle length and mother corm dimension on stigma and corm yield, no studies have yet been conducted to evaluate how the combination of these experimental factors mentioned above affects the morphological traits of flowers and the quality of saffron.

Spice quality depends on different parameters as moisture content, total ash, presence of adulterans, flower residue or foreign matter, and mainly on the composition and content of three bioactive compounds, as crocin, picrocrocin and safranal (D'Auria et al., 2004). These latter are responsible for the yellow color, bitter taste and unique aroma, respectively. These secondary metabolites are spectrophotometrically determined according to specifications recommended by the ISO 3632 normative at 440 nm (coloring strength), at 257 nm (the wavelength of picrocrocin maximum absorbance) and at 330 nm (the wavelength of safranal maximum absorbance) ISO 3632-1 (2011). Actually this normative is the most used and at the international level classifies saffron into three categories based on the specified physical and chemical parameters (Alonso et al., 2012)

The quality of spice is influenced by many parameters, mainly by the pre- and post-harvest practices (Cardone et al., 2020a; Fallahi et al., 2020) and represents an important requirement that allows to fetch a higher commercial value in the market and satisfy consumer preferences (Mohammadi and Reed, 2020).

In saffron cultivation the farmer's purposes are to increase the stigma yield, to obtain the spice with high qualitative traits and the daughter corms with large dimension. Therefore, the aim of the present investigation was to compare the effect of annual and biennial crop cycle during the same period, using three mother corm dimensional classes (D1: 2.0–2.5 cm, D2: 2.6–3.5 cm and D3: 3.6–4.5 cm), on morphological, quantitative and qualitative traits of saffron.

2. Material and methods

2.1. Site and experimental design

Two consecutive field experiments were carried out for the 2017/2018 and 2018/2019 seasons at experimental field of Muro Lucano, located in Potenza province, northwest of Basilicata region, Italy (40° 45′ N, 15° 29′ E, 450 m a.s.l), using corms from Sardinia origin (San Gavino Monreale, Medio Campidano, Italy). Each experiment was arranged in a split-plot (two factors—length of crop cycle and mother corm dimension—were applied in main plots and subplots, respectively) in a randomized complete block design (RCBD), and each experimental treatment was repeated three times. The length of crop cycle factor has two levels (annual and biennial) and mother corm dimension has three levels (D1: 2.0–2.5 cm, D2: 2.6–3.5 cm and D3: 3.6–4.5 cm). The corms belonging to D1, D2 and D3 dimensional classes have a mean weigh of 5, 12, and 25 g, respectively.

In detail, the trial was started in 2016 with annual planting of corms in order to have the biennial one in 2017. Thus, the biennial crop cycle was compared with annual one during the same period for two experimental years.

The soil has a clay loam texture (39.5% of sand, 25.9% of silt and 34.5% of clay), moderately calcareous (13.4 g kg^{-1}), with a slightly acidic pH (6.4), a low electrical conductivity (114.8 μS cm^{-1}) and a sufficient amount of organic matter (1.5 g kg^{-1}).

Prior to saffron planting, the soil was prepared by incorporating a fertilizer N:P:K (12:12:17) (YaraMila, Yara S.p.A, Milano, Italy) and corms were dipped in 0.1% solution of prochloraz to avoid fungal diseases caused mainly by *Fusarium oxisporum* f. sp. *gladioli*. On September of each experimental year (2017/2018 and 2018/2019) corms were placed on rows 50 cm apart respecting a distance of 5 cm on the row and with a depth of 15 cm, achieving a density of 40 plants m^{-2}.

Weed control was carried out by hand and no irrigation was applied during the whole crop cycle.

During the flowering period of each year, between October and November, flowers were collected by hand, in the early hours of each day. Successively, the harvested flowers were taken to a 'Vegetable Crops and Floriculture' laboratory of the University of Basilicata, where stigmas were separated manually from the rest of flower and dried in a forced air oven at low temperature (40–45 °C for 24 h). Dry stigmas were stored in closed glass jars in suitable condition (room temperature of 19 ± 3 °C) and kept in the dark until qualitative analysis which were carried out at biochemical laboratory of the Institute of Methodologies for Environmental Analysis of the National Council of Research.

The following parameters were recorded on 10 plants per each experimental thesis: length of stigmas and stamens (mm); fresh and dry weight of stigmas, stamens and tepals (mg); number of harvested flowers per corm and per m^2, stigma yield (kg ha^{-1}), leaf number per plant and leaf length (cm). At the end of crop cycle, during senescence phase, the corms were lifted from the soil and number of daughter corms for mother corm, daughter corm mean weight (g), total daughter corm weight (g $plant^{-1}$), daughter corm horizontal diameter (cm), replacement corm yield (t ha^{-1}) and the date of senescence were recorded.

To allow the flower formation the conditions of corm storage were: room temperature of 25 ± 2 °C and darkness. For each crop cycle, climatic data were collected by using a weather station equipped with temperature and relative humidity probes (CS500-L- modified version of Vaisala's 50Y Humitter, Campbell Scientific Inc, Utah, USA) and with a TE525 precipitation sensor (Texas Electronics, Texsas, USA) to measure the rainfall. Collected data were recorded by a CR 10x data-logger (Campbell Scientific Inc, Utah, USA) in 2017/2018 and 2018/2019.

2.2. Spectrophotometric analysis of saffron extract

To determine the saffron quality, samples of spice produced in 2017 and 2018 were analyzed in triplicate according to the ISO 3632 normative (ISO 3632-1, 2011), which is the internationally accepted reference specification used at commercial level.

As reported in our previous study (Cardone et al., 2020b), 500 mg of powdered samples were passed through a 0.5 mm sieve, transferred into a 1000 mL volumetric flask with 900 mL of distilled water. The obtained aqueous solution was stirred for 1 h in the dark and then brought to 1000 mL with distilled water. This extract was diluted (1:10 v/v) with deionized water and filtrated with polytetrafluoroethylene (PTFE) filters (15 mm diameter and 0.45 μm pore size). The results of the ISO 3632 (ISO 3632-1, 2011) parameters were expressed as values of 440 nm or coloring strength, values of 330 or aroma strength, and values of 257 or flavor strength, using an UV–Vis spectrophotometer (Ultrospec 4000, Amersham Pharmacia Biotech, Milan, Italy) according to the following equation:

$$A_{1cm}^{1\%}\ (\lambda max) = \frac{(D^{*}20,000)}{(100 - H)} \quad (1)$$

where: D is the absorbance at 257, 330 and 440 nm; 20,000 is dilution factor of the total extract considering the amount of saffron sample; H is the moisture and volatile matter content, expressed as a mass fraction. H was determined, placing 2.5 ± 0.001 g of each saffron samples in an oven from 103 ± 2 °C for 16 h and it was calculated as the percentage of the initial weight of the sample according to the formula:

$$\mathrm{H} = (\mathrm{m}_0 - - \mathrm{m}_1)^{*}(100 / \mathrm{m}_0)\% \quad (2)$$

where: m_0 is the mass, in grams, of the saffron portion before drying and m_1 is the mass, in grams, of the dry residue.

2.3. Statistical analysis

Before performing analysis of variance (ANOVA), Shapiro-Wilk ($p \leq 0.05$) and Levene ($p \leq 0.01$) tests were applied to test normality and homogeneity of variances, respectively. The obtained data were then submitted to ANOVA with subsequent comparison of the means using the Student–Newman–Keuls (SNK) test at $P \leq 0.05$.

Data were subjected to factorial analysis of variance, considering crop cycle length, mother corm dimension and experimental year as sources of variation. All statistical procedures were computed using the software RStudio: Integrated Development for R, version 1.0.136 ((R Core Team, 2020).

3. Results and discussion

3.1. Climatic data

Climate parameters (monthly rainfall, mean, maximum and minimum air temperature) from September to May, when the growth of the saffron occurred, are reported in Table 1.

The climate of Muro Lucano site is subhumid, according to the De Martonne climatic classification (Bove et al., 2005). The average annual rainfall of two experimental years is 678.1 mm, the average annual air temperature is 10.5 °C, the minimum air temperature is 6.2 °C and the maximum air temperature is 16.0 °C (Table 1). In detail, the first experimental year was rainier and colder than the second one. In 2017/2018 rainfall was more concentrated during the vegetative period, from December to May, with 452.8 mm. Meanwhile, the second experimental year was characterized by higher rainfall and temperatures during the flowering period (October-November) compared to the first one (Table 1).

3.2. Effect of crop cycle length and corm dimension on flower traits in two years

The ANOVA results showed significant effects of crop cycle length and corm dimension, as well as their interaction, on most of the flower traits. No significant effects of crop cycle length on tepal dry weight and of corm dimension on stigma and stamen length were found (Table 2).

With respect to the crop cycle length, the annual cycle produced bigger and heavier flowers with longer stigmas and stamens compared to biennial one (Table 2). Consequently to this result, 161 and 147 flowers were needed to obtain 1 g of dried stigmas in biennial and annual crop cycles, respectively (Table 2).

During the biennial crop cycle, a greater crop density increased the intraspecific competition between plants which reduced the availability of resources, such as water, nutrients, and solar radiation (Craine and Dybzinski, 2013). Competition for resources and growing space among plants could have generated abiotic stress for plants producing smaller and lighter flowers characterized by shorter stigmas (Cardone et al., 2019).

Considering the single effect of corm dimension on flower traits, D3 class showed the highest values (Table 2). In particular, all flower traits increased as increasing the dimension of mother corm, except for the stigma and stamen length. Big corms have more carbohydrate reserves which positively affected the morphological traits of flower. In this regard, Amirnia et al. (2014) reported that the corms with a weight of 12 g

Table 1
Monthly minimum, mean and maximum air temperature and total rainfall recorded during two growing seasons (2017/2018 and 2018/2019) in Muro Lucano.

	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Mean (Sep-May)	Mean (Dec-May)
2017/2018											
T.min (°C)	11.9	8.3	4.8	1.6	3.4	1.0	3.6	8.9	11.3	6.1	5.0
T.mean (°C)	16.3	13.1	8.3	4.6	6.5	4.0	7.1	13.5	15.4	9.9	8.5
T.max (°C)	22.1	19.1	12.5	8.1	10.5	8.3	11.3	19.2	20.8	14.7	13.0
Rain (mm)	107.8	60.6	93.8	99.2	56.4	99.8	104.4	53.2	39.8	715.0	452.8
2018/2019											
T.min (°C)	14.6	11.4	7.2	2.0	0.5	1.8	5.0	5.4	8.8	6.3	3.9
T.mean (°C)	19.9	16.2	10.6	6.3	4.1	6.8	9.7	12.1	13.0	11.0	8.7
T.max (°C)	26.6	24.3	17.6	13.2	9.0	13.6	15.0	17.9	18.5	17.3	14.5
Rain (mm)	26.2	120.6	130.6	35.2	116.8	37.2	53.4	37.8	83.4	641.2	363.8
Two-year average	13.2	9.9	6.0	1.8	2.0	1.4	4.3	7.2	10.1	6.2	4.5
	18.1	14.6	9.5	5.5	5.3	5.4	8.4	12.8	14.2	10.5	8.6
	24.4	21.7	15.1	10.7	9.8	11.0	13.2	18.6	19.7	16.0	13.8
	67.0	90.6	112.2	67.2	86.6	68.5	78.9	45.5	61.6	678.1	408.3

Table 2
Effect of crop cycle length (C), corm dimension (D), and their interaction on flower traits of saffron in two years.

Experimental factors[(1)]	Stigma length (mm)	Stigma fresh weigh (mg)	Stigma dry weight (mg)	Stamen length (mm)	Stamen fresh weight (mg)	Stamen dry weight (mg)	Tepal fresh weight (mg)	Tepal dry weight (mg)
Crop cycle length (C)								
Annual	39.8	39.1	6.8	23.5	48.6	9.3	305.0	34.1
Biennial	36.8	35.8	6.2	19.4	31.1	8.8	271.1	33.3
Significance[(2)]	*	*	*	*	**	*	*	ns
Corm dimension (D)								
D1	37.7	34.7 b	6.2 b	20.5	33.0 b	8.3 b	255.8 b	29.6 b
D2	39.1	39.7 a	6.6 a	21.7	42.0 ab	8.9 ab	292.5 a	34.6 ab
D3	38.3	38.0 a	6.7 a	21.9	44.5 a	10.0 a	315.8 a	37. 0 a
Significance	ns	*	*	ns	*	*	**	**
Year (Y)								
2017/2018	38.6	37.3	6.6	21.8	40.8	9.6	296.7	34.2
2018/2019	38.1	37.6	6.4	21.0	38.9	8.5	279.4	33.2
Significance	ns	ns	ns	ns	ns	*	*	ns
Interactions								
C × D	*	*	**	ns	**	*	**	*
C × Y	ns	ns	ns	ns	ns	ns	ns	ns
D × Y	ns	ns	ns	ns	ns	ns	ns	ns
C × D × Y	ns	ns	ns	ns	ns	ns	ns	ns

(1) Mean values followed by a different letter are significantly different at $P \leq 0.05$, according to SNK test.
(2) *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; ns, no significant difference.

showed an increasing of fresh and dry weight of stigmas and flowers, compared to corms weighing 6 g. Similarly to this study, Branca and Argento (2010) also observed that the stigma dry weight increased from 0.0034 g in small corms (<20 g) to 0.0052 g in big corms (>40 g).

The results reported in Fig. 1A-D demonstrated the significant interactive effects of experimental factors on some morphological traits of flower. The highest stigma length, dry weight of stigmas, stamens and tepals were obtained by corms belonged to D3 class, planted in annual crop cycle. In contrast, the lowest values of stigma length and stigma dry weight were obtained by corms belonged to D3 class planted in biennial crop cycle (Fig. 1A-B), and the lowest values of stamen and tepal dry weight were found in D1 corms planted in annual crop cycle (Fig. 1C-D).

Among the morphological traits of saffron flower, stigma dry weight is the most important parameter that influences the stigma yield. The mean value of the stigma dry weight between annual and biennial crop cycle was in accordance with study of Temperini et al. (2009), who reported a mean value of 7.1 mg flower^{-1}. Both in 2017 and 2018, no significant differences were found in flower traits, except for stamen dry

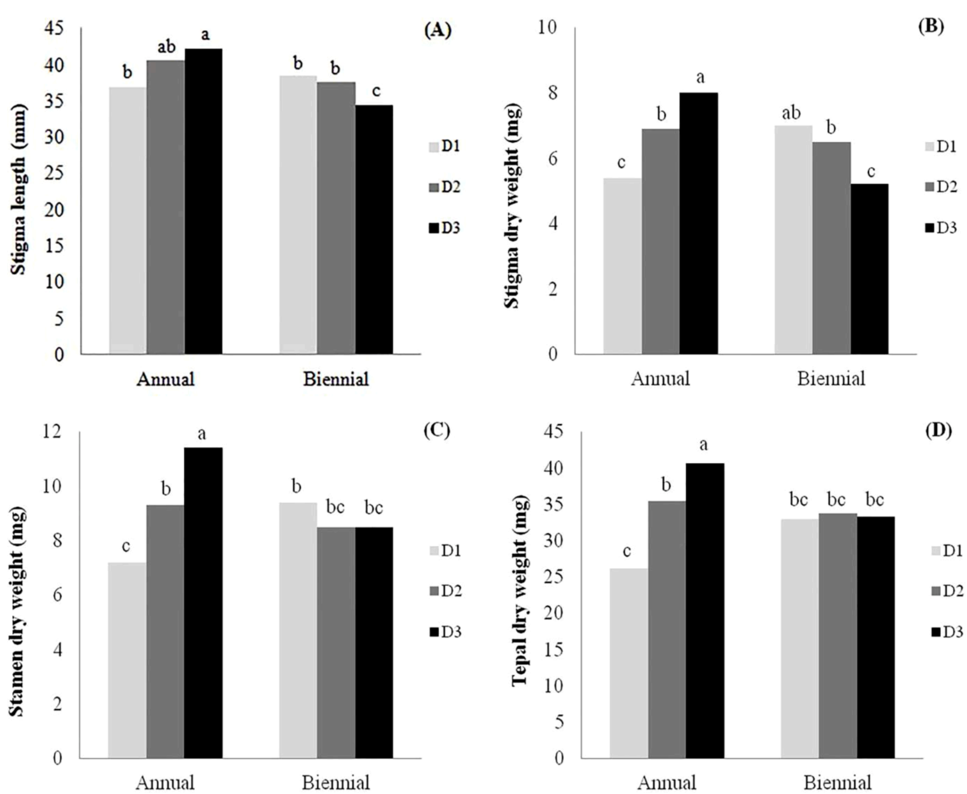


Fig. 1. Interactive effect of "crop cycle length × corm dimension" on the stigma length (A), dry weight of stigmas (B), stamens (C) and tepals (D); different letters above columns indicate significant differences at $P \leq 0.05$, according to SNK test.

weight and tepal fresh weight (Table 2). However, the values of these traits were higher in the first experimental year (2017/2018) compared to ones obtained in the second experimental year (2018/2019). The flower dimension could be favored by the climatic conditions recorded in the first experimental year, such as lower air temperature during flowering period (10.7 °C) compared to that registrated in the second experimental year (13.5 °C). Low temperatures could have contributed to decrease the dark respiration rate of plants and so the increase in flower traits according to study of Shin et al. (2001).

3.3. *Effect of crop cycle length and corm dimension on saffron yield in two years*

Table 3 shows that the crop cycle length and corm dimension significantly influenced the flowering time, production of flowers and spice. In general, the flowering occurred after 41 days from the corm planting. In detail, the flowering started before in biennial crop cycle and from corms belonging to D2 and D3 classes (Table 3).

The positive correlation between horizontal diameter and weight of corms with flowering and spice yield has been reported in many studies (de Juan et al., 2009; Renau-Morata et al., 2012; Amirnia et al., 2014; Koocheki and Seyyedi, 2015; Khorramdel et al., 2015; Bayat et al., 2016; Fallahi et al., 2017). Gresta et al. (2008) studied the effect of mother corm on saffron traits in Mediterranean environment and reported that the big corms (dimensional class: 3.5–4.5 cm) produced the highest saffron yield and replacement of progeny corms. Koocheki et al. (2016) found the maximum flower number and stigma yield when corms weighting more of 10 g were planted because of the greater food reserve content in large corms compared to small corms.

Large corms of D2 and D3 classes have more flower buds and the ability to produce more flowers already in the first cultivation year.

In general, the highest number of flowers per m^2 was recorded by corms belonged to D3 class and by corms planted in biennial crop cycle (Table 3). The number of flowers per m^2 observed in the biennial crop cycle was significantly higher by three times in comparison with that one recorded in the annual crop cycle (Table 3).

The highest stigma yield was observed in the biennial crop cycle and in corms belonged to D3 and D2 classes, while the lowest spice production was recorded in annual crop cycle from corms belonged to D1 dimensional class (Table 3).

The earliness of flowering, flowering interval, flower number and stigma yield were significantly affected by interaction between crop cycle length and corm dimension (Table 3). The earliest and widest flowering occurred in corms of D3 class planted in a biennial crop cycle in both the experimental years (data not shown). Probably the earliness of flowering observed in biennial crop cycle can be attributed to factors such as soil temperature and humidity which have stimulated the growth of saffron plants (Molina et al., 2005).

Stigma yield is a complex parameter determined by the interaction of different factors, mainly by corm geographical origin and dimension, environmental conditions (Douglas et al., 2014; Cardone et al., 2019), physical and chemical properties of soil (Cardone et al., 2020b), cultivation methods (Renau-Morata et al., 2012), planting density (Rezvani-Moghaddam et al., 2013), fertilization (Jami et al., 2020; Koocheki and Seyyedi, 2015; Ghanbari et al., 2019) and irrigation (Cardone et al., 2020a). The significant and positive effect of crop cycle length on stigma yield has been reported in literature by some authors (Temperini et al., 2009; Branca and Argento, 2010).

Stigma yield increased by 72.5% from annual to biennial crop cycle (Table 3) and this ratio is in according with findings of Goliaris Goliaris (1999), who showed that the stigma yield in Greece varied from 3 kg ha^{-1} in the first year and 10 kg ha^{-1} in the second year. Temperini et al. (2009) reported stigma yields values of 7.2 and 15.2 kg ha^{-1} for the one- and two-year cycle treatments, respectively. Higher values were obtained in a three year-research of McGimpsey et al. (1997), who reported that saffron production increased from 0.2 g m^{-2} in the first year to 1.66 g m^{-2} in the second production year in New Zealand.

Significant interactive effects of crop cycle length and corm dimension on the flower production and stigma yield were found (Fig. 2A and B). The highest flower production (311.8 m^{-2}) (Fig. 2A) and spice yield (20.7 kg ha^{-1}) were noted in "biennial crop cycle × D2 class" interaction (Fig. 2B).

All corm dimensional classes produced an increase of spice production from annual to biennial crop cycle: D1 class showed an increasing of yield from 0.45 kg ha^{-1} to 7.01 kg ha^{-1}; D2 class exhibited an increasing of yield from 2.95 to 20.72 and D3 from 8.26 to 14.60 kg ha^{-1} (Fig. 2B).

3.4. *Effect of crop cycle length and corm dimension on saffron quality in two years*

Besides to stigma yield also the quality is an important factor which influences the economic value of this spice. Many authors have studied the effect of corm geographical origin (Cardone et al., 2021; Macchia et al., 2013), climatic conditions (air temperature, light radiation, precipitation, altitude) (Zarinkamar et al., 2011; Cardone et al., 2019), physical and chemical soil properties (Cardone et al., 2020a), fertilization (Caser et al., 2019), planting method (Yarami and Sepaskhah, 2016), drying methods (Pardo et al., 2002; Tong et al., 2015; Chen et al.,

Table 3
Effect of crop cycle length (C), corm dimension (D), and their interaction on flowering and saffron yield in two years.

Experimental factors[(1)]	Days to flowering (d)	Flowering interval (d)	Flower number per corm	Flower number per m^2	Stigma yield (kg ha^{-1})
Crop cycle length (C)					
Annual	44.3	12.4	0.82	54. 9	3.9
Biennial	38.2	20.6	3.30	220.2	14.1
Significance[(2)]	*	**	**	**	**
Corm dimension (D)					
D1	45.2 a	7.2 b	0.79 b	53.3 b	3.7 b
D2	39.4 b	20.3 a	2.67 a	178.0 a	11.8 a
D3	39.0 b	22.1 a	2.72 a	181.3 a	11.4 a
Significance	*	**	**	**	**
Year (Y)					
2017/2018	40.5	15.8	2.03	135.2	8.9
2018/2019	42.0	17.2	2.10	139.9	9.1
Significance	ns	ns	ns	ns	ns
Interactions					
C × D	*	**	**	**	**
C × Y	ns	ns	ns	ns	ns
D × Y	ns	ns	ns	ns	ns
C × D × Y	ns	ns	ns	ns	ns

(1) Mean values followed by a different letter are significantly different at $P \leq 0.05$, according to SNK test.
(2) *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; ns, no significant difference.

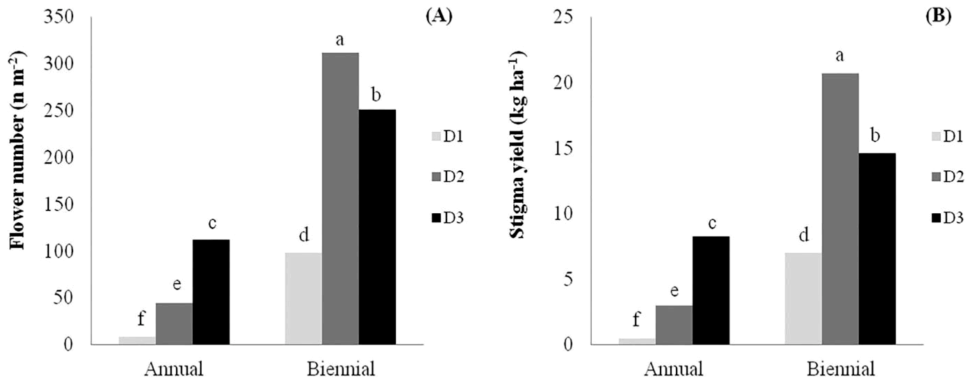


Fig. 2. Interactive effect of "crop cycle length × corm dimension" on the flower number (A) and stigma yield (B); different letters above columns indicate significant differences at $P \leq 0.05$, according to SNK test.

2020) and storage conditions (Maggi et al., 2010) on the main bioactive compounds which contribute to quality, but no data are reported on the effect of crop cycle length (annual *vs* biennial) and its combination with corm dimension on spice quality.

Table 4 shows the qualitative classification of the spice samples examined in this study according to the ISO 3632–2, (2010), ISO 3632-1, (2011). Qualitative traits (color and taste) were significantly and positively influenced by crop cycle length ($P \leq 0.01$), except for the aromatic power. The highest values of coloring ($A_{1\,cm}^{1\,\%}$ 440 nm) and bittering ($A_{1\,cm}^{1\,\%}$ 257 nm) powers were recorded in spice obtained from corms planted in biennial crop cycle (Table 4).

Generally, secondary metabolites play a major role in the adaption of plants to the environment and in overcoming stress conditions (Akula and Ravishankar, 2011). Koocheki et al. (2016) reported that the maximum contents of crocin and picrocrocin were obtained under stress condition such as no irrigation treatment. The biennial crop cycle has probably favored the diffusion of abiotic and biotic stress conditions determining the increase of secondary metabolites, such as crocin and picrocrocin, which contributed to improve the spice quality. Also the decrease of macroelements availability into the soil could have favored a greater accumulation of secondary metabolites. In this context, (Yarami and Sepaskhah, 2016) found that the lowest level of fertilizer (cow manure) increased the coloring power of spice.

This finding is consistent with our previous observations (Cardone et al., 2019), which showed that the spice with highest coloring power was obtained during the second cultivation year.

No significant effect of mother corm dimension on spice quality was found (Table 4). This result is in agreement with study of Gresta et al. (2008), who found no correlation between two corm dimensions (big corms with horizontal diameter of 3.5–4.5 cm and small corms with horizontal diameter 2.5–3.5 cm) and ISO values. Similarly, Koocheki et al. (2016) observed that crocin and picrocrocin were not affected by three sizes of mother corm (≤5 g, 5.1–10 g and >10 g). The same authors indicated that safranal did not depend on other factors, as mother corm weight and cultivation year.

Significant interactive effects were found for the coloring and bittering powers (Fig. 3A and B). The "biennial crop cycle x D2 class" combination obtained the highest coloring (306.3 $A_{1\,cm}^{1\,\%}$ 440 nm) and bittering (116.2 $A_{1\,cm}^{1\,\%}$ 257 nm) powers of spice, while the lowest values of these qualitative parameters were obtained by "annual crop cycle x

Table 4
Effect of crop cycle length (C), corm dimension (D), and their interaction on saffron qualitative traits in two years.

Experimental factors[1]	Qualitative traits					
	$A_{1\,cm}^{1\,\%}$ [3] (440 nm)	ISO [4] reference (crocetin esters)	$A_{1\,cm}^{1\,\%}$ [5] (257 nm)	ISO [6] reference (picrocrocin)	$A_{1\,cm}^{1\,\%}$ [7] (330 nm)	ISO [8] reference (safranal)
Crop cycle length (C)						
Annual	280.53	I	107.57	I	23.68	I
Biennial	304.84	I	114.53	I	24.55	I
Significance[2]	**		**		ns	
Corm dimension (D)						
D1	287.85	I	108.85	I	24.02	I
D2	294.38	I	112.36	I	24.29	I
D3	295.82	I	111.94	I	24.04	I
Significance	ns		ns		ns	
Year (Y)						
2017/2018	283.76	I	109.59	I	23.06	I
2018/2019	301.61	I	112.51	I	25.17	I
Significance	**		ns		ns	
Interactions						
C × D	*		*		ns	
C × Y	**		ns		ns	
D × Y	ns		ns		ns	
C × D × Y	ns		ns		ns	

[1] Mean values followed by a different letter are significantly different at $P \leq 0.05$, according to SNK test. [2] *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; ns, no significant difference. (3) Absorbance of 1% aqueous saffron extract at 440 nm. (4) ISO reference for crocetin esters: I category $A_{1\,cm}^{1\,\%} \geq 200$, II category $A_{1\,cm}^{1\,\%} \geq 170$, III category $A_{1\,cm}^{1\,\%} \geq 120$. (5) Absorbance of 1% aqueous saffron extract at 257 nm. (6) ISO reference for picrocrocin: I category $A_{1\,cm}^{1\,\%} \geq 70$, II category $A_{1\,cm}^{1\,\%} \geq 55$, III category $A_{1\,cm}^{1\,\%} \geq 40$. (7) Absorbance of 1% aqueous saffron extract at 330 nm. (8) ISO reference for safranal: I, II and III category $A_{1\,cm}^{1\,\%}$ minimum 20 and maximum 50.

D1 class" interaction (Fig. 3A and B).

The coloring power of spice was significantly influenced by the experimental year ($P \leq 0.01$) (Table 4). During the second experimental year (2018/2019) the corms produced a spice characterized by higher values of coloring and bittering powers (Table 4). Higher air mean temperature recorded during flowering period in 2018/2019 (9.3 °C) compared to one of the first year (6.6 °C) favored the accumulation of secondary metabolites as crocin and so the increasing of spice color (Cardone et al., 2019). Aromatic power was not influenced by experimental factors and their interaction (Table 4).

In general, all samples belonged to first qualitative category according ISO 3632 references. This result confirms what is reported in our previous study (Cardone et al., 2019), showing that the pedoclimatic conditions of Basilicata environment are suitable for saffron cultivation and for obtaining a spice with high quality.

3.5. Effect of crop cycle length and corm dimension on leaf and daughter corms traits in two years

Table 5 shows that crop cycle length and corm dimension influenced significantly the leaf and daughter corm traits. No significant effect of cultivation year was found for all traits, except for leaf length and total daughter corm weight (Table 5).

Daughter corm multiplication occurs after flowering period, from middle of November to April, reaching the maximum values in March and April (Renau-Morata et al., 2012). This stage can be affected by environmental conditions such as temperature, rainfall and humidity (Renau-Morata et al., 2012; Rahimi et al., 2017), mother corm dimension and origin (Bayat et al., 2016), planting densities (Koocheki et al., 2014) and agronomic practices (Seyyedi et al., 2018).

The highest values of leaf length and total daughter corm weight were obtained in 2017/2018 cycle (Table 5) due to higher rainfall (452.8 mm) compared to that recorded in 2018/2019 cycle (363.8 mm) (Table 1). The increasing of leaf length in the first cultivation year allowed a greater accumulation of reserves and weight in daughter corms (Renau-Morata et al., 2012).

Leaf number of the plants developed from corms planted in biennial crop cycle was increased by two folds as compared to those planted in annual crop cycle (Table 5). This finding is due to the increase of daughter corms and their buds during the previous crop cycle of saffron in according to literature (Douglas et al., 2014; Lopez-Corcoles et al., 2015).

The highest daughter corm number per mother corm, and the lowest daughter corm horizontal diameter and yield were obtained in the biennial crop cycle (Table 5). A negative correlation between daughter corm number and diameter was also found in others studies (Gresta et al., 2008; Douglas et al., 2014; Khorramdel et al., 2015).

As shown in Table 5, the increasing of mother corm dimension was resulted as rising of leaf number, daughter corm number per mother corm and daughter corm yield.

Leaf number of plants developed from corms of D3 class was increased by three folds as compared with corms belonged to D1 class (Table 5). These results are in agreement with Renau-Morata et al. (2012) who indicated that the number of leaves increased from 29 in corms with a weight of 6–12 g to 53 in corms with a weight of 30–36 g. By contrast, leaf length, daughter corm mean weight and horizontal diameter decreased with increasing mother corm dimension (Table 5).

Significant interaction between experimental factors was found for all traits regarding the growth of leaves and daughter corms ($P \leq 0.05$; $P \leq 0.01$). The highest leaf number per plant (93.2) was recorded when corms of D3 class were planted in biennial crop cycle (data not shown).

The significant interactions for the horizontal diameter, mean and total weight, and yield of daughter corms were reported in Fig. 4A–D. The "annual crop cycle x D1 class" interaction showed the highest daughter corm mean diameter (3.4 cm) and unitary weight (19.7 g) (Fig. 4A and B).

Daughter corm yield of D3 class decreased from 35.95 t ha^{-1} in annual crop cycle to 26.17 t ha^{-1} in biennial crop cycle and consequently the daughter corm diameter decreased from 2.60 cm to 1.60 cm, obtaining so more small corms not able to produce flowers in the successive crop cycle (Fig. 4B–D). These findings suggest that the planting of D3 corms in biennial crop cycle showed the worst performance in terms of daughter corm production. These results are in agreement with Koocheki et al. (2019), who reported that the percentage of replacement corms with a weight of more than 6 g increased when large mother corms (8–12 g) were planted for two years.

These results are in contrast with Husaini et al. (2010) who suggests that saffron fields should be lifted after every 4–5 years in Kashmir because the maximum corm yield (16.1 t ha^{-1}) was reached after 4 years. This contrasting result is due probably to the lack of evaluation of interaction between mother corm dimension and crop cycle length.

To make a better evaluation of daughter corm production, a distribution (n m^{-2}) in six categories according to diameter and weight was reported in Tables 6 and 7.

"Annual crop cycle × D3 class" interaction, followed by "Annual crop cycle × D2 class", resulted as the best agronomic management in terms of daughter corms production with a diameter from 3.1 to 4.5 cm (Table 6). This suitable daughter corms multiplication of D2 and D3 classes allowed to obtain the increase of flower number and stigma yield during the biennial crop cycle (Fig. 2A and B). Although D3 class obtained the highest number of harvested daughter corms per m^2 with a diameter of 4.1–4.5 cm and weigh greater than 25 g (Table 7), produced less flowers than D2 class. This result could be due to fact that corms of sufficient size to flower produced from D2 corms would have mostly two main buds, while those from D3 corms, produced from auxillary buds, might have a lower percentage of corms with more than one bud.

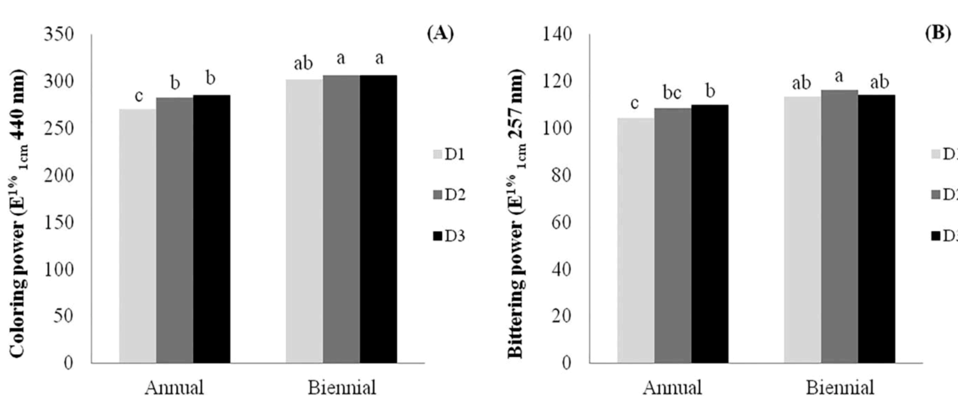


Fig. 3. Interactive effect of "crop cycle length × corm dimension" on the coloring (A) and bittering (B) powers; different letters above columns indicate significant differences at $P \leq 0.05$, according to SNK test.

Table 5
Effect of crop cycle length (C), corm dimension (D), and their interaction on leaf and daughter corms traits in two years.

Experimental factors [1]	Leaf number per plant	Leaf length (cm)	Daughter corm number per mother corm	Daughter corm mean weight (g)	Total daughter corm weight (g plant^{-1})	Daughter corm horizontal diameter (cm)	Daughter corm yield (t ha^{-1})
Crop cycle length (C)							
Annual	32.6	44.9	3.9	13.9	40.5	2.9	26.7
Biennial	66.8	45.9	10.0	3.9	28.8	1.9	19.1
Significance [2]	**	ns	**	**	*	**	*
Corm dimension (D)							
D1	25.7 c	46.6 a	3.0 c	12.5 a	23.1 c	2.8 a	15.2 c
D2	46.4 b	46.5 a	6.1 b	8.0 ab	33.9 b	2.3 ab	22.3 b
D3	77.1 a	43.3 b	11.9 a	6.4 b	47.1 a	2.1 b	31.1 a
Significance	**	*	**	*	**	*	**
Year (Y)							
2017/2018	49.9	47.3	6.7	9.3	36.8	2.4	24.3
2018/2019	49.5	43.6	7.3	8.6	32.6	2.4	21.5
Significance	ns	**	ns	ns	*	ns	Ns
Interactions							
C × D	**	*	**	**	**	**	**
C × Y	ns	ns	ns	ns	ns	ns	Ns
D × Y	ns	ns	ns	ns	ns	ns	Ns
C × D × Y	ns	ns	ns	ns	ns	ns	Ns

[1] Mean values followed by a different letter are significantly different at $P \leq 0.05$, according to SNK test.
[2] *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; ns, no significant difference.

Similar results have been observed by three-year study of Koocheki et al. (2019) who showed that the highest number of large daughter corms (>9 g) has been obtained by planting medium size mother corms (6–8 g) and lifting at the end of the first growing season. Torricelli et al. (2019) also found that the lifting and selection of daughter corms at the end of annual crop cycle, typical phases of Abruzzo region (Italy), allowed to obtain bigger corms.

Increase in crop cycle length and mother corm dimension enhanced the number of daughter corms per m^2 with a diameter from 0.1 to 2.5 cm and weight from 0.1 to 10 (Tables 6 and 7). Saffron corms with these characteristics are not able to flower because the minimum diameter to obtain one flower corm^{-1} is greater than 2.5 cm (Kumar et al., 2008; Cardone et al., 2020a). In biennial crop cycle, D1 class was resulted as the most productive in terms of daughter corms with a diameter from 3.1 to 4 cm and a weight of 15.1–20 g (Tables 6 and 7).

From an economic point of view, the purchase of flowering corms in the first year of saffron cultivation represents one of the major inputs. Other costs are related to the planting, lifting and cleaning of the corms,

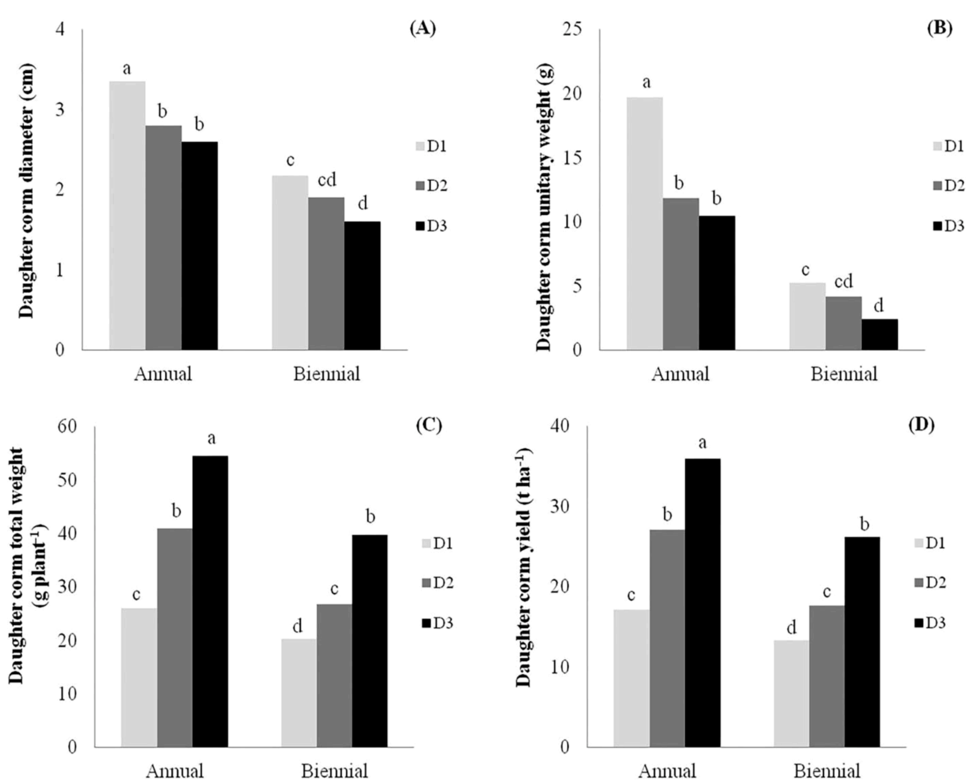


Fig. 4. Interactive effect of "crop cycle length × corm dimension" on the daughter corm diameter (A), unitary (B) and total weight (C), and yield (D); different letters above columns indicate significant differences at $P \leq 0.05$, according to SNK test.

Table 6
Number of harvested daughter corms per m^2 in different diameter classes in relation to crop cycle length (C), corm dimension (D), and their interaction in two years.

Experimental factors [(1)]	0.1-2 cm	2.1-2.5 cm	2.6-3 cm	3.1-3.5 cm	3.6-4 cm	4.1-4.5 cm
Crop cycle length (C)						
Annual	52.1	30.6	17.2	24.4	22.4	8.7
Biennial	266.0	106.0	22.8	5.6	1.6	0.0
Significance[(2)]	**	**	*	**	**	**
Corm dimension (D)						
D1	60.0 c	32.6 c	8.4 b	11.3 b	8.7 b	4.7 ab
D2	130.9 b	52.0 b	25.5 a	17.4 a	13.5 a	3.2 b
D3	286.2 a	114.2 a	26.2 a	16.3 a	13.8 a	5.2 a
Significance	**	**	**	*	*	*
Year						
2017/2018	145.1	56.5	24.9	14.8	12.1	5.7
2018/2019	173.0	76.1	15.1	15.3	11.9	3.0
Significance	**	**	**	ns	ns	*
Interactions						
Annual × D1	13.3 e	4.7 e	4.7 d	13.3 c	14.7 a	9.3 a
Annual × D2	46.7 d	33.7 d	20.7 b	27.3 b	25.0 a	6.3 b
Annual × D3	96.3 c	53.3 c	26.3 ab	32.7 a	27.7 a	10.3 a
Biennial × D1	106.7 c	60.6 bc	12.1 c	9.3 cd	2.8 c	0.0 c
Biennial × D2	215.2 b	70.4 b	30.3 a	7.5 d	2.0 c	0.0 c
Biennial × D3	476.0 a	175.0 a	26.0 ab	0.0 e	0.0 d	0.0 c
Significance	**	**	**	**	**	**

(1) Mean values followed by a different letter are significantly different at $P \leq 0.05$, according to SNK test.
(2) *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; ns, no significant difference.

Table 7
Number of harvested daughter corms per m^2 in different weight classes in relation to crop cycle length (C), corm dimension (D) and their interaction in two years.

Experimental factors [(1)]	0.1-5 g	5.1-10 g	10.1-15 g	15.1-20 g	20.1-25 g	> 25 g
Crop cycle length (C)						
Annual	56.7	35.9	19.9	17.6	16.3	9.1
Biennial	316.8	59.6	6.1	1.6	0.0	0.0
Significance[(2)]	**	*	**	**	**	**
Corm dimension (D)						
D1	58.2 c	28.5 c	8.7 c	7.7 b	7.0 b	5.3 a
D2	151.1 b	42.7 b	13.3 b	12.0 a	9.8 a	3.2 b
D3	351.0 a	72.0 a	17.0 a	9.0 ab	7.7 b	5.2 a
Significance	**	**	**	*	*	*
Year (Y)						
2017/2018	171.5	44.1	13.3	8.2	8.8	5.9
2018/2019	202.0	51.4	12.6	10.9	7.6	3.2
Significance	*	*	ns	*	ns	*
Interactions						
Annual × D1	13.3 e	6.7 e	4.7 d	10.7 c	14.0 b	10.7 a
Annual × D2	49.7 d	39.0 d	21.0 b	24.0 a	19.7 a	6.3 b
Annual × D3	107.0 c	62.0 b	34.0 a	18.0 b	15.3 b	10.3 a
Biennial × D1	103.0 c	50.4 c	12.7 c	4.8 d	0.0 c	0.0 c
Biennial × D2	252.5 b	46.5 c	5.5 d	0.0 e	0.0 c	0.0 c
Biennial × D3	595.0 a	82.0 a	0.0 e	0.0 e	0.0 c	0.0 c
Significance	**	*	**	**	**	**

(1) Mean values followed by a different letter are significantly different at $P \leq 0.05$, according to SNK test.
(2) *, Significance at $P \leq 0.05$; **, significance at $P \leq 0.01$; ns, no significant difference.

which often occur manually in small companies. Therefore, to amortize these costs, it is recommended to adopt a two-year cycle using corms with a minimum diameter of 2.0–2.5 cm and a maximum of 2.6–3.5 cm.

4. Conclusion

In saffron market, the most gain comes from the sale of spice and daughter corms. The price of spice depends mainly on its qualitative traits and that of daughter corms depends on their diameter. This study demonstrated that the evaluation of combination between crop cycle length and corm dimension is necessary in the saffron agronomic management and it can be taken into account in order to achieve optimum saffron yield and quality.

The highest saffron yield and quality were found when D2 dimensional class (2.5–3.5 cm) was used for biennial crop cycle, meanwhile the highest daughter corms production was obtained when corms belonged to D3 class (3.6–4.5 cm) were planted in annual crop cycle.

Regarding the qualitative parameters, coloring and bittering powers increased in biennial crop cycle, meanwhile aromatic power was not influenced by saffron cycle length. Considering the high nutritional aspect of vegetables observed under biotic (presence of weeds or pathogens) and abiotic stress conditions (low macronutrient and micronutrient availability) (Kyriacou and Rouphael, 2018), further studies are necessary to evaluate the nutritional quality of this spice obtained under different crop cycles, determining mineral, protein, lipid and vitamin content and phytochemical concentrations as carotenoids and flavonoids.

In addition, the effect of experimental factors on saffron by-products such as tepals, stamens and little corms (D1 class), is noteworthy. Big flowers with heavier tepals and stamens, produced by D3 corms in annual crop cycle, can be use to maximize the profitability because of their importance in pharmaceutical and cosmetic industries.

Furthermore, the tepals represent a very important part of the flower from ornamental point of view too. Corms belonged to D1 class, with a horizontal diameter between 2.0 and 2.5 cm, are more suitable for biennial crop cycle as they obtained the lowest percentage of daughter corms with a diameter from 0.1 to 2 cm and a weight from 0.1 to 5 g. In this way, these latter corms can be valorized by their planting in a biennial crop cycle, avoiding the buying of corms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Mr. Rocco Pirrone and Miss Maria Montesano for their support and assistance in collecting climatic and agronomic data.

References

Ahrazem, O., Rubio-Moraga, A., Castillo-López, R., Mozos, A.T., Gómez-Gómez, L., 2010. *Crocus sativus* pathogens and defence responses. Funct. Plant Sci. Biotechnol. 81–90. Special issue: Saffron. London, UK, Global Science Books.

Akula, R., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6 (11), 1720–1731.

Alonso, G.L., Zalacain, A., Carmona, M., 2012. Saffron. Handbook of Herbs and Spices. Woodhead Publishing, pp. 469–498.

Amirnia, R., Bayat, M., Tajbakhsh, M., 2014. Effects of nano fertilizer application and maternal corm weight on flowering at some saffron (*Crocus sativus* L.) ecotypes. Turkish J. Field Crop. 19 (2), 158–168.

Bagur, M.J., Salinas, G.L.A., Jiménez-Monreal, A.M., Chaouqi, S., Llorens, S., Martínez-Tomé, M., Alonso, G.L., 2018. Saffron: an old medicinal plant and a potential novel functional food. Molecules 23 (1), 30.

Bayat, M., Amirnia, R., Tajbakhsh, M., Ramezani, M., 2016. Evaluation of saffron ecotypes for stigma yield and yield components using different maternal corm weights. J. Plant. Physiol. Breed. 6 (1), 53–64.

Bove, B., Brindisi, P., Glisci, C., Pacifico, G., Summa, M.L., 2005. Climatic indicators of desertification in Basilicata, Italy. Forest@ 2, 74–84.

Branca, F., Argento, S., 2010. Evaluation of saffron pluriannual growing cycle in central Sicily. Acta Hort. 850, 153–158.

Cardone, L., Castonuovo, D., Perniola, M., Cicco, N., Candido, V., 2019. Evaluation of corm origin and climatic conditions on saffron (*Crocus sativus* L.) yield and quality. J. Sci. Food Agric. 99, 5858–5869.

Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Candido, V., 2020a. Saffron (*Crocus sativus* L.), the king of spices: an overview. Sci. Hort. 272, 109560.

Cardone, L., Castronuovo, D., Perniola, M., Scrano, L., Cicco, N., Candido, V., 2020b. The influence of soil physical and chemical properties on saffron (*Crocus sativus* L.) growth, yield and quality. Agronomy 10, 1154.

Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Molina, R.V., Renau-Morata, B., Nebauer, S.G., Candido, V., 2021. *Crocus sativus* L. ecotypes from mediterranean countries: phenological, morpho-productive, qualitative and genetic traits. Agronomy 11 (3), 551.

Caser, M., Victorino, Í.M., Demasi, S., Berruti, A., Donno, D., Lumini, E., Bianciotto, V., Scariot, V., 2019. Saffron cultivation in marginal Alpine environments: how AMF inoculation modulates yield and bioactive compounds. Agronomy 9 (1), 12.

Chen, D., Xing, B., Yi, H., Li, Y., Zheng, B., Wang, Y., Shao, Q., 2020. Effects of different drying methods on appearance, microstructure, bioactive compounds and aroma compounds of saffron (*Crocus sativus* L.). LWT Food Sci. Technol. 120, 108913.

Craine, J.M., Dybzinski, R., 2013. Mechanisms of plant competition for nutrients, water and light. Funct. Ecol. 27, 833–840.

D'Auria, M., Mauriello, G., Rana, G.L., 2004. Volatile organic compounds from saffron. Flavour Frag. J. 19 (1), 17–23.

de Juan, J.A., Córcoles, H.L., Muñoz, R.M., Picornell, M.R., 2009. Yield and yield components of saffron under different cropping systems. Ind. Crops Prod. 30 (2), 212–219.

Douglas, M.H., Smallfield, B.M., Wallace, A.R., McGimpsey, J.A., 2014. Saffron (*Crocus sativus* L.): the effect of mother corm size on progeny multiplication, flower and stigma production. Sci. Hortic. 166, 50–58.

18 European Saffron White Book, 2006. Problems and Strategies for improving the quality and strengthen competitiveness. In: López Córcoles, H., Carmona, M., López Fuster, P., Alonso, G., et al. (Eds.), Interreg IIIC.

Fallahi, H.R., Aghhavani-Shajari, M., Sahabi, H., Feizi, H., 2017. Mother corm weight and soil amendment improves the vegetative and reproductive growth of saffron (*Crocus sativus* L.). J. Med. Spice Plants (Zeitschrift für Arznei-& Gewürzpflanzen) 22 (3), 110–114.

Fallahi, H.R., Mahsa Aghhavani-Shajari, M., Sahabi, H., Behdani, M.A., Sayyari-Zohan, M.H., Vatandoost, S., 2020. Influence of some pre and post-harvest practices on quality of saffron stigmata. Sci. Hortic. 278, 109846.

Ghanbari, J., Khajoei-Nejad, G., van Ruth, S.M., Aghighi, S., 2019. The possibility for improvement of flowering, corm properties, bioactive compounds, and antioxidant activity in saffron (Crocus sativus L.) by different nutritional regimes. Ind. Crops Prod. 135, 301–310.

Goliaris, A.H., 1999. Saffron. Saffron cultivation in Greece. CRC Press, pp. 78–89.

Gresta, F., Avola, G., Lombardo, G.M., Siracusa, L., Ruberto, G., 2009. Analysis of flowering, stigmas yield and qualitative traits of saffron (Crocus sativus L.) as affected by environmental conditions. Sci Hortic. 119 (3), 320–324.

Gresta, F., Lombardo, G.M., Siracusa, L., Ruberto, G., 2008. Effect of mother corm dimension and sowing time on stigma yield, daughter corms and qualitative aspects of saffron (*Crocus sativus* L.) in a Mediterranean environment. J. Sci. Food Agric. 88, 1144–1150.

Grilli Caiola, M., Canini, A., 2010. Looking for saffron's (*Crocus sativus* L.) parents. Funct. Plant Sci. Biotechnol. 4 (2), 1–14.

Husaini, A.M., Hassan, B., Ghani, M.Y., Teixeira da Silva, J.A., Kirmani, N.A., 2010. Saffron (*Crocus sativus* Kashmirianus) cultivation in Kashmir: practices and problems. Funct. Plant Sci. Biotechnol. 4, 108–115.

ISO 3632-1, 2011. Saffron (*Crocus Sativus* L.): Specifications. International Standards Organization, Switzerland, Geneva (2003 revised 2011).

ISO 3632–2, 2010. (*Crocus Sativus* L.): Test Methods. International Standards Organization, Switzerland, Geneva (2003 revised 2010).

Jami, N., Rahimi, A., Naghizadeh, M., Sedaghati, E., 2020. Investigating the use of different levels of Mycorrhiza and Vermicompost on quantitative and qualitative yield of saffron (*Crocus sativus* L.). Sci. Hortic. 262, 109027.

Khorramdel, S., Eskandari, Nasrabadi, S., Mahmoodi, G., 2015. Evaluation of mother corm weights and foliar fertilizer levels on saffron (*Crocus sativus* L.) growth and yield components. J. Appl. Res. Medic. Arom. Plants 2, 9–14.

Koocheki, A., Ebrahimian, E., Seyyedi, S.M., 2016. How irrigation rounds and mother corm size control saffron yield, quality, daughter corms behavior and phosphorus uptake. Sci. Hort. 213, 132–143.

Koocheki, A., Moghaddam, P.R., Aghhavani-Shajari, M., Fallahi, H.R., 2019. Corm weight or number per unit of land: which one is more effective when planting corm, based on the age of the field from which corms were selected? Ind. Crops Prod. 131, 78–84.

Koocheki, A., Seyyedi, S.M., 2015. Relationship between nitrogen and phosphorus use efficiency in saffron (*Crocus sativus* L.) as affected by mother corm size and fertilization. Ind. Crops Prod. 71, 128–137.

Koocheki, A., Seyyedi, S.M., Eyni, M.J., 2014. Irrigation levels and dense planting affect flower yield and phosphorus concentration of saffron corms under semi-arid region of Mashhad, Northeast Iran. Sci. Hortic. 180, 147–155.

Kumar, R., Singh, V., Devi, K., Sharma, M., Singh, M.K., Ahuja, P.S., 2008. State of art of saffron (*Crocus sativus* L.) agronomy: a comprehensive review. Food Rev. Int. 25 (1), 44–85.

Kyriacou, M.C., Rouphael, Y., 2018. Towards a new definition of quality for fresh fruits and vegetables. Sci. Hortic. 234, 463–546.

Lopez-Corcoles, H., Brasa-Ramos, A., Montero-García, F., Romero-Valverde, M., Montero-Riquelme, F., 2015. Phenological growth stages of saffron plant (*Crocus sativus* L.) according to the BBCH scale. J. Agric. Res. 13 (3).

Macchia, M., Ceccarini, L., Molfetta, I., Cioni, P.L., Flamini, G., 2013. Studies on saffron (*Crocus sativus* L.) from Tuscan Maremma (Italy): effects of geographical origin, cultivation environment and drying method on volatile emission. J. Food Sci. Technol. 48 (11), 2370–2375.

Maggi, L., Carmona, M., Zalacain, A., Kanakis, C.D., Anastasaki, E., Tarantilis, P.A., Polissiou, M.G., Alonso, G.L., 2010. Changes in saffron volatile profile according to its storage time. Food Res. Int. 43, 1329–2133.

McGimpsey, J.A., Douglas, M.H., Wallace, A.R., 1997. Evaluation of saffron (*Crocus sativus* L.) production in New Zealand. NZ J. Crop. Hort. Sci. 28, 159–168.

Mohammadi, H., Reed, M., 2020. Saffron Marketing: Challenges and Opportunities, Eds: Koocheki, A. Khajeh-Hosseini, M., Saffron, Woodhead Publishing, 22, pp. 357–365, https://doi.org/10.1016/B978-0-12-818638-1.00022-8.

Molina, R.V., Valero, M., Navarro, Y., Guardiola, J.L., Garcia-Luis, A., 2005. Temperature effects on flower formation in saffron (*Crocus sativus* L.). Sci. Hortic. 103 (3), 361–379.

Mzabri, I., Addi, M., Berrichi, A., 2019. Traditional and modern uses of saffron (*Crocus sativus*). Cosmetics 6 (4), 63.

Nehvi, F.A., Ajaz, A., Lone, A.A., Khan, M.A., Makhdoomi, M.I., 2010. Comparative study on effect of nutrient management on growth and yield of saffron under temperate conditions of Kashmir. Acta Hort. (ISHS) 850, 165–170.

Pardo, J.E., Zalacain, A., Carmona, M., Lopez, E., Alvarruiz, A., Alonso, G.L., 2002. Influence of the type of dehydration process on the sensory properties of saffron spice. Ital. J. Food Sci. 14, 413–422.

R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing,. Vienna, Austria.

Rahimi, H., Shokrpour, M., Tabriziraeini, L., Esfandiari, E., 2017. A study on the effects of environmental factors on vegetative characteristics and corm yield of Saffron (*Crocus sativus*). Iran J. Hortic. Sci. 48, 45–52.

Renau-Morata, B., Nebauer, S.G., Sánchez, M., Molina, R.V., 2012. Effect of corm size, water stress and cultivation conditions on photosynthesis and biomass partitioning during the vegetative growth of saffron (*Crocus sativus* L.). Ind. Crops Prod. 39, 40–46.

Rezvani-Moghaddam, P., Khorramdel, S., Shabahang, J., Amin Ghafouri, A., 2013. Evaluation of planting method, corm weight and density effects on growth characteristics and yield of saffron (*Crocus sativus* L.). J. Agroecol. 3 (1), 52–68.

Seyyedi, S.M., Ebrahimian, E., Rezaei-Chiyaneh, E., 2018. Saffron daughter corms formation, nitrogen and phosphorous uptake in response to low planting density,

sampling rounds, vermicompost and mineral fertilizers. Commun. Soil Sci. Plant. 49 (5), 585–603.
Shajari, M.A., Moghaddam, P.R., Ghorbani, R., Koocheki, A., 2020. The possibility of improving saffron (*Crocus sativus* L.) flower and corm yield through the irrigation and soil texture managements. Sci. Hortic. 271, 109485.
Shin, H.K., Lieth, J.H., Kim, S.H., 2001. Effects of temperature on leaf area and flower size in rose. Acta Hort. 547, 185–191.
Temperini, O., Rea, R., Temperini, A., Colla, G., Rouphael, Y., 2009. Evaluation of saffron (*Crocus sativus* L.) production in Italy: effects of age of saffron fields and plant density. J. Food Agric. Environ. 7, 19–23.
Tong, Y., Zhu, X., Yan, Y., Liu, R., Gong, F., Zhang, L., Hu, J., Fang, L., Wang, R., Wan, P., 2015. The influence of different drying methods on constituents and antioxidant activity of saffron from China. Int. J. Anal. Chem. https://doi.org/10.1155/2015/953164.
Torricelli, R., Yousefi Javan, I., Albertini, E., Venanzoni, R., Hosseinzadeh, Y.G., 2019. Morphological and molecular characterization of Italian, Iranian and Spanish saffron (*Crocus sativus* L.) accessions. Appl. Ecol. Environ. Res. 17, 1875–1887.
Yarami, N., Sepaskhah, A.R., 2016. Effect of irrigation water salinity, manure application and planting method on soil ions variation and ions uptake by saffron (Crocus sativus L.). Int. J. Plant Prod. 10 (2), 197–219.
Zarinkamar, F., Tajik, S., Soleimanpour, S., 2011. Effects of altitude on anatomy and concentration of crocin, picrocrocin and safranal in *Crocus sativus* L. Australian J. Crop Sci. 5, 831–838.