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Research Article

The effects of mother corm weight and cultivation systems on morphophysiological characteristics of saffron (*Crocus sativus* L.)

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Abstract

This study aimed to investigate the effects of different cultivation and mother corm weight on certain morphological and physiological characteristics of saffron (*Crocus sativus* L.). This experiment was conducted as a completely randomized factorial design with three replications. Experimental treatments include three types of cultivation, such as field, aeroponic, and 1/2 Murashige and Skooge (MS). and three levels of corm weights of <4 g, 4-9 g, and 9-23 g. The results revealed that the cultivation had significant ($P \leq 0.01$) effects on all studied traits except for leaf carotenoid. Safranal content was highest in the field, followed by 1/2 MS and aeroponic. Maximum crocin and picrocrocin levels in the stigma are desirable, such as with aeroponic and 1/2MS cultivation, respectively. The highest fresh weight of flower, dry weight of flowerand stigma dry weight were related to 1/2 MS cultivation. The effect of the mother corm weight was also significant on picrocrocin at the $P \leq 0.05$ level and on all traits at the $P \leq 0.01$ level except for the root fresh weight and stigma safranal content. Investigation of interaction effects of cultivation type and mother corm weight showed that the best efficiency of the saffron crops in terms of yield was related to the heavier than 9 g mother corm weight and 1/2 MS cultivation, such as flower number, flower fresh weight, flower dry weight, stigma fresh weight, stigma dry weight, and picrocrocin content. Therefore, to produce organic saffron and increase its yield, it is recommended to cultivate mother corms heavier than 9 g in the 1/2 MS cultivation.

Keywords: Cultivation, Morphological, Physiological, Saffron

Introduction

Saffron (*Crocus sativus* L.) is a perennial species from the iris family Iridaceae that has spread in low-rain regions of Iran that have cold winters and hot summers. The plant is known as an expensive spice species with many applications in the pharmaceutical and food industries (Gresta *et al.*, 2008; Alizadeh *et al.*, 2025a). Saffron is useful for human health due to having three main compounds: safranal, crocin, picrocrocin (Cardone *et al*., 2020).

The main challenge of saffron production in Iran is the yield gap due to the high difference between the mean yield and the attainable yield. The mean yield of saffron is 3.96 kg/ha in Iran, significantly differing from, for example, 15 kg/ha in Spain and 9 kg/ha in Pakistan (Behdani *et al*., 2008). It is crucial to check different ways of agronomic management for improving saffron yield. Indeed, traditional practices must be replaced with modern management practices for increasing saffron production and export, according to the medicinal and economic importance of saffron, finding ways to increase the quality and production per unit area is important, It seems that the use of new technologies such as aeroponics and hydroponics in production can increase the yield of saffron. Soilless farming is one of the new methods of crop production in the world and will be a valuable solution for producing quality and sustainable products throughout the year in areas that face adverse weather conditions or limited land and resources for crop production. In this method, plants are grown in the air without using a growing medium instead of soil (Lakhiar *et al*., 2020).

In the soilless culture of saffron using such substrates as peat moss, cocopeat, perlite, mineral wool, and vermiculite, the plant yield and water use efficiency are increased significantly (Frederic *et al.*, 2000; Alizadeh *et al.*, 2025b; Molina *et al*., 2005; Savvas, 2003; Shojaei *et al*., 2019; Souret and Weathers, 2000).

Majid *et al*. (2021) found that hydroponic systems in temperate regions produced higher yields in a shorter period. Additionally, Brockhagen *et al*. (2021) demonstrated that growing spinach, strawberries, and brassica in a soilless system significantly reduced the water requirements. Atzori *et al.* (2019) and Silva *et al.* (2020) reported that chicory exhibited increased water use efficiency in hydroponics compared to traditional

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soil cultivation. Feyzizadeh *et al*. (2023) reported that the type of cultivation has an effect on plant parameters, including plant length, leaf area, dry and wet weight of leaves, dry weight of corms, diameter of corms, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids.

The use of small mother corms is one of the reasons for the low yield of saffron plants in traditional Iranian farms. In other countries, to avoid this issue, large and healthy corms from 4 years farms are used for planting in new farms. In addition to determining the amount of flowers and yield in the first year, these corms produce new corms that will serve as the seed of the plant in the second year, and the newly produced corms will successively affect the yield in subsequent years (Amirshekari *et al*., 2006). The effect of mother corm size on increasing traits related to saffron economic performance (stigma weight, flower weight, and number of flowers) in soil cultivation has also been reported by other researchers (Koocheki *et al*., 2016; Hassanzadeh Aval *et al.*, 2014).

However, no research has been conducted so far on the importance of the size of mother corms in soilless cultivation of saffron. According to research, the weight of the mother corm is one of the determining factors in achieving the desired yield in saffron cultivation. Planting mother corms with the right weight will improve the growth and yield of saffron.

Despite the fact that Iran has the largest area of saffron cultivation and production in the world, the yield per unit area of this product is very low compared to other producing countries; Therefore, solutions that lead to higher yields in a shorter period of time will prevent the wastage of water, land, and capital of saffron growers. Cultivation systems under controlled conditions can replace conventional saffron cultivation methods. Therefore, considering that in soilless cultivation, flower production is fundamentally dependent on the conditions of the mother corm used, determining the range of corm weights used for optimal flower production and also achieving maximum new corm yield is of great importance. Using small mother corms increases the risk of reducing flower yield and also producing daughter corms unsuitable for use in future years, and on the other hand, using larger mother corms than required can lead to higher costs for producers; therefore, in the present study, the effect of mother corm weight on traits related to three types of saffron cultivation has been studied. No studies have compared aeroponic, 1/2MS, and field cultivation for saffron with varying corm weights.

Materials and methods

In August 2019, saffron corms with a weight of 4-23 g were selected. Then, their tunica layers were removed, and the broken, pest-infected, wormy, fungi-infected ones were discarded. The remaining corms were disinfected with 1% sodium hypochlorite and were stored at 5˚C in a refrigerator.

This experiment was conducted as a completely randomized factorial design with three replications, including three types of culture, such as aeroponic, 1/2 Murashige and Skooge (1962) MS, and field cultivation, and three levels of corm weights at <4 g, 4-9 g, and 9-23 g. The number and density of the culture were different based on the mother corms weight. Mother corms from the beginning of the experiment until the height of the central tubes grew about 2 to 3 cm at a temperature of 17˚C and 70% humidity; they grew without receiving light. After this stage, the temperature of the environment for the growth of saffron seeds was gradually changed to 12˚C and humidity to 85%. By starting the temperature and humidity change process, the corms were exposed to light with a photoperiod of 12 hours of light, and in these conditions, the flowers were preserved until the end of the harvesting period (Souret and Weathers, 2000).

A substrate used in this research is 1/2 MS. It was first developed and used for tobacco cultivation. Though designed for tobacco, its culture medium has many applications for many other plants. This liquid is composed of three categories of nutrients, micronutrients, and other supplements, which are combined in certain ratios, as seen in Table 1 (Kamali, 2020). Trays in the size of 25×15×5 cm were provided, and saffron corms were placed inside them based on weights, while in 1/2 MS liquid they were immersed, the humidity and temperature conditions were the same as aeroponic cultivation.

A parcel of land was separated from the research farm of the Yazd University (54° 21' 31" E and 31° 49' 46" N) and prepared for field cultivation. The average annual soil temperature in this area was between 15-17°C, and the mean humidity of the soil was 15-20%. The physical and chemical properties of farm soil are shown in table 2. The electrical conductivity (EC) and the pH of the soil were determined with an EC meter and a pH meter on the saturated soil paste (Page *et al*., 1982). Some other characteristics of the soil, including organic matter content, were determined by the Walkley-Black method (Nelson and Sommers, 1983). Total nitrogen content was estimated by the Kjeldahl method (Chapman and Pratt, 1961), potassium content by flame photometry (Chapman and Pratt, 1961), phosphorous content by Olsen's (1954) method, calcium and magnesium contents by complexometry, and sodium content by flame photometry on the saturated soil paste. The physical and chemical properties of farm soil are shown in table 2.

With the onset of flowering in late November 2019, the daily flower harvesting operations were carried out, and immediately after counting the number of flowers and their components, they were weighed using a precise digital scale with a sensitivity of 0.0001 grams. To reach the dry weight, each treatment was separately dried at room temperature, and after weighing the dried saffron components, they were stored in clean plastic containers.

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Table 1. Characteristics and amount of soluble elements of 1/2 Murashige-Skooge (ppm) (Kamali, 2020).

NH_4NO_3	KNO_3	$CaCl_{202}H_2O$	$MgSO_{207}H_2O$	$KHPO_2$	$MnSO_{20}4H_2O$	H_2BO_2	KI	$Na_2MoO_402H_2O$
825	950	220	185	85	11.15	3.1	0.415	0.125
$CoCl_{20}6H_2O$	$ZnSO_{20}7H_2O$	$FeSO_{20}7H_2O$	$CuSO_45H_2O$	Glycine	Thiamin. HCL	$Na_{20}EDTA$	Myo-inositol	Nicotinic acid
0.0125	4.3	13.9	0.0125	2	0.1	18.6	100	0.5

Table 2. Characteristics and amount of elements of soil

Soil texture	EC($dS.m^{-1}$)	pH	OM %	TN(%)	P(ppm)	K(ppm)	Ca(ppm)	Mg(ppm)	Na(ppm)
Sandy loam	2.43	7.74	0.31	0.02	8.54	201.83	293.91	246.10	122.33

Photosynthesizing pigments, including chlorophylls, were measured by Arnon's (1967) method, for which 0.2 g of plant leaves was washed with distilled water, crushed in a mortar, and added with 5 mL of 80% acetone. Then, it was infiltrated through filter paper and centrifuged at 3500 rpm for 15 minutes. Next, a spectrophotometer (SPECORD 210, Analytik Jena, 210, Germany) model was calibrated with 80% acetone, and the absorbance of the samples was read at 663 and 645 nm. Finally, Eqs. (1)-(3) were used to determine the contents of photosynthesizing pigments:

(1) Chlorophyll *a* ($mgg^{-1}fw$) = (19.3 × A664 – 0.86 × A645) × 20/100 × g plant

(2) Chlorophyll *b* ($mgg^{-1}fw$) = (19.3 × A645 – 3.6 × A663) × 20/100 × g plant

(3) Total Chlorophyll = Chl *a* + Chl *b*

The chemical analysis of stigma, including crocin, picrocrocin, and safranal, was performed as per the national saffron standard of Iran (INSO, 2013). To this end, 50 mg of the powdered saffron sample was poured into a 100-ml volumetric flask, and 900 ml of sterilized twice distilled water was added to it. The samples were shaken with a magnetic blender at 1000 rpm for one hour. Then, the volume of the solution was adjusted to 100 ml by adding twice distilled water and was centrifuged at 4000 rpm for 15 minutes. Next, 20 ml of the solution was adjusted to 200 ml, and its absorbance was read at 200-700 nm. The absorbance at 257, 330, and 440 nm represented picrocrocin, safranal, and crocin, respectively, whose quantities were calculated by Eq. (4) (INSO, 2013).

(4) E=D×10000 /m(100-H)

In which *D* represents the absorbance read with an Analytic Jena spectrophotometer, *m* represents the weight of the saffron sample (g), and *H* represents the moisture and volatiles content (% w/w). To measure the moisture content, the stigmas were first crushed in a mortar. Then, after the powdered sample was weighed, it was oven-dried at 45°C for 24 hours and re-weighed. The moisture content was calculated by Eq. (5).

(5)

$$\text{Moisture content (\%)} = \frac{\text{Fresh sample weight-Dry sample weight}}{\text{Fresh sample weight}} \times 100$$

Data were subjected to a two-way analysis of variance, and means were compared with the Duncan Multiple Range test. Statistical data analysis and drawing of the graphs were performed using SPSS (version 20) and Excel software, respectively.

Results

Table 3 presents the analysis of variance (mean squares) for the effects of mother corm weight and cultivation systems on some morphological and yield traits, and table 4 presents the results for physiological traits of the saffron stigmas and leaves based on the analysis of variance. The effect of the substrate was significant on fresh and dry root weight, flower number, flower fresh and dry weight, stigma fresh and dry weight, stigma crocin, safranal, and picrocrocin contents, chlorophyll *b* content, and total chlorophyll content at the P≤ 0.01 level and on chlorophyll *a* content. The comparison of means revealed that the effect of mother corm weight was significant on root dry weight, flower number, flower fresh and dry weight, stigma fresh and dry weight, stigma crocin content, chlorophyll *a* and *b* contents, total chlorophyll content, and carotenoid content at the P≤ 0.01 level and on sigma picrocrocin content at the P≤ 0.05 level. However, it did not bring about a significant change in stigma safranal content (Tables 3 and 4). The interaction of the substrate and mother corm weight was also significant for all traits, except for the carotenoid and stigma safranal contents.

Root weight: As seen in Table 3, the effect of the substrate was significant (P≤0.01) on the root fresh and dry weight. The effect of the mother corm weight was insignificant on the root fresh weight but significant (P≤0.01) on the root dry weight. Also, both the fresh and dry weights of the roots were significantly (P≤0.01) influenced by the interactive effects of the substrate and mother corm weight. The comparison of means revealed that the highest root fresh weight was related to the aeroponic system and the lowest to the 1/2MS (Figure 1A). The highest root dry weight was obtained from the aeroponic system, not differing from the soil culture and 1/2MS significantly. The lowest was obtained from the mother corms that were < 4 g in weight (Figure 1B).

Flower yield: As is evident in table 3, the effects of both substrate and mother corm weight were significant (P≤0.01) on the fresh and dry weight.

Based on the comparison of means, the highest flower number and fresh/dry weight were observed in plants grown from mother corms >9 g in the 1/2 MS substrate, and the lowest flower number and fresh/dry weight were observed in plants grown from mother corms <4 g in the aeroponic system (Figure 2A and 2B).

Stigma yield: Stigma production is the main goal of saffron cultivation. According to table 3, the cultivation systems had a significant effect (P≤0.01) on both

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Table 3. Analysis of variance of the effects of mother corm weight and cultivation systems on some morphological traits of saffron yield (per corm)

Source of variation	df	Root fresh weight(g)	Root dry weight(g)	Weight of fresh flower(g)	Weight of dry flower(g)	Weight of fresh stigma(g)	Weight of dry stigma(g)
Cultivation type (A)	2	13.22**	1.05**	39.11**	4.37**	4.91**	0.009**
Corm weight (B)	2	0.002^{ns}	0.17**	56.56**	6.68**	3.96**	0.030**
(A)× (B)	4	5.74**	0.57**	3.81**	0.14**	0.66**	0.002**
Error	18	0.490	0.009	0.164	0.004	0.056	0.008

***and ** indicates significant at the 0.05 and 0.01, probability level, respectively; ns= no significant difference.**

Table 4. Analysis of variance of the effects of mother corm weight and cultivation systems on some physiological traits of stigmas and leaves of saffron.

Source of variation	df	Stigma Crocin ($E_{1cm}^{1\%}$ 440 nm)	Stigma Safranal ($E_{1cm}^{1\%}$ 330 nm)	Stigma Picrocrocin ($E_{1cm}^{1\%}$ 257 nm)	Chl a	Chl b	T chl	Carotenoid
					(mgg^{-1} Fw)			
Cultivation type (A)	2	9320.037**	543.749**	448.245**	15.460*	261.668**	200.710**	488965.690^{ns}
Corm weight (B)	2	84.593**	0.003^{ns}	0.785*	68.556**	194.073**	501.430**	3222686.580**
(A)× (B)	4	59.870**	0.008^{ns}	0.970**	13.438**	110.388**	83.373**	258438.207^{ns}
Error	18	0.481	0.004	0.077	2.541	10.093	14.196	128403.517

*** and ** indicates significant at the 0.05 and 0.01, probability level, respectively; ns= no significant difference.**

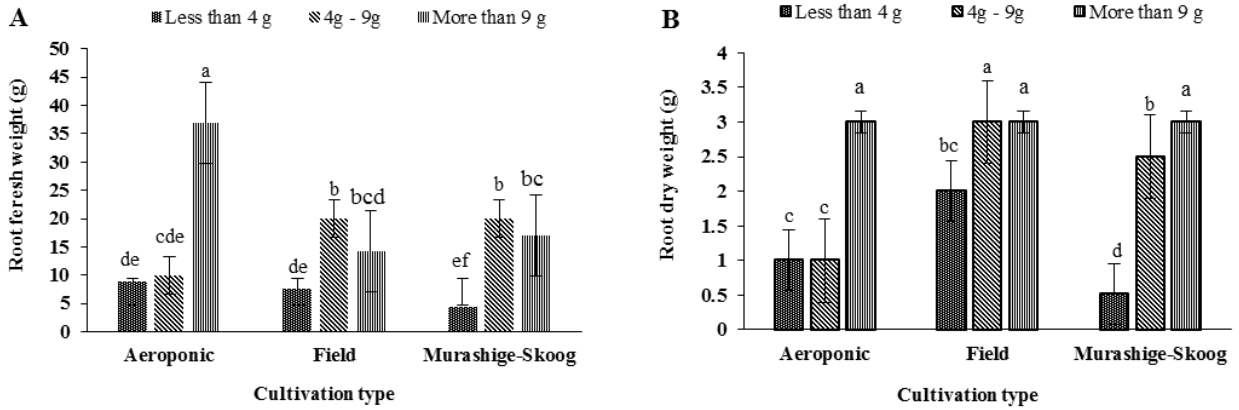

Figure 1. Interaction effects of cultivation type and mother corm weight on the weight of fresh root (A) and weight of dry root (B) of saffron (*Crocus sativus* L.). (Means with at least one common letter are not significantly different by Duncan multiple range test at P ≤ 0.05).

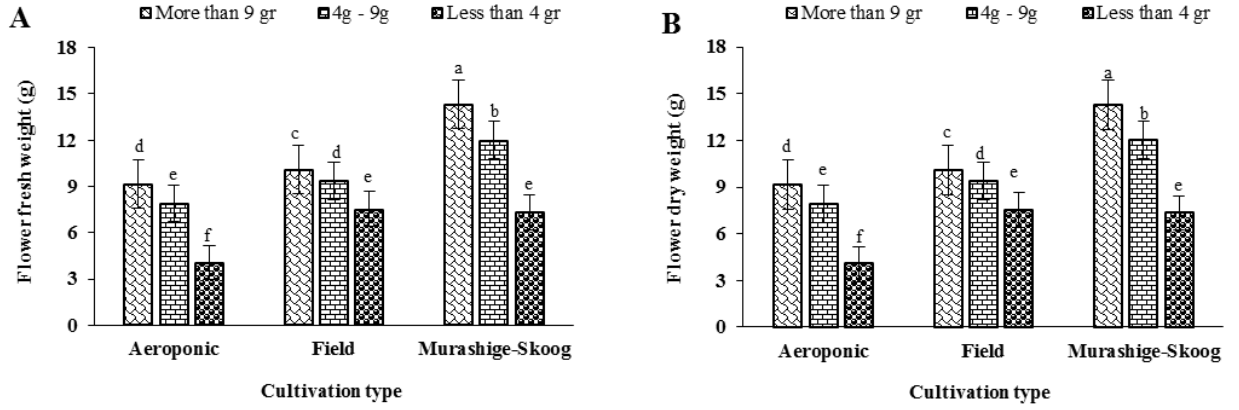

Figure 2. Interaction effect of cultivation type and mother corm weight on the weight of fresh flower (A) and weight of dry flower (B) of saffron (*Crocus sativus* L.). (Means with at least one common letter are not significantly different by Duncan multiple range test at P ≤ 0.05).

stigma fresh and dry weight, the mother corm weight had a significant effect (P≤0.01) on both stigma fresh and dry weight. The interaction of substrate and mother corm weight was also significant (P≤0.01) for both stigma fresh and dry weight.

The highest stigma fresh weight was obtained from

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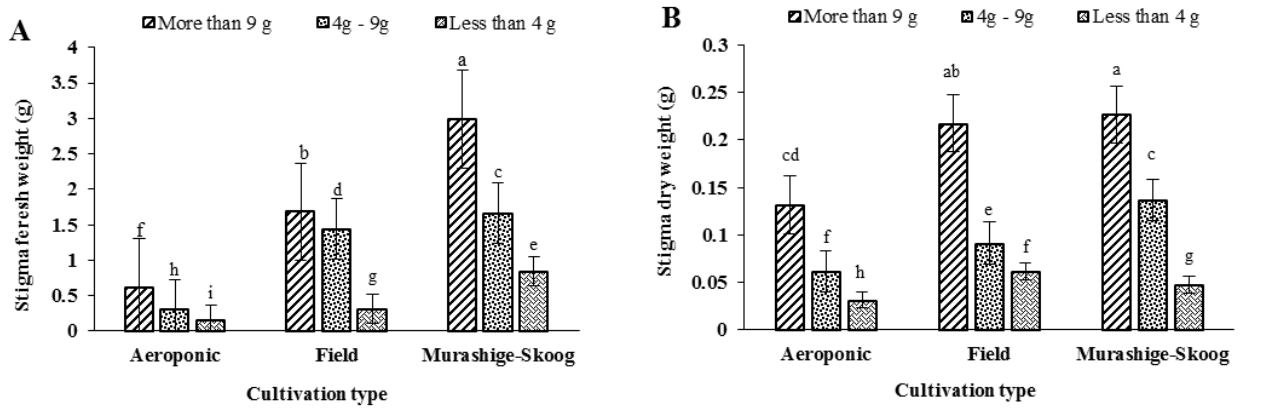

Figure 3. Interaction effect of cultivation type and mother weight corm on: weight of fresh stigma (A) and weight of dry stigma (B), of saffron (*Crocus sativus* L.). (Means with at least one common letter are not significantly different by Duncan multiple range test at (P ≤ 0.05).

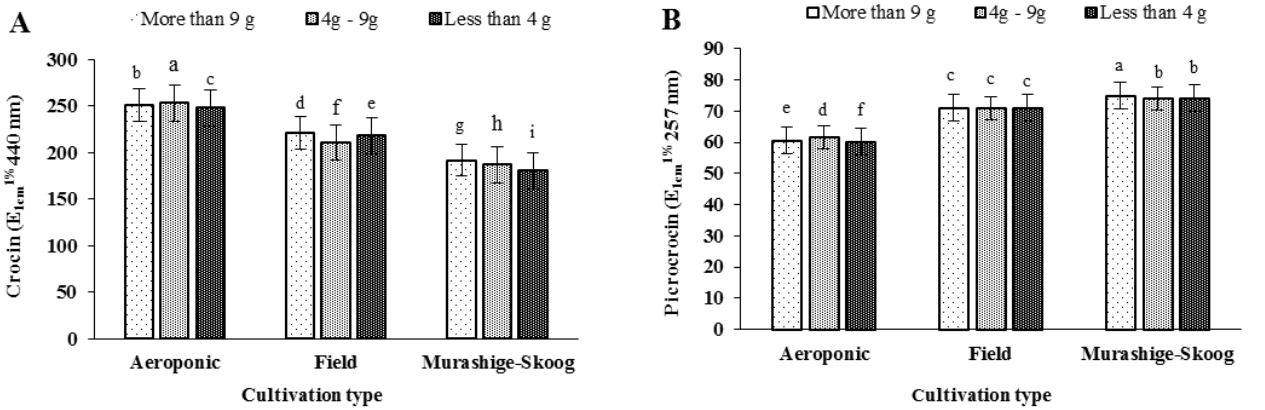

Figure 4. Interaction effects of cultivation type and mother corm weight on crocin (A) and picrocrocin (B) of saffron (*Crocus sativus* L.). (Means with at least one common letter are not significantly different by Duncan multiple range test at P ≤ 0.05).

plants grown with moderate- to high weight mother corms (>4 g) in the 1/2MS substrate; the lowest stigma fresh weight was from the interaction of the aeroponic system and <4 g mother corms (Figure 3A).

The maximum stigma dry weight was obtained from the 1/2MS substrate with >9 g mother corms, not significantly different from the soil culture (Figure 3B).

The result explains that the 1/2MS substrate, which contains easily available nutrients like phosphorus, allows better nutrient uptake by the plant roots and results in increased stigma growth and yield.

Larger mother corms have higher nutrient reserves, faster emergence, and better utilization of resources, leading to improved reproductive growth and higher stigma production. The positive correlation between flower number and stigma weight suggests that the plant does not change the photosynthate allocation to the stigma under different conditions.

Physiological traits: Table 4, presents that the effect of the cultivation type had a significant effect (P≤0.01) on the stigma content of the three key bioactive compounds: Safranal, Crocin and picrocrocin. The mother corm weight had a significant effect (P≤0.01) on the stigma crocin content, also significant (P≤0.05) on the stigma picrocrocin content. However, the mother corm weight did not had a significant effect on the stigma safranal content.

The interaction between cultivation type and mother corm weight was examined for the stigma biochemical composition. The highest stigma crocin content was obtained from the aeroponic system across all three mother corm weight ranges, the next highest crocin content was from the soil culture, while the lowest was from the 1/2MS substrate (Figure 4A). The higher stigma crocin content in the higher stigma crocin content in the aeroponic system may be associated with the relative nutrient poverty of this substrate. This could have caused some nutrient deficiencies, particularly of nitrogen, leading the plants to increase the synthesis of active compounds like crocin as an adaptive response to environmental stress.

According to the Figure 4B, the highest picrocrocin was obtained from the 1/2MS medium, this was attributed to the higher availability of key micronutrients like iron, zinc, manganese and copper in the 1/2 MS medium, which can act as enzyme activators and influence saffron metabolism.

The soil culture was associated with the second-highest picrocrocin content and the soilless with the corm weight of < 4 g was ranked the lowest (Figure

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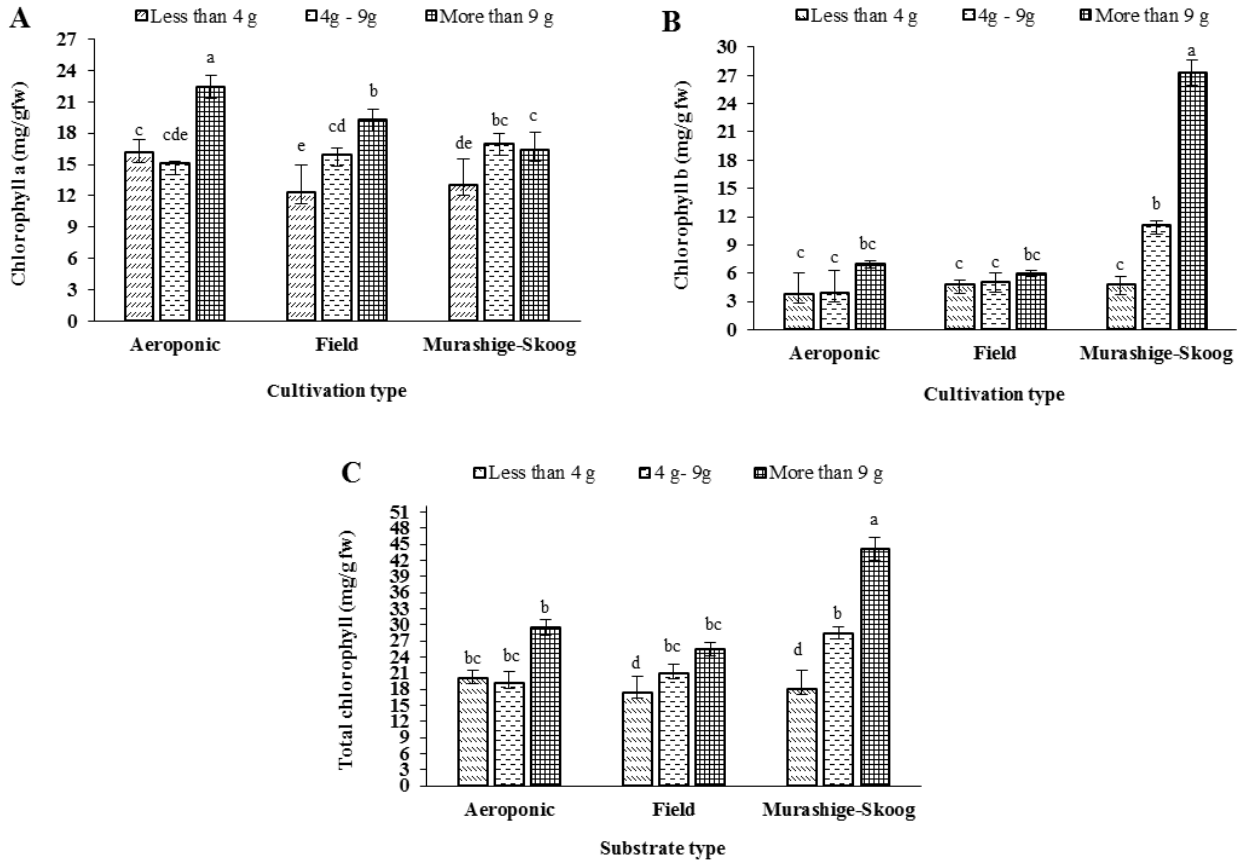

Figure 5. Interaction effects of cultivation type and mother corm weight on chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) of saffron (*Crocus sativus* L.). (Means with at least one common letter are not significantly different by Duncan multiple range test at (P ≤ 0.05).

4B). The soil culture was associated with the second-highest picrocrocin content and the soilless with the corm weight of < 4 g was ranked the lowest (Figure 4B).

Leaf physiological traits: Based on table 4, the leaf carotenoid content was not significantly affected by the cultivation type but was significantly (P≤0.01) affected by mother corm weight. Heavier mother corms can better supply the plant's nitrogen requirements, leading to increased photosynthetic pigments like carotenoids.

Carotenoids act as antioxidants and play important roles in photosynthesis, photoreception, and protecting cells from damage. Various factors like nutrition, mother reserves, climate, fertilization, and agronomic practices can influence carotenoid quantity and quality.

According to table 4, the cultivation type had a significant (P≤0.01) effect on leaf chlorophyll a, chlorophyll b, and total chlorophyll, and mother corm weight also significantly (P≤0.01) influenced these chlorophyll parameters.

The highest chlorophyll a was in the aeroponic system with more than 9 g of mother corms, while the lowest was in the soil culture with less than 4 g of mother corms (Figure 5A), and the highest chlorophyll b was obtained from more than 9 g of corms in the 1/2 MS cultivation type, which is rich in micronutrients like Fe, Zn, and P for chlorophyll (Figure 5B). The highest total chlorophyll was in more than 9 g mother corms grown in 1/2 MS; the lowest was in less than 4 g mother corms (Figure 5C).

Discussion

Root weight: The severe decline in root growth despite the direct access to nutrients in the 1/2MS culture can be associated with the limited depth of the root growth chamber or inadequate oxygen supply to the roots, which causes hypoxia and the loss of root growth (Samarghandian and Farkhondeh 2020; Souret and Weathers 2000; Drew 1997; Schwarz 1995). Mechanical resistance may also hinder root growth in hydroponics (Zobel, 1991). It was reported that roots faced with a mechanical barrier were shorter, thicker, and more irregular than roots grown in the soil (Hakimzadeh Ardakani *et al*., 2023). The role of roots in water and nutrient uptake depends on the dispersion of root systems and the efficiency of individual roots (Fathizad *et al*., 2022). The significant decline in the root dry matter in all three studied culture media implies the dependence of the roots as an important plant component on the culture; this response of the saffron as compared to its soil culture has already been reported for beans grown in hydroponic and aeroponic systems (Bennie, 1991). The decline in the root growth of the saffron plants grown in the soilless culture may be

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related to the light exposure of the corms, given the fact that light is known as a root growth inhibitor (Poovaiah and Reddy, 1991; Cavusoglu *et al.*, 2009).

In this experiment, it was observed that the saffron plants cultivated in the aeroponic system adopted a proper strategy by producing thinner and capillary roots. This means that the auxiliary roots of the saffron plants kept on moisture uptake with minimum energy consumption.

Aeroponics maximizes oxygen exposure to roots by delivering nutrients via fine mist, avoiding waterlogged conditions that cause hypoxia in traditional hydroponics or soil systems (Lachguer *et al*., 2025).

Studies show that dissolved oxygen levels in aeroponic systems are critical for root health, with optimal concentrations (~8 mg/L) promoting aerobic respiration and preventing metabolic stress (Soffer and Burger, 1988). The study Homes (1979) has dealt with abscisic acid that accumulates in plants during stresses as the main reason for the decrease in root thickness and the production of more root hairs.

Aeroponics facilitates unobstructed root elongation and branching due to the absence of physical barriers like soil or aggregate media. For example, cassava roots in aeroponics showed accelerated storage root bulking, attributed to unrestricted secondary xylem expansion (Selvaraj *et al*., 2019).

Hypoxia-sensitive species like tomato and muskmelon developed denser root hairs in aeroponics, likely as a compensatory mechanism to increase surface area for nutrient uptake under stress (Czernicka *et al*., 2022).

While aeroponics inherently avoids hypoxia, studies on *Syzygium kunstleri* (a flood-tolerant woody plant) revealed that primary and secondary aerenchyma (air-filled tissues) form in hypoxic conditions to facilitate oxygen transport. However, aeroponics eliminates the need for such adaptations by maintaining high root-zone oxygenation (Sou *et al*., 2021).

Keita *et al*. (2022) used aeroponics to correlate root hypoxia with photosynthetic damage in *Capsicum*, showing leaf abscission as a survival strategy.

Aeroponic cassava systems identified auxin's role in storage root development, bypassing hypoxia-induced constraint (Selvaraj *et al*., 2019).

Flower yield: Since saffron flowers appear before their other organs, the flower formation and economic yield of saffron depend on the corm accumulated in the previous year so that corms in the next year transfer their surplus photosynthates to the underground parts to be reserved for forming new corms and initiating flowers. Obviously, petal yield increases with the increase in flower number, and finally, the flower weight increases. This increased number of flowers and the resulting increase in the flower's fresh and dry weight in the 1/2MS can be attributed to the positive effect of the mother corm weight along with nutrient availability. Since the generation of the shoot and reproductive organs is influenced by root uptake-related activities, water and nutrient mobilization rate, and photosynthesis, adequate nutrients increase the photosynthesis rate and consequently increase crop yield. On the other hand, the weight of the mother corm plays a key role in saffron flowering, which is related to the greater nutrient reserve of heavier corms. This reserve supplies the photosynthate requirement of the plant after dormancy and at the early growth stage.

Considering the nutrient solution used in soilless systems, which contains macronutrients (NH_4NO_3, $KHPO_2$, KNO_3, $CaCl_{202}H_2O$, KI) micronutrients ($MgSO_{207}H_2O$, $MnSO_{20}4H_2O$, $CoCl_{20}6H_2$, $ZnSO_{20}7H_2O$, $FeSO_{20}7H_2O$, $CuSO_45H_2O$) and other supplements (nicotinic acid, myo-inositol, glycine, and thiamine), the plant has easily been able to meet its needs in cationic forms. The special role of hormones in the growth of the flowers the plant, as well as the production of proteins necessary for the production of cell components and enzymes, has been completely effective in improving performance.

Additionally, the availability of adequate nutrients in the 1/2MS substrate, along with the positive effect of larger mother corm size, resulted in the highest flower yields. In contrast, the lower flower yields in the aeroponic system with smaller corms were likely due to insufficient nutrient reserves and suboptimal growth conditions.

The findings are consistent with previous studies on the influence of corm size on saffron flowering; the emergence percentage, the number of leaves, and the number of flowers are all functions of corm diameter. An increase of 1 cm in the corm diameter can triple saffron production (Molina *et al.*, 2010). In a study in Turkey, Cavusoglu *et al.* (2009) addressed the effect of different saffron corm sizes in greenhouse conditions and revealed that flower number and flowering time depended on the mother corm size. Our results regarding the flower yield of the saffron are consistent with those reported by (Joushan, 2020; McGimpsey *et al*., 1997; Pandy and Srivastava, 1979).

Stigma yield: The result explains that the 1/2MS cultivation, which contains easily available nutrients like phosphorus, allows better nutrient uptake by the plant roots and results in increased stigma growth and yield.

Nardi *et al*. (2022) presented an optimized protocol for the production of pharmaceutical-grade saffron, capable of yielding 3.2 g/m^2 (i.e., more than three times more than field production under optimal conditions), which met the combined standards for the treatment of macular degeneration, using hydroponic cultivation.

Feyzizadeh *et al*. (2023) reported that the type of cultivation has an effect on plant parameters, including plant length, leaf area, dry and wet weight of leaves, dry weight of corms, diameter of corms, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids.

Larger mother corms have higher nutrient reserves, faster emergence, and better utilization of resources, leading to improved reproductive growth and higher

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stigma production. The positive correlation between flower number and stigma weight suggests that the plant does not change the photosynthate allocation to the stigma under different conditions. Overall, the findings highlight the importance of optimizing both the substrate conditions and mother corm size to achieve maximum stigma yield in saffron cultivation. The interactive effects of these two factors were critical in determining the final stigma production.

Gresta *et al.* (2008) recorded big mother corms increased the total weight of progeny corms by 16.6%, and Poggi *et al.* (2010) found the large corms were three times more productive for saffron production. Mahmoudi *et al.* (2025) concluded that corms of 8-10 g size show optimum flowering.

This result can be ascribed to the differences between the substrates in water retention capacity, nutrient release rate, and physical attributes (Savvas, 2003). Ait-aubahou and El-otmani (1999) state that saffron plants do not need abundant nutrients, but the achievement of optimal yield requires proper physical features of the substrate for corm growth and propagation. A research study in France on cultivating saffron in hydroponic and soil culture conditions revealed that the stigma fresh and dry weights and the growth of daughter corms were higher in the soil culture than in the hydroponic system (Souret and Weathers 2000). In a similar study, Gholizadeh *et al*. (2016) reported that the effect of corm weight was significant on the stigma length, so the highest mean stigma length was produced by the corms that were heavier than 12 g. Likewise, Alipoor Miandehi *et al*. (2015) stated that the saffron corm weight had a significant effect on the stigma length so that the stigmas grown from larger corms were lunge.

Physiological traits: The interaction of cultivation and mother corm weight was examined for the stigma biochemical composition. The highest stigma crocin content was obtained from the aeroponic system across all three mother corm weight ranges; the next highest crocin content was from the soil culture, while the lowest was from the 1/2MS substrate.

The higher stigma crocin content in the aeroponic system may be associated with the superior aroma and color consistency (crocin) due to precise environmental control (Locatelli *et al*., 2025). The amount of crocin in soil culture decreased, which could be due to the effects of esters, such as strong winds.

Moslemi *et al.* (2021) observed that the significant decrease with increasing salt concentration (150 mM NaCl) reduced crocin levels.

Tajik *et al.* (2002) reported that the plants grown from corms that were placed at the soil surface had less access to minerals and nutrients, so they employed the mechanism of reducing the activity of biosynthesis enzymes, which increased the synthesis of stigma crocin. On the other hand, Jalali *et al.* (2022) observed that the stigmal crocin content increased with increasing the planting depth and reducing the temperature, showing that the saffron crop quality and its chemical composition depended on the growth environment.

Some studies report crocin stability under stress, while others note declines, possibly due to cultivar differences or measurement methods (Mena-Garcia *et al*., 2023)

A study on Ukrainian saffron found crocin content (another key bioactive compound) ranged from 18-33%, further highlighting the variability in saffron composition (Mykhailenko *et al*., 2021).

Yield and quality of saffron (*Crocus sativus* L.) in response to underwater stress and relative to the control treatment: increases in crocin content were recorded by 4.6% (Shamshiri *et al*., 2025).

Picrocrocin is a bitter-tasting glycoside compound found in saffron that contributes to its flavor profile. The highest picrocrocin was obtained from the 1/2 MS medium; this was attributed to the higher availability of key micronutrients like iron, zinc, manganese, and copper in the 1/2 MS medium, which can act as enzyme activators and influence saffron metabolism.

In agricultural optimization, controlled hormone treatments can enhance picrocrocin, while optimal soil altitude maximizes crocin (Mena-Garcia *et al.*, 2023).

The soil cultivation was associated with the second-highest picrocrocin content, and the aeroponic with the corm weight of less than 4 g was ranked the lowest; the reason for this could be the texture of the farm soil. Sandy-loam soils with good drainage favor higher metabolite yields compared to clay-heavy soils (Huang and Hartemink, 2020).

In a study on the effect of planting depth and the control of summer temperature on the quantitative and qualitative yield of saffron, Razavian *et al*. (2019) observed that the highest picrocrocin content was obtained from those planted at the depth of 10 cm and that the picrocrocin content was lower at higher depths. Enhances picrocrocin and safranal under optimal irrigation (70% FC), but crocin trends are not specified (Ziaei *et al*., 2024).

The effect of the substrate was significant ($P \leq 0.01$) on the stigma safranal content. But the mother corm weight had no significant effect on this trait. The safranal content was in the order of field > 1/2MS > aeroponic system.

Saffron (*Crocus sativus* L.) produces its signature aroma and flavor primarily from safranal, a volatile terpenoid compound. This research shows that soil-grown saffron often contains higher safranal levels than hydroponic or tissue-cultured saffron, and soil microbes appear to play a key role (Kumar *et al*., 2009).

Safranal is derived from carotenoids (like zeaxanthin) via enzymatic breakdown. Soil microbes enhance this process through rhizobacteria (e.g., *Pseudomonas*, *Bacillus)*; *mycorrhizal* fungi secrete enzymes that stimulate carotenoid precursor synthesis (e.g., lycopene, β-carotene) and upregulate carotenoid cleavage dioxygenases (CCDs), which convert zeaxanthin to safranal.

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On the other hand, in traditional cultivation, the saffron plant may be exposed to uncontrollable environmental stress. Therefore, beneficial microbes trigger mild oxidative stress, signaling the plant to produce more secondary metabolites (like safranal) as a defense mechanism, and fungal interactions (e.g., *Trichoderma*) mimic pathogen attacks, further boosting safranal synthesis.

Studies report 20–30% higher safranal in mycorrhiza-treated saffron (Cardone *et al.*, 2020).

Bernhoft (2008) observed that plants increased their active ingredients when exposed to environmental stresses.

Phosphate-solubilizing microbes improve phosphorus uptake, critical for terpenoid pathways, and siderophore-producing bacteria enhance iron availability, which influences pigment and aroma compound development.

The 1/2 MS substrate likely increased safranal synthesis by providing nutrients that enhance photosynthesis and photosynthate production; therefore, photosynthesis and photosynthate production have a direct relationship with essential oil synthesis (Ait-aubahou and El-otmani, 1999; Lopez *et al*., 2011).

Soilless systems (1/2MS and aeroponic) have lower safranal because controlled environments minimize the biotic/abiotic stress that stimulates secondary metabolites.

Lack of microbial interactions reduces enzymatic triggers for safranal production, and in vitro cultures often use synthetic auxins/cytokinins, which may disrupt natural terpenoid pathways. Soil-grown saffron consistently outperforms hydroponics in safranal content (Gresta *et al*., 2008). Organic saffron with richer microbial activity shows higher safranal than chemically fertilized crops (Kalantar *et al*., 2020).

Leaf physiological traits: The highest total chlorophyll was in more than 9 g mother corms grown in 1/2 MS; the lowest was in less than 4 g mother corms. Corms are underground storage organs that accumulate carbohydrates and nutrients, including nitrogen (Dewir *et al.*, 2022). Larger corms typically have greater N reserves, which are critical for chlorophyll synthesis (since chlorophyll molecules contain N in their porphyrin ring). Nitrogen is a key component of chlorophyll (Chl *a* and Chl *b*) and photosynthetic enzymes (e.g., Rubisco). Plants with more N can synthesize more chlorophyll, leading to darker green leaves and greater light absorption capacity.

More chlorophyll enhances light harvesting, increasing the rate of photon capture for the light-dependent reactions of photosynthesis.

Enhanced photosynthesis produces more photoassimilates (sugars), which can be stored in corms, promoting further growth and N storage—creating a positive feedback loop. Sugarcane with 30% higher Chl content led to 15% greater *P* and 22% larger corms (Inman-Bamber *et al.*, 2011).

Studies on taro (*Colocasia esculenta*) and crocus (*Crocus sativus* L.) show that larger corms correlate with higher leaf N, chlorophyll content, and photosynthetic rates (Aytekin and Acikgoz, 2008; Katrina *et al*., 2020). N-deficient plants exhibit lower chlorophyll (SPAD values) and reduced photosynthesis, confirming the N-Chl-P linkage (Peng *et al.*, 1993).

Larger corms have more nutrient reserves, better root growth, and higher N uptake efficiency, leading to increased chlorophyll synthesis. In soilless systems, the readily available N forms (ammonium, nitrate) can be more efficiently utilized by the plant compared to the soil-dependent N mineralization process. Nitrogen is the primary constituent of all amino acids in proteins and fats, which act as the structural components of chloroplasts and finally increase the chlorophyll content (Arisha and Bradisi, 1999). An increase in plant chlorophyll content as a result of the use of nutrient media has been reported by many researchers (Azizian *et al*., 2018; Gholinegad *et al*., 2014).

Avarseji *et al*. (2013) reported that the application of further potassium to the Hoagland solution in the hydroponic system could alleviate the adverse effects of salinity on the vegetative growth of saffron.

Leaves from corms grown from heavier than 9 g mother corms had the highest carotenoid content, while leaves from corms grown from 4 g mother corms had the lowest. Larger mother corms can better supply the plant's nitrogen requirements, leading to increased photosynthetic pigments like carotenoids.

Carotenoids act as antioxidants and play important roles in photosynthesis, photoreception, and protecting cells from damage. Various factors like nutrition, mother reserves, climate, fertilization, and agronomic practices can influence carotenoid quantity and quality.

The amount and diversity of secondary metabolites in plants depend on various factors such as climatic conditions, fertilization, and agronomic practices (Lopez *et al.*, 2011). The application of thiamine significantly increased the leaf carotenoid content of *Petunia* hybrid (Salehi *et al.*, 2016) and *Glycyrrhiza glabra* (Soltani *et al.*, 2017). Many studies have shown that the application of fertilizers increased carotenoid content.

Gholinegad *et al.* (2014) reported that the highest leaf carotenoid content of *Borago officinalis* was obtained from the compost substrate. Perez *et al*. (2007) reported that the application of organic fertilizers to peppers increased their fruit carotenoid content. According to the work done by Copetta *et al*. (2011) and Perkins-Veazie, 2007, they dealt with the carotenoid content of tomatoes increasing with the application of organic fertilizers.

Conclusion

As one of the most expensive spices in the world, saffron and its constituents, such as crocin, crocetin, and safranal, have shown various biochemical and pharmacological functions. The study examined various

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cultivation systems and mother corm weights for saffron production in dry climates. The results revealed that the cultivation systems and mother corm weights significantly impact the morpho-physiological characteristics. Using small mother corms increases the risk of reducing flower yield and also producing daughter corms unsuitable for use in future years, and on the other hand, using larger mother corms than required can lead to higher costs for producers; therefore, it is recommended to use mother corms that are heavier than 9 g. Soilless techniques could enable the large-scale, sustainable production of saffron bioactives without the constraints of traditional cultivation. This can also facilitate trade and regulatory compliance for saffron producers and manufacturers; replication of the study in other labs or environments would help strengthen confidence in the results. In total, since saffron production had a higher yield in the Murashige-Skoog cultivation, it is recommended to consider cultivating mother corms of more than 9 g in the 1/2 MS cultivation. Thus, saffron cultivation performed under controlled conditions 1/2 MS can be a convenient approach because plant growth and application of nutrition medium are both controlled, and it is possible to achieve a higher yield and a better quality. While Murashige-Skoog culture has high setup costs, its superior efficiency, scalability, and disease-free output make it highly profitable for commercial agriculture, biotech, and high-value plant production. This study did not investigate the economic factors associated with producing saffron (*Crocus sativus* L.) in various cultivation systems, which is an important area to explore in future research.

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