



## Production systems and methods affect the quality and the quantity of saffron (*Crocus sativus* L.)

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### Abstract

**Aim of study:** To compare the flowering of saffron between open field and controlled environment and to study the possibility of saffron transplanting.

**Area of study:** University of Birjand (Iran)

**Material and methods:** In a first experiment, saffron yield and quality produced by traditional production system (TPS) and by soilless one (SPS) were compared. In a second experiment, the effects of the production method, by direct planting (DP) or by transplanting plant (TP) in open field were studied.

**Main Results:** Percentage of flowering corms grown by SPS was 39% and 65%, while by TPS was 6% and 56% in 2018 and 2019, respectively. Flower and stigma yields were significantly higher by SPS than by TPS. Stigma obtained from SPS had higher *L* (lightness) and crocin. Safranal content was higher in stigma produced by TPS. Leaf and root numbers and corm weight were higher for SPS, but after transplanting there was better status for DP than for TP. At the end of the first growing season (2018-19), mean replacement corms weight (4.4 vs 3.0 g), replacement corms yield (21.3 vs 12.8 g per plant), weight of main replacement corm (11.7 vs 6.0 g) and number of large replacement corms (0.6 vs 0.1 corms per plant) for DP were significantly higher than for TP. However, during the second growing season (2019-20), the plants in TP plots improved their performances.

**Research highlights:** Saffron production was more favorable under controlled environment. Transplanting is possible, but there is a need to improve methods to gain more favorable results.

**Additional keywords:** crocin; flower; hydroponics; safranal; stigma; transplanting.

**Abbreviations used:** DP (direct planting); PM (production methods); PS (production systems); SPS (soilless production system); TP (transplanting); TPS (traditional production system)

**Authors' contributions:** MAS and HRF came up with the idea for the experiment. MAS, HRF and HS designed and performed the experiment. HK performed qualitative analysis of samples. HRF and HK analysed the data. HRF and FB coordinated the research project and paper writing. All authors read and approved the final manuscript.

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### Introduction

Saffron (*Crocus sativus* L.) plant is native to the Mediterranean region and is cultivated mostly in Iran, which has near 90% of the world production (Behdani & Fallahi, 2015). Saffron growing zones are spread from 10°W to 60°E longitude and 29-42°N latitude, between Central

Asia in the east to Spain in the west. This plant can be cultivated in very diverse environmental conditions, but the best climate for its growing is the Mediterranean climate (Koocheki & Khajeh-Hosseini, 2020). Saffron stigma and corolla have many applications in cosmetic, food, health and medical industries (Koocheki *et al.*, 2019). Crocin (responsible for the colour) and picrocrocin (responsible

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for the taste) are two main components in stigma, which constitutes 6-16% and 1-13% of saffron's dry matter, respectively. In addition, safranal (responsible for the aroma) is the most important compound among more than 160 volatile components and represents 30-70% of essential oil and 0.001–0.006% of saffron dry matter (Kiani & Minaei, 2016).

Saffron botanically is an annual plant, because each mother corm lasts for only a single year. Flowering occurs in autumn before, concurrent or even after leaf appearance. The replacement of the initial corm and its propagation occurs after flowering up to the end of the growing season in mid-spring, when real dormancy of produced corms/cormlets starts, and it continues with pseudo-dormancy during the summer season (Koocheki *et al.*, 2019).

The saffron has been traditionally grown by perennial growing cycle and its yield has a strong correlation with the corm size (Branca & Argento, 2010). The only method of traditional saffron cultivation is its production under open field conditions. Recently, however, mainly due to climate change outcomes attention has been paid to its production under controlled conditions (Behdani & Fallahi, 2015). In the last two decades, decrease in precipitation, increased temperature during flower initiation stage and consequently the abortion of some initiated flowers, delay in supplying the proper temperature for flower emergence in autumn, poor soils quality which renders difficulty the flower emergence; have led progressively to the decline in the flowering capacity of saffron in several countries (Fallahi *et al.*, 2015, 2018a). Accordingly, its production under controlled environment conditions could represent a possible solution for reducing the above-cited problems, due to the lack of soil, the proper temperature levels, and providing appropriate water availability in this production system (Behdani & Fallahi, 2015). Saffron production under the controlled environment based on the hydroponic method leads to an increase in water use efficiency, which is a great advantage in areas affected by drought stress (Sadeghi, 2013). The microclimate and nutrition of the plants can also be carefully controlled, resulting in higher yield and perhaps better product quality (Mollafilabi, 2014). Preventing the need for the labor force in a short of time (by extending the flowering period), lower flower contamination, faster and easier harvesting of flowers, reducing the prevalence of weeds and pathogens and releasing large volumes of water and land for the planting of other plants are the other benefits of hydroponically saffron production (Behdani & Fallahi, 2015).

Saffron corm has two separated stages to flower. The first stage is called flower initiation, which takes place during pseudo-dormancy stage in summer. Under the controlled environment, corms must be stored in dark conditions (to avoid etiolation, and the disproportionate growth of leaves and floral tube) for 55-150 days (the best: 90-120 days), at ~23-27°C (the best: 25 °C) and rela-

tive humidity of ~85%, for proper flower initiation (Molina *et al.*, 2004; Alonso *et al.*, 2012; Mollafilabi, 2014). The levels of CO (carbon monoxide, which should not exceed 2500 ppm) and ethylene (which can break corm dormancy) are the other important factors during this stage (Alonso *et al.*, 2012). The second stage of flowering is named flower emergence or flower appearance. During this stage, flowers appear on the surface of the soil and naturally happen around mid-autumn, what mainly depends on temperature and soil humidity. For proper performance of this stage under the controlled environment, it is necessary to reduce the temperature (~15-17 °C), provide water and light with an interval of ~8 h light and 16 h darkness (Molina *et al.*, 2004; Behdani & Fallahi, 2015).

Mollafilabi (2014) concluded that the real dormancy period of corms (around June) is the best time for corm/cormlets digging up, which are going to be used in saffron cultivation under the controlled environment. The same author also found that 85-90 days is an appropriate duration for corm incubation during summer. Sadeghi (2013) also found that application of coco-peat around the incubated corms and incubation period of 60-150 days can improve saffron flowering under controlled conditions. Maggio *et al.* (2006) revealed that saffron flowering in a soilless system was higher in perlite compared to the mixture of peat and perlite as growing media. Besides, saffron yield in glasshouse and growth chambers was double of those obtained in traditional field cultivation. Molina *et al.* (2004) also reported that with the planting density of 457 corms m<sup>-2</sup>, it is possible to produce 855 kg of saffron spice in one hectare of a greenhouse. Fallahi *et al.* (2017a) observed that flowering in the soilless culture of saffron was 6.6 times higher than its soil cultivation in the field. Souret & Weathers (2000) observed that stigma yield and quality of saffron were similar in three production systems (aeroponics, hydroponics, and soil culture). Poggi *et al.* (2010) found that stigma obtained from the controlled environment was superior in yield and quality to that produced in the field. García-Rodríguez *et al.* (2017) reported that cold storage and the incubation of corms imposed a negative effect on the quality of stigma.

After completing the second stage of flowering under the controlled environment, saffron corms most probably can be transplanted in the field for the production of replacement corms (Alonso *et al.*, 2012; Sadeghi, 2013). At the end of the growing season in mid-spring when leaves become senescent, large produced corms will be used for starting a new cycle of flowering under the controlled environment (Sadeghi, 2013). However, so far, this procedure has not been fully investigated. Molina *et al.* (2010) reported that leaves of transplanted plants grown in the greenhouse were longer but had lower photosynthesis rate than the field-grown ones and finally obtained lower number of corms at the end of the growing season. Overall, despite the feasibility of saffron production under

the controlled environment, there are still doubts about the quality of produced stigma, as well as about the possibility of corm/plant transplanting in soilless systems. This study aimed to compare stigma yield and quality produced under natural or controlled conditions and to investigate the possibility of transplanting those corms, which flower in soilless conditions.

## Material and methods

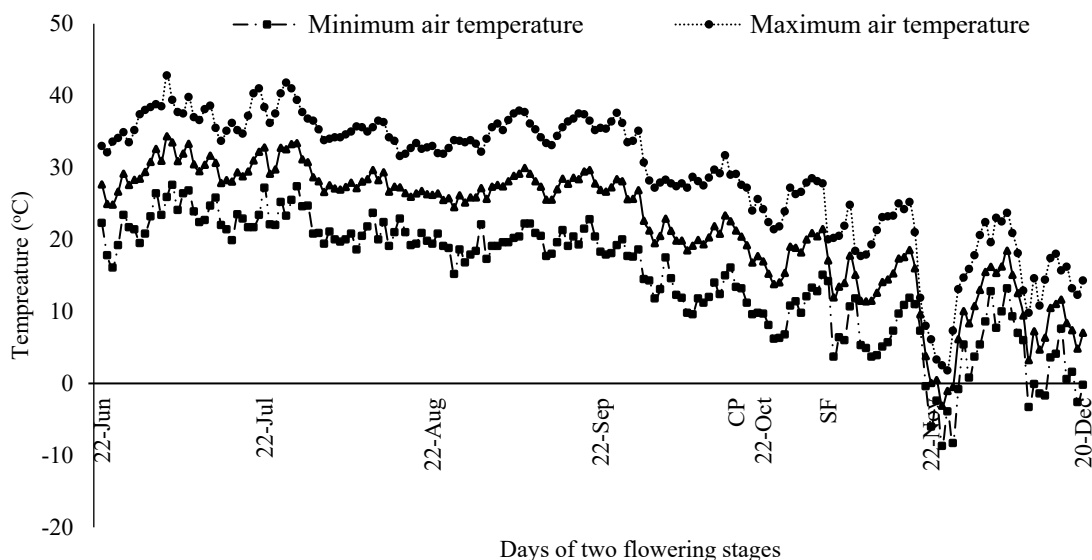
### Saffron yield and quality in relation to the production system

In this experiment, the effect of production systems (PS) and of its environmental conditions was evaluated on the yield and quality of saffron during flowering seasons of 2018 and 2019. The treatments were corm planting under traditional production systems (TPS) into the soils or under soilless production systems (SPS). In previous studies (Molina *et al.*, 2004; Sadeghi, 2013; Mollafilabi, 2014) on soilless production of saffron, both flower initiation (during summer) and flower emergence (during mid-autumn) stages occurred under controlled conditions. However, in the present study, the first stage of flowering took place in the soil in open field conditions, and only the second stage of flowering occurred in a controlled environment. Therefore, the flower initiation stage of all corms was completed under field conditions in Sarayan Faculty of Agriculture, University of Birjand, Iran. Daily air temperatures during this stage are presented in Fig. 1.

Corm batches were lifted from the soil on 15<sup>th</sup> October 2018 and 2019, then corms were separated and those which were healthy and weighed 7-9 g (2.62 cm horizon-

tal diameter), selected to be used for establishing the experiment. Each PS had three replications. In each replication, 100 corms were planted on October 17, in 90×30×15 cm trays or 1×1m plots into the soil. Plant density of 370 corms per m<sup>2</sup> was used in trays related to SPS, whereas for TPS the corms were placed at 10 cm distances between and along the single rows reaching the crop density of 100 corms per m<sup>2</sup>. In SPS, since the corms are only in the planting trays during the flowering period and the corm propagation does not occur in this environment, high planting density considered for the proper use of space. While in TPS, in addition to the flowering stage, the corms go through the vegetative growth stage and the production of replacement corms in the same environment, and planting density should be reduced (Behdani & Fallahi, 2015). However, to accurately compare the two treatments, the number of corms planted in each plot (for TPS) was equal to the number of corms planted in each planting tray (for SPS). The characteristics of soil used in the TPS plots are presented in Table 1, while for SPS the corms were grown in absence of soil on a linen fabric below corms plus 4 g synthetic superabsorbent (Table 1) per tray to hold water and nutrients for roots.

In both planting environments, the first irrigation (necessary for flower bud sprouting and flower emergence) was done on the 20th October. After corms irrigation, experimental trays were transferred to a room with a temperature of 18±1°C during days and 12±1°C during nights (similar to daily temperature trend in nature). The photoperiod was set with 8 hours of light and 16 hours of darkness (Molina *et al.*, 2004); while in TPS corms were grown under natural temperature and photoperiod (Fig. 2). The light intensity in SPS was around 26 μmol m<sup>-2</sup> s<sup>-1</sup>, which was provided by fluorescent lamps. Under SPS,



**Figure 1.** Air temperature trend during both flower initiation and flower emergence stages (2018). CP= Corm planting in field or flowering room; SF=start of flowering

**Table 1.** Soil characteristics of the field utilized for the traditional production system (TPS) and the main properties of superabsorbent polymer used for soilless production system (SPS)

Year	Soil texture	Phosphorous (ppm)	Organic carbon (%)	Total nitrogen (%)	pH	EC (dS m <sup>-1</sup> )
2018 <sup>[1]</sup>	Loam	4.9	0.21	0.019	8.10	3.02
2019 <sup>[2]</sup>	Loamy sand	4.7	0.35	0.018	8.03	3.00
Super absorbent polymer characteristics						
Density (g cm <sup>-3</sup> )	Grain size (mm)	Water holding capacity (g g <sup>-1</sup> )	Maximum durability (yrs)	pH	EC (dS m <sup>-1</sup> )	
1.1-1.5	0.5-1	330	7	7.4	1.75	

<sup>[1]</sup> First flowering season (autumn 2018). <sup>[2]</sup> Second flowering season (autumn 2019)

corms were irrigated every two days with 1% solution, which was prepared with Dalfard 15® chemical fertilizer (a specific fertilizer for saffron containing 12% N, 8% P<sub>2</sub>O<sub>5</sub>, 4% K<sub>2</sub>O, 2000 ppm Fe, 1000 ppm Zn, 1000 ppm Mn, and 500 ppm Cu). For TPS there was no irrigation up to the end of the flowering stage, but a crust breaking was applied five days after pre-flowering irrigation to help easier flower emergence through the soil.

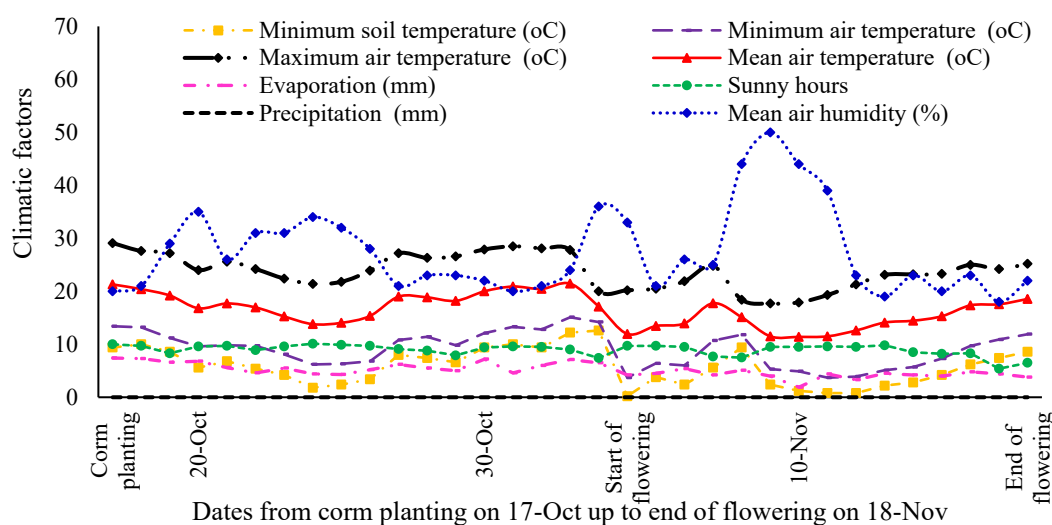
During the flowering season (started from 4<sup>th</sup> of November), flowers were counted and harvested daily by hand, and then were transferred to the laboratory for measurements of their length and weight. After that, the style and petals were separated and dried under room temperature (~25°C) at shade for 10 days. At the end of the flowering period (~two weeks), the daily sum of flowers weight was considered as flower yield. Moreover, total dried styles and petals were weighed with a precision of 0.001 g and considered as their yields.

The dried stigmas were used for qualitative evaluation including picrocrocin, crocin, and safranal content and Hunter's color parameters. ISO 3632 was used for picrocrocin, safranal, and crocin content measurement using

the UV-vis spectrometric method (ISO/TS 3632, 2011). Their contents were expressed as direct readings of the absorbance of 1% aqueous solution of dried stigma at 257, 330, and 440 nm, respectively. In addition, saffron classification was done based on ISO normative (ISO/TS 3632, 2011), which classifies the saffron into three categories according to crocin, picrocrocin, and safranal compounds. Color parameters (Hunter color values *L*, *a*, and *b*) of stigma were determined using a colorimeter (TES 135, Shenzhen Youfu Tools Co., Ltd., Taiwan) (Fallahi *et al.*, 2017b; Khayyat *et al.*, 2018).

### Saffron vegetative growth in relation to the production method

Corms which flowered in trays by SPS (in the first experiment), were transplanted to plots in the open field beside the plots of the TPS, on the 25<sup>th</sup> of December. Accordingly, we compared two production methods (PM): (1) saffron growing in the natural environmental conditions by direct planting (DP), (2) saffron grown in SPS



**Figure 2.** Climatic parameters for traditional production system (TPS) from corm planting date up to the end of flowering (2018)

and after in TPS by transplanting (TP). The soil used was similar for both PS (Table 1).

For comparison of vegetative traits of saffron grown by the two PM, six sampling dates were considered during the vegetative growth stage on the 25<sup>th</sup> of December 2018 and on 25<sup>th</sup> January, 15<sup>th</sup> February, 6<sup>th</sup> March, 9<sup>th</sup> April, and 4<sup>th</sup> May 2019. On each sampling date, four plants were removed from the soil of each plot, and then they were washed. The measured traits were: number of leaves per plant, leaf length, cataphyll (leaf sheath=white and non-photosynthetic leaves) length, number of fibrous roots per plants, mean length of roots, the total length of roots per plant, number of contractile roots per plant, the weight of mother corm, number of replacement corms per plant, weight of all replacement corms per plant and weight of main replacement corm (the largest corm in each plant, which has the most flowering capacity). In addition, at the end of the growing season, the replacement corms of five plants were removed from the soil to compare their final growth status concerning the PM. Then, the number of replacement corms in different weight groups (<3, 3-6, 6-9, 9-12, and >12 g), mean weight of replacement corms, main replacement corm weight (largest corm), and total yield of replacement corms per plant, were determined. However, because the produced replacement corms in both PMs were not in the appropriate size at the end of the first growing season (3 and 4.4 g for TP and DP, respectively), the experiment was continued for another growing season (2019-20). Our hypothesis was the new replaced

corms weight would be more favorable, if the transferred plants stay in the same field for another year. At the end of the second growing season corm growth parameters, including their number and weight, were again measured in both PM.

## Data analysis

In both experiments, SAS 9.2 was used for data analysis. The *t*-test was used to compare results between PS and PM, in terms of qualitative and quantitative parameters.

## Results and discussion

### Saffron yield and quality in relation to the production system

#### Flowering traits

There were significant differences between all flowering parameters of saffron planted in the two PS in both flowering seasons (Table 2). In the first flowering season, by fixing the planting density in the planting plots and trays, while only 6% of planted corms produced flower by TPS, the flowering percent for SPS was 38.6%. The considerable higher flowering percentage in SPS compared with TPS is due to optimal environmental conditions,

**Table 2.** Effect of production system on saffron flowering descriptors during flowering seasons of 2018 and 2019.

Flowering parameters	Year	SPS	TPS	<i>t</i> value	<i>p</i> value
Percentage of flowered corms	2018	38.66	06.00	-13.59	0.0002
	2019	65.20	56.70	-8.89	0.0009
Flower length (cm)	2018	8.48	5.25	-103.89	0.0001
	2019	7.47	5.67	-5.75	0.0045
Mean flower weight (g)	2018	0.43	0.34	-8.14	0.0012
	2019	0.41	0.36	-3.13	0.0353
Flower yield (g per 100 planted corms) <sup>[1]</sup>	2018	16.80	02.03	-24.09	0.0016
	2019	26.70	20.60	-2.79	0.0495
Stigma length (cm)	2018	6.00	3.67	-11.11	0.0072
	2019	2.51	2.34	-2.54	0.0542
Pistil fresh yield (g per 100 planted corms)	2018	1.86	0.62	-72.96	0.0001
Pistil dry yield (g per 100 planted corms) <sup>[1]</sup>	2018	0.263	0.025	-85.58	0.0001
	2019	0.416	0.324	-5.13	0.0068
Petal dry weight (g per 100 planted corms) <sup>[1]</sup>	2018	1.80	0.20	-31.38	0.0001
	2019	2.81	2.30	-3.88	0.0178

SPS: soilless production system. TPS: traditional production system. For each descriptor, the value of *p* represents the significant differences between two planting systems. <sup>[1]</sup>These 100 corms were planted in 0.27 m<sup>2</sup> (30×90 cm trays) and 1 m<sup>2</sup> (100×100 cm plots) for SPS and TPS, respectively. Therefore, to report yield values in SPS based on unit area, the table numbers must be multiplied by 3.7.

especially proper temperature, during the flower emergence stage (Koocheki & Khajeh-Hosseini, 2020) and the absence of soil physical resistance against flower emergence (Behdani & Fallahi, 2015). It has been reported that about 70% of saffron flowering variation is determined with temperature parameters. A relative increase in air temperature during flower emergence stage, will postpone blooming and will reduce the percentage of flowered corms (Askari-Khorasgani & Pessarakli, 2019; Rezvani Moghaddam, 2020). In the present study, air temperature on some days was not optimal for saffron flower emergence, which resulted in lower flowering in TPS. In addition, soil structure of the experimental plots in the first year of study was not suitable enough for flower emergence (Table 1). These two factors probably caused a severe difference in the flowering percentage of corms between the two PS.

The flowering percentages for the second growing season were 65% and 56% for SPS and TPS, respectively (Table 2). Considerable increase in flowering of TPS in the second flowering season compared with the first flowering season is probably related to the better soil properties used as corms substrate in the second growing season, where the soil was lighter with higher organic carbon and slightly less electrical conductivity (Table 1). In support of this hypothesis, Fallahi *et al.* (2018b) concluded that saffron flowers emerged more easily when corm planting was done in a soil with a lighter texture and higher organic matter content. The results of Cardone *et al.* (2020) also revealed that saffron corm planting in a loam and sandy-loam soil, not very calcareous, with a sub-alkaline and neutral pH, low electrical conductivity with a content of organic matter between 5.46 and 8.67 g kg<sup>-1</sup>, is more favorable to improve its growth and yield. In addition to soil properties, the mean air temperature during the flowering stage was around 1.2 °C lower in the second flowering season, which is considered as a motivation factor in flower emergence (Behdani & Fallahi, 2015).

Higher flowering of saffron under the controlled environment can be caused by lack of heat stress during flower initiation stage (around mid-summer), proper temperature to stimulate flower emergence (in mid-fall), and removal of soil physical resistance against flower emergence through the soil (Molina *et al.*, 2004; Fallahi *et al.*, 2018a). Considering that in the present research, in both PS, the plants at the flower initiation stage were grown under open field conditions, higher flowering under SPS mainly arises from the last two reasons. Assuming that almost all corms can produce flower when both flowering stages (flower initiation and flower emergence) be passed under a well-controlled environment (Behdani & Fallahi, 2015), we found that about 10-30% of the increase in flowered corms in SPS is related to providing optimal environmental conditions during flower emergence stage. Accordingly, it is possible to gain a higher flowering percentage, if mother corms get out from the soil during

the real dormancy period and then kept under appropriate climatic parameters up to the end of the both flowering phases (Mollafilabi, 2014). As reported by Molina *et al.* (2010), 1.5 flowers per corm were produced, when corms were kept in 25 °C for 105 days to pass their flower initiation stage (formation of flower primordia) and then were transferred to a growth chamber with appropriate temperature (17 °C). Compared with Molina *et al.* (2010), the flowering capacity of corms under SPS was lower (0.38 and 0.65 flowers per corm in the first and the second flowering seasons, respectively). One reason for this difference is the lower weight of the corms (7-9 g with 2.62 cm horizontal diameter), which were used in the present study. However, the more important reason is that in their experiment both flowering stages were carried out under favorable controlled conditions.

Molina *et al.* (2010) stated that it is possible to gain 600-700 kg ha<sup>-1</sup> spice during 8-9 harvests from September to May under greenhouse conditions, when all flowering stages were passed under an optimum controlled environment. Mollafilabi (2014) also reported that 1.38 flowers per corm (average weight of 15.5 g) were produced when saffron corms were kept under controlled conditions for 90 days during the flower initiation stage and then transferred to the flowering room during flower emergence stage. Poggi *et al.* (2010) kept saffron corms under the controlled climate during both flowering stages and then harvested 1.08 and 3.01 flowers per corm, when the weight of planted corms was 10 and 20 g, respectively. Maggio *et al.* (2006) planted corms with a diameter of 3 cm in greenhouse (ambient temperature in the dark conditions under black mulching during incubation and natural sunlight during flowering) and growth chamber (25 and 17 °C during flower initiation and flower emergence stages, respectively and artificial light during flowering). Their results revealed that the number of flowers per corm was 4.5 and 4.7 for the greenhouse and growth chamber, respectively, when perlite was used as substrate. These values were 4.9 and 5.4, respectively, when vermiculite was used as corms substrate. They also observed that the mean greenhouse temperature (average minimum and maximum daily temperatures during the corm incubation period, from the 10<sup>th</sup> August to 1<sup>st</sup> of November, were 16 and 31 °C, respectively) was very similar to the saffron requirements. This shows that in some areas high yields of saffron can be obtained under cold greenhouses, with no need to create facilities to control environmental factors.

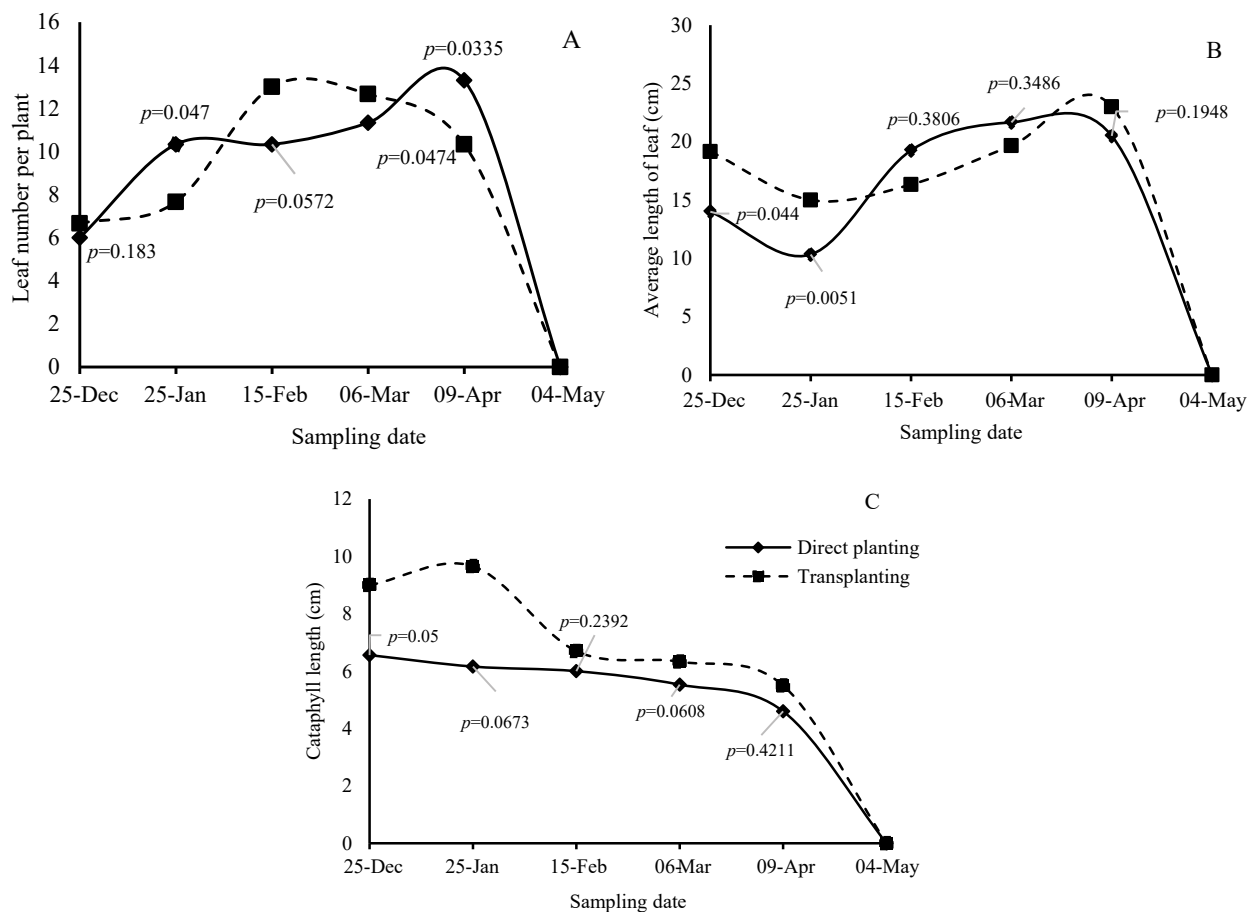
In the first and the second flowering seasons, flower length (39 and 31%, respectively), flower weight (26 and 14%, respectively), and stigma length (63 and 7%) in SPS were higher than the ones produced under TPS (Table 2). This observation probably arises from the lack of physical resistance of the planting bed against the emergence of flowers in SPS. In a previous study on saffron, also the

positive role of reduction in soil resistance against flower emergence has been proved (Aghhavan-Shajari *et al.*, 2015). One probable reason for longer flowers under SPS is due to longer cataphylls (Fig. 3C), the organs that the flowers within them reach to the top of the soil. The final outcome of improved reproductive growth of saffron under SPS was observed as increased fresh flowers (727 and 30% for the first and the second flowering seasons, respectively) and dry pistil yields (952 and 28% for the first and the second flowering seasons, respectively), in comparison with TPS, which mainly was resulted from an increase in the percentage of flowered corms (Table 2). Poggi *et al.* (2010) similarly reported that stigma production per corm under SPS conditions was twice higher than that obtained in the field. They also reported that alternated flowering and higher corm density (6-9 times) under controlled conditions remarkably increase the greenhouse production per  $m^2$ . In the study of Mollafilabi (2014), mean flower weight of saffron in the controlled environment was 0.53 g, which is around 22% higher than in our observations. Similarly, flower yield was around 4 times higher than of those obtained in our

study. This differences on one hand is due to more corm weight used in their experiment (15.5 g vs 8 g in ours) and on the other hand again shows the vital role of flower initiation stage on flowering parameters, because in their study both stages of flowering passed out under appropriate condition in the controlled environment. Considering heat stresses occurred during the flower initiation stage (mid-summer) (Fig. 1), which was higher than the results of Molina *et al.* (2004, 2010) on the appropriate temperature for this stage ( $\sim 25^\circ C$ ), the appropriate results of saffron production in SPS, especially in areas with hot summers, certainly will gain when both stages of flowering passes out under controlled conditions.

### Quality of stigma in relation to the production system

The production system had a significant effect on  $L$  and a color parameters, and also safranal and crocin content of stigma at least during one flowering season (Table 3). Stigmas obtained from SPS had higher  $L$  and crocin



**Figure 3.** Effect of production method of saffron on number of leaves (A), leaf length (B) and cataphyll length (C) during its vegetative growth stages. The green parts of leaf considered in measurements. Date of transplanting: 25<sup>th</sup> December 2018 (The first sampling was also done on this day). For each descriptor and sampling date, the value of  $p$  represents the significant differences between two planting methods based on  $t$ -test.



**Table 3.** Effect of production system on saffron stigma quality during flowering seasons of 2018 and 2019.

	<i>L</i>		<i>a</i>		<i>b</i>		Safranal ( $\lambda_{1cm}^{1\%}$ ) (330 nm) <sup>[1, 4]</sup>		Crocin ( $\lambda_{1cm}^{1\%}$ ) (440 nm) <sup>[2, 4]</sup>		Picrocrocin ( $\lambda_{1cm}^{1\%}$ ) (257 nm) <sup>[3, 4]</sup>
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2019
SPS	44.2	49.95	20.1	16.46	8.67	8.16	44.2	40.13	182.7	186.2	78.7
TPS	27.9	48.66	27.9	10.58	8.72	7.60	54.2	47.33	170.7	170.9	77.1
<i>t</i> value	-3.93	-0.52	3.25	-5.57	0.07	-0.41	7.27	4.69	-0.95	-3.89	-0.89
<i>p</i> value <sup>[5]</sup>	0.0170	0.6304	0.0315	0.0051	0.9477	0.7008	0.0019	0.0093	0.3945	0.0176	0.320

SPS: soilless production system. TPS: traditional production system. <sup>[1]</sup> Absorbance of 1% aqueous saffron extract at 330 nm. <sup>[2]</sup> Absorbance of 1% aqueous saffron extract at 440 nm. <sup>[3]</sup> Absorbance of 1% aqueous saffron extract at 257 nm. <sup>[4]</sup> Based on ISO normative, stigmas produced in both production system belonged to II, I and I categories in terms of crocin, picrocrocin and safranal content, respectively (see more information in Cardone *et al.*, 2020). <sup>[5]</sup> For each descriptor, the value of *p* represents the significant differences between the two planting systems.

values while safranal content was significantly lower in comparison to TPS (Table 3). This means that stigma produced under TPS was some darker in appearance but had a higher aroma. There is low information about the quality of saffron produced in SPS. In a primary research on saffron, it was reported that crocin and crocetin content were higher in stigma obtained by aeroponically grown corms, while picrocrocin was a little bit greater in soil culture (Souret & Weathers, 2000). In the study of García-Rodríguez *et al.* (2017) the corms were first kept in ultra-low oxygen cooling chambers for 40 and 70 days, then transferred to different incubation periods (0, 30, and 60 days) and finally were grown in hydroponics in a flowering room. Their results showed that the cold storage and the incubation time provided a negative effect on the quality parameters including safranal, crocin, and picrocrocin contents than control treatment (corm planting in the flowering room at 18 °C without any ultra-low oxygen or incubation pre-treatment). In their study, there was no control field-grown treatment. However, safranal content of hydroponics samples was higher, while picrocrocin content was similar to those reported in other open field studies. Finally, they stated that some treatments were able to provide stigma of high quality according to the ISO standard. Mollafilabi (2014) also found that saffron produced in stone wool and peat moss beds under the controlled environment, was graded as excellent according to Iranian national standards for stigma quality. Poggi *et al.* (2010) compared the quality of stigma produced under greenhouse with others obtained in field and concluded that crocin, picrocrocin, and safranal contents of saffron produced in the chamber was better (values ~20% higher) according to ISO 3632-1 specification. In the present study, based on ISO normative, stigmas produced in both production systems belonged to II, I, and I categories in terms of crocin, picrocrocin, and safranal contents, respectively (Table 3). Overall, it seems that saffron production under SPS can meet the indices defined in saffron quality standards, although more studies are required to judge accurately.

### Saffron growth in relation to the production method

#### The aerial parts growth trend

Based on t-test results, there were significant differences between DP and TP methods in terms of the number of leaves per plant in most sampling dates during saffron growing season. Production of new leaves in DP continued up to early April, while the maximum number of leaves in TP was obtained in mid-February (Fig. 3A). Transplanted plants had significantly more leaf length up to late January, but after that, there were no significant differences between the two planting methods (Fig. 3B). In a similar study on saffron, leaves of the transplanted plants were longer with higher leaf area but had a lower photosynthesis rate than the DP ones (Molina *et al.*, 2010). In both DP and TP treatments, the highest values of leaf length were obtained in early April; then, simultaneously with the onset of leaf aging, showed a sharp decreasing trend (Fig. 3B). In saffron, at the end of the growing season simultaneous with an increase in temperature and decrease in soil moisture, photoassimilates reallocate from the leaves to the new cormlets and the leaves start to senescence (Lopez-Corcoles *et al.*, 2015). The change in sink-source relations and reallocation of photoassimilates during the last growing phase of saffron has also been reported by Behdani *et al.* (2016) and Fallahi & Mahmoodi (2018a). Similar to our findings, Gholami *et al.* (2017) in an open-field study found that saffron leaf length reached its highest value (from 7 to 40 cm) between 30 and 110 days after emergence, while during the two last months of the growing season was constant. Reduction in leaf length during the first month of sampling (Fig. 3B) is due to the high production rate of new leaves during the early phase of vegetative growth (Behdani & Fallahi, 2015). Cataphyll length in TP was higher than in DP throughout the vegetative growth stage, although this difference was statistically significant only at the first sampling date (Fig. 3C). These white membranous

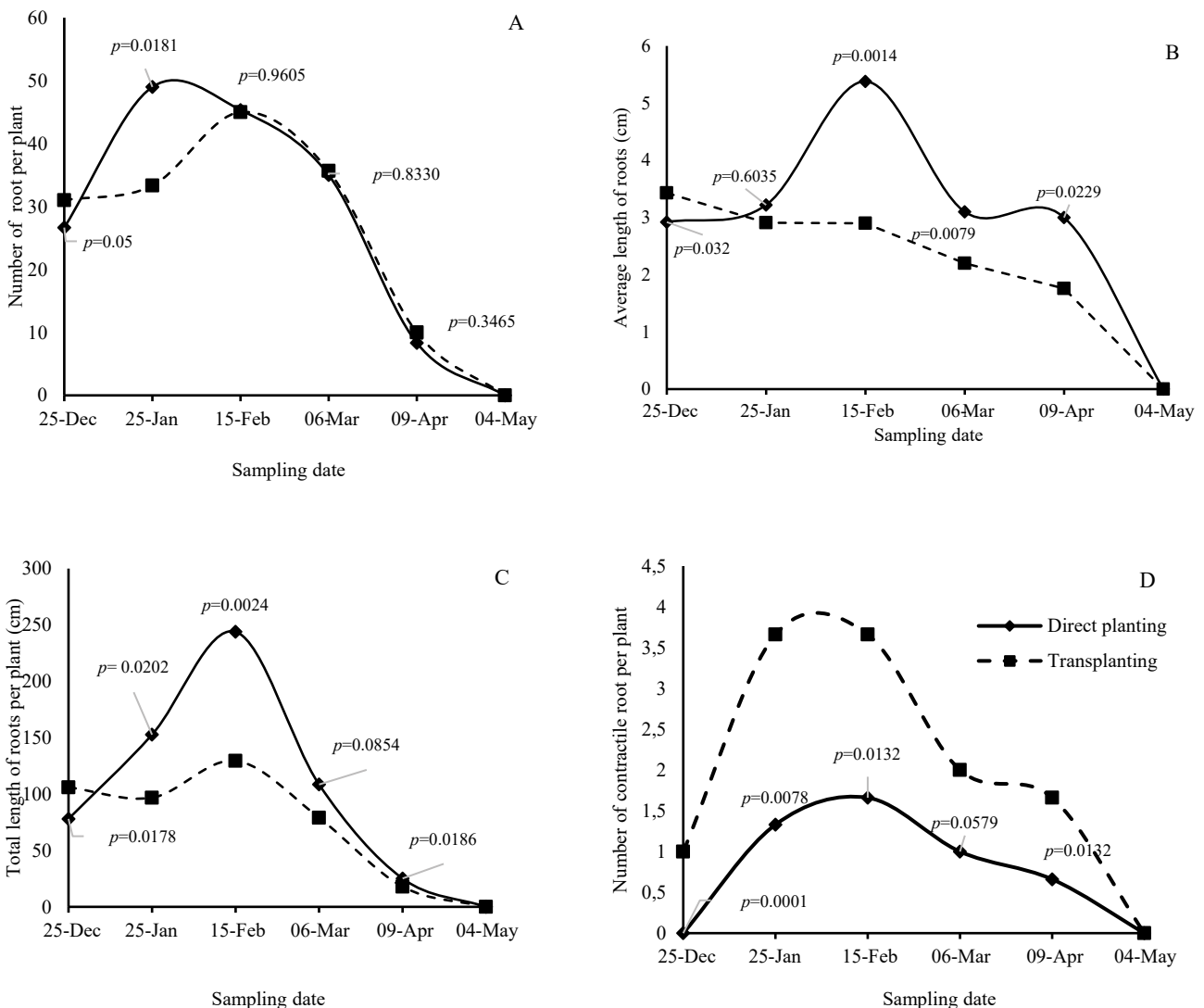


structures cover and protect the true green leaves of saffron as such as the vegetative apex. Behdani & Fallahi (2015) reported that early irrigation causes cataphylls to manifest before blooming therefore, more water availability in the TP (when they were under controlled condition) may be the reason for longer cataphylls.

### Saffron root growth in relation to the production method

The effect of the planting method was significant on the number of roots per plant for the first (transplanting day) and the second sampling dates. TP plants showed more roots than DP ones, and the latter showed the same amount of roots one month after transplanting. This index

had similar amounts in the two production systems, after mid-February (Fig. 4A). In relative similarity with the findings of Renau-Morata *et al.* (2012), the trend of new root production during vegetative growth was increasing up to February and then followed by a rapidly decreasing trend in both methods of planting (Fig. 4A). Accordingly, Fallahi & Mahmoodi (2018b) found that foliar nutrition is more effective than soil application of nutrients, when the growth of the root system starts to decrease. Average root length had a different trend between the two production methods. Corms that emerged under the controlled environment had longer roots (in transplanting day) but their enlargement stopped after transplanting, while root the length in corms that were planted directly in the soil increased up to the mid-February and then decreased (Fig. 4B). Therefore, the root was longer in the controlled



**Figure 4.** Effect of production method of saffron on the number of roots (A), root length (B), total roots length (C) and number of contractile roots (D) during its vegetative growth stage. Date of transplanting: 25<sup>th</sup> December 2018 (The first sampling was also done on this day). For each descriptor and sampling date, the value of p represents the significant differences between two planting methods based on *t*-test.

environment but up to a short period after transplanting. Accordingly, it seems that transplanting caused a stress, which resulted in a reduction in root system growth. Souret & Weathers (2000) stated that root development of saffron under hydroponic conditions decreases if oxygen supply for the root system is low or if the root is exposed to light. Overall, root length -especially during February and March with significant differences- was higher in DP during most part of the vegetative growth stage (Fig. 4B).

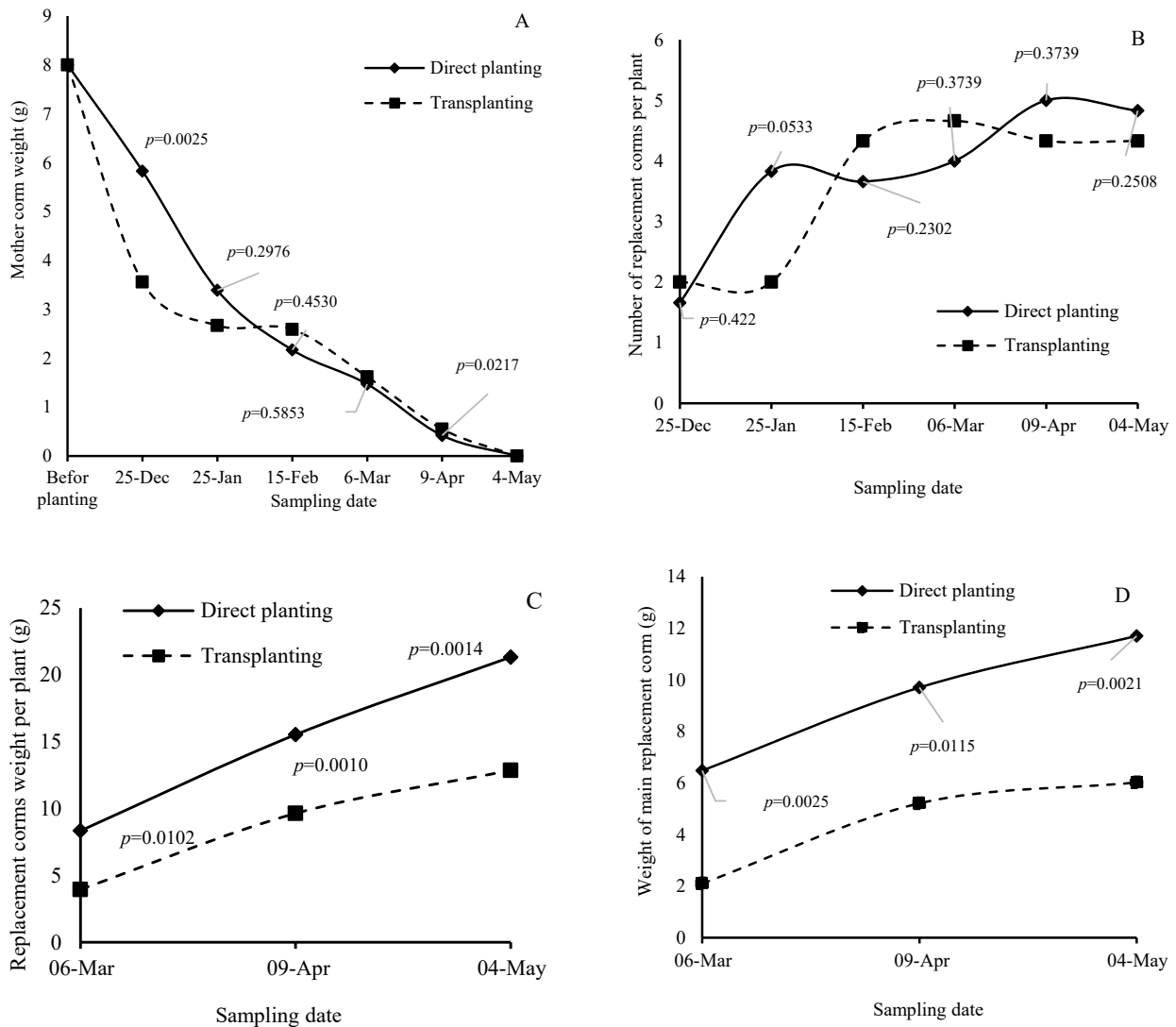
Root number and length, calculated as total root length per plant, showed an increasing trend up to mid-February and then decreased for both DP and TP. Although total length of the root system was higher under the controlled environment compared with DP, the TP showed reduced values of the above-cited characteristics, especially during January and February, when these differences were different significantly (Fig. 4C). The number of contractile roots had a similar trend between the two methods of saffron planting, but with higher amounts in TP during the whole plant growing season. The differences between the two planting methods were significant during plant growing season and the highest values were gained during February (Fig. 4D). Souret & Weathers (2000) reported that saffron plants were grown hydroponically or aeroponically had shorter and thicker roots and more contractile roots than soil cultured plants. They stated that the unusual morphology of roots in their experiment was due to mechanical impedance associated with the NFT (nutrient film technique) channel in hydroponic and the wall of mist chamber in aeroponics culture systems. Moreover, they said that contractile roots production, which reduces corms growth, may be related to planting depth and light intensity. In another study, the high soil humidity, inappropriate planting date, and also untimely irrigation (delayed spring, summer, and early fall irrigations) were considered as the reasons for the increase in contractile roots development in saffron (Koocheki *et al.*, 2016).

#### *Saffron corm growth in relation to the production method*

Differences between the two planting methods in terms of mother corm weight were significant only in the first sampling date (25<sup>th</sup> December = transplanting day). After two months from the first irrigation time, 55.5 and 27.2% of mother corm reservoirs were depleted by both TP and DP, respectively (Fig. 5A). The main result of this event was an increase in flowering percent in SPS (Table 2), because flowers produce only by relying on planted corms reserves (Koocheki *et al.*, 2019). Results of Renau-Morata *et al.* (2012) under field conditions also revealed that 20-30% of the mother corm reservoirs are used for the flowering and the emergence of the roots and leaves. However, after transplanting of those corms that

were flowered under controlled condition (TP), there was no significant difference between them and the DP ones in terms of mother corm weight (Fig. 5A). Mother corm reservoirs were used to produce flowers, leaves, and roots (Behdani *et al.*, 2016). Rapid breakdown of reservoirs of mother corms in soilless culture led to further growth of aerial (Figs. 3A, B, C) and underground (Figs. 4A, B, C, D) parts of saffron at the beginning of the growing season. This is a useful occurrence because a higher rate of vegetative growth after flowering will lead to further growth of replacement corms, provided that this condition was continued after transplanting.

Corm proliferation affected negatively by transplanting, so that, number of corms per plant in DP and TP were 3.83 and 2, respectively, one month after transplanting which were, respectively, 70% and 0%, higher than the date of transplanting. However, these significant differences disappeared in the next sampling dates during vegetative growth and reached to 4.83 and 4.33 replacement corms per plant for DP and TP, respectively, at the end of the growing season (Fig. 5B). The highest rate of corm proliferation for DP was during January, but for TP was with a one-month delay, during February (Fig. 5B), which indicates a stress imposition on the transplanted plants exactly after transplanting. This time delay in corm proliferation resulted in a less significant yield of replacement corms (Fig. 5C) and lower weight of main replacement corm (Fig. 5D) in TP compared with the DP, during the last two months of the growing season. Really, the active period for corm filling in TP was one month shorter with a delay in corm proliferation. The yield of replacement corms per plant and the weight of the main replacement corm at the end of the growing season for DP were 66% and 94% higher than TP, respectively (Fig. 5C, D). Accordingly, transplanting of corms that flowered under the controlled environmental conditions could not produce replacement corms with the desired weight which can be used in the new flowering cycle under SPS. In our research, there was no specific nutritional management for transplanting treatment despite low availability of nutrients in the soil (Table 1). Therefore, it seems that we need an appropriate nutrition program both before and after the TP or we should try to find new strategies to reduce the negative effect of stress caused by transplanting. Similarly to Behdani *et al.* (2016), our results also revealed that the last two months of vegetative growth, when the production rate of new corms was very slow (Fig. 5B), is a critical period for replacement corms growth (Fig. 5C, D). In the study of Fallahi & Mahmoodi (2018a) also the highest rate of corm proliferation (mid-February up to mid-March) was observed before the highest rate of corm filling (End-March up to mid-May). Renau-Morata *et al.* (2012) found that in saffron when leaf and root development ends, the exponential growth stage of replacement corms starts. In another study on saffron, also the highest



**Figure 5.** Effect of production method on the mother corm weight (A), number of replaced corms (B), weight of all replaced corms (C) and weight of main replaced corm (D) during saffron vegetative growth stage. Date of transplanting: 25<sup>th</sup> December. For each descriptor and sampling date, the value of p represents the significant differences between two planting methods based on *t*-test.

crop growth rate was obtained during the last two months of the growing cycle (Fallahi & Mahmoodi, 2018b). Gholami *et al.* (2017) also reported that rapid replacement corms growth starts about 80 days after leaves emergence.

#### *Final growth of replacement corms in relation to the production method*

The planting method had a significant effect on most criteria related to the final growth status of replacement corms (Table 4). Replacement corm growth parameters in terms of number, weight, and percentage of large produced corms in DP were higher than TP method at the end of the first growing season (Table 4). In a similar study on saffron, Molina *et al.* (2010) concluded that the plant dry weight and number of replacement corms at the end

of the vegetative growth stage in spring, were higher in the field-grown plants compared to TP ones. Moreover, the replacement corms in TP plants were so small, which was unlikely to produce flowers in the following season. The same authors also found that lower photosynthesis in transplanted plants may be the reason for that observation. In their study, although transplanting of plants from the growth chamber to the field resulted in a higher photosynthetic rate, it did not reach that of the DP plants. In addition, stomatal conductivity in the field-grown plants was considerably higher than in those that flowered in the controlled environment (Molina *et al.*, 2010). Totally, at the end of the first growing season we found that without a proper strategy, transplanting of corms that flowered under controlled conditions cannot lead to a favorable outcome in terms of replacement corms growth. Therefore, we guessed that more favorable results would be achieved

**Table 4.** Effect of production method of saffron on final growth status of replacement corms at the end of first (2019) and second (2020) growing seasons

Parameter	Weight Group	Year	DP	TP	value	p value
Number of replacement corm per plant in different weight groups	<3 g	2019	3.16	2.50	-2.00	0.1161
		2020	8.20	2.04	11.58	0.0003
	3-6 g	2019	0.31	1.15	25.00	0.0016
		2020	0.10	2.12	-4.48	0.0110
	6-9 g	2019	0.48	0.31	-2.24	0.0890
		2020	0	0	-	-
	9-12 g	2019	0.27	0.27	0.00	1.00
		2020	0	0	-	-
	>12 g	2019	0.6	0.1	-8.66	0.0131
		2020	0	0	-	-
Total	2019	4.83	4.33	-1.34	0.2508	
	2020	8.30	4.16	7.57	0.0016	
Mean replacement corm weight (g)	2019	4.41	3.00	-10.11	0.0005	
	2020	1.36	3.45	-6.46	0.0030	
Yield of replacement corms per plant (g)	2019	21.33	12.86	-7.83	0.0014	
	2020	11.30	13.83	-1.93	0.1259	
Weight of main replacement corm (g)	2019	11.70	6.02	-7.05	0.0021	
	2020	2.62	5.52	-8.97	0.0009	

DP: direct planting. TP: transplanting. For each descriptor and for each year, the value of *p* represents the significant differences between two planting methods based on *t*-test results.

if the transplanted plants stay in the same field for another year. At the end of the second growing season, although the mean replacement corm weight in TP increased by 15% compared with the end of the first growing season (Table 4), but the results were still not as desired. It should be noted that no new strategy like nutritional management was also applied during the second growing season. In addition, in the DP method the mean weight of replacement corms at the end of the second growing season reduced severely mainly due to increasing competition for resources resulted from a considerable increase in the number of replacement corms (72%) per plant in that year compared with the end of the first growing season (Table 4). These results are in a good agreement with those reported by Fallahi *et al.* (2018b) who revealed that the end of the first growing season is more suitable time for obtaining big replacement corms when large mother corms (6 g) are used for planting, while the end of the second flowering season is a more favorable time to obtain big replacement corms, when planted mother corms are small (<4 g).

Overall, although we found that saffron transplanting is possible; but more researches are needed on how it is possible to overcome the problem of this method. In a recent study on saffron transplanting, Fallahi *et al.* (2021a) for increasing the weight of replacement corms, used the

inputs (such as water and fertilizers) in large quantities, compared to the conventional (traditional) saffron cultivation. The results showed that at least one third of the replacement corms yield in transplanting method was at the desired weight. The same authors also proposed that other items such as transplanting depth, soil organic matter content, soil texture, transplanting as basin or in-furrow, etc. might probably affect the success of saffron transplanting. Maggio *et al.* (2006) found that a mixture of peat and perlite as substrate was more suitable for increasing saffron corm growth under cold glasshouse, compared with pure perlite. It should be noted that variation in nutrients, pH, and EC could strongly affect saffron growth in soilless systems than soil-based culture due to the buffering capacity of soils. Therefore, precise regulation of these factors in the planting bed of SPS is crucial for optimal plant growth (Askari-Khorasgani & Pessaraki, 2019). In another study on saffron, corms that were flowered in incubator were transferred to pots filled with perlite, in an open room. The hypothesis was that it is possible to obtain replacement corms with the suitable weight, by improving the physical properties of the planting bed. However, the production of contractile roots, early drying of the aerial parts (in mid-March) and no full allocation of mother corm reservoirs to plant vegetative growth,

led to the rejection of the hypothesis (Fallahi *et al.*, 2021b). The same authors proposed that combined application of organic and mineral substrates and corm planting from the beginning in non-soil substrates (to remove the stress caused by transplanting) could probably lead to more favorable results.

In conclusion, the articulated plan of trials carried out provides information on the effects of production systems and methods for saffron production. The great interest for reducing the surface and to increasing the yield and the quality of the products suggested using soilless growing in some growth stage. The traditional production system in the open field could be integrated during the first growing cycle from the soilless cultivation of the corms and increase the production of propagation organs during the following years. Saffron had a much higher flowering capacity and stigma yield in SPS than in the TPS under field conditions. In the SPS, the value of the chromatic parameter L increased but the values registered for a chromatic parameter and for the safranal content decreased compared with TPS. The SPS supported much better growth of saffron plant, both for epigeous and hypogeous organs, but transplanting it in the open field reduced all these parameters compared with the direct planting method.

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